QUANTITATIVE EPIDEMIOLOGICAL STUDIES ON RECURRENT AIRWAY OBSTRUCTION IN THE HORSE POPULATION OF GREAT BRITAIN USING A RISK-SCREENING QUESTIONNAIRE

by

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ABSTRACT

The principal aim of this study was to investigate the epidemiology of recurrent airway obstruction (RAO) in horses in Great Britain using a risk-screening questionnaire (RSQ). Three processes were used to aid construction of the RSQ for RAO, namely: a review of the scientific literature, a survey of equine practitioners in the UK and a modified Delphi consultation with experts in the field of RAO. The Delphi process helped generate many of the questions for inclusion in the RSQ for RAO. The quantitative outputs provided estimates of the probabilities of a horse having RAO, for each particular piece of historical information or clinical sign; this provided one method of scoring the RSQ.

The RSQ for RAO was a short and simple questionnaire for completion by horse owners. Analysis of an owner completed RSQ, using the Delphi scoring method, provided a score (between 0 and 1) indicating the likelihood of a horse having RAO. The RSQ was validated against a reference standard of a veterinary diagnosis that included the use of respiratory cytology. A receiver operating characteristic (ROC) curve was used to select a positive cut-off of 0.87 for the RSQ for RAO; this suggested that the RSQ had a sensitivity of 0.83 and specificity of 0.85 for the diagnosis of apparent RAO (compared to all other diagnoses). An alternative method of scoring the RSQ was investigated using Homogeneity Analysis by means of Alternating Least Squares (HOMALS procedure). After comparing the two methods it was elected to use the Delphi scoring system when the RSQ was employed in future surveys. A survey of horse owners in Great Britain was conducted using a horse health questionnaire that incorporated the RSQ for RAO. The aim of the study was to estimate the prevalence of RAO in the horse population of Great Britain and to investigate possible risk factors. The study sample was selected using a geographically stratified two-stage cluster sampling of horse owners from throughout Great Britain. The first stage was the random selection of veterinary practices followed by a random selection of their clients; 1431 questionnaires were mailed. Using the RSQ the estimated true prevalence of RAO in the horse population of Great Britain was 14%.

The demographic information, generated by the questionnaire, enabled investigation of risk factors associated with the disease using multilevel, multivariable logistic regression. Two models were constructed. The first related to host and environmental risk factors and the second explored the effect of early life factors. The host and environmental model identified an increased risk of RAO in association with increasing age and the horse residing in an urban or semi-urban environment. There were also some associations that were contrary to what would be expected from knowledge of the aetiology of RAO. In particular, horses fed soaked (wet) hay had increased odds of having RAO, whilst horses fed dry hay had decreased odds. The early life model identified an increased risk of a horse having RAO if its owner had acquired them after the age of two years or that in early life it had been fed hay or had a respiratory infection.

The final stage of the study was to develop and assess an educational package for horse owners regarding the disease. This was done by the creation of booklets concerning airway health and one concerning ragwort poisoning. Copies of one or other of the booklets were randomly mailed to horse owners who had been recruited during the horse health survey. The impact of this educational intervention was assessed using a mailed questionnaire. A greater impact was made on different groups within the study. For example, owners of horses that had been designated as positive for RAO by the RSQ in the previous survey were more likely to indicate that the respiratory booklet had contained information of which they were previously unaware. In conclusion, RAO appears to be worryingly prevalent in the horse population of Great Britain; a real concern in terms of welfare. Much can be done to alleviate this chronic disease by controlling a horse's environment to reduce respiratory challenge. Greater emphasis could be placed on assisting horse owners in making this transition by providing detailed guidance.

DECLARATION

I, Joel Hotchkiss, declare that the work in this thesis is original, was carried out solely by myself or with due acknowledgements. It has not been submitted in any form for another degree or professional qualification.

Joel Hotchkiss

Parts of this thesis have been accepted for publication or presentation elsewhere.

ABSTRACTS

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LIST OF ABBREVIATIONS

95%CI	95% confidence interval
AIC	Akaike information criterion
AUC	Area under the curve
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BETA	British Equestrian Trades Association
BEVA	British Equine Veterinary Association
COPD	Chronic obstructive pulmonary disease
DEFRA	Department for Environment, Farming and Rural affairs
ECRHS	European Community Respiratory Health Survey
GAM	Generalised additive model
GLMM	Generalised linear mixed model
HORFH	Home of Rest For Horses
HOMALS	Homogeneity analysis by means of alternating least squares
IAD	Inflammatory airway disease
ICC	Intraclass correlation coefficient
ISAAC	International Study of Asthma and Allergies in Childhood
IUATLD	International Union against Tuberculosis and Lung Disease
IQR	Interquartile range
OR	Odds ratio
PQL	Penalised quasi-likelihood estimates

RAO	Recurrent airway obstruction
RCVS	Royal College of Veterinary Surgeons
ROC	Receiver operating characteristic
RSQ	Risk-screening questionnaire
s.d.	Standard deviation
s.e.	Standard error
SPAOPD	Summer pasture associated obstructive pulmonary disease
TDM	Total design method
Th2	T-helper 2 cells
TTA	Transtracheal aspirate
TW	Tracheal wash
TWF	Tracheal wash fluid
UK	United Kingdom
R_L	Total resistance
C_{dyn}	Dynamic compliance
TBFV	Tidal breathing flow-volume
FEFV	Forced expiratory flow-volume
Max ΔPpl	Maximal intrapleural pressure change
$PaCO_2$	Arterial carbon dioxide partial pressure
PaO_2	Arterial oxygen partial pressure

CHAPTER 1

LITERATURE REVIEW

Section 1.1. Historical Review

A respiratory disease of horses with striking similarities to the disease known today as recurrent airway obstruction (RAO) has been recognised at least since the days of Aristotle in 333 BC. His treatise "Historia Animalism" (333 BC) described an incurable disease affecting the horse characterised by a "drawing in of the flank" (Smith, 1976). Through the subsequent centuries a disease known as 'broken wind' has been reported (mainly through plagiarism) from Hemerius (400 AD) to Blundeville, Markham and De Grey in the 1600s (Smith, 1976). They describe pathology, such as air pipes clogged by "gross humour," and clinical signs that a modern day clinician would recognise as RAO. Some of these authors even recommended that the best diet for such a horse would be hay sprinkled and moistened with water in winter, grass in summer and that the door of a stall should be left open in summer! In some ways our recognition and management of this disease has not advanced greatly.

Section 1.2. Terminology

In more recent times, the disease has been associated with a plethora of names including pursey, broken wind, chronic emphysema, chronic pulmonary disease, chronic bronchiolitis/bronchitis, heaves, small airway disease, inflammatory airway disease, hay sickness and, in recent decades, fairly uniformly as chronic obstructive pulmonary disease (COPD). Emphysema was a widely used term, because the lungs of affected horses often failed to collapse when the thorax was opened at post mortem. However, this is thought to be as a result of bronchospasm and mucus plugging rather than due to destruction of the alveolar septa that support the bronchioles, which is the true definition of emphysema (Lowell, 1964, Thurlbeck and Lowell, 1964, Nicholls, 1978). It was two of these authors who may have been the first to suggest the disease was a result of the inhalation of an agent, possibly *Aspergillus,* and that it was reversible (Lowell, 1964, Thurlbeck and Lowell, 1964).

The use of the term COPD was particularly problematic in comparative pulmonary research as it also applies to an unrelated condition in humans characterised by a persistent and progressive limitation of expiratory airflow; whereas the condition in equids is far more analogous to the asthma syndrome where airflow limitation is variable and reversible (Jeffery, 1994). Prompted by the confusion surrounding the terminology and the various definitions used to identify this disease, an International Workshop of Equine Chronic Airway disease was held at Michigan State University in June 2000 in an attempt to clarify the situation and develop a unified approach to future research (Robinson, 2001b). This committee recommended that the terms Recurrent Airway Obstruction or 'heaves' should be used for describing the mature horse with airway obstruction that is reversed by a change in environment or use of bronchodilators (Robinson, 2001b). The participants established the status of knowledge regarding the disease and suggested criteria for defining the RAO phenotype (section 1.7).

Section 1.3. Aetiology and Pathogenesis

RAO is a syndrome affecting equids that encompasses clinical signs ranging from mild exercise intolerance to extreme respiratory distress. It is believed to be the manifestation of a hypersensitivity to dust, moulds and spores in the environment of a susceptible horse (McPherson *et al.*, 1979b, McGorum *et al.*, 1993d). It is rare in climates where animals are housed outside all year round and is common in climates where horses are stabled and fed hay for long periods of time (Derksen, 1991b); this is particularly the case in temperate climates (like the UK) where horses are housed for the winter period.

The main pathological feature of RAO is a diffuse, chronic bronchiolitis characterised by bronchiolar epithelial cell hyperplasia, goblet cell metaplasia, airway smooth muscle hypertrophy, peribronchial lymphocytic infiltration, airway luminal neutrophils, excess mucus production and acinar over inflation (Nicholls, 1978, Derksen *et al.*, 1985a, Naylor *et al.*, 1992). Histological lesions have a high correlation with clinical signs in horses with RAO (Kaup *et al.*, 1990, Naylor *et al.*, 1992).

RAO is thought predominately to be a delayed type hypersensitivity response, although an immediate type response is also probably involved (Robinson *et al.*, 1996). This hypersensitivity leads to bronchoconstriction, accumulation of mucoid secretions in the airways and mural inflammation that culminates in airway obstruction (Robinson *et al.*, 1996). There is strong support for an immune mediated process, rather than a non-specific response (Halliwell *et al.*, 1979, Mair *et al.*, 1988, McGorum *et al.*, 1993b), involving at least a partial local immune response (Winder and von Fellenberg, 1986, Watson *et al.*, 1997). Evidence for immediate type hypersensitivity includes the presence of increased local IgE concentrations (Halliwell *et al.*, 1993, Schmallenbach *et al.*, 1998). It also is likely that T-helper 2 cells (Th2) and Th2-type cytokines (such as interleukin-4 and interleukin-5) play a significant role in RAO, these cells and mediators being central to an immediate type response (Franchini *et al.*, 1998, Aggarwal and Holmes, 1999, Horohov, 2000, Lavoie *et al.*, 2001, Cordeau *et al.*, 2004). However, other researchers have not identified a cytokine profile of a Th2 response in horses with RAO (Giguere *et al.*, 2002, Ainsworth *et al.*, 2003).

The presence of a delayed hypersensitivity type reaction is suggested by the identification of increased histamine concentrations in bronchoalveolar lavage fluid (BALF) several hours after challenge (not immediately) (McGorum *et al.*, 1993c). There is also evidence for the involvement of Type 1 helper cells that are involved in delayed type hypersensitivity (Halliwell *et al.*, 1993, Aggarwal and Holmes, 1999, Giguere *et al.*, 2002). The neutrophilia in BALF may be partly explained by the identification of the two cytokines, interleukin-8 and leukotriene B_4, in high concentrations in BALF; both are chemotactic for neutrophils (Franchini *et al.*, 2000, Lindberg *et al.*, 2004). The bronchospasm that occurs during RAO crisis is mediated totally via cholinergic (muscarinic) mechanisms (Broadstone, 1988, Robinson, 2001c).

Stabling of horses with RAO in an unsuitable environment (typically a poorly ventilated stable with straw bedding) and the feeding of dry hay will cause signs of disease to become apparent (McPherson *et al.*, 1979b, McGorum *et al.*, 1993d). Allergens, such as *Faenia rectivirgula* and *Aspergillus fumigatus*, present in forage or bedding have been shown to induce disease in susceptible horses (McPherson and Thomson, 1983, Clarke, 1987b, Derksen *et al.*, 1988). Antigen challenge results in recruitment of neutrophils, eosinophils and lymphocytes to the lungs (Derksen *et al.*, 1985c, McGorum *et al.*, 1993d). A number of fungal and thermophilic actinomycete spores have been implicated (table 1.1).

Table 1.1. Fungal and thermophilic actinomycete spores that have been identified as allergens associated with RAO.

Allergen	Reference
Aspergillus fumigatus	(McPherson *et al.*, 1979b, Halliwell *et al.*, 1993, McGorum *et al.*, 1993d, Schmallenbach *et al.*, 1998, Eder *et al.*, 2000)
Faeni rectivirgulia (formerly *Micropolyspora faeni*)	(McPherson *et al.*, 1979b, Derksen *et al.*, 1988, Halliwell *et al.*, 1993, McGorum *et al.*, 1993d, Bureau *et al.*, 2000)
Thermoactinomyces vulgaris	(McGorum *et al.*, 1993d, Bureau *et al.*, 2000)
Alternaria alternata	(Eder *et al.*, 2000)

Allergen exposure has been demonstrated to stimulate the accumulation of radiolabelled neutrophils in horse's lungs (Fairbairn *et al.*, 1993). Many other stimuli may also be involved, including endotoxins, which are present in a stabled horse's environment (McGorum, 1998, Tanner *et al.*, 1998, Pirie *et al.*, 2001). The airways of horses with RAO also become hyperresponsive to non-specific stimuli, thus inhalation of irritants may exacerbate or prolong clinical signs (Robinson *et al.*, 1996, Derksen, 1999). It is also postulated that hot/humid air and cold air may exacerbate or stimulate RAO episodes (Beech, 1989a). 3-Methyl-indole is known to cause a clinical and histopathological condition similar to RAO (Derksen *et al.*, 1982b, Beech, 1989a) and has been administered to foals to induce chronic bronchiolitis in studies of the disease (Nicholls, 1978). Airway obstruction can be replicated in experimental designs by aerosol administration of ovalbumin subsequent to sensitising a horse to the compound (Derksen *et al.*, 1982a, Connally and Derksen, 1994, Bowles *et al.*, 2002).

Dust particles, viruses, bacteria and allergens constantly challenge the surface area of the lung, which is covered in most places by only a single layer of epithelial cells. The epithelial lining of the airway provides the basis for complex defensive mechanisms that provide protection against the potentially harmful effects of respirable debris (Buechner-Maxwell, 1993). These pulmonary defences were probably adequate until domestication resulted in many horses being kept under crowded conditions, in poorly ventilated buildings and fed fodder containing up to 5,000 respirable particles/mg of feed (Clarke and Madelin, 1987). Aerosols are groups of particles that remain suspended in the air for prolonged periods. These can be subdivided into large aerosols (greater than 10μ) that settle/impact in the upper respiratory tract/trachea and small particles that impact by sedimentation in the small airways and gas exchange region. Of these small particles, those less than 0.5μ may remain suspended and are exhaled, whereas particles between 1 μ and 5μ in size penetrate deep within the lung (Derksen, 1991a, Buechner-Maxwell, 1993). Additional irritants to a horse's respiratory tract are noxious gases, for example ammonia, that can accumulate with poor drainage and stable hygiene (Clarke, 1987b).

Section 1.4. Clinically Important Historical Information

1.4.1. Housing

There is a strong link between housing and the disease of RAO. This is not least because when horses are stabled they can be exposed to high airborne dust concentrations, and therefore exposure to potential allergens and non-specific stimuli (Clarke, 1987b, Webster *et al.*, 1987, Woods *et al.*, 1993). An impression can be obtained of the aerosol challenge a horse is subjected to in a particular stable using sampling devices (Clarke and Madelin, 1987, Swain, 2004). The total airborne dust concentration in a straw and hay management system can be in the region of 2.55mg/m^3; creating a minimal dust environment can reduce this burden to 0.53mg/m^3 (Woods *et al.*, 1993). Housing healthy, young horses has a direct effect on the respiratory system, causing inflammation (Holcombe *et al.*, 2001), reflected by neutrophilia in BALF samples (Tremblay *et al.*, 1993). Airway inflammation and mucus accumulation are common in asymptomatic, well-performing sport horses (Gerber *et al.*, 2003).

It is important to consider the breathing zone of a horse, i.e. the dust concentrations in the region of a horse's nostrils (McGorum, 1998). Good ventilation and stable design can reduce the burden of dust in the breathing zone but not when a horse has its muzzle buried in dusty hay when feeding; hay is a major source of respirable spores (Webster *et al.*, 1987, Woods *et al.*, 1993). The British climate ensures that the moisture content of hay is sometimes higher than would be desired. This allows large numbers of thermophilic fungi to develop during the initial curing phase of the hay resulting in a heavy spore burden (Clarke, 1989). During 'mucking out' there can be a 50-fold increase in respirable dust particles within a stable compared to quiet times (Clarke *et al.*, 1987). In a case series of 300 horses (published in 1995) with chronic pulmonary disease referred to a clinic, 96.6% were fed hay and 73.6% were bedded on straw (Dixon *et al.*, 1995b). This compares to the results of a questionnaire survey carried out in the second half of the 1990s in a similar geographical area that suggested 87% of all horses were fed hay at some point during the year and 50% of stabled horses were bedded on straw (Mellor *et al.*, 2001).

1.4.2. Age

RAO is generally believed to be a disease of older horses with prevalence increasing with age (McPherson *et al.*, 1979b, Couetil and Ward, 2003) or, at least, the disease only becomes apparent or is easily detected above a certain age. Various authors have confirmed this with their investigations of clinical case series (table 1.2).

Table 1.2. Published age ranges, or averages, of case series of horses affected by RAO.

Author(s) (year of publication)(reference)	Number of Horses	Age (years)
(Lowell, 1964)	6	14 to 20
(Gillespie, 1966)	11	7 to 17
(Gerber, 1973)	207	Mean = 9.5
(McPherson *et al.*, 1979a)	38	6 to 10
(Asmundsson *et al.*, 1983)	15	6 to 20
(Pearson and Riebold, 1989)	18	Mean = 11
(Vrins *et al.*, 1991)	69	Over 5
(Naylor *et al.*, 1992)	18	Mean = 12
(Dixon *et al.*, 1995b)	148	9

1.4.3. Previous/antecedent respiratory infections

In the human field, respiratory infections, including viral and mycoplasmal, precipitate wheezing in many patients with asthma (Busse, 1988, Lemanske, 2003). Not only do viral respiratory infections provoke attacks of asthma but they may also be a pivotal component in the development of bronchial hyperresponsiveness and asthma. There is some evidence that this applies to horses and pulmonary infection (in particular viral) has been implicated as a trigger factor in the induction of RAO (Gerber, 1973). Antibodies to *Micropolyspora faeni* and *Aspergillus fumigatus* have been found to be increased in the BALF of horses with signs of acute respiratory viral infection (Halliwell *et al.*, 1993). It is also thought that virus-induced hyperresponsiveness may exacerbate attacks of RAO in susceptible horses (Robinson *et al.*, 1996). Evidence for viral involvement includes higher levels of influenza A haemagglutination inhibiting activity in RAO affected than in control animals (Thorsen *et al.*, 1983). A French study also suggested that equine influenza virus might promote other respiratory disease in horses (Chabchoub *et al.*, 1994). It is interesting to speculate that the absence of equine influenza virus may account for the apparent absence of RAO in Australia and New Zealand. However, climatic factors almost certainly have a role in these regions. In guinea pigs (often used in models of asthma) viral infection causes transient airway obstruction and non-specific hyperresponsiveness to inhaled agents (Antoon *et al.*, 1995). Some authors have reported a history of a previous febrile illness prior to presentation for investigation of RAO (Muylle and Oyaert, 1973, McPherson *et al.*, 1978). However, in a case series including 148 horses with RAO, less than 20% had a history of antecedent respiratory infection (Dixon *et al.*, 1995b). It may be that a viral infection appears to "trigger" RAO in a particular animal, not only because it is a common occurrence in a horse's life, but also because it may have exacerbated existing sub-clinical RAO, which has then become detectable by the owner for the first time. Horses with RAO usually have a normal haematological profile and plasma fibrinogen concentration (Mair, 1987, Beech, 1989a), suggesting systemic inflammation is not a component of the disease at the time of diagnosis.

1.4.4. Other factors

Gender appears to have no influence on the disease occurrence in horses (McPherson *et al.*, 1979a). There has been limited evidence that females may be at a greater risk (Couetil and Ward, 2003). Recent vaccination does not have any consequences to the respiratory system in normal or RAO horses (Dixon *et al.*, 1996). The influence of past or present parasite burden of a horse, and therefore any deworming regimen, on the occurrence of RAO is unknown, although there is evidence of an effect in human asthma (Buijs *et al.*, 1994, Lynch *et al.*, 1997).

Section 1.5. Clinical Signs

RAO is characterised clinically by recurrent airway obstruction and physiologically by hypoxaemia, decreased dynamic compliance, increased pulmonary resistance, prolongation of nitrogen washout and, frequently, by airway inflammation and bronchospasm (Derksen *et al.*, 1985b, Robinson *et al.*, 1996, Derksen *et al.*, 1999). The clinical signs of RAO are well recognised and include a chronic cough, nasal discharge, dyspnoea and exercise intolerance (Alexander, 1959, Littlejohn, 1980, Mair, 1987). In one study when horses with

RAO were placed in a natural challenge environment (small, poorly-ventilated box, bedded on deep litter straw and fed poorly-saved hay), they showed clinical pulmonary disease within 5 hours, including some of the following signs: coughing, increased rate and depth of respiration, double expiratory lift ('heave'), nasal discharge, increased and abnormal tracheal and lung sounds (McGorum *et al.*, 1993b). All these signs reverted to normal when these horses were moved to a controlled environment with a remission rate of 2-6 weeks. The clinical signs present depend on the individual horse and the environment it has been kept in within the last month or so. Horses may be termed as being in 'crisis' or 'remission' depending on whether or not the disease has recently been exacerbated.

1.5.1. Cough

Often the first sign of RAO is a soft spontaneous cough and it is the most common historical clinical complaint (in around 80% of referred cases) (Naylor *et al.*, 1992, Dixon *et al.*, 1995b, Robinson, 2001c). Coughing should be regarded as strong evidence of inflammation of the distal airway (Dixon, 1997). Investigation of a limited number of horses in a research herd has suggested that coughing is a sensitive and specific indicator that horses, when sampled, will have greater than 20% neutrophils in bronchoalveolar lavage fluid (Robinson *et al.*, 2003). This author postulated that inflammation causes an increase in the sensitivity of cough receptors. He also highlighted that there is a strong correlation between cough and the quantity of mucus in the trachea and that this accumulation probably acts as a physical stimulus to coughing. The mucus induced in stabled horses with RAO has increased viscoelasticity and is difficult to clear by mucociliary action alone (Gerber *et al.*, 2000). It has even been suggested that coughing could be used as a surrogate marker for increased pleural pressure change, an indirect indicator of airway obstruction, and hence a marker of airway dysfunction severity (Robinson *et al.*, 1999).

When does a horse become a coughing horse? One approach in racehorses with lower airway disease was to define it as more than 4 coughs recorded in one day (Burrell *et al.*, 1996). Alternatively, in RAO, one investigator defined coughing as being present if reported during the week preceding or during the examination (Dixon *et al.*, 1995a). Other authors have graded the frequency of coughing from no cough (0), infrequent cough (+) and frequent cough (++) (Matthews *et al.*, 1988). Coughing may occur in intermittent paroxysmal bouts (Beech, 1989a) or, in less severely affected animals, it may only be associated with activity or during feeding and mucking out (Robinson, 2001d). To estimate coughing frequency, the number of coughs should be counted for at least the period of an hour (Robinson *et al.*, 2003). A cough may be defined as chronic if it had persisted for longer than 1 month (Derksen *et al.*, 1989). In one series of referred cases of RAO, 71% had coughed for more than 3 months, with an average of 15 months (McPherson *et al.*, 1978). Other authors have found that intermittent coughing for more than 3 months was a primary sign of RAO (Littlejohn, 1980). A simple diagnostic technique employed by some clinicians is to compress the cranial trachea/larynx of horses in an attempt to induce coughing (Cook and Rossdale, 1963, Robinson, 2001d).

1.5.2. Nasal discharge

The presence of a nasal discharge has frequently been identified in RAO. Variable quantities and qualities have been described, but it is often mucopurulent in character (Beech, 1989a, Aviza *et al.*, 2001). In Dixon's study of referred cases of RAO, only 54.1% had a nasal discharge in the week preceding or during the examination, making it a less reliable indicator than coughing (Dixon *et al.*, 1995b). Occasionally this mucopurulent discharge may be coughed out through the mouth and collect outside the stable door. A chronic cough and bilateral nasal discharge are reasonable clinical indicators of chronic pulmonary disease (McPherson *et al.*, 1978, Dixon *et al.*, 1995b).

1.5.3. Exercise intolerance

Exercise intolerance is a common historical (or clinical) finding. The horse often has a poor recovery rate, evidenced by prolonged increase in respiratory rate and dyspnoea following cessation of exercise (Muylle and Oyaert, 1973, McPherson *et al.*, 1978, Robinson, 2001c). Two investigators found an incidence in their case series of just over 50% (Naylor *et al.*, 1992, Dixon *et al.*, 1995b). Exercise tolerance testing suggests that RAO has a negative effect on performance potential, especially when in crisis (Persson and Lindberg, 1991, Art *et al.*, 1999). The exact mechanism of exercise intolerance is unknown. A number of explanations have been suggested for how the disease has this effect, including: 1) limiting pulmonary ventilation and oxygen uptake, 2) compensatory mechanisms are overwhelmed during exercise, 3) mechanical work of breathing is higher, inducing respiratory muscle fatigue, 4) higher levels of blood lactate lead to an earlier occurrence of metabolic acidosis during exercise and 5) peripheral muscle fatigue (Persson and Lindberg, 1991, Art *et al.*, 1998). Horses with RAO do appear to compensate for their impaired lung function by respiratory, cardiovascular and haematological alterations (Persson and Lindberg, 1991, Art *et al.*, 1998). The limited use of a bronchodilator prior to exercise causes no apparent improvement in exercise tolerance (Bayly *et al.*, 2002).

1.5.4. Respiratory rate

The respiratory rate can be increased in cases of RAO to compensate for the impaired lung function, especially when in crisis. There is an argument that the term "breathing rate" (the lungs working) should be used instead of "respiratory rate" (complex rate of oxygen consumption etc.) (Hinchcliffe and Byrne, 1991). The normal respiratory rate of a horse is approximately 8 to 16 breaths per minute, but it is extremely variable between individuals; for example it may become increased with the stress of examination (Dixon *et al.*, 1995b). An increased respiratory rate is therefore an inconsistent finding depending on the severity of disease at the time examined and the individual (Kvart *et al.*, 1987). Some investigators have found the rate to be increased: mean = 22 breaths per minute (Pearson and Riebold, 1989) mean = 25 breaths per minute (Littlejohn, 1980); others have not: mean = 16 breaths per minute (Dixon *et al.*, 1995b). Respiratory rate is not closely correlated to the degree of respiratory obstruction in RAO (Robinson *et al.*, 2000). During exercise, the respiratory rate of an RAO horse is often increased and remains rapid for an abnormally long time post exercise (Muylle and Oyaert, 1973, Littlejohn *et al.*, 1982).

1.5.5. Expiratory effort: abdominal lift and nostril flare

A principal effect of lower airway obstruction is to cause an increase in the effort required for expiration (Lowell, 1964). This is often termed 'dyspnoea', although this describes a sensation of difficult or laboured breathing, something that animals cannot communicate (Hinchcliffe and Byrne, 1991). Breathing patterns suggestive of 'dyspnoea' should be described objectively e.g. as gasping or breathing with exaggerated inspiratory and expiratory effort. It is usually characterised by a more obvious abdominal component to expiration resulting in an exaggerated double expiratory effort. The resistance of the bronchioles is small and obstruction in these airways must be massive before 'dyspnoea' is encountered (Derksen *et al.*, 1982c). Normal horses have a biphasic-breathing pattern; expiration is passive until the lungs reach the relaxation volume (functional residual capacity) then expiratory thoracic and abdominal muscles are recruited to decrease lung volume even further (Derksen and Robinson, 1980, Petsche *et al.*, 1994). This biphasic pattern is lost or abandoned in horses with RAO and uniphasic peaks of airflow occur at the end of inhalation and at the start of exhalation (Gillespie, 1966, Petsche *et al.*, 1994, Robinson *et al.*, 1999). Recruitment of abdominal muscles during exhalation is often accentuated in lung disease and this clinical sign can give the clinician an indication of airway obstruction (Derksen, 1991a). In long-standing and severe cases, it can cause hypertrophy of the abdominal musculature and the development of a heave line (Cook, 1976, McPherson *et al.*, 1978). Expiratory effort correlates best with objective intrapleural pressure as a measure of the severity of bronchial obstruction (Erichsen *et al.*, 1994). Dixon found in his case series that 43.1% had signs of 'dyspnoea' when at rest (Dixon *et al.*, 1995b). Another group observed a 68% incidence in their cases of RAO (Aviza *et al.*, 2001).

Respiratory distress is further demonstrated by the flaring of nostrils, which implies an increased effort of breathing (Gillespie and Tyler, 1969) (Naylor *et al.*, 1992, Robinson *et al.*, 2000). Nostril flaring is a means of decreasing the resistance provided by the upper airway (Robinson, 1976). Movement of the anus with respiration is also an indicator of increased respiratory effort (Gillespie and Tyler, 1969, Littlejohn, 1980, Naylor *et al.*, 1992, Traub-Dargatz *et al.*, 1992, Ammann *et al.*, 1998). When any of these clinical signs of respiratory distress, i.e. abdominal effort, flared nostrils and exaggerated anal movement, are obvious in resting horses, the airway obstruction is quite severe (Robinson *et al.*, 1994b).

1.5.6. Cardiovascular signs

Heart rate may be increased in some cases, especially in acute crisis (Gillespie and Tyler, 1969, Robinson *et al.*, 2001). However, in most subjects the heart rate is unaffected (Littlejohn, 1980, Kvart *et al.*, 1987). During exercise the heart rate is often increased above what would be expected in a normal animal (Persson and Lindberg, 1991, Art *et al.*, 1998). Horses demonstrating clinical signs have pulmonary hypertension associated with systemic arterial hypoxia (Dixon, 1978, Benamou *et al.*, 1998). This does not appear to have any significant consequences to the heart as the hypertension is reversible on remission (Dixon *et al.*, 1982). Cyanosis implies severity as it indicates an extreme hypoxaemia and/or poor circulation/hypoxia (Robinson *et al.*, 2001).

1.5.7. Thoracic auscultation

Thoracic auscultation is an essential part of many clinicians' examination protocol. Normal lung sounds, best described as breath sounds, accompany air movement through the bronchial tree and are loudest on inspiration. These are often difficult to hear in normal animals (Curtis *et al.*, 1986). It is not clear what generates these sounds but they may be produced by oscillation of respiratory tissue and by rapid fluctuations of gas pressure (turbulence) (Roudebush, 1982). Increased breath sounds may be detected in exercising animals and certain disease states. Adventitious or abnormal lung sounds are extra, superimposed sounds that are best described as crackles (discontinuous, non-musical, intermittent and discrete) or wheezes (continuous musical or whistling sounds) (Roudebush, 1982, Kotlikoff and Gillespie, 1983). Horses with RAO generally have wheezes and crackles during expiration (Gillespie and Tyler, 1969, McPherson *et al.*, 1978, Pearson and Riebold, 1989). During exhalation, airway diameter decreases and some airways may even close (collapse) at low lung volumes. The explosive equalisation of pressure following the reopening of these airways is thought to produce crackles (Roudebush, 1982). Wheezes are believed to occur as a result of air passing through airways, narrowed by bronchospasm and respiratory secretions, causing a regular vibration of the airway wall and the associated thick secretions (Kotlikoff and Gillespie, 1983).

Thoracic auscultation is a relatively insensitive assessment of chronic pulmonary diseases. Dixon *et al.* (1995b) found only 46.9% of horses with pulmonary disease had abnormal findings. In another study, abnormal lung sounds were detected in 69% of horses with COPD (Naylor *et al.*, 1992). In one retrospective study, 50% of cases had detectable crackles and 59% had wheezes (Aviza *et al.*, 2001). In some animals, regions of silence may be detected, 29% in one case series, as airways are so obstructed that there is insufficient air movement to generate sound (Naylor *et al.*, 1992). In severe cases, wheezing referred from deeper in the lung may be heard over the trachea and even at the nostrils. (Robinson, 2001d). Tracheal auscultation following nasal occlusion was abnormal in 63.1% of RAO cases in Dixon's study, although it was also a subjective procedure with disagreement between interpreters (Dixon *et al.*, 1995b).

1.5.8. Thoracic auscultation with the use of a rebreathing bag

The use of a rebreathing bag facilitates auscultation by causing deep breathing and, if it induces coughing, suggests respiratory disease may be present (Beech, 1989a). An example of the technique is to place a 40L bag over the muzzle and hold it in place for one to five minutes (Naylor *et al.*, 1992). Normal horses have a good tolerance to rebreathing, subjectively this can be defined as a slow increase in minute ventilation and ventilatory efforts while rebreathing with no coughing or other signs of distress (Davis *et al.*, 1998). The use of a rebreathing bag is more sensitive than auscultation alone. In Naylor's series of cases, abnormal lung sounds were detected in 88% horses when a rebreathing bag was used compared to 69% without (Naylor *et al.*, 1992). Alternatively, auscultation can be performed before and after exercise or after nostril occlusion (McPherson *et al.*, 1978, Marti *et al.*, 1991).

1.5.9. Other clinical signs

Percussion of the lung fields has historically been used to aid in the clinical examination of RAO (Roudebush and Sweeney, 1990). Findings include an elevation of the lung fields and a decrease in resonance (Littlejohn, 1980, Klein and Deegen, 1986). However, its interpretation is difficult and many believe it to be of no value (Cook, 1976). Lobeline, a respiratory stimulant, induces forced breathing and has been used to aid the detection of abnormalities on auscultation (Klein and Deegen, 1986, Bracher *et al.*, 1991, Franchini *et al.*, 2000, Marlin *et al.*, 2000). Weight loss can be a feature of severe RAO. Perhaps persistent coughing and respiratory distress interferes with feeding, or the effort associated with breathing results in escalated energy demands (Mazan *et al.*, 2003, Mazan *et al.*, 2004). This is not something widely reported from European sources and perhaps reflects the severity of disease encountered in North America, indeed euthanasia as a consequence of a horse being diagnosed with RAO would probably be foreign to most clinicians in the UK (Aviza *et al.*, 2001, Couetil and Ward, 2003).

1.5.10. Clinical scoring systems

Clinical scoring systems have been used by various authors for grading the severity of disease and particularly for the assessment of response to therapy in drug trials (appendix A1.1). These clinical scores are often based exclusively on subjective clinical observation. Scores are assigned for individual parameters and the sum of these scores is used to define the presence and/or severity of RAO. One of the first uses of a scoring system in RAO was by Naylor *et al.* (1992), who used a combination of scores of respiratory rate, effort and auscultation to grade severity of RAO. He found a close correlation between clinical score and histopathology of lung biopsies; in the majority of cases clinical examination was sufficient in the diagnosis of RAO (Naylor *et al.*, 1992). Ancillary tests increased diagnostic precision but only made a major contribution in 2 out of 18 cases. In the same year, another scoring system was used in the evaluation of three drugs for the treatment of RAO (Traub-Dargatz *et al.*, 1992). In a trial of the efficacy of the bronchodilator clenbuterol, a 'heavey' score system was used based on expiratory effort alone, as it correlated best with objective intrapleural pressure as a measure of the severity of bronchial obstruction (Erichsen *et al.*, 1994). Tesarowski *et al.* (1996) devised a more complex weighted system based on one used in foals published by Hoffman *et al.*, 1992. In a later study, this system had a sensitivity and specificity of 66.7% and 85.7%, when a cut-off score of 6 was used (Couetil *et al.*, 2001). However, relatively low animal numbers were used and no clear 'gold standard' was used for comparison. This paper also identified that obstruction, as determined by lung function tests, could be significant before the clinical score system identified abnormality (Couetil *et al.*, 2001).

Robinson proposed a subjective rating of airway obstruction, although initially he was less than convinced as to its usefulness, suggesting that the score indicated alteration in breathing strategy and that it was poorly correlated with measures of airway obstruction (Robinson *et al.*, 1994b). Later work, using a large number of observations of each horse in the study, did demonstrate that this clinical scoring system could detect airway obstruction in RAO-affected horses (Robinson *et al.*, 2000). Robinson highlighted that there were limitations, with considerable overlap of lung function even between the highest and lowest scores. This scoring system was based on just nostril flare and abdominal lift (indicating increased respiratory effort), each having possible scores of 1 to 4 and the total possible score of 8. The system was used in a study of summer pasture associated obstructive pulmonary disease (SPAOPD), using parameters for both medial and lateral nasal flare (Seahorn *et al.*, 1997). The system has since been widely used or adapted by various authors (Rush *et al.*, 1998b, Olszewski *et al.*, 1999, Henrikson and Rush, 2001). These types of scoring system, utilising subjective scoring of abdominal lift and nostril flare, have never been properly validated for RAO, including for inter- and intra- observer variability. In SPAOPD, a high correlation has been found between this type of clinical score system and pleural pressure measurements and both these provided valid estimates of disease severity when compared to histopathology of lung biopsies (Costa *et al.*, 2000). Clinical scores that indicate obstruction have been included as possible criteria for defining RAO, but only if validated (Robinson, 2001b).

Section 1.6. Diagnostic Tools

A number of diagnostic tools have been developed to aid the clinician in the diagnosis of RAO. These tests can be broadly divided into two main areas: the assessment of lung function, and the resultant effect on respiration, and the qualitative or quantitative assessment of inflammation of the lower respiratory tract. A presumptive diagnosis of RAO is often based on the history and clinical signs alone and further investigation is only pursued if there is failure to respond to treatment and/or changes in management.

1.6.1. Endoscopy

Endoscopy permits visualisation of the respiratory tract to the level of the bifurcation of the trachea (and further if a longer endoscope is available)(Sweeney *et al.*, 1992b, Smith *et al.*, 1994). The normal trachea has no visible secretions and the presence of mucoid, mucopurulent to purulent fluid, which often pools at the thoracic inlet, is a sensitive indicator of pulmonary inflammation (Whitwell and Greet, 1984, Robinson *et al.*, 1996). The quantity of mucopus may be so copious as to result in it being visible in the nasopharynx and larynx, further indication of pulmonary inflammation (Dixon, 1997). Grunig *et al.* (1989), although finding the amounts of respiratory secretion visible in the trachea correlated to the maximum changes of pleural pressure in horses with chronic pulmonary disease, warned care should be taken in interpretation as inflammation may be due to other causes and might not be accompanied by dyspnoea. The appearance of the tracheobronchial mucosa can also be scrutinised for inflammation, hyperaemia and oedema. Oedema of the carina at the tracheal bifurcation is believed to imply pulmonary inflammation (Dixon, 1997). Finally, the upper trachea of the normal horse is relatively insensitive and the presence of an endoscope rarely causes coughing. Horses with RAO often cough on introduction of an endoscope into the trachea, suggesting inflammation of the tracheal mucosa and distal airways (Dixon, 1997). This inflammation of the upper respiratory tract may be secondary to persistent coughing (Dixon *et al.*, 1995c).

A number of authors have devised schemes for scoring the quantity of exudate in the tracheal lumen as a result of airway inflammation (table 1.3), particularly in racehorses (Whitwell and Greet, 1984, Burrell, 1985, Chapman *et al.*, 2000, Christley *et al.*, 2001). Other authors have devised similar systems for RAO (Bracher *et al.*, 1991, Nyman *et al.*, 1991, Dixon *et al.*, 1995a) and some also assessed the tracheal and bronchial mucosa for evidence of inflammation, bluntness of carina, or erythema (Traub-Dargatz *et al.*, 1992, Dixon *et al.*, 1995a). Dixon *et al.* (1995c), in a series of 300 referred cases of pulmonary disease, identified that 96% of horses had respiratory secretions of grade 1 or more present and that presence of a cough was related to the respiratory secretion score. Endoscopic scoring of mucus accumulation has been demonstrated to have excellent interobserver agreement and moderate horse-related variance, as well as correlation with neutrophilic airway inflammation (Gerber *et al.*, 2004). A simple scoring system for severity of disease that combined the quantity of exudate in the trachea with observations of clinical signs (coughing, abnormal respiratory sounds and 'dyspnoea') demonstrated that, as severity increased, the proportion of neutrophils in BALF also increased (Vrins *et al.*, 1991).

Table 1.3. Two published scoring systems for scoring the quantity of exudate in the tracheal lumen identified endoscopically.

Description	Grade
(Burrell, 1985) – for respiratory inflammation in racehorses	
Isolated globules	1
A thin continuous stream < 15 mm wide	2
A thick continuous stream > 15 mm wide	3
(Nyman *et al.*, 1991) – for RAO	
Isolated spots of mucoid secreta	Mild
A continuous string of mucoid secreta	Moderate
Excessive, widespread mucoid secreta	Severe

1.6.2. Tracheal wash

The sampling of airway secretions is commonly performed to assess inflammation of the respiratory tract. Fluid is collected principally from two sites: the trachea (tracheal wash) and the bronchioles (bronchoalveolar lavage). Two methods for sampling secretions accumulated in the trachea have been developed and both usually involve the introduction of 20 to 30ml of physiological fluid to perform a tracheal wash (TW) to assist in the collection. The percutaneous, transtracheal aspiration (TTA) method involves the passage of a sterile polyethylene tube into the distal trachea via a needle passed into the tracheal lumen (Mansmann and Knight, 1972, Beech, 1975). The second method utilises the biopsy channel of a fibre optic or video endoscope to pass a catheter into the trachea once the instrument has been guided into the tracheal lumen (Greet, 1982). If a TTA has been performed correctly then there should be no sample contamination; this cannot be said for the endoscopic method, as there will inevitably be some nasopharyngeal contamination. To attempt to address this problem of obtaining a sterile sample, a number of methods have been developed, including the plugging of catheter tips with agar (Whitwell and Greet, 1984). More recently, the use of protected aspiration catheters for performing tracheal washes has been shown to be reliable for the sterile recovery of respiratory secretion samples for cytology and culture (Darien *et al.*, 1990b, Christley *et al.*, 1999). However, the validity of the procedure has been questioned (Darien *et al.*, 1990a, Racklyeft, 1990). Tracheal secretions have been sampled using catheter brushes for both culture (Darien *et al.*, 1990b, Grandguillot *et al.*, 1991) and mucus collection (Gerber *et al.*, 2000). The endoscopic collection method is quickly performed and should have few or no complications, whereas with TTA, careful preparation is required and subcutaneous emphysema or superficial infection can occur (Beech, 1981). Some authors directly aspirate secretions as a result of concerns regarding dilution factors (Grunig *et al.*, 1989) and some only wash when there are little or no respiratory secretions present in the trachea (Dixon *et al.*, 1995a).

Samples obtained by tracheal washing (if collected appropriately) can be submitted for culture (bacterial, fungal or viral), cytology and macroscopic examination for *Dictyocaulus arnfieldi* larvae. The results of cytological examination can be expressed as total nucleated cell counts and the relative proportions of cell types either by absolute cell counts, semi-quantitatively or as a percentage in the form of differential cell counts. Examples of semi-quantitative systems include those of Whitwell and Greet (1984) where the relative proportions of cells was graded from +++ (the predominant cell type) to 0 (not more than two cells present), and Bracher *et al.* (1991) who used a scale of + (very few cells) to ++++ (large number of cells). When calculating the cell proportions, epithelial cells are often excluded, as their numbers can be very variable and leukocyte ratios, not total cell ratios, are used (Dixon *et al.*, 1995c). A tracheal wash from a normal horse would be expected to be relatively clear (little or no mucus) with the cellular content principally made up of macrophages; neutrophils should contribute less than 15% of the total white cell count (Mair *et al.*, 1987, Dixon *et al.*, 1995c). In horses kept indoors, up to 20% neutrophils can be recovered (Dixon, 1997). Horses with RAO are generally considered to have increased neutrophil proportions and increased mucus (Nuytten *et al.*, 1983); one study identified a median neutrophil proportion of 90% (range 14 to 100%) (Dixon *et al.*, 1995c) and another a mean percentage of 62 % (range 7 to 96%, including epithelial cells) (Mair, 1987). Therefore, caution should be used, as results are variable depending on the individual horse and the management it has been recently subjected to for example housing, transport and activity. Horses recently transported to a clinic will usually have had their heads elevated, and possibly endured poor ventilation. This results in the accumulation of inflammatory exudate (Racklyeft and Love, 1990, Raidal *et al.*, 1995). It may be that tracheal wash samples should be obtained after exercise, as specimens collected then are more likely to reveal airway disease (Roberts *et al.*, 1997, Martin *et al.*, 1999). Cytological examination of tracheal secretions may be good for the classification of the degree of RAO (Bracher *et al.*, 1991).

Further scoring systems to grade the severity of airway tract inflammation in horses have been created by combining endoscopic findings and the results of cytological examination of tracheal aspirates (Whitwell and Greet, 1984). This inflammatory score system has been further developed, principally for racehorses (Burrell *et al.*, 1996, Mills *et al.*, 1996, Wood *et al.*, 1997). These score systems rely on the uncertain effect of dilution on quantitative cell counts (Mair, 1987). A similar score system has been applied to horses with chronic pulmonary disease (Votion *et al.*, 1999a). Other scoring systems that utilise various combinations of clinical signs, endoscopic scores, arterial oxygen measurements and cytology scores have been described (Grunig *et al.*, 1988, Winder *et al.*, 1990, Herholz *et al.*, 2002).

1.6.3. Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) is widely used for the diagnosis of RAO and is often considered to be the closest available 'gold standard'. Composition of BALF is uniform throughout the lungs of both normal horses and horses with symptomatic RAO therefore, a single BALF sample is representative of the entire lung in these horses (McGorum *et al.*, 1993e). It should not be considered to be a completely innocuous procedure in horses with RAO (Traub-Dargatz *et al.*, 1992). Two methods are practised either endoscopically guided or using a blindly placed catheter (appendix A1.2). The majority of published papers favour the use of endoscopy and an attempt to achieve a consensus for the most appropriate method has been made (section 1.7). The majority of methods involve sedating and then twitching a horse before passing an endoscope via the nasopharynx. As the endoscope is advanced beyond the tracheal bifurcation, diluted local anaesthetic can be simultaneously sprayed to minimise coughing and distress. The tip of the scope is advanced into a main-stem bronchus until it becomes wedged, usually in the right caudodorsal region of the lung. The scope can be guided, within reason, in any direction desired to sample different regions of the lung. A variable volume of physiological fluid (200 to 500ml, warmed to 37°C) is then infused via the biopsy channel before being harvested either using syringes or a suction device. Warmed fluid slightly increases the yield of respiratory cells (in humans) and local anaesthetic is not thought to affect the cells retrieved (Reynolds, 1987). It must be ensured that a large enough volume is used to ensure a true bronchoalveolar lavage is performed and not a bronchial lavage (Vrins *et al.*, 1991).

A blind BAL can be performed utilising a narrow siliconised catheter passed into the distal airways (Fogarty, 1990a, Fogarty, 1990b). Following restraint using a twitch alone or with sedation, a short, wider diameter tube is first passed via the nasopharynx into the proximal trachea. This facilitates the passage of the narrower, more flexible BAL catheter as it is advanced until it becomes wedged. A blindly passed catheter usually samples the caudodorsal region of the lung (Fogarty, 1990b, McKane and Rose, 1993). A variable volume of physiological fluid (60 to 180ml) is then infused before aspirating. Often a cuffed sampling tube is used to increase the quantity of lavage fluid recovered (McKane *et al.*, 1993). It must be ensured that the cuff is not over inflated to minimise bronchial mucosa pressure necrosis. Pulmonary segment over-distension should be avoided by using a relatively small volume of lavage fluid (Fogarty, 1990a). This technique has the advantage of not requiring expensive and bulky equipment. There is no risk of transmitting pathogens between horses as individual catheters can be used rather than relying on the lengthy, and relatively inefficient, sterilisation of a single endoscope.

BALF can be assessed grossly before cytological examination. A frothy appearance to collected samples denotes surfactant is present, indicating the alveoli have been lavaged (Reynolds, 1987). Some authors have graded the gross characteristics of samples for their colour, turbidity and mucus content (McKane *et al.*, 1993). Before cytological examination samples are usually filtered to remove mucus and then cells are concentrated by centrifugation. Nasopharyngeal bacterial contamination prevents the culture of BALF (McGorum and Dixon, 1994). Cytological examination of BALF usually comprises total cell counts and differential cell counts. In normal horses, lymphocytes and macrophages predominate with neutrophils comprising less than 10% of cells (table 1.4).

Table 1.4. Published normal differential cell counts identified in BALF (mean values).

	(McGorum *et al.*, 1993f)	(Mair *et al.*, 1987)	(Naylor *et al.*, 1992)	(Lapointe *et al.*, 1994)	(Vrins *et al.*, 1991)
Neutrophil (%)	**1.4**	**6.2**	**4.4**	**2.4**	**3.6**
Macrophage (%)	58.5	70.3	48.2	25.6	65.1
Lymphocyte (%)	31.8	7.6	38.8	71.4	28.9
Eosinophil (%)	0	1.0	1.3	0.0	0.1
Mast cells (%)	7.4	0.6	7.3	0.5	1.4
Epithelial cells (%)	0.5	14.3	-	-	0.8

Horses with RAO typically have an increase in the percentage of neutrophils on cytological analysis, which in severely affected animals may exceed 50%; these cells are not degenerate (table 1.5). This profound neutrophilia may be identified in both tracheal wash fluid (TWF) and BALF (McGorum *et al.*, 1993d). It should be noted that increased neutrophil numbers and increased mucus are normal non-specific responses of the airways to many agents (Robinson, 2001b). For example, acute viral infection may cause a transient neutrophilia in respiratory secretions (Dixon *et al.*, 1995c). Initially this can make diagnosis a problem but in chronic post viral infection, a normal BALF neutrophil count is expected (McGorum and Dixon, 1994). Neutrophilia also occurs in bacterial pulmonary disease, which is rare in the UK (Beech, 1975, Mair, 1987). Clinical examinations, the absence of bacteria (plus other abnormalities) on cytological examination and negative tracheal wash cultures enable pulmonary infection to be discounted. Some authors have detected an increase in total cell count when a horse with RAO has an exacerbation (Mair, 1987, Traub-Dargatz *et al.*, 1992, McGorum *et al.*, 1993d). Others have found that there is no increase in total cell counts on induction of heaves (Derksen *et al.*, 1985c, McGorum *et al.*, 1993e, Tremblay *et al.*, 1993, Lapointe *et al.*, 1994, Rush *et al.*, 1998c).

Table 1.5. Published proportion of neutrophils in BALF from normal horses and horses diagnosed with RAO.

Reference	Normal Horses % (range) or ± s.d.	RAO Horses % (range) or ± s.d.
(McGorum *et al.*, 1993f)	1.4	-
(McGorum *et al.*, 1993e)	-	59 to 64.2 (5.7 to 95.3)
(Mair *et al.*, 1987)	6.2	-
(Derksen *et al.*, 1989)	8.9	-
(Sweeney *et al.*, 1994)	2.1 to 5.4	-
(Naylor *et al.*, 1992)	4.4	58.8 (17 to 82)
(Lapointe *et al.*, 1994)	2.4 ± 2.0	-
(Sandersen *et al.*, 2001) During crisis	0.33 ± 0.51	71.0 ± 12.5
(Sandersen *et al.*, 2001) After crisis	-	14.6 ± 5.4
(Traub-Dargatz *et al.*, 1992)	4 (0 to 20)	66 (18 to 95)
(Vrins *et al.*, 1991)	3.6 ± 2.2	16.5 to 39.4
(Tremblay *et al.*, 1993) Before stabling	-	29.4
(Tremblay *et al.*, 1993) With stabling	-	71.6
(Dixon *et al.*, 1995c)	-	20.5 (5 to 98)

Confident comparison of published data is difficult in the absence, until recently, of a standardised BAL technique. The volume of fluid used affects the total cell count, absolute cell count and concentration of cells recovered and there may even be a difference between the left and right lung (Sweeney *et al.*, 1992a). To combat the dilutional effect of a BAL, investigators have used the concentration of albumin and urea as references (McGorum *et al.*, 1993f). Care has to be taken if samples are to be collected repeatedly as a BAL induces a localized pulmonary neutrophil influx that persists for at least 48 hours, characterised by an increase in the number of neutrophils (Sweeney *et al.*, 1994, Lindberg *et al.*, 2004).

Repeated sampling by other investigators at 3 to 7 day intervals did not identify this as a problem (Derksen *et al.*, 1985c, Clark *et al.*, 1995). When exposed to a stable environment with straw bedding and hay forage, horses with RAO have increased neutrophils in BALF, which decrease when pastured again (Derksen *et al.*, 1985c, Tremblay *et al.*, 1993). Clinical remission when at pasture can take 2-6 weeks with the BALF neutrophilia being the last to revert (McGorum *et al.*, 1993b). Even when in remission and demonstrating no detectable signs of obstruction, horses with RAO may still have slightly increased neutrophils (Grunig *et al.*, 1989, Kirschvink *et al.*, 1999, Jackson *et al.*, 2000). Normal horses do have an increase in neutrophil cell proportion in BALF when housed, but to a far lesser degree than RAO horses (Lapointe *et al.*, 1993). Transport of animals prior to BAL can have an effect, resulting in a neutrophilia (Hobo *et al.*, 1997). Exercise has been shown to increase the proportion of neutrophils in BAL samples (Roberts *et al.*, 1997).

BALF neutrophilia is generally believed to be the most useful diagnostic indicator of equine RAO, being considerably more sensitive than clinical examination, arterial blood gas analyses and pulmonary mechanics testing (McGorum and Dixon, 1994). A good correlation has been found between the cells in BALF and generalised pulmonary cellular patterns identified on histopathological examinations (Fogarty, 1990b, Naylor *et al.*, 1992). The latter of these papers identified that BALF had a poor correlation with severity of clinical signs on initial presentation, although decreases in neutrophil percentages did parallel improvement in clinical condition (Naylor *et al.*, 1992). In one investigation the percentage of neutrophils (not count) was highly sensitive, but not very specific, for RAO (Couetil *et al.*, 2001). Eosinophilia in TWF and BALF is an inconsistent finding in horses with RAO, possibly reflecting different pathophysiological mechanisms (Derksen *et al.*, 1985c). This can also occur as a consequence of parasitic pulmonary infestation (MacKay and Urquhart, 1979) or it may be idiopathic (Dixon *et al.*, 1992). An increase in mast cell population may be identified and may reflect hyperreactivity (Derksen *et al.*, 1989).

There is often no correlation between BAL and TW sampling techniques as transtracheal aspirates may not be representative of the cell population in lower airways (Derksen *et al.*, 1989, Traub-Dargatz *et al.*, 1992, Malikides *et al.*, 2001, Hughes *et al.*, 2003). However, some investigators have found to the contrary with close correlation between TW and BAL (Dixon *et al.*, 1995c). TWF often has large quantities of mucin strands and large numbers of degenerate cells. Therefore, a BAL may be more appropriate as the samples obtained contain cells that are more easily identified, there is less variability in cell populations and they are representative of diffuse pulmonary disease (Larson and Busch, 1985, Derksen *et al.*, 1989, Dixon *et al.*, 1995c). Despite this, tracheal samples can be easily collected, permit both cytopathological and microbiological investigations and are often sufficient in initial clinical examinations; they are also representative of the whole lung (Whitwell and Greet, 1984, Mair, 1987). On consideration of the limitations of these two procedures, perhaps it is best to follow the advice of Malikides *et al.* (2003) and investigate inflammation of the entire lung using both.

1.6.4. Arterial blood gas measurements

Arterial blood gas measurements are a clinically useful diagnostic tool, although allowances must be made for the environment (for example temperature and barometric pressure) and sub-clinical horses may alter "normal" horse results (Davis *et al.*, 1998)(table 1.6). Arterial oxygen partial pressure (PaO_2) is a simple test of pulmonary function and is often less than the threshold of 85 mmHg in horses suffering from RAO (McPherson *et al.*, 1978, Davis *et al.*, 1998). Hypoxaemia may be a reflection of poor gas exchange in the terminal alveoli as a result of poor lung function and could explain the degree of respiratory distress seen in acute RAO. Ventilation-perfusion mismatching and increased dead space in the lungs of RAO horses may be important factors (Nyman *et al.*, 1991). The degree of gas exchange compromise is correlated with the severity of bronchiolitis and clinical signs (Nyman *et al.*, 1991). Horses with RAO kept in a reduced dust environment had a greater PaO_2 at rest and when exercised up to 5 m/s compared to when they were kept in poor conditions (Kvart *et al.*, 1987). This test and other pulmonary function tests, including responses to treatment, are relatively insensitive compared to sampling of respiratory secretions (McGorum and Dixon, 1994, Robinson, 2001b). One investigator found no significant difference between healthy horses and those with sub-clinical or mild RAO (Bracher *et al.*, 1991). Dixon identified that 72% of his RAO cases were hypoxaemic ($PaO_2 < 85$mmHg) (Dixon *et al.*, 1995a).

Table 1.6. Published arterial oxygen partial pressures in normal and diseased horses.

	Normal PaO_2 (mmHg)	Threshold PaO_2 classified as abnormal by investigators (mmHg)
(Davis *et al.*, 1998)	98 to 106	61 to 100
(Bracher *et al.*, 1991)	-	< 90
(Mair, 1987)	84	< 84
(Pearson and Riebold, 1989)	-	< 83
(Dixon *et al.*, 1995a)	-	< 82
(McPherson *et al.*, 1978)	91	≤ 82
(Tesarowski *et al.*, 1996)	-	82

Arterial carbon dioxide partial pressure ($PaCO_2$) is less useful than arterial oxygen concentration and is very variable (Art *et al.*, 1998) (Kvart *et al.*, 1987), although it does tend to be higher when a horse is in RAO crisis (Tesarowski *et al.*, 1996, Art *et al.*, 1999). The two measurements of PaO_2 and $PaCO_2$ can be combined to calculate the arterial alveolar difference (or gradient) (Maisi *et al.*, 1994). Normal values for this gradient should fall between 7 and 10 mmHg (Tulamo and Maisi, 1997, Sasse, 2001).

1.6.5. Pulmonary function tests

Pulmonary function tests are important for demonstrating the respiratory compromise of horses suffering from RAO. They are particularly utilised in experimental investigation of the disease, as well as in its diagnosis. Traditionally, pulmonary function tests have included measurement of pleural pressure changes (indirect) using an oesophageal balloon catheter and flow rates (along with inspiratory and expiratory volumes) using a face mask/tracheostomy (via a flow transducer and a pneumotachograph) (Gillespie, 1966, Muylle and Oyaert, 1973). The data generated can then be used to calculate the most widely quoted parameters: total resistance (R_L), dynamic compliance (C_{dyn}) and maximal intrapleural pressure change (Max ΔPpl). Dynamic compliance is defined as the volume change per unit change in intrapleural pressure. Resistance is defined as the pressure change per unit change in flow (Stadler and Deegen, 1986). Flow and volume results can be plotted to create tidal breathing flow-volume (TBFV) loops to help in the detection of airway obstruction (Connally and Derksen, 1994). Although, like other pulmonary function tests it is relatively insensitive, the TBFV loops of horses with heaves tend to have a characteristic appearance with loss of a biphasic pattern (Petsche *et al.*, 1994).

Pulmonary function tests are too insensitive and the results too variable to be useful in detecting mild or moderate lung disease (Derksen *et al.*, 1982c, Beech, 1989a, Dixon, 1997). For a reliable diagnosis using pulmonary function tests obstruction needs to be so severe that measurement becomes redundant as the clinical signs will have become so obvious (Robinson *et al.*, 2000). In addition, pulmonary function does vary throughout a 24-hour period, with maximal deterioration at night, and there is considerable inter-individual and intra-individual variation (Stadler and Deegen, 1986, Jean *et al.*, 1999). Pulmonary function tests can be useful for monitoring an individual's response to treatment, particularly bronchodilators (Derksen *et al.*, 1982c, Jean *et al.*, 1999). Max ΔPpl is a particularly simple means of assessing this response and helps demonstrate the reversibility of the bronchoconstriction in RAO (see later). The functional impairment may take longer to resolve than clinical signs (Votion *et al.*, 1999a). Few clinicians have the full plethora of pulmonary function tests available as the equipment is expensive, trained personnel are required and new clinical cases require training prior to the measurements being made (Beech, 1989a).

In an attempt to address some of these problems, less invasive methods have been developed for measuring pulmonary function. Total respiratory impedance can be measured using forced oscillatory respiratory mechanics (Young and Hall, 1989, Van Erck *et al.*, 2004). Impedance is a sum of three independent factors: resistance, elastance (recoil of lungs during inflation) and inertance (force required to accelerate a column of air) (Hoffman and Mazan, 1999). Forced oscillatory mechanics have been used for bronchoprovocation testing using histamine (Hoffman *et al.*, 1998, Mazan *et al.*, 1999) and methacholine (van Erck *et al.*, 2003). To circumvent the inability of animals to perform forced expiration, a vital lung function test in humans, a technique has been developed which can be performed on a sedated horse. This manoeuvre requires the mechanical ventilation of the horse prior to maximal expansion of the lungs, then exposure to a vacuum; this can be used to generate forced expiratory flow-volume curves (FEFV) (Couetil *et al.*, 2000). It can be used to detect peripheral airflow obstruction and use in a limited number of animals suggests it may be a relatively specific/sensitive test for RAO (Couetil *et al.*, 2001). Multiple-breath nitrogen washout is a little-used technique for pulmonary function testing (Gallivan *et al.*, 1990). Ultrasound-spirometry/capnography has been used as a simple, non-invasive test of pulmonary function (Davis *et al.*, 1998, Herholz *et al.*, 2001). This method has been demonstrated to be sensitive enough to distinguish between healthy horses and horses in RAO remission (Herholz *et al.*, 2003a, Herholz *et al.*, 2003b).

1.6.6. Pleural pressure changes

Pleural pressure changes can be measured in isolation as a simpler measurement of pulmonary function, and usually this information is presented as a Max ΔPpl. This can be calculated using one of two methods that measure the difference between the atmospheric and pleural pressures. Direct measurement requires the placement of blunt needles, connected to transducers, into the pleural space (Muylle and Oyaert, 1973, Derksen and Robinson, 1980). Alternatively, an indirect, less invasive technique measures oesophageal pressure changes, as this tends to reflect local changes in pleural pressure (McPherson and Lawson, 1974, Derksen and Robinson, 1980, Deegen and Klein, 1987). Measurements are made using an oesophageal balloon catheter, connected to a pressure transducer and recorder, positioned in the mid-thoracic oesophagus (Gillespie, 1966, Robinson *et al.*, 1993). Healthy horses have been reported as having a Max ΔPpl = 3.7mmHg (McPherson *et al.*, 1978), indicating that the pressure differences necessary for ventilation of normal airways are relatively small.

In diseased horses, the increased resistance associated with airway obstruction forces greater pressure changes for effective movement of air. Thus, the Max ΔPpl is increased; a cut off point of $\geq$ 6 mmHg (8 cmH_2O) has been taken as indicating an abnormal finding

(McPherson *et al.*, 1978, Mair, 1987). Many other researchers have used a positive cut-off of greater than either 15 cmH_2O (11 mmHg) (Robinson *et al.*, 1993, Rush *et al.*, 1998a, Lavoie *et al.*, 2002) or 20 cmH_2O (Derksen *et al.*, 1999, Robinson *et al.*, 2000) to identify airway obstruction in horses for inclusion in studies. In severely 'dyspnoeic' horses suffering acute exacerbations, Max ΔPpl can increase to as high as 67 mmHg in some individuals (Pearson and Riebold, 1989). In a case series of 148 horses with RAO, only 48% had Max ΔPpl > 6 mmHg (Dixon *et al.*, 1995c). As with other function tests, Max ΔPpl tends only to detect RAO cases with severe mechanical pulmonary dysfunction that could be deduced by clinical observation alone and therefore is of limited use in diagnosis (Dixon *et al.*, 1995c, Robinson *et al.*, 2000). Nonetheless, increased Max ΔPpl, together with a history of chronic respiratory disease (> than 2 months), is highly suggestive of RAO (Dixon *et al.*, 1995c) and is a good overall reflection of the severity of lung disease in horses with the disease (Robinson *et al.*, 1999).

1.6.7. Natural straw/hay challenges (provocation)

Challenging horses with mouldy hay and straw combined with housed conditions and observing the consequent clinical signs of obstruction and/or alterations in lung function measurements has been used to identify animals with RAO for diagnostic and experimental purposes (Lowell, 1964, Robinson *et al.*, 1993, Dixon *et al.*, 1995b). In one study of 5 horses with RAO (in remission), a 6-hour challenge with hay/straw increased the mean Max ΔPpl from 7.0 to 11.2 cmH_2O after 24 hours and the mean respiratory rate from 20.6 to 25.6 per minute after 6 hours (Rickards *et al.*, 2000). Following challenge, the reversibility of any induced obstruction is often demonstrated by administering a bronchodilator such as atropine (Obel and Schmetterlow, 1948, Muylle and Oyaert, 1973, Benamou *et al.*, 1998). This is often used for selection of principals for use in studies. The use of atropine may not induce a complete reversal of airway obstruction due to irreversible damage and/or mucus plugging (Jean *et al.*, 1999). Another bronchodilator that can be used in this way is clenbuterol. The concept of reversibility can be extrapolated to a diagnostic process used by many veterinary practitioners, i.e. response to therapy or change in management following a preliminary diagnosis of RAO (Gerber, 1973, Clarke, 1987a). The use of nebulised hay dust suspensions as a superior provocation method has been investigated (Pirie *et al.*, 2002).

1.6.8. Histamine or methacholine challenge (provocation)

Aerosol administrations of histamine (which can also be administered IV) and methacholine (a muscarinic agonist) have been used for testing for non-specific airway hyperreactivity (also known as bronchoprovocation) (Obel and Schmetterlow, 1948, Derksen *et al.*, 1985a, Armstrong, 1986). Methacholine causes bronchoconstriction via vagal reflexes, whereas histamine may act by direct action on smooth muscle (Broadstone, 1988). These tests are widely used in human medicine for diagnosing asthma (Beasley *et al.*, 1998), although they are widely recognised to be far from perfect with many healthy individuals reacting (Josephs *et al.*, 1989, Britton, 1998). Hyperreactivity during acute exacerbations of RAO, but not during remission, is demonstrated by the results of pulmonary function tests, including a reduction in dynamic compliance and an increase in pulmonary resistance following aerosol administration (Derksen *et al.*, 1985a, Armstrong, 1986). Some researchers believe this test can detect horses in remission (van Erck *et al.*, 2003), but also that it demonstrates that normal horses can be hyperreactive (Doucet *et al.*, 1991); airway hyperresponsiveness is not exclusive to the disease RAO (Klein and Deegen, 1986, Hoffman, 2000).

1.6.9. Lung biopsy

A definitive diagnosis is possible pre-mortem by obtaining a biopsy of lung tissue. This may be performed using a percutaneous blind technique and a 'Tru-Cut' instrument (Raphael, 1981). Naylor *et al.* (1992) used this technique in a prospective survey of 18 horses referred to his clinic with RAO. Sixteen of these horse had biopsy findings consistent with RAO, namely bronchiolar goblet cell metaplasia, bronchiolar luminal exudate accumulation, peribronchiolar lymphoplasmacytic cell infiltration and accumulations of neutrophils, that were closely correlated to a clinical score given to the horse at admission (Naylor *et al.*, 1992). An alternative method to obtain a biopsy sample is thoracoscopy (Peroni *et al.*, 2000, Peroni *et al.*, 2001, Lugo *et al.*, 2002). It is also possible to biopsy airway mucosa via endoscopy, although this technique has not yet been demonstrated to be useful in the investigation of RAO (Watson *et al.*, 1993, Wilson *et al.*, 1993, Buechner-Maxwell *et al.*, 1996).

1.6.10. Radiography and nuclear imaging

Thoracic radiographs have been used to help in the diagnosis of RAO, although the need for specialist radiographic equipment limits its availability. The acquisition of consistently interpretable views of the thorax is fraught with difficulty. It has been reported that in normal to advanced cases, a variable, mild interstitial pattern of increased lung density or peribronchial reaction can be identified (McPherson *et al.*, 1978, Mair, 1987, Beech, 1989a). Other authors have found that radiography inconsistently detected pulmonary disease (Dixon *et al.*, 1995c). Bronchovascular, interstitial changes and sometimes lung hyperinflation, are not pathognomonic for RAO. Further imaging techniques such as nuclear imaging have utilised inhaled and injected radioisotopes to investigate lung perfusion and ventilation, principally in relation to the study of the delivery of inhaled pharmacological agents to the lungs (Beech, 1989a, Willoughby *et al.*, 1991, Viel, 1994, Votion *et al.*, 1997, Votion *et al.*, 1998, Rush *et al.*, 1999a, Votion *et al.*, 1999a). Scintigraphic alveolar clearance rate has been shown to be a sensitive indicator of lung damage and may be better at detecting subclinical RAO than clinical examination, pulmonary function tests and cytology of BALF (Votion *et al.*, 1999b).

1.6.11. Intradermal skin testing

Intradermal antigen testing using aqueous extracts of *Micropolyspora faeni, Thermoactionmyces vulgaris and Aspergillus fumigatus* as a means of investigating RAO, although initially thought to hold some promise (Eyre, 1972, McPherson *et al.*, 1979b), has more recently been shown to be of limited value in distinguishing RAO sufferers from healthy animals (Evans *et al.*, 1992, McGorum *et al.*, 1993a). However, patterns of response to large banks of antigens may be of some use in identifying horses with non-specific hypersensitivity (Jose-Cunilleras *et al.*, 2001, Lorch *et al.*, 2001). The presence of serum precipitating antibodies to fungal and thermophilic actinomycete antigens has been investigated as a possible aid in the diagnosis of RAO (Lawson *et al.*, 1979). However, these antibodies are also present in clinically healthy horses and are of little value (Madelin *et al.*, 1991).

1.6.12. Breath collection and breath condensate collection

A non-invasive technique has been developed in the human field for assessing airway inflammation involving the collection of breath and breath condensates (Kharitonov and Barnes, 2001). Markers of inflammation that have been measured in exhaled breath include nitric oxide, carbon monoxide and hydrocarbons (Bernareggi and Cremona, 1999). Exhaled breath condensates have been analysed for a large number of inflammatory markers including hydrogen peroxide, eicosanoids and for the direct measurement of pH (Antczak *et al.*, 1997, Montuschi *et al.*, 1999, Kostikas *et al.*, 2002). These have been found to be particularly useful in the assessment of asthma, COPD, cystic fibrosis and acute respiratory distress syndrome. This technology has been used to assess calves (Reinhold *et al.*, 2000) and initial work has been commenced on investigation of the usefulness of this technique in horses (Deaton *et al.*, 2001, Fey *et al.*, 2001, Reinhold, 2001, Wyse *et al.*, 2004a).

Section 1.7. Definition of the Recurrent Airway Obstruction Phenotype

What constitutes a diagnosis of RAO depends on the disease definition that a clinician or investigator utilises. In studies of the disease and investigation of therapies, authors hopefully state their definition of RAO, i.e. the criteria that an animal is required to satisfy before it can be classified as a principal. These criteria vary greatly and can rely on historical information, clinical signs and specific diagnostic tests including respiratory cytology, endoscopy, pulmonary function tests, arterial oxygen partial pressure and thoracic radiographs. These criteria are often applied when a horse is in a state of RAO crisis, induced using hay/straw provocation, following, or reverting to, clinical remission, either at pasture or in a controlled environment. An attempt at consensus on the matter was made at the International Workshop of Equine Chronic Airway Disease (Robinson, 2001b). The participants suggested criteria for defining the RAO phenotype:

1. Demonstrated reversible lower airway obstruction induced by an environmental challenge, as documented by either lung function or a defensible clinical score.
2. Airway obstruction is reversible by use of a bronchodilator or by return to a nonchallenge environment.
3. An increase in BALF neutrophils during environmental challenge.
4. During environmental challenge there is evidence of airway obstruction determined either by a Max ΔPpl greater than 15 cmH_2O (2 kP) or by use of a validated clinical score. Airway obstruction must be accompanied by more than 25% neutrophils in BALF.
5. Before environmental challenge, control horses must have Max ΔPpl less than 10 cmH_2O, or an equivalent validated clinical score, and less than 10% neutrophils in BALF. After environmental challenge, the control horses should have Max ΔPpl less than 10 cmH_2O.
6. Because neutrophil count is sensitive to the collection method, BALF analysis is recommended for detecting pulmonary neutrophilia in horses with heaves. These guidelines should be followed: use a sampling tube 10 to 13 mm diameter; if sampling frequently, use an endoscope to sample different sites of the lung; if blind sampling by a BAL tube, allow 7 days between sample collection; the volume of lavage fluid should range from 250 to 500 ml. Dilutional effects may be problematic, but no standardised means of adjusting BALF constituents has been determined.

Section 1.8. Therapy and Management

1.8.1. Environment

The cornerstone of treatment for RAO is the reduction in allergen exposure by altering environmental management (Jackson *et al.*, 2000). Ideally this would involve the affected animal no longer being housed. However, in most situations housing is required to some degree; this should be kept to a minimum. When stabled, the management system should be altered to incorporate minimal dust exposure and good ventilation (Thomson and McPherson, 1984, Clarke, 1987b). Hay, not bedding, is thought to be the major source of respirable spores (Webster *et al.*, 1987, Vandenput *et al.*, 1998a) and soaking hay is aimed at reducing this challenge (Clarke, 1993). Prolonged and excessive soaking of hay does reduce its nutritional value (Warr and Petch, 1992). Soaking leaches minerals and, if it is for as long as 12 hours, there is significant loss of water-soluble carbohydrates and nitrogen compounds (Blackman and Moore-Colyer, 1998). Somewhere between 10 and 30 minutes soaking (or steaming) is sufficient to reduce greatly the respirable dust dose (Moore-Colyer, 1996, Blackman and Moore-Colyer, 1998). However, wetting of hay may be insufficient, especially if it dries out during feeding (thus releasing more spores), and feeding haylage may be more appropriate (Thomson and McPherson, 1983, Dixon *et al.*, 1995d). Changing housed RAO horses from silage to hay increases the percentage of neutrophils in BAL fluid substantially (Franchini *et al.*, 2000). The feeding of complete pelleted feeds to replace forage has been utilised to good effect (Thomson and McPherson, 1984, Jackson *et al.*, 2000). Low dust bedding such as wood shavings, paper or cardboard also may be advantageous (Thomson and McPherson, 1984, Burrell *et al.*, 1996, Vandenput *et al.*, 1998b, Kirschvink *et al.*, 2002). Paper and shavings have been shown to have lower concentrations of airborne endotoxin than straw (Tanner *et al.*, 1998). It is usually considered important to change the environment of neighbouring horses, although this may not necessarily be the case (Robinson, 2001a). The proximity of any forage store and muckheap should also be considered.

1.8.2. Therapeutics

Although theoretically environmental control should be sufficient, in many cases medical therapy is instigated to speed remission of clinical signs or where complete control of the environment is impossible. Oral prednisolone has been used to reduce the inflammatory response (Beech, 1989b, Rush, 2001). Corticosteroids reduce production of inflammatory mediators, such as leukotrienes, as well as inhibiting cell immigration (including that of neutrophils) into the lungs of horses with RAO (Lapointe *et al.*, 1993, Derksen, 1999). Systemic corticosteroid therapy should be used with caution to minimise the possibility of inducing laminitis, adrenal insufficiency or the exacerbation of concurrent infections because of the associated immunosuppressive effects (Eyre *et al.*, 1982, Rang and Dale, 1991, Mair, 1996a). The use of inhaled corticosteroids enables these problems to be circumvented (Ammann *et al.*, 1998, Rush *et al.*, 1998a, Rush *et al.*, 1998c, Rush *et al.*, 1999b, Giguere *et al.*, 2002). Anticholinergic bronchodilators, such as atropine, can be utilised in the treatment of severe, acute exacerbations of RAO. Unfortunately, prolonged use is prevented by the serious side effects of anticholinergic drugs, such as ileus (Ducharme and Fubini, 1983). To avoid the side effects of systemic use of this drug, inhaled ipratropium provides an effective alternative (Robinson *et al.*, 1993, Robinson *et al.*, 1994a, Duvivier *et al.*, 1997, Duvivier *et al.*, 1999, Bayly *et al.*, 2002). The β_2-adrenergic agonist, clenbuterol is licensed for use in the UK and helps to reduce the airway obstruction caused by constriction of airway smooth muscle (Traub-Dargatz *et al.*, 1992, Erichsen *et al.*, 1994, Kearns *et al.*, 2001). The bronchodilator also increases mucociliary clearance and may have anti-inflammatory actions (Dixon, 1992). Persistent small airway obstruction occurs following bronchodilator therapy as a result of airway inflammation and excess mucus (Robinson *et al.*, 1996). Other β_2 bronchodilators are available for inhalational use (Tesarowski *et al.*, 1994, Derksen *et al.*, 1999, Rush *et al.*, 1999a).

Dembrexine may be beneficial as it may decrease the viscosity of mucus and improve mucokinesis (Dixon, 1992). Nebulised sodium cromoglycate has been shown to have some prophylactic effects, possibly by preventing mast cell degranulation (Thomson and McPherson, 1981, Soma, 1987). Recently, phosphodiesterase inhibitors, such as pentoxyfylline, have been investigated for their therapeutic potential (Chilcoat *et al.*, 2002, Leguillette *et al.*, 2002).

Section 1.9. Differential Diagnoses

1.9.1. Summer pasture associated obstructive pulmonary disease

In 1983 a similar condition to RAO was first reported that occurred when horses were at pasture in the Southeastern region of the United States and was termed summer pasture associated obstructive pulmonary disease (SPAOPD) (Beadle, 1983). Following this report, Beadle co-authored a retrospective study of SPAOPD cases from 1983 to 1991 (Seahorn and Beadle, 1993) and two cases were documented as occurring in the UK, with a suggestion that other similar cases had occurred in the past (Dixon and McGorum, 1990b). SPAOPD is clinically indistinguishable from RAO except that the allergen is thought to be present in the pasture environment (Dixon and McGorum, 1990b). The increase in the commercial farming of oil seed rape (*Brassica* spp.) has been suggested as a factor in the apparent emergence or increased awareness of SPAOPD in the UK (Dixon and McGorum, 1990a, Hackett, 1990). The pollen of oil seed rape has been reported as being a potent allergen in humans (Parratt *et al.*, 1990). A study of six ponies pastured near to oil seed rape was inconclusive, although these were horses that had RAO, not SPAOPD, i.e. they developed reversible obstructive airway disease when housed in a poorly ventilated stable containing mouldy hay and straw (McGorum and Dixon, 1992). Horses at pasture are exposed to an almost infinite number of allergens or irritants from grass and tree pollens to chemicals used in arable farming and other environmental air pollution depending on location (Hannant, 1988, Mazan *et al.*, 2001). Horses with SPAOPD may have long-term airway hyperresponsiveness that results in response to a wide range of specific (e.g. moulds or pollen allergens) and non-specific triggers (e.g. cold air, dry air, exercise, irritant dusts) (McGorum and Dixon, 1999). It has been suggested that horses with pre-existing RAO and/or a recent episode of viral respiratory disease may have a predilection for developing SPAOPD (Hackett, 1990). In one review of eighteen cases of SPAOPD (mean age 15.3 years), eleven horses also showed clinical signs of RAO; nine of these had been diagnosed at least one year before recognition of the summer condition (Mair, 1996b). In the University of Edinburgh review of 300 referred cases of respiratory disease, 14 cases suffered from both RAO and SPAOPD and just two had only SPAOPD (Dixon *et al.*, 1995a).

Seahorn *et al.* (1996) performed a mail survey of veterinarians practising in Louisiana, USA, regarding SPAOPD and determined that the disease was similar in all aspects to RAO, apart from precipitating factors. For example, more mature horses were affected (mean 12.6±5.49 years) with similar clinical signs: dry cough, slight serous nasal discharge, laboured expiratory effort and flaring nostrils. Clinical scoring systems using these observations (including assessment of medial and lateral flare of nostrils) have been developed for assessing SPAOPD (Seahorn *et al.*, 1997)(appendix A1.1). These clinical scoring systems can provide valid estimates of SPAOPD severity (Costa *et al.*, 2000).

1.9.2. Other differential diagnoses

Differential diagnoses for RAO include many diseases that cause inflammation of the airways. Acute respiratory diseases include post-viral airway disease (McGorum and Dixon, 1994), acute eosinophilic interstitial pulmonary disease (Dixon *et al.*, 1992), smoke inhalation, and pulmonary oedema (cardiogenic) (Mair and Lane, 1996). Bacterial infections causing bronchopneumonia and pleuropneumonia will present not only with respiratory symptoms, but also signs of infection, e.g. pyrexia, abnormal haematological findings and, more specifically, degenerative neutrophils in bronchoalveolar lavage fluid (McGorum and Dixon, 1994). These pulmonary diseases often have a history that is suggestive of an infectious origin, for example recent group diseases, lymphadenitis, ocular discharge etc (Dixon *et al.*, 1995a). Chronic interstitial pulmonary diseases that lead to lung fibrosis or diffuse granuloma formation will present with classical signs of RAO but these animals will not respond to a bronchodilator (Derksen *et al.*, 1982d, Robinson, 2001d). In interstitial disease (or pleuritis) it is inspiration that is restrictive, not expiration as in RAO (Beech, 1989a). *Dictyocaulus arnfieldi* infestation can cause very similar clinical signs to RAO, although usually there is a sudden onset (often in more than one horse) with recent exposure to pasture grazed by donkeys and absence of effective deworming (MacKay and Urquhart, 1979). In such cases, tracheal wash samples will often identify an eosinophilia and the presence of larvae (George *et al.*, 1981). Pulmonary neoplasia, for example mediastinal lymphoma with associated pleural effusion, can present with similar clinical signs to RAO. A syndrome has been recognised of lower inflammatory airway disease (IAD), particularly in younger horses and an attempt to define it has been made (Robinson, 2003). Principally, the disease occurs in racehorses and there is great debate as to the possible causes including persistent/recurrent viral infection, post-viral or environmental challenge and bacterial or mycoplasmal infections (Wood *et al.*, 1993, Burrell *et al.*, 1996, Chapman *et al.*, 2000). There is inflammation of the lower respiratory tract although, unlike RAO, conventional measurements of pulmonary function are unaltered (Robinson, 2001b). It has been proposed that IAD might be an early stage of

RAO (Viel, 1997, Hare and Viel, 1998); to date the relationship between the two diseases is unknown and would seem unlikely, not least because of the lack of recurrence of the IAD (Christley, 2000, Robinson, 2001b).

Section 1.10. Incidence or Prevalence of Recurrent Airway Obstruction

RAO is believed to be a common cause of chronic coughing in horses in the UK (McPherson and Thomson, 1983). However, the true prevalence of the disease in the UK is unknown. A Swiss investigator identified an incidence of 54% in his home country, using criteria based on clinical examination, endoscopy, tracheal wash cytology and arterial blood gases (Bracher *et al.*, 1991). Sasse and co-workers investigated the incidence of chronic pulmonary disease in a survey of 16 large riding schools in Holland and reported their findings at an international symposium on equine chronic pulmonary disease in Hanover in 1985 (Dixon, 1986). They identified chronic pulmonary disease in one third of all horses examined endoscopically, as manifest by excessive and mucopurulent respiratory secretions in the trachea. Using similar criteria, 22% of Standardbreds (total number of horses 965) had COPD (MacNamara *et al.*, 1990). No inference for the UK horse population can be taken from these studies. Not only were the populations not representative or comparable, but also there are problems of disease definition in terms of recent recommendations (Robinson, 2001b). Although reports based on referred populations cannot be extrapolated to the general population, they may give a general impression of occurrence. From 300 referred cases to a University clinic, of which 270 had respiratory disease, 148 (54.8%) had RAO (Dixon *et al.*, 1995a). In another study of cases referred to Bristol University, of 106 horses with chronic pulmonary disease, 91 were diagnosed as having COPD (Mair, 1987). Both these studies emphasise that RAO is probably one of the commonest respiratory disease in the UK. A sentinel practice-based survey in northern Britain conducted in the mid nineties identified that 4% of horses in that region were suffering from RAO (Mellor *et al.*, 2001). The incidence may vary from year to year depending on the weather, for example affecting stabling periods or the quality of harvested hay (Cook and Rossdale, 1963). In the UK, one clinician has suggested that many practitioners believe there is an increasing incidence (Mair, 1995).

Section 1.11. The UK Equine Population

There are no reliable data describing the size, composition and geographical distribution of the entire British equine population. At present, the size of the horse population can only be estimated. The Department for Environment, Farming and Rural affairs (DEFRA) conduct a yearly census of agricultural holdings, and in 2003 the number of horses identified on these establishments was 299,886. This is undoubtedly a gross underestimate. In 1996, a survey of horse owners in Scotland and the five most northern counties of England estimated that the horse population in this region was approximately 3 times the figure purported by the government agency for this area (Mellor *et al.*, 1999). Following the suggestion by Mellor to extend this ratio to the rest of the UK the total population of horses could be estimated to be 899,658. This figure is remarkably similar to the population estimate of 900,000 made in 1999 by a British Equine Trade Association (BETA) survey (Anon, 1999). The introduction of a mandatory passport scheme for all horses and the establishment of a National Equine Database may in the future allow access to accurate data on the UK horse population (Great Britain. Parliament, 2004).

The management of horses and ponies in the UK is generally dictated by the purpose for which they are kept, who owns them, geographical location and season. The majority of horses in the UK do have access to grazing but most are also stabled for at least part of the day at some time throughout the year (Mellor *et al.*, 2001). Racehorses in training tend to be kept stabled, with little or no access to pasture (Jones *et al.*, 1987). The construction of stables varies greatly from open fronted boxes to American style barns and the ventilation and drainage in these different types of stables varies greatly. Most horses have bedding provided and it is believed that straw is still the most commonly utilised bedding material (Harris, 1999). A wide variety of alternatives exist including wood shavings, rubber matting, paper and commercial products. As most horses do not have continuous access to pasture they require supplementary feeding, usually consisting principally of forage (hay or haylage) as well as a highly variable (in quantity and ingredients) bucket feed that might consist of cereals, pelleted feeds, sugar beet and supplements.

Section 1.12. Asthma

Asthma is a syndrome occurring in humans that is strikingly similar to RAO; in fact the latter has been used as a model of asthma (Snapper, 1986, Bureau *et al.*, 2000, Turlej *et al.*, 2001). A definitive definition of asthma is constantly being debated. A World Health Organisation definition is as follows (Beasley *et al.*, 1998): 'Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils and T lymphocytes. In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough particularly at night and/or early in morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli'. Diagnosis of asthma is not straightforward and many methods are employed including history, physical examination, bronchoprovocation tests, spirometry and breath condensate collection (Thiadens *et al.*, 1998, Wolf *et al.*, 1999). Bronchial hyperresponsiveness using methacholine (or histamine) has been considered to represent the 'gold standard' marker for asthma, although it is neither wholly sensitive nor specific for asthma (Pattemore *et al.*, 1990, Sears, 1997a, Beasley *et al.*, 1998). Spirometry measures airflow variability and, principally, serial peak flow measurements (Beasley *et al.*, 1998).

CHAPTER 2

A CONFIDENTIAL SURVEY OF THE OPINIONS OF EQUINE PRACTITIONERS, IN THE UNITED KINGDOM, ON ISSUES ASSOCIATED WITH DIAGNOSIS AND TREATMENT OF RECURRENT AIRWAY OBSTRUCTION

Part 1: Application and Evaluation of a Mailed Questionnaire

Section 2.1.1. Introduction and Aims of Study

As part of the development of a risk-screening questionnaire (RSQ) for recurrent airway obstruction (RAO), veterinary surgeons in the United Kingdom were surveyed regarding the disease. The study aimed to investigate how equine practitioners diagnosed RAO, particularly what they regarded to be pertinent historical and clinical information, as well as treatment and management for the condition. This kind of data collection can be conducted by mail (and now the internet), telephone or personal interview. These methods may also be used in combination. Whatever technique is employed, there are implications for the survey's costs, response and completion rates, and reliability and validity of a survey (Harewood *et al.*, 2001, Kellerman and Herold, 2001).

Mailed surveys are generally less costly and more immediate than other methods. There are concerns that self-administered mailed surveys may have lower response rates than direct questioning (Battistutta *et al.*, 1983). Allaying these fears, a recent review identified that the response rates of mail surveys of physicians compared favourably with those from telephone and personal interview surveys (Kellerman and Herold, 2001). To ensure high response rates it is well recognised that a number of simple procedures are required in the design and administration of a questionnaire. The total design method (TDM) encompasses many of these approaches, stipulating questionnaire format as well as implementation procedures (Dillman, 1983). The limitation imposed by the "one size fits all" approach of TDM to survey research has been recognised by the original author who now adopts a more tailored design method that depends on the survey context (Dillman, 1999). Modifications of TDM have been widely used in medical, business, social and veterinary fields, including equine veterinary science (Doherr *et al.*, 1998, Christley *et al.*, 2000b).

In this study, a mailed survey using a modified TDM approach was selected to collect information from equine practitioners in the United Kingdom. Heavy emphasis was placed on achieving high response rates, as it is hoped in doing so non-response (or selection) bias is minimised. However, only a 100% response rate will guarantee its absence. Therefore, it is essential that the possibility and extent of non-response bias be investigated when a survey is conducted (Sheikh and Mattingly, 1981, Barclay *et al.*, 2002). The aim of this study was to investigate the effectiveness of this technique for surveying this sample population and attempt to identify and assess non-response bias.

Section 2.1.2. Materials and Methods

2.1.2.1. Selection of study sample (survey population)

The target population for the survey was UK veterinary surgeons that predominately worked with horses. A database of equine practitioners was created using information from a variety of sources. A total of 302 veterinary surgeons and their practice addresses were identified in this process.

Initially, the Royal College of Veterinary Surgeons (RCVS) database of practices for 2001 was searched to identify practices that stated they offered veterinary services for horses; all branch practices were excluded. Individual veterinary surgeons were identified working at these practices (either as partners or as assistants) by cross-referencing with:

1. Veterinary Surgeons listed as working at a practice in the RCVS directory of practices 2001 (Anon, 2001b).
2. Holders of RCVS Certificates in the equine disciplines, particularly internal medicine and practice as listed in the RCVS register of members 2001 (Anon, 2001d).
3. Practice websites that listed staff and their clinical interests.
4. Attendees at British Equine Veterinary Association (BEVA) annual congress 2001 (Anon, 2001a).

Particular attention was paid to the 77 practices that stated they worked exclusively with horses in the RCVS database. In addition, practices were targeted that were on the BEVA list of practices willing to take emergencies (Anon, 2001c). The final database of contacts was managed using Microsoft Excel 2000.

2.1.2.2. Construction of questionnaire

A list of topics that encompassed all possible information that the proposed questionnaire could address was created by review of the scientific literature. Topics focused on how RAO is diagnosed and treated by veterinary surgeons, including case histories, results of clinical examination, further diagnostic tests and case management or therapy. Questions were then phrased that would elicit this information using self-administration. The questions were then placed in a logical order, as would be perceived by a clinician, starting from historical information and terminating with therapy. The format and final content of the questionnaire was designed, edited and pre-coded using a data capture software program, TELE*form* Elite v8. This software allowed fields (such as tick boxes) to be predefined. Subsequently, following scanning of the completed questionnaires, the software was able to recognise which field had been completed. The software was also able to recognise printed text (letters and numbers).

It was predetermined that the questionnaire would be limited to an A4 8-page booklet. The cover page aimed to create a strong identity, emphasising the affiliations (University of Glasgow and The Home of Rest for Horses (HORFH)) and title of the questionnaire (Recurrent Airway Obstruction in Horses-A Confidential Survey of Equine Practitioners). The cover page also contained a contact address, instructions for completion of the questionnaire, the deadline for return of the questionnaire and a unique code number to identify the respondent. In addition to these instructions there were definitions of the disease RAO, including a guide to terminology. It was emphasised that it was the respondent's personal opinion that was required and that all responses were confidential. An opportunity was given for respondents who believed that they were unable to answer the questionnaire, because they had limited or no clinical interaction with horses, to indicate so by ticking a box on the front cover and returning the questionnaire uncompleted. Apart from the cover page title, an Arial size 10-type font was used throughout the questionnaire.

The back cover page (page 8) of the questionnaire booklet was kept as empty as possible with just an open box giving the opportunity to provide additional comments regarding the questionnaire. There was also a closed-ended question asking if the participant would be willing to take part in a future survey. The page concluded with instructions on returning the questionnaire and the extension of gratitude to the respondent. The remaining six inner pages of the booklet were devoted to the actual questionnaire. A copy of the finished questionnaire is available in appendix A2.1.1.

There were four sections, each one began on a new page and facing pages were utilised when more than one page was required (table 2.1.1). The list of all possible questions had to be edited to fit the available space, avoiding overcrowding of each page. Overall, a total of 13 closed-ended questions were included. Boxes were provided for participants to place a tick or cross in to indicate their responses. Nine of the questions had an empty "other" field as the last option. This gave the opportunity for respondents to provide additional criteria with a space for a written explanation (known as other/specify). Six of the closed-ended questions had different criteria grouped together, as a grid, to save space (known as grouped questions). These questions utilised faint shading to help guide the completion of the form. Four separate open-ended response questions were used. Many of the open-ended questions provided constrained print comb style boxes for insertion of one letter or number at a time. Others utilised open boxes that permitted free-written text.

Table 2.1.1. Questions in the mailed survey on diagnosis and treatment of RAO.

Question	Number of categories
Section 1 Professional details	
1.1 Proportion of total workload with horses	4 pre-coded, multiple choice
1.2 Other species making up workload*	4 pre-coded, multiple choice
1.3 Type of practice*	5 pre-coded, multiple choice
1.4 Type of horses worked with (proportion)*	6 open ended
Section 2 Historical information related to RAO	
Presenting clinical signs – 6 criteria	
Medical history – 5 criteria	
Feeding management – 3 criteria	5 pre-coded, importance scale
Stable management – 4 criteria	
Other please specify – 1 criterion	
Section 3 Diagnosis of RAO	
3.1 Diagnostic tests - 12 criteria* (with ranking)	5 pre-coded, frequency scale
3.2 Stage of diagnosis confident of RAO	4 pre-coded, multiple choice
3.3 Clinical signs association with severity–12 criteria*	4 pre-coded, severity scale
3.4 Endoscopic signs association with severity–4 criteria	
Section 4 Management and therapy of RAO	
4.1 Management practices – 9 criteria	4 pre-coded, frequency scale
Hay soak time	Open ended
Type of bedding*	6 pre-coded, multiple choice
4.2 Therapeutics – 11 criteria*	4 pre-coded, frequency scale
4.3 Administration of clenbuterol	3 pre-coded, multiple choice
4.4 Prevalence of RAO increased	4 pre-coded, multiple choice
Back page	
Additional comments	Open ended
Participate in a further survey	2 pre-coded, multiple choice

*An extra criterion is provided as "Other – please specify"

2.1.2.3. Pre-testing of questionnaire

The questionnaire was tested on 9 clinicians working in the Division of Equine Clinical Studies, University of Glasgow Veterinary School and one visiting equine veterinary surgeon. None of the test participants had previously read the questionnaire. No warning was given prior to administration of the questionnaire. Respondents were asked to complete the questionnaire as if they were working at their previous practice job; no verbal guidance was given. All participants took less than 15 minutes to complete the questionnaire and item omission was minimal. Participants did not find the layout of the questionnaire difficult to follow or too tedious. A minority of participants did suggest additional criteria in the "other" categories but these were considered to be infrequently used options. No omissions of major criteria were identified. Final changes stimulated by this process were correction of typographical errors and minor design-orientated changes; two questions were slightly rephrased.

2.1.2.4. Final questionnaire design

The questionnaire was professionally printed black on white 80g A3 paper and collated, folded and wire stitched to form an A4 8-page booklet (appendix A2.1.1). The front covers were pre-numbered from 1 to 302. A Royal Mail Freepost Response Service was created so that pre-paid (second-class) return envelopes could be provided. C5 white self-seal envelopes were printed professionally with the unique Freepost address and the University of Glasgow crest (appendix A2.1.2). Recipients were unaware that returns were to be second-class.

A cover letter was written inviting recipients of the questionnaire to participate in the survey (appendix A2.1.3). The letter was printed black on white A4 Division of Equine Clinical Studies, University of Glasgow Veterinary School headed notepaper. The green logo of the HORFH was printed alongside the University colour crest. A size 11 Arial font was used throughout. The postal date was included. The letter explained the nature of the questionnaire and who was conducting and funding the survey. The overall study aim, to investigate the prevalence of RAO in the UK horse population, was also explained. The importance of responding to the survey and its confidentiality was emphasised. The inclusion of the pre-paid return envelope was highlighted and a deadline (14 days after mailing) for return of the completed questionnaire was given.

2.1.2.5. Distribution of the questionnaire

The initial mailing included a cover letter individually addressed to the recipient, one pre-paid return envelope and a questionnaire printed with the code number corresponding to that person. This survey pack was placed in a white C4 envelope, with address window, that had been pre-printed on the reverse with the return address. Each cover letter was printed with the individual's address so that it was visible through the window of the postal envelope. Individual addresses and salutations were created using the mail merge option of Microsoft Word 2000 linked to the database of address contacts. The author and the Head of the Division of Equine Clinical Studies (Professor Sandy Love) signed each letter in blue pen. The questionnaires were all sent on the same day, first class, using automatic postal franking.

The questionnaire was mailed on Monday, 27th May 2002 (table 2.1.2). The deadline for return of completed questionnaires was given as Monday, 10th June 2002. All non-respondents on 24th June 2002 were sent a second identical questionnaire (with hand written code numbers on front cover) on Tuesday, 25th June 2002; no deadline date was printed on the front cover. A cover letter with similar layout to the first one was included (appendix A2.1.4), as well as a pre-paid return envelope. The letter repeated information about the affiliations of the study, its purpose and its confidentiality. It was emphasised how important it was that people completed the questionnaire. It was reported that, to date, there had been an excellent response to the survey. The recipient was reminded that if they did not believe they were able to complete the questionnaire due to lack of contact with equidae that they should return the questionnaire uncompleted. No deadline for return was given. The author signed the letters in blue pen and the survey pack was mailed in an identical manner to the first mailing.

Table 2.1.2. Important dates in the distribution of the practitioner questionnaire.

Week	Day	Event
1	**0**	First mailing of questionnaire Monday, 27th May 2002
2	**-**	Queen's Golden Jubilee Holiday (National Holiday) Monday, 3rd June and Tuesday, 4th June 2002
3	**14**	Return Deadline as stated in cover letter Monday, 10th June 2002
5	**29**	Second Mailing of questionnaire to non-responders Tuesday, 25th June 2002
7	**42**	Reminder postcard sent to non-responders Monday, 8th July 2002
14	**96**	Final deadline, questionnaires received after this date not included in analyses Saturday, 31st August 2002

On Monday, 8th July 2002, 14 days after the second mailing, a reminder postcard was sent to the remaining non-responders. This postcard reminded recipients that they had previously been sent a questionnaire on RAO and encouraged the return of a completed questionnaire (appendix A2.1.5). The opportunity was given for additional questionnaires to be requested, full contact details were provided. The cards were printed with the University crest and the HORFH logo. Postcards were personally addressed as previously using mail merge. They were printed (in house) black on white card 105 mm x 148 mm. An Arial size 11-type font was used. Half of the recipients were randomly selected (using a function of Microsoft Excel 2000) to receive postcards signed, in blue pen, by the author. The remainder received identical but unsigned cards. All the postcards were mailed second-class using automatic postal franking. A final deadline of 31st August 2002, 96 days after the first mailing, was selected.

2.1.2.6. Processing of returned questionnaires

Returned questionnaires were stamped with the date of arrival and the spine of the booklet removed to separate the pages. Questionnaires were then scanned, as a batch, using a fujitsu fi-4110cu image scanner. The resultant images were then imported into the data capture software (TELE*form* Elite v8). The software processed the images automatically extracting the data from the completed fields before verification and correction by the operator. This included manual entry of free text information. The actual scanned image of each page was visually compared with the image generated by the software (with its interpretation of the responses) before the data were exported into Microsoft Excel 2000.

2.1.2.7. Analysis

Responses were considered useable if at least one question was answered. The return of a blank questionnaire, with or without the decline box on the front cover completed, was interpreted as being a decline to participate. The study sample was described using four demographic variables. Firstly, postcodes were used to divide the sample into nine geographic regions. Secondly, the sample was broken down into the institutions where veterinary qualifications had been obtained (university of graduation i.e. veterinary school). This was determined from the individual's entry in the RCVS register of members (Anon, 2001d). The categories used were the five UK universities and an "other" category for international veterinary schools. Thirdly and fourthly, the population was also examined as to how long each individual had been qualified and whether or not they possessed further professional qualifications; again this was achieved by referring to the RCVS register of members (Anon, 2001d).

The overall response rates (return proportion and response proportion) were calculated using all responses (including those who declined participation). However, the completion proportion (number of completed questionnaires) was also calculated. In the assessment of non-response bias, those who provided only completed responses and non-responders were included. Responders that declined to participate in the survey were excluded (although their inclusion gave similar results). Bias was investigated by the use of publicly available data that permitted the comparison of responders and non-responders according to five demographic variables obtained from the RCVS register of members (Anon, 2001d); gender, veterinary school, time since qualification, geographic location and further professional qualifications. The time taken to respond was also assessed using these five demographic variables and using information provided by the responders. For some analyses, the number of years since qualification was divided into five-year bands, plus a final band that spanned 36 to 50 years. Similarly, prior to analysis, the measure of proportion of workload made up by horses was converted into a dichotomous variable, representing those respondents for whom horses comprised less than 90%, and those for whom horses made up between 90 and 100%.

Comparisons between groups were made using Mantel-Haenszel chi-square analysis. Where the variable was ordinal, trend chi-square analysis was also performed. These statistical tests were performed using Epi Info v6.04d. Cox regression (or proportional hazards model) and Kaplan-Meier survival curves were used to compare the probability of responding, for different groups, depending on the time to respond (S-Plus 2000 v3). The relationship between the times taken to respond, in days, between different groups were investigated using Mann-Whitney test (U)(two-sample rank test) for two populations and Kruskal-Wallis (H) for more than two populations (Minitab v12.21). Significance for all analyses was taken as $p < 0.05$.

Section 2.1.3. Results

2.1.3.1. Description of study population

A total of 302 veterinary surgeons (240 (79.5%) men and 62 (20.5%) women) were selected as the study sample. The geographic spread of the sample across the UK is shown in table 2.1.3, divided into 9 regions. The most common geographic location was the South and South-East of England with 86 veterinary surgeons on the mailing list. Northern Ireland had the fewest representatives with only four equine practitioners. Table 2.1.4 lists the study sample as divided by veterinary school of qualification.

Table 2.1.3. Geographic location of veterinary surgeons that received the questionnaire (n = 302).

	Number of recipients	Proportion of total recipients (%)
Scotland	31	10
Wales	10	3
Northern Ireland	4	1
North East England	26	9
North West England	27	9
South West England	53	18
South and South East England	88	29
Midlands	28	9
Eastern England	35	12

Table 2.1.4. Individuals in the practitioner survey sample grouped according to their veterinary school of graduation (n = 302).

	Number of recipients	Proportion of total recipients (%)
Bristol	38	13
Cambridge	48	16
Glasgow	27	9
Liverpool	50	17
London	53	18
Edinburgh	52	17
International veterinary schools*	34	11

*Other included: Dublin (11), Massey (4), Ghent (3), Pretoria (4), Sydney (3), Melbourne (1), Uppsala (2), Zaragoza (1), Munich (1), Liege (1), Murdoch (1), Hannover (1) and Utrecht (1).

The mean number of years qualified was 17.7 years (standard deviation (s.d.) = 9.5 years). The minimum number of years qualified was 2 years and the maximum was 46 years (figure 2.1.1). It was identified that 221 (73.2%) of individuals had no further professional qualifications. Of the veterinary surgeons that had further professional qualifications, 74 (24.5%) had equine specific further qualifications.

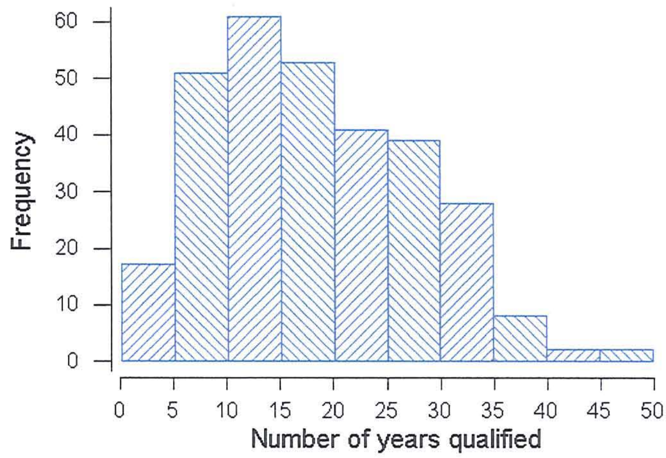

Figure 2.1.1. Histogram of the distribution in the population of veterinary surgeons by the number of years they had been qualified.

2.1.3.2. Description of response rate

The total number of questionnaires sent in the first mailing was 302. The response rate is summarised in table 2.1.5 and figure 2.1.2 (appendix A2.1.6). Prior to the second mailing 49.0% (148/302) recipients had returned their questionnaire (this included individuals who declined to participate). The remaining 51.0% (154/302) who had not responded were sent the second mailing. Of these, 33.4% (101/302) had still not responded by the time the reminder postcard was sent to them.

Table 2.1.5. Response rate to each stage of the practitioner survey.

	Cumulative Frequency	Proportion (%)	95% Confidence interval (%)
Response by first mailing deadline	117	38.7	32.7 to 43.6
Response prior to second mailing	148	49.0	43.4 to 54.6
Response prior to postcard	201	66.6	61.2 to 71.9
Total response	242	80.1	75.6 to 84.6

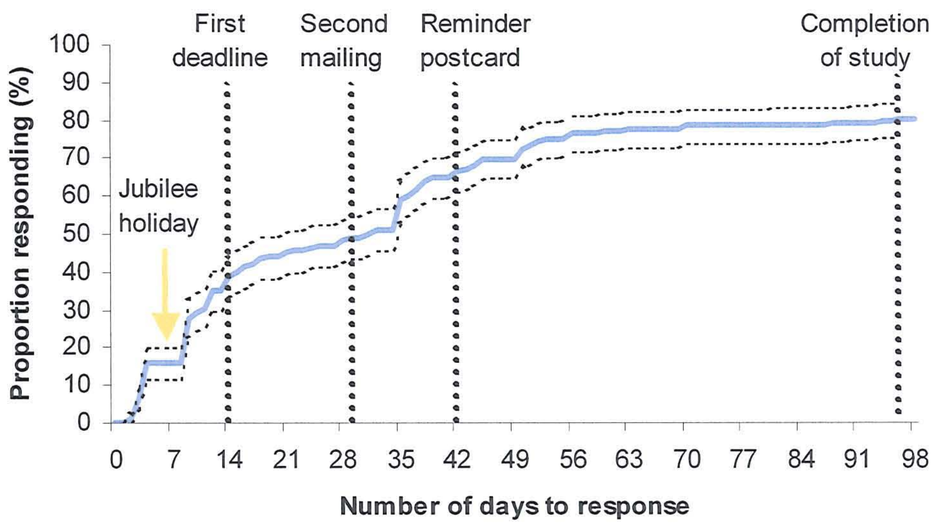

Figure 2.1.2. The cumulative return rate over time for the practitioner survey.
The arrows represent the jubilee holiday (Monday, 3rd to Tuesday, 4th June 2002), first deadline (Monday, 10th June 2002), second mailing (Tuesday, 25th June 2002), reminder postcard (Monday, 8th July 2002) and completion of study (Saturday 31st August 2002). Dotted lines represent 95% confidence interval.

Table 2.1.6 and figure 2.1.3 summarise return patterns to the two survey mailings, including the proportion of returns with which participation was declined. There were no surveys returned because of an incorrect address. Reasons for declining participation in the survey included moving abroad (n = 1), disagreeing in general with the principle of surveys (n = 1), retirement (n = 2) and the remainder (n = 11) did not believe they did enough equine work to participate in the survey, i.e. they had probably been contacted in error.

Table 2.1.6. Mailing numbers, return categories and observed return, response and completion proportions of the practitioner survey.
95%CI = 95% confidence interval.

Return category	Returned (of 302)	
	Number	Percentage (%) (95%CI)
First Mailing	148	49.0 (43.4 to 56.5)
Second Mailing	94	61.0 (55.5 to 66.5)
Participation declined	15	5.0 (2.5 to 7.4)
Returned proportion (also response proportion)	242	80.1 (75.6 to 84.6)
No return by end of study period	60	19.9 (15.4 to 24.4)
Completion proportion (usable surveys)	227	75.2 (70.3 to 80.0)

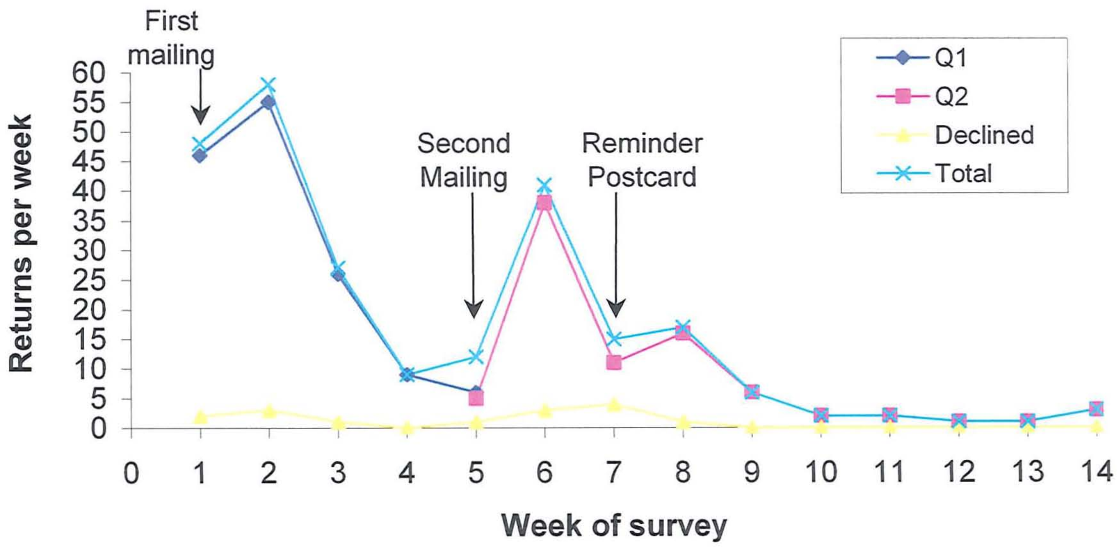

Figure 2.1.3. Return frequencies over time for the practitioner survey.
The arrows indicate the three mailings, first mailing Monday, 27/05/02, second mailing Tuesday, 25th June 2002 and the postcard reminder Monday, 8th July 2002. Total: total number of returns regardless of reason; Q1 or Q2: mailing 1 or 2 returned; Declined: participation rejected by returning an uncompleted questionnaire.

The first mailing elicited 6/148 (4.05%) rejections of participation in the survey compared to 9/94 (9.57%) in the second mailing. As the number of interventions required to get a response increased, there was a trend for the proportion of respondents declining participation also to increase (χ^2 for linear trend 3.8, p = 0.05)(table 2.1.7).

Table 2.1.7. Table comparing the number of questionnaires returned completed per intervention to the number returned uncompleted (indicating a rejection of participation).

	Completed questionnaires	Declined participation	Proportion declined (%)
First mailing	142	6	4
Second mailing	49	4	8
Postcard reminder	36	5	14

Table 2.1.8 gives median time and range (in days) between the date of each mailing and the arrival of a response. The median time for all returns of the questionnaire was 15 days from day zero. More than 90 % of the returns had been received by the end of the eighth week of the survey (day 55). Three questionnaires were returned after completion of the study (45, 46 and 111 days after study completion). These were not included in analyses.

Table 2.1.8. Return times for the practitioner survey.

Return mailing	**Number returned**	**Return** (days)	
		Median	Range
Questionnaire 1 (first mailing)	148[a]	15	2 to 29
Questionnaire 2 (second mailing)	94[a]	10	2 to 67
Total response (both mailings)	242[b]	15	2 to 96

[a] Days to return counted from the mailing day of that specific questionnaire (first mailing at day 0, second mailing day at day 30); all responses were counted

[b] Days to return counted from date of first mailing (day 0); all responses were counted.

The response to the reminder postcards is shown in table 2.1.9. The signing of reminder postcards was associated with a higher response (χ^2_1= 3.89, p = 0.048). The ratio of odds for responding following receiving a signed reminder postcard and an unsigned postcard was 2.27 (95% confidence interval (95%CI) = 1.00 to 5.12).

Table 2.1.9. Return of questionnaires (completed and uncompleted) following mailing of postcards to non-responding veterinary surgeons, half of the cards were signed.

	Response	No response
Signed postcards	26	26
Unsigned postcards	15	34

The sending of a signed reminder postcard increased the risk at any given time of the recipients responding by 1.85 (95%CI = 0.98 to 3.49) times, although this was not statistically significant p = 0.059 (appendix A2.1.7). Figure 2.1.4 is a Kaplan-Meier survival curve for return of questionnaires by recipients of signed and unsigned postcards. The corresponding log rank test indicated that the difference between the two groups approached significance (Logrank = 3.69 for 1 degree of freedom, p = 0.05).

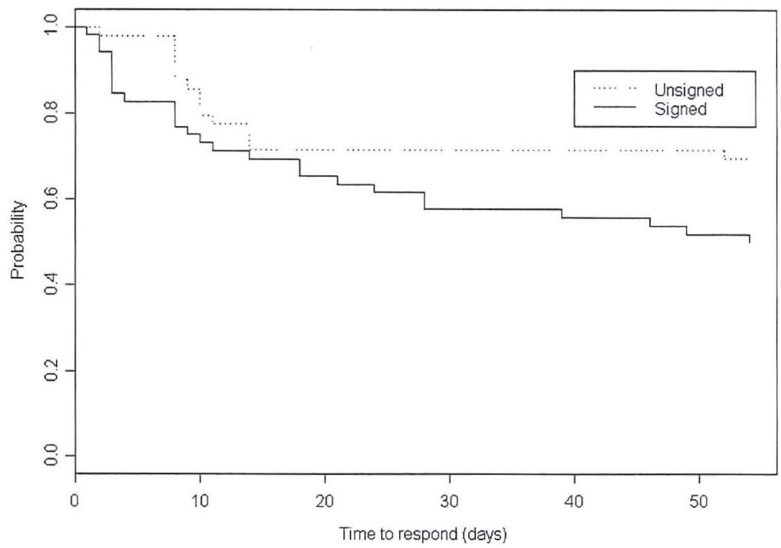

Figure 2.1.4. Kaplan-Meier survival curve for return of questionnaires by recipients of signed and unsigned reminder postcards.

2.1.3.3. Factors associated with response – information in the public domain

The following factors associated with response were calculated using the 227 completed (useable) returned questionnaires and the 60 non-responders. Approximately equal proportions of men (179 (78.2%; 95%CI = 72.8 to 83.5%)) and women (48 (82.8%; 95%CI = 73.0 to 92.5%) responded to the survey ($\chi^2_1 = 0.59$, p = 0.4). There was no significant difference between the two genders for the number of days taken to respond (U = 19692, p = 0.1). There was no significant effect of University of graduation on the proportions responding, which ranged from 78 to 83% ($\chi^2_6 = 0.64$, p = 1.0)(appendix A2.1.8), or the mailing stage response occurred ($\chi^2_{18} = 13.8$, p=0.7)(figure 2.1.5; appendix A2.1.9). The time, in days, to respond was also not significantly different between veterinary schools (H= 5.87, DF = 6, p = 0.4; appendix A2.1.10).

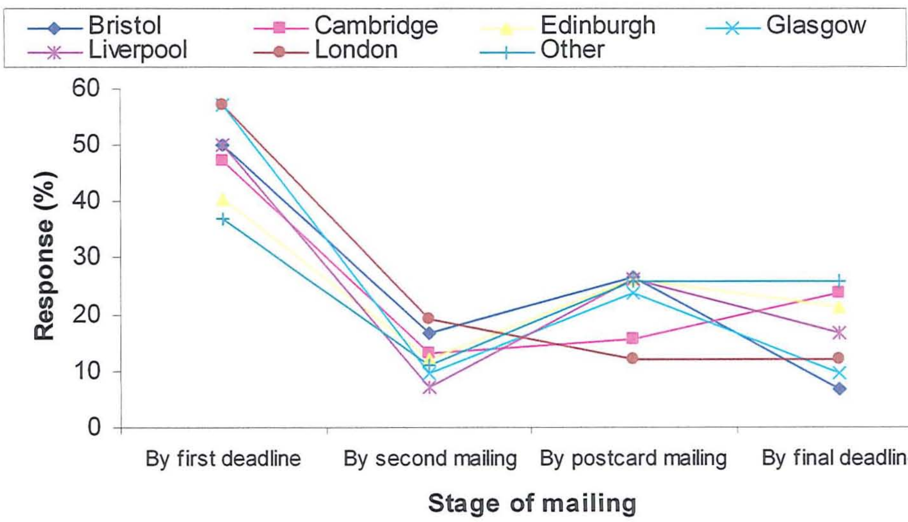

Figure 2.1.5. Response pattern for each of the five UK universities and overseas veterinary schools (other) for each stage of questionnaire mailing.

How long a person had been qualified did not affect whether or not they would respond (t = -0.29, p = 0.8) or the number of days it took to get a response (H = 8.56, df = 7 p =0.3; appendix A2.11). Veterinary surgeons who had been qualified for between 21 and 25 years and 31 to 35 years were least likely to reply (figure 2.1.6); but this was not significant (χ^2 for linear trend = 0.16, p = 0.7; appendix A2.1.12). The stage of mailing at which a recipient responded was not different between different band groupings of years qualified (χ^2 for linear trend = 23.6, p = 0.3; appendix A2.1.13).

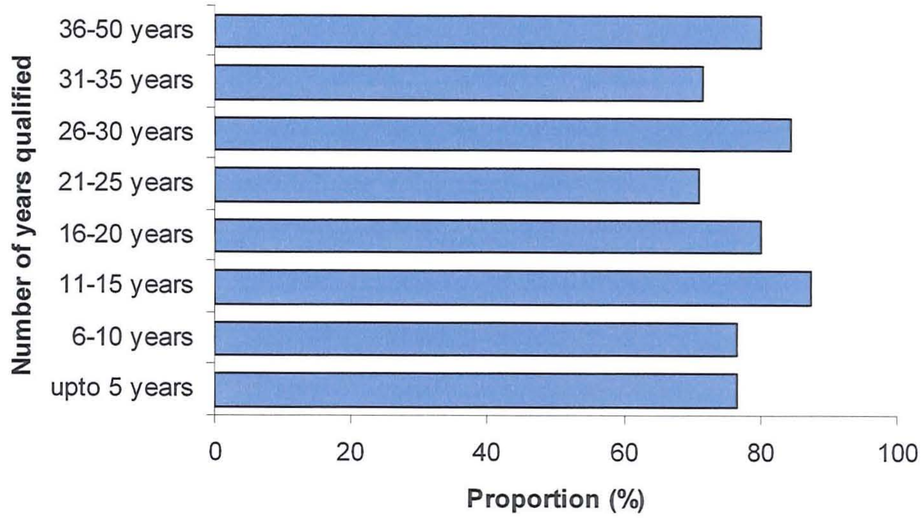

Figure 2.1.6. Response by the number of years since qualification as a veterinary surgeon (years since registered with RCVS).
Number of years qualified: divided into five-year bands except for last band spanning 36 to 50 years.

All holders of the RCVS certificate in equine internal medicine responded (15/15) and almost all holders of the certificate in equine practice (40/44). Overall, veterinary surgeons with further professional qualifications were considerably more likely to respond than those without (χ^2_1 = 6.452, p = 0.01)(table 2.1.10). Equine practitioners with further qualifications responded to earlier mailings than those with no further qualifications (χ^2 = 2.64, p = 0.01)(table 2.1.11).

Table 2.1.10. Table of the proportion of veterinary surgeons responding by ownership, or not, of a professional qualification.

	Response	No response
No further professional qualifications	159	51
Further professional qualifications	68	8

Table 2.1.11. Table of the proportion of practitioners responding at different stages of mailing by ownership, or not, of a professional qualification.

Stage of response	Further professional qualifications	No further professional qualifications
By first mailing deadline	38	73
By time of second mailing	9	22
By time of postcard mailing	14	35
By final deadline	7	29

A veterinary surgeon with a further professional qualification was 1.24 (95%CI = 1.07 to 1.43) times more likely to respond at any given time than someone who had not obtained such a qualification (appendix A2.1.14). Kaplan-Meier curves for time to return of questionnaire (figure 2.1.7) were significantly different (Logrank test = 8.81, one degree of freedom, p = 0.003) for those with further qualifications compared to those without.

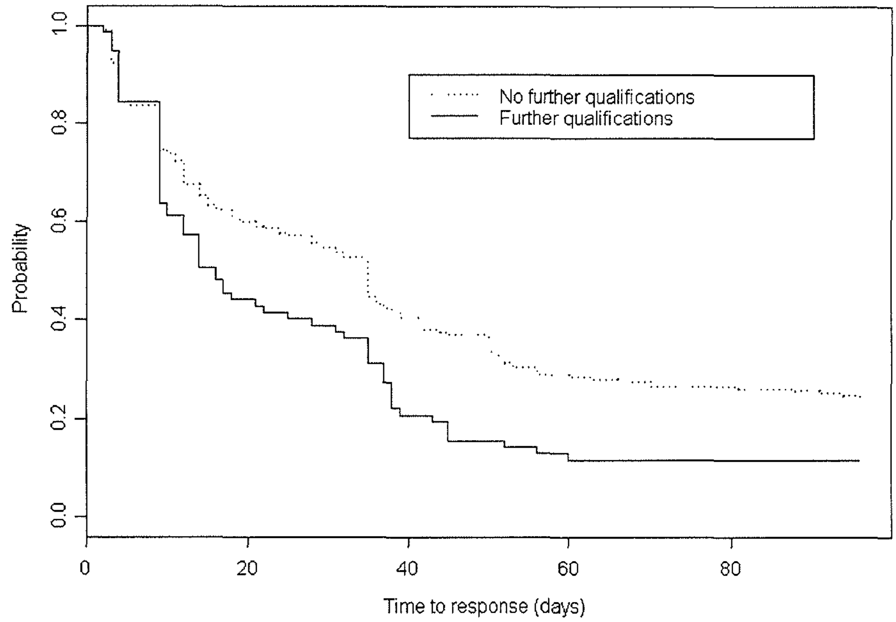

Figure 2.1.7. Kaplan-Meier survival curve for time to response (return) of questionnaire depending on possession of further professional qualifications.

Most of the UK regions had similar response proportions (appendix A2.1.15). Statistical analysis was limited because the population numbers in Northern Ireland were low. When each region was compared against the remainder of the UK, only Scotland had a significantly higher response proportion ($\chi^2_1 = 6.74$, p = 0.01)(table 2.1.12). The odds ratio for each region responding compared to Scotland was relatively low (χ^2 for linear trend = 12.8, p = 0.0003)(table 2.1.13).

Table 2.1.12. Proportion of practitioners responding from Scotland compared to the rest of the United Kingdom.

	Response	No response
Scotland	31	1
Rest of UK	199	59

Table 2.1.13. Proportion of practitioners responding from each region compared to the response proportion from Scotland.
95%CI = 95% confidence interval.

Region	Response	No response	Proportion responding	Odds ratio	95%CI
Scotland	28	1	0.97	1.00	
Eastern England	36	4	0.90	0.32	0.03 to 3.04
Midlands	21	4	0.84	0.19	0.02 to 1.80
Wales	7	2	0.78	0.13	0.01 to 1.58
North West	19	6	0.76	0.11	0.01 to 1.02
South & South East	63	21	0.75	0.11	0.01 to 0.84
South West	34	13	0.72	0.09	0.01 to 0.76
North East	17	7	0.71	0.09	0.01 to 0.77
Northern Ireland	2	2	0.50	0.04	0.00 to 0.59

The response pattern was similar for most of the regions apart from Wales (figure 2.1.8; appendix A2.1.16). The time to response (in days) was different between the regions of the UK; H = 16.3, p = 0.04 (table 2.1.14). Recipients in Wales took significantly longer to respond than recipients in the North East and Scotland for example.

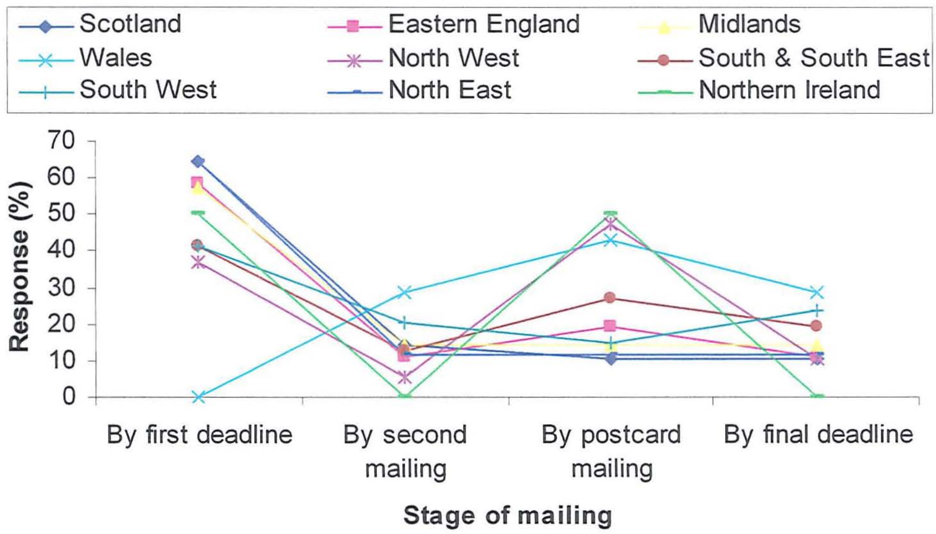

Figure 2.1.8. Proportion of response by practitioners, depending on the regional location, for each stage of the questionnaire mailing.

Table 2.1.14. Summary of time to response by practitioners for the nine UK regions.

Region	Median time to response (days)	Range (days)
North East	9	2 to 60
Scotland	11	3 to 94
Midlands	11	4 to 88
Eastern England	12	3 to 91
Northern Ireland	19	3 to 35
South West	22	3 to 70
South & South East	28	2 to 66
North West	35	2 to 66
Wales	36	24 to 96

2.1.3.4. Responder provided factors associated with time to respond

Greater than half the responders (67.3%; 95%CI = 61.2 to 73.4%) indicated that they spent over 90% of their working day dealing with horses (figure 2.2.1). In fact, the majority of respondents (59.3%; 95%CI = 52.6 to 65.4%) indicated that they worked exclusively with horses. Respondents working predominately with horses returned their completed questionnaires slower than veterinary surgeons that worked less than 90% of their time with horses ($\chi^2_1 = 32.3$, $p < 0.0001$)(table 2.1.15). Veterinary surgeons who worked for less than 90% of their time with horses responded in a median of 11 days compared to a median of 18.5 days for practitioners who worked greater than 90% of their time with horses. The time to response was significantly different between these two groups, $U = 7451.5$, $p = 0.02$.

Table 2.1.15. Table comparing early return of a questionnaire compared to late return by the proportion of equine work carried out by responding practitioners.

	Proportion of workload made up by horses	
	Less than 90%	**Greater than 90%**
Early return (before second mailing)	54	88
Late return (after second mailing)	21	164

Scotland had the smallest proportion of veterinary surgeons that worked for greater than 90% of their time with horses (64.7%; 95%CI = 47.0 to 82.4%)(figure 2.1.9). This was significantly different from the rest of the UK regions combined ($\chi^2_1 = 11.1$, $p = 0.001$)(table 2.1.16a).

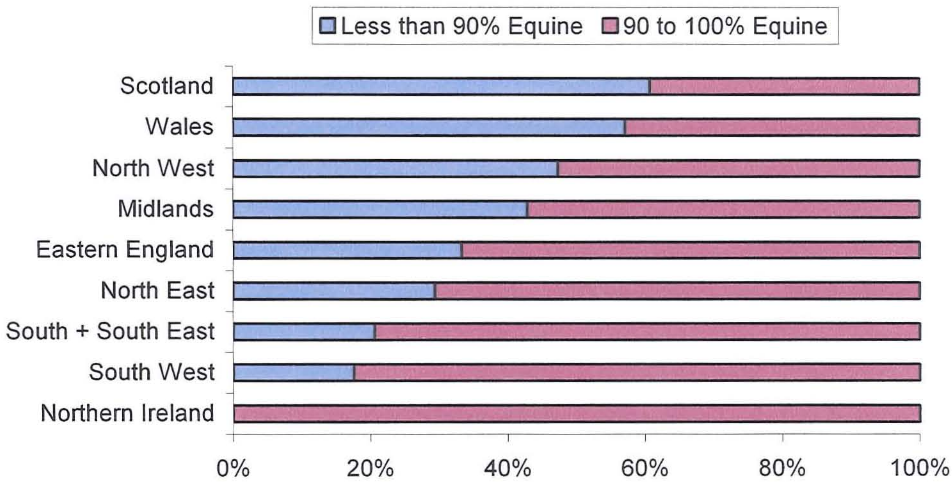

Figure 2.1.9. Proportion of practitioners' workload that was horses within the nine UK regions.

Table 2.1.16. Table of (a) Geographic location and (b) possession of further professional qualifications according to the proportion of equine workload.

a.		
	Scotland	**Rest of the UK**
Less than 90% Equine	17	58
90 to 100% Equine	11	141
b.	**Further professional qualifications**	**No further professional qualifications**
Less than 90% Equine	13	62
90 to 100% Equine	55	97

In the sample selected, the proportions of veterinary surgeons that worked for greater than 90% of their time with horses varied between the universities of graduation ($\chi^2_6 = 13.8$, $p = 0.03$)(figure 2.1.10). For example, greater proportion of graduates from Bristol University worked predominately with horses (85.7%; 95%CI = 72.8 to 98.7%) than graduates from Glasgow (52.3%; 95%CI = 31.0 to 73.7%) or London (52.5%; 95%CI = 37.0 to 68.0%).

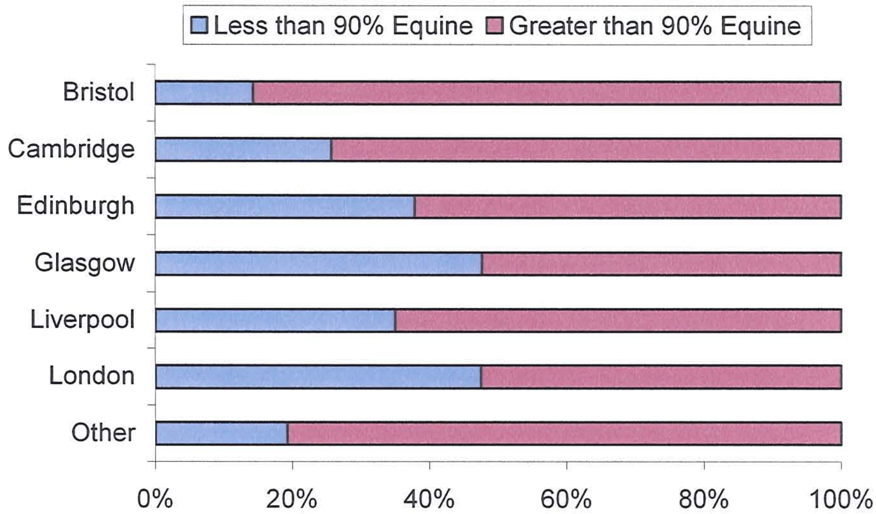

Figure 2.1.10. Bar chart of the proportion of time (less than or greater than 90%) that respondents qualifying from different veterinary schools spent working with horses. Other = International veterinary schools

Responders who had qualified more recently tended to work predominately with horses ($U = 10058$, $p = 0.001$ (adjusted for ties)). Median time since qualified for veterinary surgeons working for greater than 90% of time with horses was 14 years compared to 20 years for practitioners that worked less with horses. Holders of a further professional qualification worked, more often than not, predominately with horses ($\chi^2 = 8.47$, $p = 0.004$)(table 2.1.16b).

The type of equine practice that a responder worked in did not affect how long it took them to respond ($U = 16979$, $p = 0.8$). There was no difference between the types of equine practices respondents worked in and their geographical location (χ^2 for linear trend = 12.6, $p = 0.08$), university of graduation (χ^2 for linear trend = 9.3, $p = 0.2$) or number of years qualified ($U = 16660$, $p = 0.3$). Practitioners with further professional qualifications were more likely to work in practices that did some referral work or exclusively did referral work ($\chi^2_1 = 23.8$, $p < 0.001$)(table 2.1.17).

Table 2.1.17. The possession of a further professional qualification by whether or not a respondent did a component of referral work

	First opinion work only	First opinion with some referral work or exclusive referral work
Further professional qualifications	29	39
No further professional qualifications	121	38

2.1.3.5. Cost of survey and item omission

The cost per useable returned questionnaire was £2.40. Costs are summarised in appendix A2.1.17, labour costs (the principal investigator) were not included.

The items omitted by respondents in their returned questionnaires are summarised in table 2.1.18. Omissions were rare in first part of questionnaire detailing personal information and historical questions related to RAO. Questions related to diagnostic tools were well answered apart from questions related to pulmonary function tests (omission rate = 7.0%) and challenge provocation tests (omission rate = 5.7%) that accounted for 71% of the omissions in that category. 10% of the respondents did not perform the ranking task as requested in the second part to the question. Questions related to clinical signs had a high omission rate in relation to certain questions, the following 5 accounting for 75% of the omissions: rate of respiration (20 to 30), cough when squeeze trachea/larynx, cough when rebreathing bag applied, wheeze/crackles detected on auscultation, and regions of silence on auscultation. The latter accounting for 38 % of the omission rate from these 5 categories, this was the last question in the series. These figures were calculated after people who stated they never used a rebreathing bag had been excluded from the responses relating to the use of rebreathing bags.

Table 2.1.18. Item omissions
(SF = separate format; GF = group format).
95%CI = 95% confidence interval.

	Question subject	Percentage omission rate	95%CI (%)	Total number of possible responses
1. Professional details				
SF	1.1 Percentage equine work	0	-	227
SF	1.2 Alternative species	0.4	0.1 to 2	227
SF	1.3 Type of practice	0	-	227
SF	1.4 Type of workload	0	-	227
2. Historical				
GF	Presenting clinical signs	1.0	0.5 to 1.5	1589
GF	Medical history	1.0	0.4 to 1.6	1135
GF	Feeding management	0.6	0.02 to 1.2	681
GF	Stable management	0.3	0.03 to 0.8	908
3. Diagnosis				
GF	3.1 Diagnostic tools	1.5	1.0 to 2.0	2724
SF	3.2 Level of diagnosis	1.3	0.3 to 4.0	227
GF	3.3 Clinical signs	2.6	2.0 to 3.2	2497
GF	3.4 Endoscopic findings	7.3	5.6 to 9.0	908
4. Management & Treatment				
GF	4.1 Management	1.8	1.2 to 2.4	2043
SF	Time hay soaked for	6.2	3.1 to 9.3	227
SF	Bedding recommended	0	-	227
GF	4.2 Treatment	3.0	2.3 to 3.7	2497
SF	4.3 Stepwise	0.9	0.1 to 3	227
SF	4.4 Prevalence	0	-	227
	Overall 325 total omissions	**1.3**	**1.2 to 1.4**	**25197**

Questions relating to endoscopy were poorly answered, even after eliminating those who never used endoscopy. 42% of the respondents omitting this question used an endoscope rarely. Management and treatment were generally well answered. The question related to treatment was the most poorly answered grouped question, with a 3% item omission rate, particularly relating to the use of clenbuterol/antibiotics (3.5%) antibiotics (7.9%), inhaled agents (11.5%) and systemic anticholinergic (3.5%). Combined, these accounted for 79% of the omissions in this section.

35 individuals accounted for 58 % of the omissions, omitting to answer from 4 to 10 questions. All four questions on endoscopy were commonly omitted with 10 people omitting all four. These individuals did not reply any slower than the rest of the sample (χ^2 for linear trend = 0.67, p = 0.4) but a greater proportion did less than 90% of equine work ($\chi^2_1 = 6.3$, p = 0.01). This group of high question omitters had qualified in the more distant past (median time since qualified = 20 years) compared to more complete responders (median time since qualified = 14 years)(U = 21269; p = 0.04).

Section 2.1.4. Discussion

This survey achieved a high response rate from veterinary surgeons in the United Kingdom who worked with horses. Previous mailed surveys of equine veterinary surgeons in the UK (Price *et al.*, 2002), Australia (Christley *et al.*, 2000b) and USA (Traub-Dargatz *et al.*, 1991, Moll *et al.*, 1995, Southwood *et al.*, 1997) have all achieved lower response rates, with one having a response rate of 25%. This favourable response rate may reflect the careful survey design, a questionnaire content that struck a chord with motivated individuals or perhaps because the sample was directly selected.

The sample population studied was not randomly selected; this was partly because of the unavailability of relevant databases. However, the survey needed to be targeted at veterinary surgeons that worked predominately with horses. Less than 10% of all the UK veterinary professions' time spent working in veterinary practice is with horses (equivalent to approximately 1000 full time veterinary surgeons) (Anon, 2002). It would have been costly (and largely irrelevant to the majority of recipients) if the survey had been mailed to a randomly selected sample of all veterinary surgeons. It is important to emphasise that none of the recipients were selected on their geographic location, university of graduation or number of years qualified. However, bias in selection cannot be ignored as a result of the personal knowledge of the compiler as this was used to guide the process (for example previous work colleagues, previous job locations etc.). Actively seeking out holders of further professional qualifications may have selected for veterinary surgeons with more motivation and who worked in particular types of practices. Named veterinary surgeons (rather than just mailing anonymous surveys to practices) were required to encourage a high response rate and to permit some knowledge of a respondent's professional experience to be assessed.

It is difficult to interpret how the sample population selected represented the target equine veterinary surgeon population, as no data are available. It is important to note that it was not the aim of this study to obtain a representative sample. The sample size chosen represents one third of the number of full time equivalents working in equine practice as estimated by the RCVS (Anon, 2002). Information is available for the general veterinary population but it would be erroneous to assume this applied to the target population. One concern is the low proportion of women in the sample (20.5%). This is partially allayed by the fact that overall, women only make up 38% of the full time veterinary practice workforce (Anon, 2002). Representatives from throughout the UK were selected with some regions contributing substantially more to the sample than others. For example the South and South East provided 29% of the sample. This is not surprising, as it is a large geographic region with large human and horse populations. One recent approximation suggested 33% of the UK horse population was in the South East of England (Leckie, 2001). Other variables such as place of university of graduation were fairly evenly spread amongst the sample.

A modification of the total design method (TDM) was used to implement the questionnaire survey (Dillman, 1983). The TDM consists of two parts, firstly identifying and designing each aspect of the survey process that may affect response in a way that maximises response rates and secondly organizing the survey effort in a way that assures that the design intentions are carried out in complete detail. Great emphasis was placed on the design of the questionnaire and how it was presented to the recipient. This included professional printing of the questionnaire booklet and the personalization of all mailings. To encourage a response, pre-paid business reply envelopes were provided; in an American survey of health professionals the use of a post-office stamped envelope was advocated (Streiff *et al.*, 2001) although a recent UK survey of the general population showed it to be of no benefit (Harrison *et al.*, 2002). Pre-paid business reply envelopes were used for their convenience and presumed cost saving.

The design of the questionnaire booklet, cover letter content (plus inclusion of mailing date, individual name and addresses, hand written signatures etc.) as well as the use of first class postage and the provision of a pre-paid reply envelope all closely followed the total design method. The main difference from the method Dillman recommended was a deviation from the strict timetable of mailings he stipulates (First mailing, postcard reminder at one week, second mailing of questionnaire at 3 weeks and a third mailing of the questionnaire by certified mail at seven weeks). The timetable used was similar to that utilised in a survey of Australian equine practitioners (Christley *et al.*, 2000b). In the present study, the postcard reminder substituted for the follow-up telephone calls used in the Australian study. The high response emphasises the importance of attention to detail encouraged by Dillman in the design and production of the complete mail-out package. However, it casts doubt on the need for strict adherence to the mailing timetable he advocates. It is likely that factors influencing response, such as return envelopes and follow-ups, are essential but different factors may be at work in different survey situations and that these are probably not stable across all populations. A final mail-out using certified mail would have been costly and was personally believed to be potentially inflammatory to recipients. The satisfactory response to this survey could reflect the high motivation of the professionals contacted as well the relevance of the subject matter to the respondent.

The response proportion obtained in this study was 80.1% (75.6 to 84.6%) while the completion proportion (number of useable surveys) was 75.2 (70.3 to 80.4%). The plateau in response caused by the Queen's golden jubilee public holiday (no postal service for 2 normal working days) was unfortunate, and could have been avoided by delaying the first mailing by two weeks. The effect on the overall response rate will never be known. Each intervention subsequent to the first mailing stimulated an increase in response as can be seen from the cumulative frequency graph (figure 2.1.2). The slowing down of the response rate just before the second mailing emphasised its necessity at that time. Avoiding anonymous mailings and the use of reminders, in particular with the inclusion of a second instrument, is believed to increase response rates (Asch *et al.*, 1997, Wensing *et al.*, 1999). The postcards also boosted the response to date and the use of signatures was demonstrated to be beneficial. Recipients of signed postcards were more likely to respond than those who received unsigned cards. The importance of handwritten signatures in survey correspondence has been highlighted in other studies (Sloan *et al.*, 1997). It might have been more logical, and potentially cheaper, to have used the postcards for the second intervention rather than the repeat mailing of the instrument; fewer of the latter expensive mail packets might have then been required for the third intervention.

Only a small proportion of veterinarians declined to participate, the majority because they believed they did not do enough equine work to complete the questionnaire. It can, therefore, be interpreted that these people had been contacted in error and could be excluded from the target population. However, the numbers involved were relatively small (n = 15) and did not greatly affect the response proportion (revised response proportion 227/287 = 79.5%). These individuals were excluded from the calculations to test for response bias. As the number of interventions increased so did the likelihood that a responder would decline to participate (table 2.4). This could infer that a proportion of the remaining non-responders were potentially not part of the target population, i.e. not working with horses to any great degree. By the final deadline, the response rate was deemed satisfactory and a decision was made to not pursue non-response, as it was believed the time and cost involved would not justify the ends.

The modified TDM was used with follow-ups and reminders in an attempt to maximise the response rate. Dillman (1983), the creator of the method, reported that all published studies using TDM have achieved a response rate of greater than 60%. In one review of five conducted surveys, for samples of 301 to 500 veterinary surgeons, a mean response proportion of 60% (s.d. = 24%) was identified (For samples of 100 to 300 the mean was 75%; s.d. = 18%) (Vaillancourt *et al.*, 1991). In a survey of Australian equine practitioners, using TDM, a response proportion of 67% was achieved (Christley *et al.*, 2000b). The threshold of what is deemed an acceptable response rate is a matter of debate. A high response rate is desirable as non-response reduces the statistical power of a study and can introduce selection bias. There are more opportunities for bias when response is low, but it does not necessarily mean the response obtained will not be representative. Similarly, a high response does not mean there is no bias; it just reduces the extent or possibility of bias (Barclay *et al.*, 2002).

In surveys of general practitioners of human medicine response rates are generally lower than other populations (Asch *et al.*, 1997). A review of general practitioner surveys indicated a mean response rate of 61% (Sibbald *et al.*, 1994). Response rate to surveys amongst general practitioners is falling even though they are important in a primary care led National Health Service that is driven by evidence based practice (McAvoy and Kaner, 1996). It has been suggested that response rates of greater than 55% are required in general practitioner surveys although 70% is commonly recommended, as most biases are believed to be minimal at this level (Ashworth, 2001). Contrary to this, one study indicated that the additional effort to raise response rate was not necessary to reduce selection bias (van der Wiel *et al.*, 2002). On the other hand other researchers state that even the high response rate of 70% is inadequate (Ashworth, 2001). This is because, in contrast to the common assumption, non-responders are often fundamentally different from responders (Armstrong and Ashworth, 2000). For example, studies of general practitioners have identified that responders are more likely to be younger, work in bigger practices, have lower case loads and hold further qualifications (Cartwright, 1978, Armstrong and Ashworth, 2000, Stocks and Gunnell, 2000). However, in certain survey situations bias has been identified with response rates of 80% or more (Sheikh and Mattingly, 1981, Partin *et al.*, 2003). In complete antithesis, studies with low response proportions may be less biased than studies with high proportions (Stang and Jockel, 2004). Studies that have directly compared the results of surveys including and excluding late responders have consistently found little impact on results (Templeton *et al.*, 1997, Field *et al.*, 2002), suggesting that the loss of difficult-to-reach physicians may not have a large biasing effect.

All these conflicting studies perhaps suggest that there is no "acceptable" response rate (apart from 100%) and emphasis should be placed on testing for bias. The first approach is to re-survey a sample of (or all) non-responders (Armstrong and Ashworth, 2000). However, this method can alter the information responders provide as they have been "interfered" with (i.e. surveyed, often with personal intervention). An alternative, and the approach used in this study, is the assessment of non-response bias using publicly available data (Barclay *et al.*, 2002). This is done by comparing non-responders to responders as well as contrasting early responders to late responders. It is also feasible in some situations to compare responders to the whole population so it can be assessed whether the data collected can be applied in general. This was not possible in this study for reasons already discussed. Bias may also be investigated by examining the difference in outcome (responses given) between early and late respondents, and its extent may be proportional to the delay in response (Sheikh and Mattingly, 1981). This approach is further investigated in chapter 2.2.

The demographics chosen for testing for non-bias were easily available and were not linked to the focus of the questionnaire. In general, there was little difference in terms of gender, veterinary school attended and the length of time since qualified between responders and non-responders. Neither did these variables affect how long it took for respondents to reply. This was encouraging as it indicated that, perhaps, the two groups consisted of similar people who would have similar opinions and therefore bias due to differential response might have been minimal. There was no difference in the propensity to respond between non-UK graduates and graduates from UK veterinary schools; amongst general practitioners of human medicine this has been found to be a significant variable with the former more likely not to respond (Barclay *et al.*, 2002). Veterinary surgeons with further professional qualifications had been actively sought and these subjects were far more likely to respond than lesser-qualified colleagues; they were also more likely to respond promptly. These individuals may be more highly motivated, prepared to think about their decision making and willing to contribute their opinions. Not surprisingly, these respondents were more likely to work with horses and/or work in a practice that did at least some referral work. Thus, the data acquired from this survey will be biased towards the opinions of practitioners that could be considered to be "equine specialists". These veterinary surgeons may have the luxury of working with higher quality facilities and perhaps even with a higher quality of horse being kept in better conditions.

There was also a subtle difference between non-responders and responders in their geographical location. Overall, there was little difference between the regions of the UK except for inhabitants of Scotland. Practitioners working in Scotland were more likely to reply than any other region. These practitioners were also least likely to have a workload of greater than 90% horses when compared to the rest of the UK. This study was conducted from a Scottish University and this may have given respondents in the same country an extra incentive to respond. Their opinions could be over-represented and, in addition, may be those of vets with less experience of working with equidae. If this regional effect is valid then the use of multi-centre collaboration for mailing out the questionnaire could be beneficial.

Practitioners who worked predominately with horses (> 90%) were slower to respond than other respondents. This may be because vets working in this kind of practice are traditionally away from the practice for a great proportion of the day. Thus, they have limited "office time" each day during which a questionnaire has to compete with their main priorities (phones calls to clients, completing vetting forms, in-patients). Veterinary surgeons who spend a greater proportion of their time at their practice (for example the 17% who also did some small animal work) may have more opportunity to complete a questionnaire. The decreased proportion of time spent working with horses by Scottish respondents may explain the high response from this region. Unfortunately, these results could be interpreted that those very busy in equine practice, and therefore potentially able to provide valuable information, might not have responded at all.

In the study sample, certain veterinary schools were associated with veterinary surgeons that worked for a greater proportion of their time with horses. It is hard to interpret the importance of this finding in light of the non-random selection of this sample from the target population, although this demographic was not selected for. It could be that certain veterinary schools could select or motivate individuals towards certain types of practice. The opinions these individuals hold on particular clinical issues may be influenced by the education they received at their alma mater.

The overall omission rate was relatively small. This was important as it minimised the amount of missing data. As the amount of missing data increases the less the final results can be expected to represent the actual study sample. A high omission rate would also suggest that large portions of the questionnaire were misunderstood. Having two parts to the same question, as in the question on diagnostic tools, is often not recommended and the high proportion of omission reflects this. Questions concerning endoscopy were also poorly answered. This may reflect a paucity of undergraduate and continuing professional training available in the specific use of, and interpretation of findings from, this diagnostic tool. There is limited literature and research into exactly what constitutes abnormality in the use of this tool. In addition, this information is not easily available to the majority of practitioners. The omission by 10 respondents of all four questions concerning endoscopy may suggest that they never used it, although they indicated otherwise earlier in the questionnaire. It is a concern that 15% of the responders accounted for over half of all the omissions and that these individuals did less equine work and had been qualified for more years. Thus, they may well be consistently different from the rest of the sample and thus provide alternative information or answers that would not be represented in the data extracted from the questionnaire. Perhaps their high omission rate related to a relative lack of interest in the subject, as they spent less time working with horses.

It is difficult to compare the cost of the survey, £2.40 per useable return, to other studies as a result of inflation, exchange rates etc. Mailed surveys are considered to be relatively cheap (Abramson and Abramson, 1999). The use of a business response service licence was convenient, although only represented a small saving of about £10 (using 446 second class stamps at 19p each on the return envelopes would have cost £86.64).

Despite having a response percentage of 80%, a figure that some authors would deem high enough to avoid bias (Martin *et al.*, 1987), there were some significant differences between responders and non-responders, thus indicating the possibility for bias. It is important to note that the demographics available were somewhat limited as tools to assess an individual's characteristics. Additionally, actual quantification of the extent of bias introduced is not possible and it may well be negligible. Some influences of bias on the actual results of the questionnaire outcome are discussed in chapter 2.2. It is important that an investigator highlights possible biases so that a reader can appropriately interpret the results from the survey in question.

CHAPTER 2

A CONFIDENTIAL SURVEY OF THE OPINIONS OF EQUINE PRACTITIONERS, IN THE UNITED KINGDOM, ON ISSUES ASSOCIATED WITH DIAGNOSIS AND TREATMENT OF RECURRENT AIRWAY OBSTRUCTION

Part 2: Results of a Survey of Equine Veterinary Surgeons

Section 2.2.1. Introduction and Aims of Study

Recurrent airway obstruction (RAO) is thought to be a relatively common cause of coughing affecting the horse population of the United Kingdom (McPherson and Thomson, 1983); it has even been suggested it is increasing in incidence (Mair, 1995). However, the true prevalence is unknown. Its importance in the equine field is perhaps demonstrated by the emphasis that appears to be placed on investigating the disease's aetiopathogenesis, diagnosis and treatment, with over 250 scientific papers published since 1970, and a dedicated international workshop (Robinson, 2001b).

In spite of the extensive research into RAO, there is no single fully-validated reference standard for diagnosis of this respiratory disease, especially in first opinion practice. The attitudes of equine veterinary surgeons towards the disease, including important historical and clinical information as well as further diagnostic tools utilised, may provide an insight into how RAO is diagnosed in the general horse population. It was hoped that a survey of this kind would provide useful information for the construction of a risk-screening questionnaire for epidemiological investigation of RAO in the UK horse population. Information was also sought for how equine practitioners managed and treated RAO. The purpose of the study was to survey equine veterinary surgeons working in the United Kingdom on their approach to the diagnosis, management and treatment of RAO.

Section 2.2.2. Materials and Methods

The design and implementation of this survey were described in chapter 2.1. In summary, the Royal College of Veterinary Surgeons (RCVS) database of practices was used to identify practices that offered care for horses. By cross-reference to a number of sources, individual veterinary surgeons were identified as working at these practices. A total of 302 veterinary surgeons were identified in the survey population. The questionnaire booklet contained four sections regarding professional details, historical information related to RAO, diagnosis of RAO and management and therapy of RAO. The term RAO was used to identify the respiratory disease characterised by reversible lower airway obstruction in mature horses induced by environmental challenge. Attention was brought to the former name used for this disease, chronic obstructive pulmonary disease (COPD), and reference made to the international workshop on equine chronic airway disease (Robinson, 2001b). All non-respondents were sent a second copy of the questionnaire 29 days from the first mailing. Fourteen days later, a postcard reminder was sent to all remaining non-responders. At the conclusion of the survey, 227 (79.1%) completed questionnaires had been obtained from 287 veterinary surgeons (after excluding vets contacted in error).

2.2.2.1. Analysis

Completed questionnaires were scanned using a fujitsu fi-41110cu image scanner and the resultant images imported into TELE*form* Elite v8 data capture software. After verification the coded data were exported into Microsoft Excel 2000. Responses to questions on historical information, diagnosis and treatment were ranked using a frequency score. The score was calculated by adding the percentage response for each category multiplied by a weighting. For historical information (questionnaire section 2.1, appendix A2.1.1) this weighting was: definitely important = 1, probably important = 0.67, possibly important = 0.33 or definitely not important = 0. For diagnosis (questionnaire section 3.1) the weighting was: always = 1, frequently = 0.75, sometimes = 0.5, rarely = 0.25 and never = 0. For management and treatment (questionnaire section 4.1 and 4.2) this weighting was: almost always = 1, frequently = 0.67, sometimes = 0.33 or never = 0. Clinical signs associated with the degree of RAO severity (questionnaire section 3.3) were changed from multiple choice, with all grades of severity that a particular sign was considered associated with by respondents, to the degree of severity respondents first associated the disease with, and therefore, by association, the subsequent disease severity. For example, it was assumed if nasal discharge was associated with mild RAO, by implication it is then associated with moderate and severe. These clinical signs were grouped by the severity most frequently selected by respondents (majority). In section 3.3 of the questionnaire, respondents who had indicated in section 3.1 that they never used a rebreathing bag to aid auscultation were excluded from the two questions concerning use of a rebreathing bag. Similarly, participants were excluded from the analysis of section 3.4 of the questionnaire if they had previously specified that they never used endoscopy.

Responses to sections one, two and three of the questionnaire were analysed for differences between demographic groups from information in the public domain i.e. geographic location, university of graduation, length of time qualified and further professional qualifications. Information provided by respondents was also used to assess differences between groups i.e. proportion of daily equine work. Only significant differences are reported. The responses for the first three sections provided by participants were also compared depending on the stage at which their questionnaire was returned (early: late response). Comparisons between groups were made using Mantel-Haenszel chi-square analysis. Where expected cell counts were less than 5, Fisher's exact test was utilised. Where the variable was ordinal, trend chi-square analysis was also performed. Epi Info v6.04d was used to conduct these statistical analyses. Significance for all analyses was taken as $p< 0.05$.

Section 2.2.3. Results

2.2.3.1. Professional details

The majority of respondents (66.9%; 95% confidence interval (95%CI) = 60.8 to 73.1%) spent over 90% of their time during their working day dealing with horses (figure 2.2.1). In fact, 59.0% (52.6 to 65.4%) stated that they worked exclusively with horses. Ninety-three respondents did not solely treat horses, for 17.2% of the total respondents (95%CI = 12.3 to 22.1%) the major component of their remaining workload was with small animals, 18.1% (95%CI = 13.1 to 23.1%) treated farm animals, 4.9% (95%CI = 2.1 to 7.6%) dealt equally with small and farm animal and less than 1% did not specify the additional type of work.

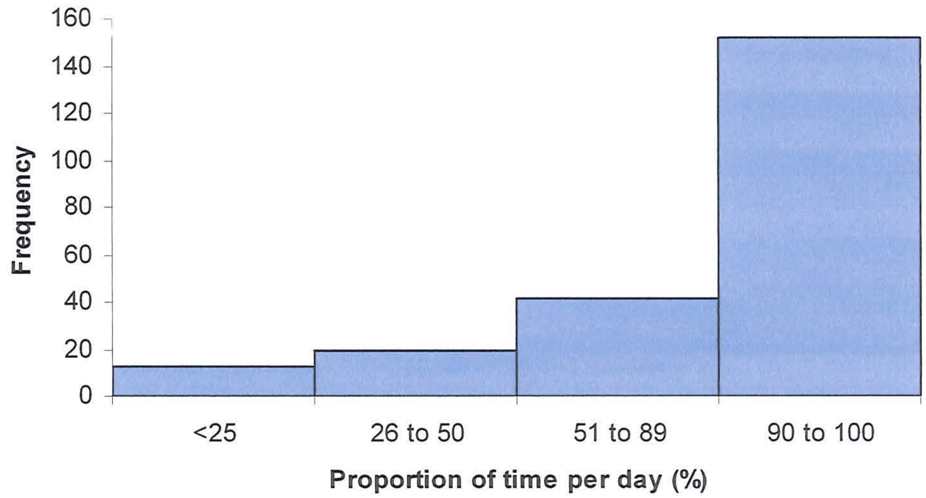

Figure 2.2.1. Frequency histogram of proportion of time respondents spent per day working with horses.

The highest proportion of equine practitioners (44.9%; 95%CI = 38.5 to 51.4%) worked in first-opinion practices with both ambulatory and clinic based services. Only 21.1% (95%CI = 15.8 to 26.5%) worked in purely first-opinion ambulatory practices and 30.8% (95%CI = 24.8 to 36.8%) did some referral work in addition to their first-opinion ambulatory and clinic based work. A minority of veterinary surgeons (3.1%; 95%CI = 0.8 to 5.3%) worked in a purely referral type environment.

Veterinary surgeons who responded to the survey worked most commonly with pleasure horses (median = 48% of the client horse population; inter-quartile range (IQR) = 25 to 60%) (figure 2.2.2). Performance horses, racehorses, show horses and studwork comprised a smaller proportion of most respondents' client base. Forty-two respondents cited that the horse population they served also comprised of other types outside of the criteria provided, the most common other horse type being donkeys (29%; 95%CI = 13.1 to 45.0%) (appendix A2.2.1).

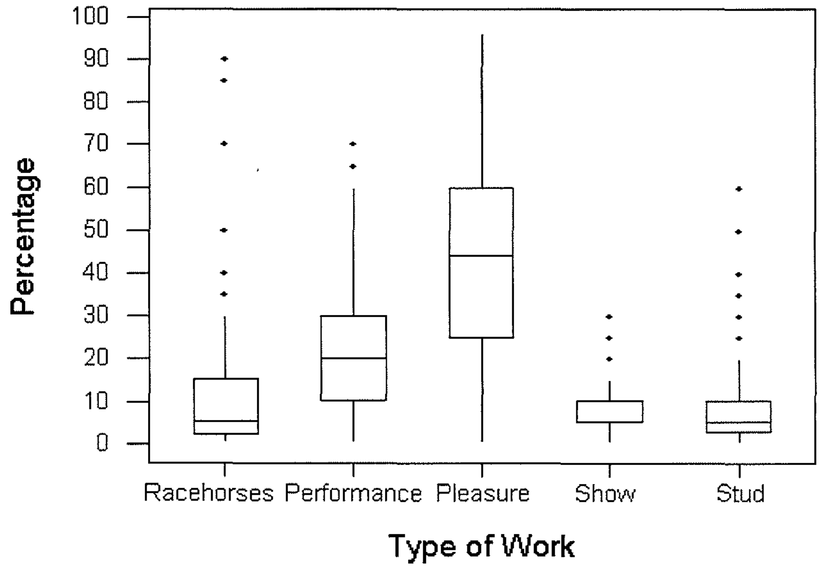

Figure 2.2.2. Box plots summarising the proportions different types of horses contributed to the client populations of all the veterinary surgeons surveyed. Whiskers represent lower limit (Q1-1.5(Q3-Q1)) and upper limit (Q3+1.5(Q3-Q1)), where first quartile = Q1 and third quartile = Q3. Outliers are plotted with asterisks (*).

2.2.3.2. Historical information related to recurrent airway obstruction

When obtaining a preliminary case history about a horse with suspected RAO, a majority of respondents believed management factors, such as ventilation (air quality in stable), type of bedding and forage, were *definitely important* (figure 2.2.3). When ranked in order, as in figure 2.2.3 with the factor considered most frequently by respondents to be *definitely important* placed first, of the first ten factors, only three were related to clinical signs, chronic cough being ranked fourth. The remainder were management factors with one relating to a previous medical history of summer pasture associated obstructive pulmonary disease (SPAOPD). A total of 97 respondents (42.7%; 95%CI = 36.3 to 49.2%) provided alternative recommendations of their own for important historical questions (table 2.2.1). Most frequently recommended as important were related to medical history, such as response to treatment and previous respiratory problems.

Table 2.2.1. Table of additional historical information to be obtained about a horse with suspected RAO, as suggested by respondents.

Additional historical information	Number of respondents
Medical History (Including previous respiratory problems , vaccination history, response to treatment, previous treatment, type of cough, when coughing occurs, age of horse)	26
Neighbouring horses management (e.g. bedding), location of hay/straw stores and muck heaps	18
Seasonal pattern or annual recurrence	18
Health of in-contact animals	8
Change in ownership, management or location	8
Geographic location/local crops	7
Quality of forage or bedding	7
Fitness/use of horse	3
Contact with donkeys	2

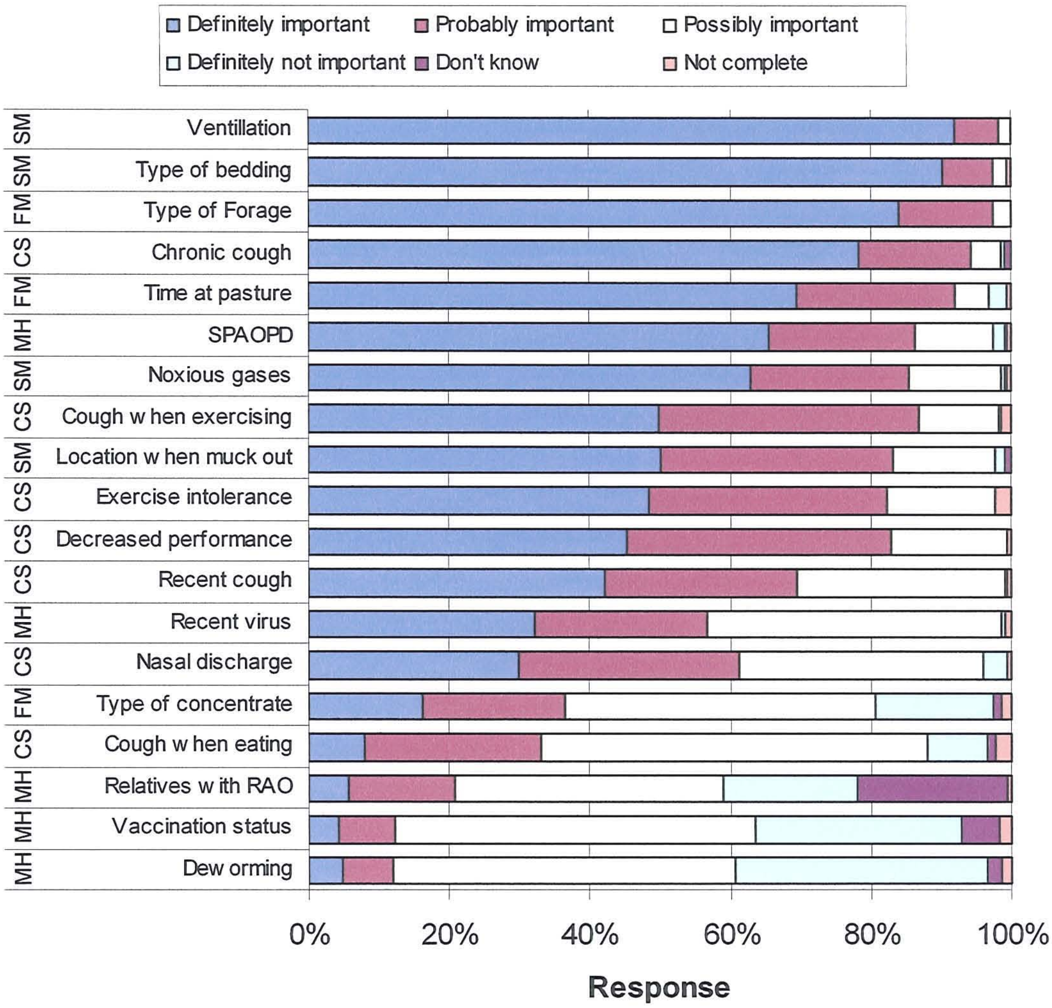

Figure 2.2.3. Cumulative responses for questions regarding the importance respondents placed on information obtained from preliminary case history of a horse with suspected RAO.
Historical information ranked with factor most frequently cited as being definitely important placed first. SPAOPD = previous diagnosis of summer pasture associated obstructive pulmonary disease. Questions regarding history were grouped under headings: **CS** = presenting clinical signs, **MH** = medical history, **FM** = feeding management and **SM** = stable management.

Comparison of the responses provided by the veterinary surgeons according to the proportion of equine work performed, possession of further qualifications and involvement in referral work did not identify any significant differences. The comparison of veterinary surgeons in Scotland to respondents from the rest of the UK did identify differences (table 2.2.2). Equine practitioners responding from Scotland were less likely to consider a previous diagnosis of SPAOPD (Fisher's exact test, $p = 0.01$), the location of a horse when its stable is mucked out ($p = 0.02$) and the exposure to noxious gases in the stable ($p = 0.02$) to be important in a preliminary case history (appendix A2.2.2).

Table 2.2.2. Preliminary case history information ranked in order of importance based on responses of equine practitioners from Scotland compared to the rest of the UK.

Rank	Scotland	Rest of the UK
1	Type of bedding	Ventilation (air quality in stable)
2	Ventilation (air quality in stable)	Type of bedding
3	Chronic cough for longer than 3 months	Type of forage
4	Type of forage	Chronic cough for longer than 3 months
5	Length of time spent at pasture	Length of time spent at pasture
6	Decreased performance	**Previous diagnosis of SPAOPD**
7	Exercise intolerance	**Drainage in stable (noxious gases)**
8	Cough when exercising	**Location of horse when mucking out**
9	**Previous diagnosis of SPAOPD**	Cough when exercising
10	Recent cough	Exercise intolerance
11	**Drainage in stable (noxious gases)**	Decreased performance
12	Recent viral respiratory infection	Recent cough
13	Nasal discharge	Nasal discharge
14	**Location of horse when mucking out**	Recent viral respiratory infection
15	Type of concentrate feed	Type of concentrate feed
16	Cough when eating	Cough when eating
17	Vaccination status	Genetic relative diagnosed with RAO
18	Deworming history	Vaccination status
19	Genetic relative diagnosed with RAO	Deworming history

Late responders considered a recent cough to be of less importance when obtaining historical information ($\chi^2_2 = 5.0$, p = 0.03) (figure 2.2.4). In contrast, responders to the two follow-up mailings were more likely to consider a previous diagnosis of SPAOPD to be *probably* or *definitely important* in comparison to responders to the first mailing ($\chi^2_2 = 5.1$, p = 0.02)(figure 2.2.4).

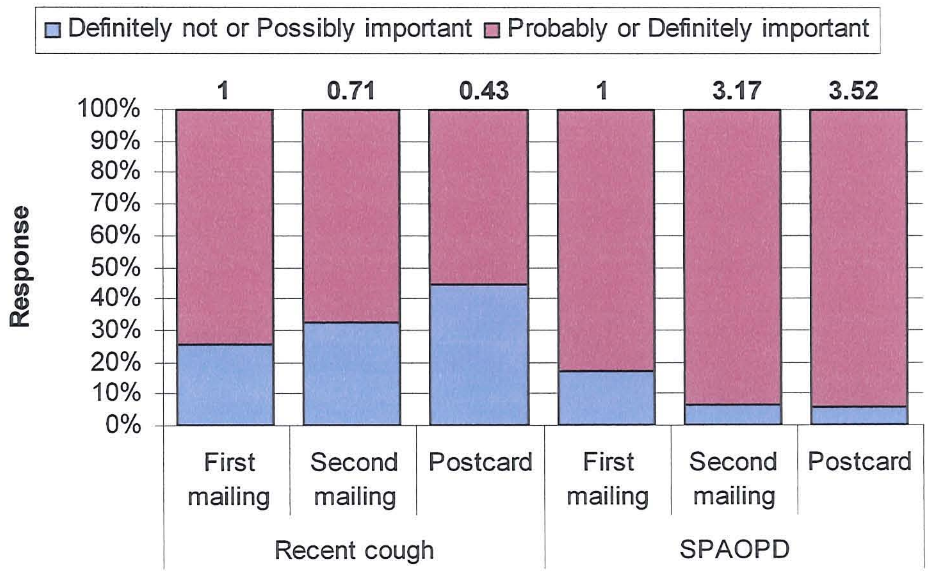

Figure 2.2.4. Comparison of the importance that early and later responders to the survey placed on a history of a recent cough and a previous diagnosis of SPAOPD in a horse with suspected RAO.
Bold numbers at top of columns indicate the odds ratio (compared to first mailing) for considering historical factors probably or definitely important according to the mailing to which response occurred.

2.2.3.3. Diagnosis of recurrent airway obstruction

Over 80% of respondents stated that they *always* used (in order of highest response first) history, auscultation (without use of a rebreathing bag) and clinical examination in the diagnosis of RAO (figure 2.2.5). Other more invasive tests were used less frequently with diagnostic tests such as endoscopy and respiratory fluid cytology *always* being used by less than 10% of the respondents and *sometimes* being used by approximately 40% of respondents.

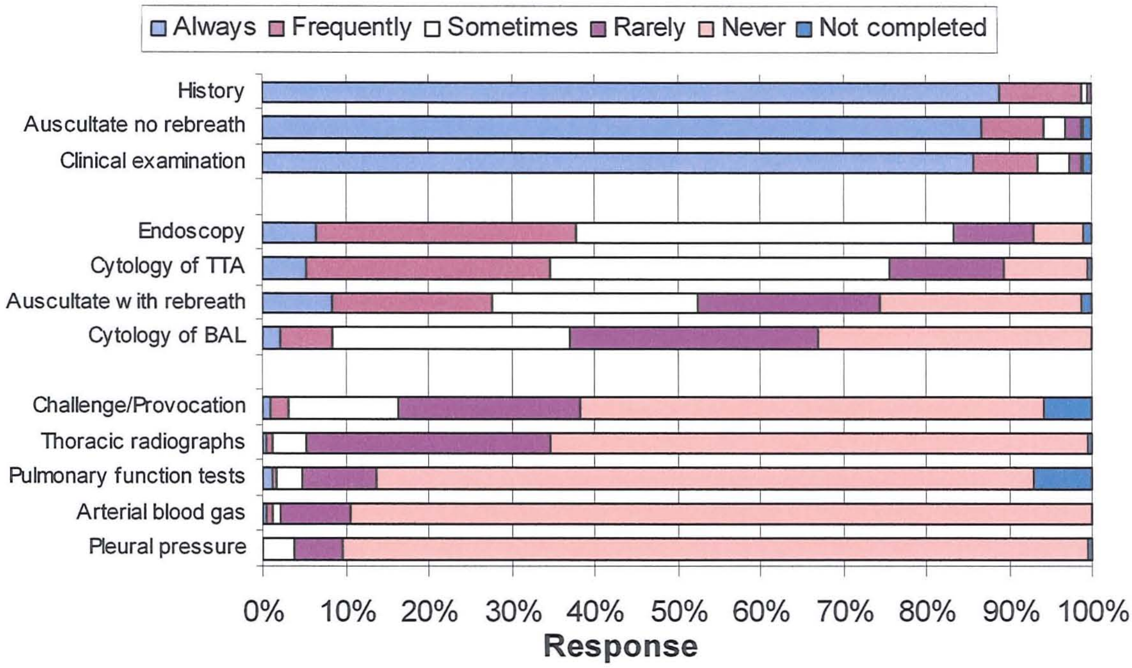

Figure 2.2.5. Cumulative responses to questions regarding how equine practitioners diagnose RAO.
TTA = transtracheal aspirates and BAL = bronchoalveolar lavage.

The respondents who answered the additional question to rank the five most important diagnostic methods in order of importance, placed the tests in a similar order to that produced by weighting of frequency of use (table 2.2.3)

Table 2.2.3. Diagnostic tests ranked in order of frequency of use compared to order of importance as ranked by responding equine practitioners.
TTA = transtracheal aspirates and BAL = bronchoalveolar lavage.

Rank	Ranking by weighting based on frequency of response	Ranking directly by respondents in order of importance
1	Obtain full history	Obtain full history
2	**Auscultation without rebreathing bag**	**Clinical examination**
3	**Clinical examination**	**Auscultation without rebreathing bag**
4	Endoscopy	Endoscopy
5	Cytology of TTA	Cytology of TTA
6	Auscultation with a rebreathing bag	Auscultation with a rebreathing bag
7	Cytology of BAL	Cytology of BAL
8	Challenge/Provocation test	Challenge/Provocation test
9	**Thoracic radiographs**	**Pulmonary function tests**
10	**Pulmonary function tests**	Pleural pressure
11	**Arterial blood gas**	**Thoracic radiographs**
12	Pleural pressure	**Arterial blood gas**

The equine practitioners also suggested a number of additional diagnostic tests not included in the list provided. The most commonly cited were post exercise auscultation/endoscopy (n = 10), the results of haematology (n = 6) and response to treatment (n = 17); five veterinary surgeons utilised response to atropine as a diagnostic test (appendix A2.2.3).

The proportion of respondents whose workload was greater than 90% equine who *frequently* or *always* used thoracic auscultation with a rebreathing bag in diagnosing RAO was 32.9% (95%CI = 25.3 to 40.4%). This was a significantly higher proportion (χ^2_1 = 4.99, p = 0.03) than vets who worked less than 90% of their time with horses (18.7%; 95%CI = 9.8 to 27.5%). The proportion of veterinary surgeons, working more than 90% of their time with horses, that used *frequently* or *always* both endoscopy (χ^2_1 = 18.2, p < 0.0001) and cytology of transtracheal aspirates (TTA) (χ^2_1 = 20.3, p < 0.0001) was also higher (figure 2.2.6).

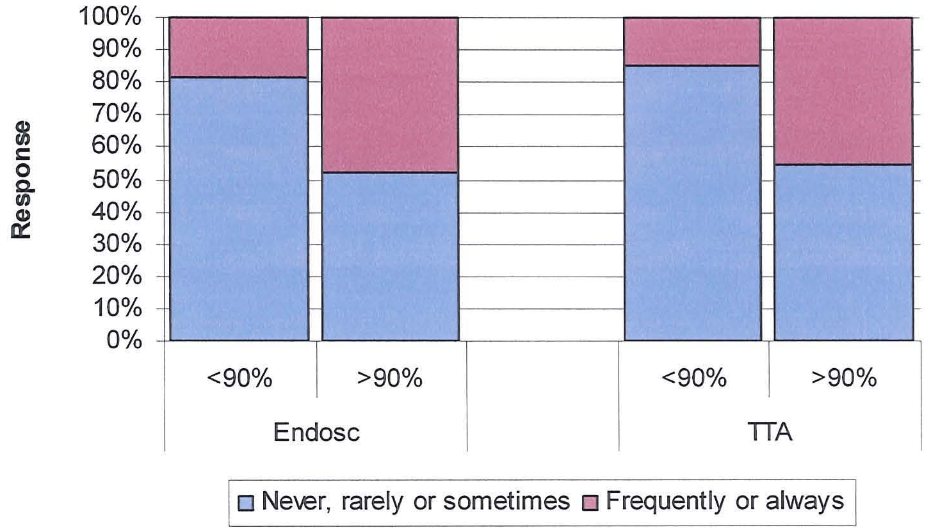

Figure 2.2.6. Comparison between respondents who did less than and greater than 90% equine work as to the frequency they used endoscopy and cytology of TTA to diagnose RAO.
<90% = Less than 90% equine work, >90% = Greater than 90% equine work.
Endosc = endoscopy of lower airway and TTA = Cytology of transtracheal aspirates.

When the use of diagnostic tests was compared depending on whether or not respondents possessed a further professional qualification, there were a number of differences. A greater proportion of veterinary surgeons with further qualifications *frequently* or *always* used endoscopy (χ^2_1 = 10.8, p = 0.001) and cytology of TTA (χ^2_1 = 11.6, p < 0.001) in the diagnosis of RAO. Greater proportions of practitioners that did some, or exclusively did, referral work reported that they used a rebreathing bag for auscultation (χ^2_1= 4.3, p = 0.04), endoscopy (χ^2_1 = 30.1, p < 0.0001), cytology of TTA (χ^2_1 = 28.2, p < 0.0001) and cytology of bronchoalveolar lavage fluid (BALF) (χ^2_1 = 7.87, p = 0.005) *frequently* or *always* in the diagnosis of RAO than practitioners in first opinion practice (figure 2.2.7; appendix A2.2.4). A significantly higher proportion of veterinary surgeons involved in referral work stated that they used routine clinical examination in the diagnosis of RAO only *never*, *rarely* or *sometimes* (Fisher's exact test, p = 0.0002). This group of clinicians was the only one to have some respondents stating that they used thoracic radiographs *frequently* or *always* (Fisher's exact test, p = 0.04).

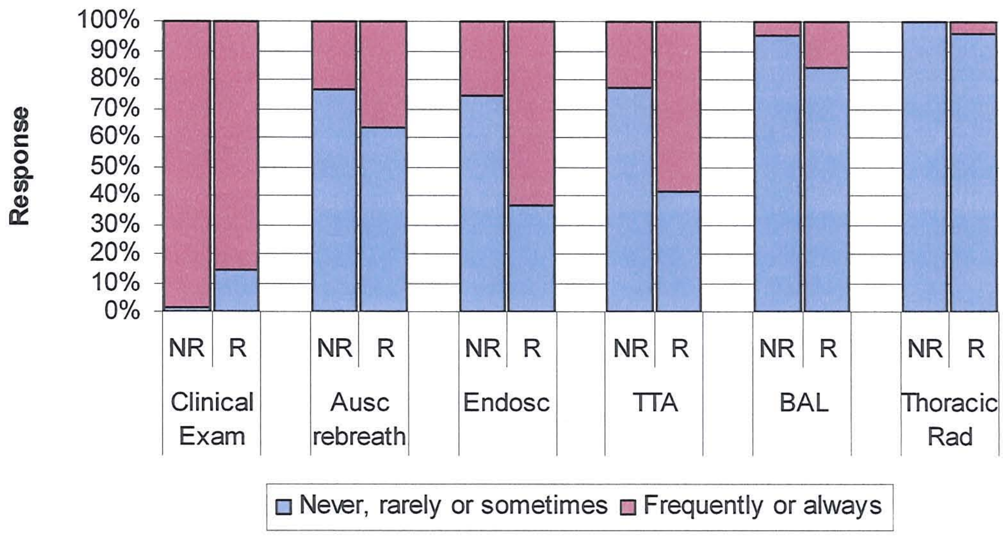

Figure 2.2.7. Comparison between respondents who did and did not have exposure to referral work as to the frequency they used various diagnostic tests.
NR = No referral work, R = referral work, Clinical exam = routine clinical examination, Ausc rebreath = Thoracic auscultation with a rebreathing bag, Endosc = endoscopy, TTA = cytology of transtracheal aspirates, BAL = Cytology of bronchoalveolar lavage and Thoracic Rad = Thoracic radiographs.

There was a trend for veterinary surgeons that responded to the later stages of the mailing to indicate that they frequently or always used endoscopy in the diagnosis of RAO (χ^2_2 = 4.4, p = 0.04)(figure 2.2.8)

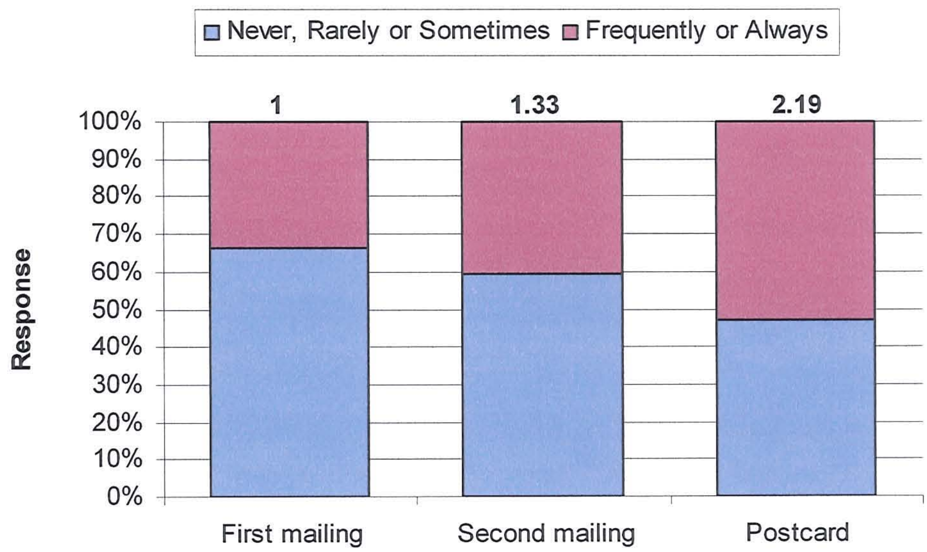

Figure 2.2.8. Comparison between the stages of mailing at which participants responded regarding how frequently they used endoscopy for the diagnosis of RAO.
Bold numbers at top of columns indicate the odds ratio for frequently using or always using endoscopy according to the mailing to which response occurred.

The majority of respondents (81%) became confident of a diagnosis of RAO using only history and clinical examination (figure 2.2.9). There was a trend for a higher proportion of clinicians who worked for over 90% of their time with equidae ($\chi^2_2 = 8.2$, $p = 0.02$) and those who had at least some referral caseload ($\chi^2_2 = 7.06$, $p = 0.03$) to report that they only become confident of a diagnosis of RAO once endoscopy and cytology had been performed (appendix A2.2.5).

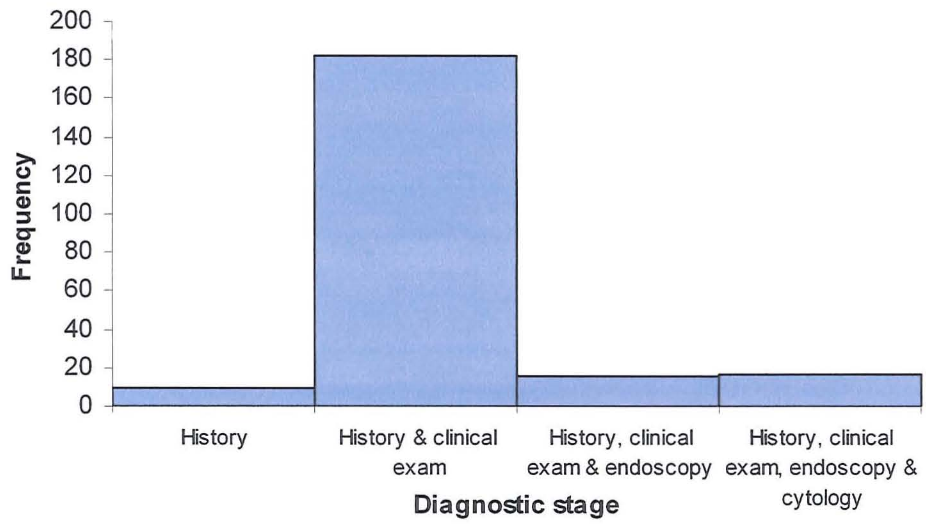

Figure 2.2.9. Frequency histogram of the diagnostic stage that respondents became confident of a diagnosis of RAO.

Respondents associated clinical signs of RAO, such as coughing when trachea squeezed and nasal discharge, predominately with, in terms of severity, *mild* RAO (figure 2.2.10). The clinical signs associated by participants with *moderate* RAO included a breathing rate of 20 to 30 breaths per minute and presence of a heave line. Signs deemed to be associated with *severe* RAO included flaring of the nostrils and a breathing rate of over 30 breaths per minute. As previously indicated, a large proportion of respondents did not use a rebreathing bag and, as a consequence, left the two related responses uncompleted (appendix A2.2.6). However, those that did use the technique related the detection of wheezing or crackles with mild disease and linked coughing on use of the bag to mild/moderate disease. Many respondents never detected regions of silence when auscultating lung fields (37.9%; 95%CI = 31.6 to 44.2%), but those that did indicated that they believed it to be associated with severe disease.

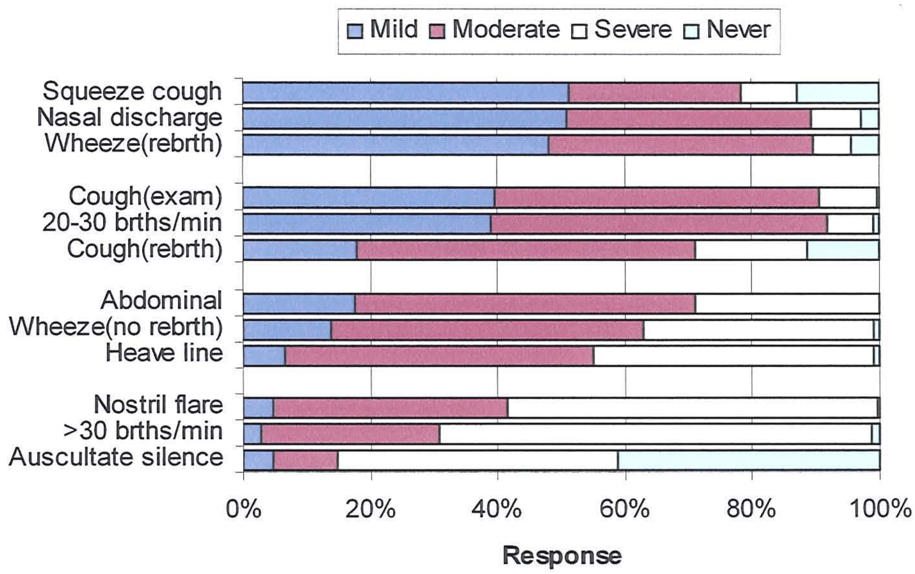

Figure 2.2.10. Clinical signs and their perceived relationship to severity of RAO from completed responses provided by participating equine practitioners.
Clinical signs ordered and grouped by the degree of severity that had the most frequent response. Responses that were left uncompleted not included in graph. Mild = mild RAO, Moderate = moderate RAO, Severe = severe RAO and Never = never observe this clinical sign.

The respondents suggested a number of additional clinical signs not included in the list provided. The most commonly cited were cough and/or nasal discharge during and/or post exercise (n = 4), poor exercise tolerance (n = 4), weight loss (n = 3), body/anal movement with respiration (n = 3) and auscultation at base of trachea (n = 3) (appendix A2.2.7). There was a significant trend for veterinary surgeons who did greater than 90% proportion of equine work to associate cough during examination ($\chi^2_2 = 6.5$, p = 0.04), the presence of a heave line ($\chi^2_2 = 6.1$, p = 0.048) and coughing when squeeze trachea ($\chi^2_3 = 12.8$, p = 0.005) with lesser degrees of disease severity than those who did less equine work (figure 2.2.11) (appendix A2.2.8).

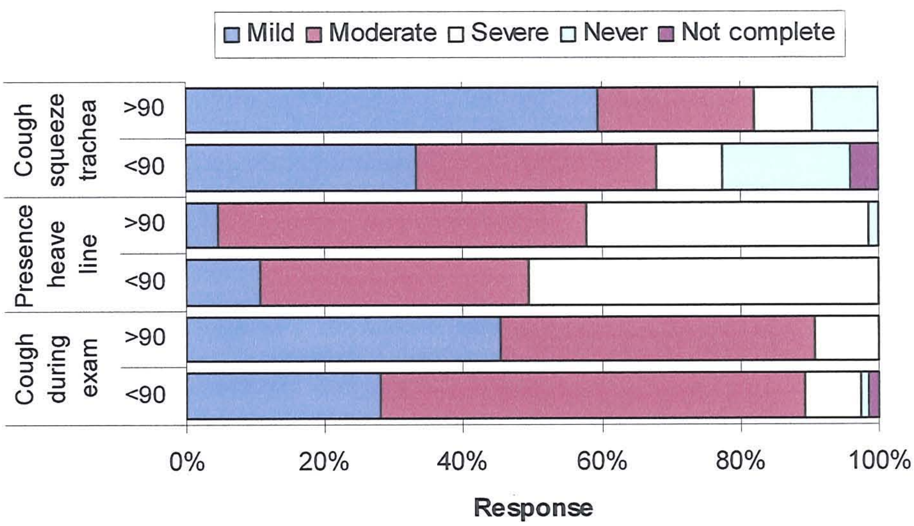

Figure 2.2.11. Clinical signs and their perceived relationship to severity of RAO as reported by respondents who did less than or greater than 90% equine work.
Mild = mild RAO, Moderate = moderate RAO, Severe = severe RAO and Never = never observe this clinical sign. <90%= Proportion of time spent working with horses less than 90% and >90% = Proportion of time spent ~~working with horses~~ greater than 90%.

Accumulation of mucus globules, as detected by endoscopy, was associated with *mild* RAO by the majority of respondents (who used endoscopy) (82.9%; 95% CI = 77.7 to 88.3%), whereas a pool of mucus was associated by most with *moderate* RAO (68.6%; 95%CI = 62.0 to 75.1%)(figure 2.2.12). Coughing whilst the endoscope was present in the trachea was believed by the majority (77.4%; 95%CI = 71.6 to 83.2%) to be related to *mild* or *moderate* RAO. Many respondents (30.2%; 95%CI = 23.8 to 36.5%) did not recognize oedema of the carina as occurring in cases of RAO.

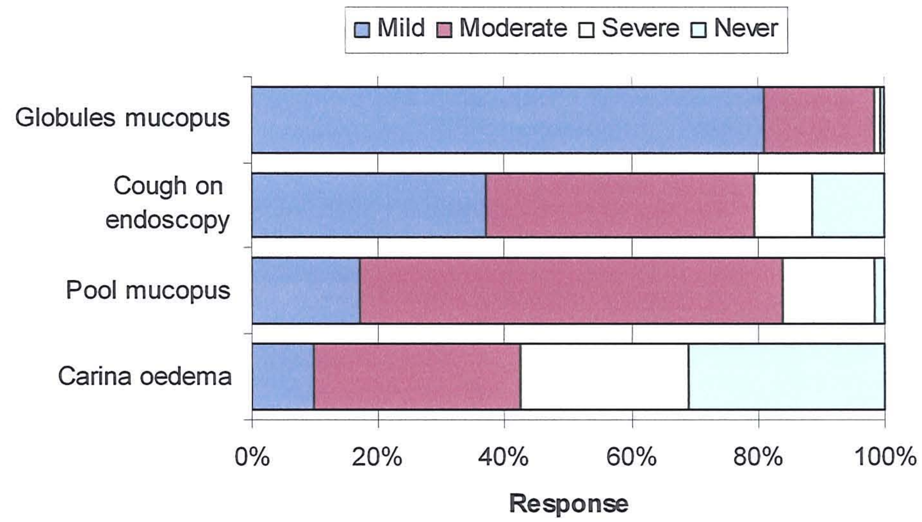

Figure 2.2.12. Endoscopic findings and their perceived relationship to severity of RAO from completed responses provided by participating veterinary surgeons. Mild = mild RAO, Moderate = moderate RAO, Severe = severe RAO, Never = never observe this clinical sign and not complete = response left uncompleted.

2.2.3.4. Management and therapy of recurrent airway obstruction

In response to the question "Once you have made a diagnosis of RAO, do you find any of the following management practices useful when treating the disease?" the equine practitioners cited those practices that reduced environmental challenge as being most useful. The change found to be most commonly useful was an increased exposure to pasture followed by a change in the type of bedding (figure 2.2.13). A change to an alternative type of forage was believed to be of more benefit than soaking hay. Logistically difficult changes, such as alteration in neighbouring horses' environment and improvement in stable location, were understandably less commonly found to be useful. The use of complete cubes, often recommended as a way to avoid use of any forage, were least commonly found to be useful.

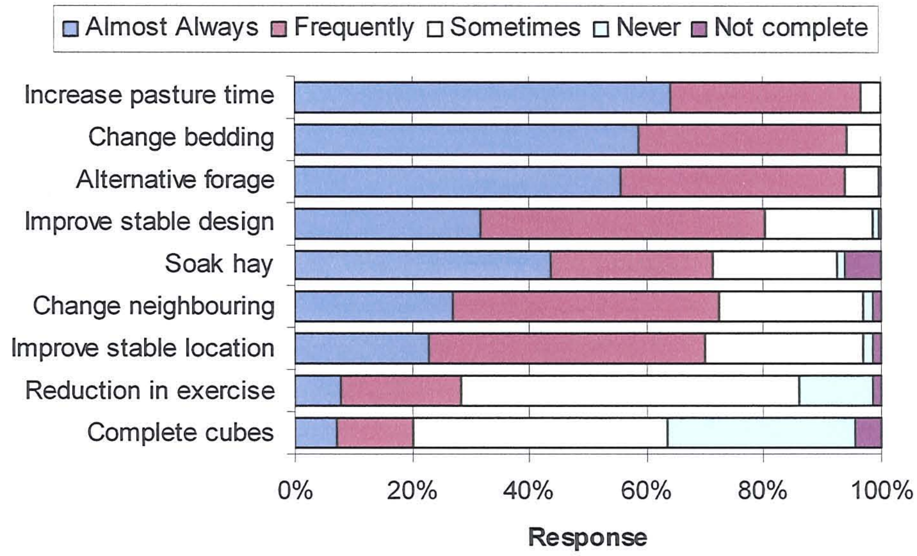

Figure 2.2.13. Management changes believed to be useful in cases of RAO as reported by responding equine practitioners.

The respondents suggested a number of other management changes that they found useful in the management of RAO including: feeding from the floor (n = 9), horse moved from stable when mucking out (n = 5) and grooming outside (n = 3) (appendix A2.2.9). The mean time respondents recommended soaking hay for was 4 hours and 30 minutes (standard deviation (s.d.) = 6h 30mins). The range was from 1 minute to 24 hours and the median soak time was 60 minutes (IQR = 30 minutes to 6 hours). Respondents tended to recommend a change to principally three types (sometimes to be used in combination) of bedding; namely shavings, rubber matting and paper/cardboard (table 2.2.4).

Table 2.2.4. Types of bedding recommended by respondents for RAO horses.

	Number of respondents recommending	Proportion (n = 227) (%)
Shavings	189	83.3
Paper/cardboard	139	61.2
Rubber matting	173	76.2
Commercial	24	10.6
Peat	31	13.7
Good quality straw	12	5.3
Other bedding*	29	12.8

*Hemp (24) (14 named a hemp commercial product), dust extracted straw (1), dust extracted or washed shavings (2), the use of field shelters (2), sisal (1) and woodchips (1).

When asked the question "How often do you prescribe each of the following medications when treating recurrent airway obstruction?" 93% of respondents stated that they *almost always* or *frequently* used bronchodilators compared to only 46% who *almost always* or *frequently* used some form of steroid medication. Only 14% stated that they used antibiotics in the treatment of RAO *almost always* or *frequently* although this figure increased to 38% when clenbuterol / trimethoprim sulphonamides combination was included. Inhaled medication was used *almost always* or *frequently* by 34% of respondents (this changed little when the use of sodium chromoglycate was excluded). The breakdown of types of therapies used, and the frequency respondents used them, is summarized in figure 2.2.14. Clenbuterol was the most frequently used medication being used almost always by over 40% of respondents.

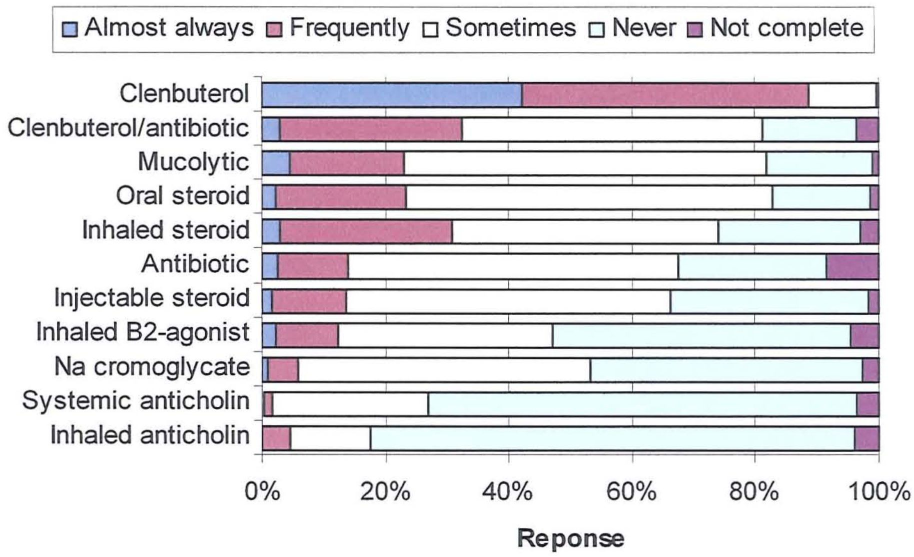

Figure 2.2.14. Respondent reported frequency of use of medications in the treatment of RAO.

Thirty-seven percent of respondents prescribed clenbuterol using stepwise incremental increases in dose to effect as opposed to 61% who did not. Of the remainder, one percent did not use clenbuterol and one percent did not complete the question. Fifty-two percent of respondents believed they had witnessed an increase in the prevalence of RAO in the previous 10 years compared to 29% who did not believe there had been an increase, 12% who did not know and 7% who were unable to answer as they had not been working for 10 years.

Ninety four percent of respondents stated that they would be willing to participate in a similar survey in the future. Only 4 respondents said no and 8 respondents did not indicate an answer (which was assumed to be no). Approximately a third of respondents (n = 71; 31%) took the opportunity to express additional information or comments, the topics are summarised in table 2.2.5.

Table 2.2.5. Additional information provided by respondents at the end of the practitioner questionnaire.

Comment/information	Number of Respondents
Criticism	3
Praise	4
Increase in SPAOPD and discussion	20 (Regional breakdown: S+SE=6, EE=4, SW=6, NW=2, Mid=1, NE=1)(all doing >50% equine)
General comments on environment and management	15
Discussion of therapeutics	14
General comments on the disease RAO	14

Section 2.4. Discussion

This author is unaware of any specific studies regarding how veterinary practitioners in the United Kingdom diagnose, manage and treat recurrent airway obstruction. The current study attempts to rectify this omission. A similar study into how Australian equine veterinary surgeons approach lower respiratory tract disease has been published (Christley *et al.*, 2000a). Although concerning different diseases, it did investigate the diagnostic procedures used to assess the respiratory system. In the current study, the redundancy of the term chronic obstructive pulmonary disease was highlighted as the decision to supersede it was only published in the UK in the months prior to the survey (Robinson, 2001b). The definition of RAO was descriptive, not quantitative, so that it could be applicable to all respondents and emphasised the recurrent, reversible nature of the respiratory disease associated with housing.

The intention was to survey practitioners who worked predominately with horses, not veterinary surgeons who only saw the occasional horse. This aim appears to have been achieved as the majority of respondents spent over 90% of their time working with horses. Very few respondents spent less than 25% of their working day with horses. It was desirable that the majority of respondents worked in first opinion practices as the cases of RAO that they encountered in their professional careers would better reflect the general horse population than perhaps the less typical and difficult cases presented for second opinions. The make-up of the UK horse population is unclear; any description of the breakdown in equine activities tends to concentrate on breeding and/or performance type sports derived from registrations with breed societies and sport governing bodies (Leckie, 2001). It is not possible to ascertain if the respondents in this survey were serving a representative sample of the horse population. However, these vets appeared to service a predominately pleasure type population rather than an "elite" sporting population. This may well be representative of the general UK population as 71% of the horse population is estimated to be unregistered with any official body, and do not fulfil the prerequisites for the majority of equine sporting activities (Leckie, 2001).

The strong link between housing and RAO (McPherson *et al.*, 1979b, McGorum *et al.*, 1993a) is emphasised by the importance that practitioners place on historical information related to management. Clinical signs, such as a chronic cough, were not considered *per se* to be as important in initial consideration of historical information. Coughing (particularly of long duration) is one of the commonest (but not specific) clinical signs of RAO, although it is not consistently present in all cases (McPherson *et al.*, 1978, Dixon *et al.*, 1995b). Perhaps this inconsistency is reflected in the fourth place ranking of chronic cough, the highest placed clinical sign. In contrast, housing and management factors which were ranked higher will, by the definition of RAO, always be involved in the occurrence of the disease in an individual horse. The presence of nasal discharge is an unreliable indicator of RAO (Dixon *et al.*, 1995b) and it is correspondingly placed low in the order of importance by equine clinicians. However, it has to be acknowledged that clinical signs such as nostril flare and abdominal movement were not included in this historical section as a consequence of a lack of space in the questionnaire. To maximise response, it is imperative that questionnaires are not overly long or crowded (Cartwright, 1978). In retrospect, this may have been an error, as information regarding the importance that vets placed on the historical reporting of such clinical signs by owners would have been interesting. These clinical signs were addressed later in the questionnaire in relation to diagnosis and many respondents believed these signs to occur in only severe cases and perhaps would not recognise owners' ability to detect these signs in milder cases.

The difference between Scottish practitioners and vets from the rest of the UK regarding the ranking of some important historical information may well be incidental. There is a possibility that it may be a reflection of the decreased proportion of equine work that vets in Scotland undertake, as outlined in chapter 2.1, resulting in a decreased familiarity with the less well-known environmental factors associated with RAO. Conversely, stabling practices may be better in Scotland, so factors like poor drainage (noxious gases) and mucking out with the horse in the stable are not common problems encountered by veterinary surgeons. As the regional incidence of SPAOPD is unknown, the influence of this disease on vets' decision-making cannot be ascertained.

Late responders to the survey were more likely to do a greater proportion of equine work than early responders (chapter 2.1). These late responders placed a different emphasis on two historical factors, recent cough and a previous diagnosis of SPAOPD. These vets may, because of their increased exposure to equidae, have higher awareness of SPAOPD, and its possible link to RAO (Hackett, 1990, Mair, 1996b), and the lack of specificity that a short period of coughing has as an indicator of RAO (McPherson *et al.*, 1978).

How veterinary surgeons actually diagnose RAO, especially in first opinion work, was of particular interest in helping the construction of the RSQ. The scientific literature, clinical text books, clinical teachers and experts tend to propose ideal methods for diagnosing RAO, including the use of diagnostic tools regarded as 'gold standards' (Robinson, 2001b). These more invasive, potentially expensive methods are essential in many situations such as research and for difficult and unresponsive cases, especially for referred cases. However, in first opinion practice a more empirical approach for the diagnosis of RAO might be adopted, relying far more on historical information and basic clinical examination.

Over 80% of all respondents indicated that they relied predominately on historical information, auscultation (no rebreathing bag) and clinical examination (e.g. temperature, heart and respiratory rate) when diagnosing RAO. The importance of history and clinical examination is not surprising. Historical information informs whether the horse has been exposed to a potentially challenging environment, duration of and type of symptoms (clinical signs) as well as the opportunity to investigate the possibility of an infective process. The importance placed on auscultation is understandable but it has to be recognised that it is probably a relatively insensitive technique (Dixon *et al.*, 1995b). The relative lack of use of a rebreathing bag to aid auscultation is disappointing even though it is well recognised to facilitate the process greatly (Beech, 1989a, Naylor *et al.*, 1992). If veterinary surgeons valued auscultation highly then perhaps rebreathing bag use would be more commonplace. This may reflect a lack of general awareness of the technique or a compliance problem, in that it may be perceived that owners would object to rebreathing bag use. It has been suggested that clinicians overestimate the usefulness of auscultation (Christley, 1998).

Respondents used endoscopy rarely in the diagnosis of most cases of RAO. However, over 80% indicated that they would perform endoscopy in some cases, suggesting that many practitioners do have this technology available to them. Similarly, many would use respiratory cytology if required in certain cases, again suggesting that they had the equipment and clinical skills required to obtain the samples. The relative lack of use of these two techniques implies that they are deemed unnecessary in the diagnosis of RAO in many cases. Respiratory cytology of samples obtained by bronchoalveolar lavage (BAL) was even less frequently used, perhaps reflecting lack of available equipment and expertise or a reluctance to perform what is an invasive procedure.

Other ancillary diagnostic tests were rarely used, including the 'gold standard' of pulmonary function tests. This probably reflects the lack of availability of equipment for these procedures for use in general practice. Pleural pressure, a relatively simple-to-perform measurement of respiratory obstruction (by indirect oesophageal measurement) was little used; there are currently no commercial products available. A simple machine (Ventigraph, Boehringher) was available in the 1980s but its fall into disuse may well imply a redundancy in the diagnosis of many cases of RAO.

Veterinary surgeons who spent more time working with horses, or did at least some referral work, were more likely to auscultate with the aid of a rebreathing bag. This increased use may be driven by clinical experience of the difficulties associated with auscultation as well as more focused equine continuing professional development. Increased clinical acumen, access to equipment and more frequent contact with difficult cases may also explain the increased use of endoscopy and respiratory cytology by practitioners who did predominately equine work, a degree of referral work or possessed equine related professional qualifications. In chapter 2.1, it was highlighted that vets with further qualifications were also more likely to work predominately with horses and do some degree of referral work. Christley *et al.* (2000a) also identified that practitioners who did a greater proportion of equine work were also more likely to use endoscopy and respiratory cytology. The increased use of BAL respiratory cytology by respondents who did at least some referral work not only may be explained by easier access to equipment and/or expertise but may also be an indication of a more complex caseload, in which a definitive diagnosis is required. Certainly, many experts prefer this technique, often in conjunction with TTA. The decreased reliance on routine clinical examination probably reflects the referral clinic situation or a different interpretation of the question; it can be argued that the results of a basic clinical examination contribute little to an actual diagnosis of RAO. Later responders to the questionnaire were more likely to use endoscopy in the diagnosis of RAO, again later responders also tended to spend the majority of their working day with horses.

The vast majority of respondents became confident of a diagnosis of RAO following only history taking and clinical examination before performing any confirmatory diagnostic techniques. Respondents from the sample population were not using the diagnostic reference standards of respiratory cytology and obstruction.

The association of tracheal squeezing (compression of the cranial trachea/larynx) to elicit a cough with mild RAO by participants demonstrates that this technique is widely used and thought to be valid. Although largely ignored by the scientific literature this clinical aid has been suggested to be, historically, pathognomonic for RAO (Cook and Rossdale, 1963) and certainly it may suggest an increased sensitivity of the cough reflex (Robinson, 2001d). Perhaps its usefulness as a diagnostic tool warrants further investigation. Some credence may be given by the greater proportion of respondents who spent more than 90% of their time working with equidae associating this clinical finding with mild RAO than those who did less horse work. Caution would dictate that predominately equine clinicians may see a wider spectrum of case severity, from sub-clinical to extremely severe, and would therefore place mild cases of RAO in the context of this wider scale of severity. The respondents that used rebreathing to aid auscultation (including cough in association with use of the bag) indicated that they found it a sensitive tool for detecting RAO at lower degrees of severity than auscultation on its own. This again emphasises what an important adjunct to auscultation this technique may be and its omission from many respondents' clinical investigations is something that continuing professional development should aim to correct.

Evidence of airway obstruction, namely increased abdominal effort, presence of a heave line and nostril flare, was principally associated with moderate to severe degrees of RAO. This is in agreement with the work of Robinson *et al.* (1994b), who identified that airway obstruction has to be relatively severe before signs of respiratory distress become obvious in a resting horse. The connection by survey participants between increased respiratory rate and greater degrees of RAO severity could follow the intuition that as lung function becomes impaired then the respiratory rate has to increase to compensate. Perhaps the virtually equal split between respondents as to whether mild RAO or moderate RAO is associated with a respiratory rate of 20 to 30 breaths per minute reflects the extreme variability of respiratory rate between individual horses (Dixon *et al.*, 1995b). The association of auscultation of silent regions with severe RAO is in agreement with Naylor *et al.*, (1992). However, its lack of recognition by many respondents either indicates that they do not encounter silent regions on auscultation in the cases of RAO in their clinical caseload, never examine cases of severe RAO or they lack the clinical acumen to detect these changes.

In accord with the scientific literature, respondents associated increasing quantities of tracheal mucus, detected on endoscopy, with increasing degrees of RAO severity (Grunig *et al.*, 1989). Coughing in response to presence of an endoscope in the trachea is thought to be a sensitive indicator of respiratory inflammation (Dixon, 1997) and it is encouraging that this is well recognised by survey participants. However, oedema of the carina was poorly recognised as occurring in cases of RAO, despite it being advocated as a marker for pulmonary inflammation (Dixon, 1997). This clinical finding is subjective and can be difficult to image, especially if an endoscope of insufficient length is used. This lack of recognition may be from reduced awareness but may also suggest that many veterinary surgeons do not find it useful in aiding diagnosis of RAO.

The respondents' approach to managing cases of RAO follows the current veterinary literature, with emphasis on improving the environment over feeding practices. It is encouraging that so many respondents were able to state that they found recommending changing stable design useful. This implies that owners are responsive to potentially permanent and costly changes to the fabric of stables. The use of alternative forage took precedent over soaking hay. The use of soaked hay is generally believed to be inferior to a shift to other types of forage, such as haylage, that represent a reduced respiratory challenge (Thomson and McPherson, 1984, Dixon *et al.*, 1995d). The proportion of participants who did not complete the question on hay soaking clouds the situation. Some of these omissions appear to be as a result of confusion due to the questionnaire layout with respondents skipping the frequency question concerning soaking of hay to complete only the section on how long they soak hay for (thus implying that they do find the management practice useful). The median soak time for hay, as recommended by participants, was 60 minutes; somewhat longer than some studies have suggested is required (Moore-Colyer, 1996, Blackman and Moore-Colyer, 1998).

The β_2-agonist bronchodilators, in particular clenbuterol, dominated the therapeutics prescribed by veterinary surgeons in the survey population. The use of anticholinergic bronchodilators was largely neglected, despite ipratropium bromide being a registered product. Steroids did hold a place in many of the veterinary surgeons' therapeutic arsenal. It was interesting that the delivery of steroids by metered dose inhalers is widely used by many veterinary surgeons, despite the relatively recent awareness in the literature of this mode of administration (Rush *et al.*, 1998a). Inhaled β_2-agonists were prescribed less commonly and this may be related to their relatively short mode of action. Antibiotics were administered perhaps because of concerns relating to secondary bacterial infection. The necessity of their use is questionable. It was disappointing that so few respondents implemented an incremental dosing, to effect, of clenbuterol, despite it being recognised that the licensed dose is ineffective in many cases of RAO (Erichsen *et al.*, 1994).

Just over half of respondents supported the claim of increasing incidence of RAO (Mair, 1995). The willingness of respondents to participate in a similar survey in the future hopefully implies that they did not find the task too arduous or intrusive. Carefully planned and targeted surveys may avoid fatigue in the profession. It is worth noting that twenty of the 227 respondents, without any prompting, indicated that they believed they had seen an increase in the number of cases of SPAOPD, with the majority of these respondents residing in the middle and south of England. This would perhaps warrant further investigation.

This is the first report of how a sample of equine veterinary surgeons working in the United Kingdom diagnose, treat and manage RAO. There were some differences in the responses given by participants depending on type and proportion of equine work, possession of further professional qualifications and promptness of response. Viewed in combination with the response patterns outlined in chapter 2.1, there evidently will be some non-response bias existing in the results of this survey. For example, if the difference between early and late responders were extrapolated to non-responders they would do more equine work and be more likely to perform endoscopy and their different experience and opinions would not be included in these survey results. However, it has to be remembered that for the vast majority of questions there were no differences between groups and therefore many of the results can be deemed to be representative of the sample population.

In conclusion, the vast majority of this selected population of equine practitioners became confident of a diagnosis of RAO following only acquisition of historical information and a basic clinical examination. Further diagnostic tests for investigating RAO were utilised relatively infrequently by participants.

CHAPTER 3

A MODIFIED DELPHI CONSULTATION OF INTERNATIONAL EXPERTS IN EQUINE RESPIRATORY MEDICINE

Section 3.1. Introduction and Aims of Study

Knowledge of important historical information and clinical signs associated with recurrent airway obstruction (RAO) is required to aid selection of the criteria, or questions, required to construct a risk-screening questionnaire (RSQ) for the disease. Although much information is available in the scientific literature, the importance that should be placed on each criteria is relatively unknown. Weighting for each criterion, that would be required for scoring the RSQ, is scarce. As a solution, a modified Delphi method was used to consult an international expert panel that had been selected on the basis of having published one or more scientific papers on RAO. Principally, the Delphi method is an iterative process designed to combine expert opinion and knowledge into a group consensus in a systematic and anonymous manner (Linstone and Turoff, 1975, Keeney *et al.*, 2001). The Delphi method has not to this author's knowledge been used in veterinary medicine before and certainly has not been applied to equine respiratory disease. The intention of this Delphi study was to identify, and quantify estimates for, the important historical and clinical signs associated with RAO.

Section 3.2. Materials and Methods

3.2.1. Selection of participants for the Delphi consultation process

It was decided that the expert panel for the Delphi consultation should consist of specialists in the field of equine respiratory medicine who were believed to have a particular interest in recurrent airway obstruction. Scientific articles (from the last 25 years) were identified from electronic bibliographic databases (namely MEDLINE, CAB Abstracts and Web of Science) using keywords, for example COPD, RAO and heaves; hand searching of reference lists from retrieved articles was also performed. The first named authors of these papers were selected and their current mailing address determined so that they could be invited to participate in the consultation process. Many of these authors had also participated in the international workshop on equine chronic airway disease (Robinson, 2001b). The identity of the participants was never revealed to the members of the panel.

3.2.2. Administration of the Delphi process

The criteria or items concerning historical and clinical signs associated with RAO included in the questionnaire for the first round of the modified Delphi process were obtained from the available scientific literature and the results of the survey of veterinary practitioners. The final criteria included in the questionnaire, as well as its format, were established by the author in conjunction with Doctor R. Christley. The 6-page questionnaire was designed, edited and pre-coded using a data capture software program, TELE*form* Elite v8. A total of 55 criteria were included with closed-ended responses on a 7-point scale that ranged from 0 to 100. Many of the items were grouped to form response grids (appendix A3.2). The questionnaire was divided into two main sections, namely historical information and clinical signs. The historical section was preceded by the question '*of all the diagnosed cases of RAO that you have observed in the past, how important did you consider the following historical information in reaching that diagnosis?*'. The scale for participants to indicate their response ranged from 0 to 100, where 0 = definitely not important, to 100 = definitely important.

The clinical signs section was further divided into three parts that related to signs associated with respiratory inflammation (cough and nasal discharge), respiratory obstruction (exaggerated abdominal effort and nostril flare) and detailed clinical examination (auscultation etc.). Clinical signs response grids were preceded by the question '*of all the diagnosed cases of RAO that you have observed in the past, approximately what percentage have exhibited each of the following signs, either during clinical examination or in the month previous to the examination, as a result of the disease?*', again a scale of 0 to 100 was provided for responses. Additionally, in part 2 of clinical signs, a response grid relating to chronicity of coughing was preceded by the questions '*of all the coughing horses that you have observed in the past, what would be the likelihood of them being diagnosed with RAO if they have been coughing for:*'. A scale of 0 to 100 was provided for this grid from 0 to 100, where 0 = could not be less likely, to 100 = could not be more likely. Opportunity was provided for participants to suggest additional items to be included in the next round of the process. They were encouraged to provide justification for their suggestions.

The questionnaire for the first round of the Delphi process was mailed on 16th October 2002 together with a letter of invitation that outlined the Delphi process and what it would entail for the respondent (appendix A3.1). An envelope was provided for returning the questionnaire if the expert accepted the invitation. Recipients in the UK were provided with a pre-paid envelope as used in the survey in chapter 2 (appendix A2.1.2). Those outside the UK received an envelope with the appropriate stamps for return from that country. A deadline of 31st October 2002 was suggested. Participants were also invited to indicate whether or not they would be willing to participate in the second round of the Delphi process using electronic mail (e-mail). If no response was obtained from invitees by the date of the mailing of the second round, one-month after the first mailing, it was assumed that the individual did not want to, or was not able to, participate.

Returned questionnaires were scanned using a fujitsu fi-4110cu image scanner and all responses were entered into Microsoft Excel 2000 via TELE*form* Elite v8 capture software.

The group responses for each criterion were summarised using the median and inter-quartile range. The second round questionnaire for the Delphi process was identical to the first round questionnaire except additional items suggested by participants were included in the appropriate sections (appendix A3.3). In addition, the group summaries from the first round for each of the criteria were provided alongside the appropriate scale. The initial responses selected by an individual were highlighted on their personalised copy of the questionnaire (figure 3.1). Additional information was also provided in the second round questionnaire in response to participants' queries (appendix A3.3).

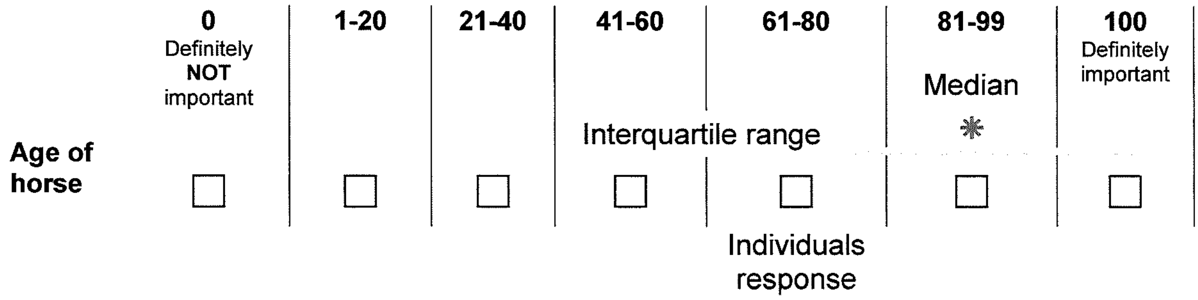

Figure 3.1. Example of the second round feedback in the Delphi consultation for one criterion showing the median and inter-quartile range of the group response in relation to an individual's response.

The second round Delphi questionnaire booklet was designed using Microsoft Word 2000 so that it could be sent (and returned) in electronic form if it had been requested. If an electronic version had not been requested the second round questionnaire was mailed as per the first round. The second round of the Delphi process was sent on 14th November 2002 with a specified deadline of 29th November 2002. The cover letter encouraged participants to reconsider their initial responses in the light of the group response (appendix A3.3). Non-responders were sent just one reminder (either letter or e-mail depending on selected medium) on 9th December 2002. If no response was obtained it was concluded to be an indication of a desire to participate no longer. Responses from the second round of the Delphi process were manually entered into Microsoft Excel 2000. The results of this second round were again summarised using medians and inter-quartile ranges. A summary of both rounds was mailed to all participants who completed both rounds of the Delphi process on 17th February 2003 (appendix A3.4).

3.2.3. Analysis

Medians and inter-quartile ranges were used to summarise the central tendency and dispersion for each criterion after every round. Consensus for each criterion was pre-defined as 75% or more of the participants placing their choice within a 3-point range, or less, of each other. Measurement error due to the variability of judgements made by humans is often assessed using reliability indices. One field in which reliability is often investigated is that of psychological measurements using questionnaire scales. These indices assess the amount of correlation, or agreement, between judges, whilst taking into account the variation between the subjects that are being measured. Two reliability coefficients (statistical indices), Cronbach's α and the average measure intraclass correlation coefficient (ICC), were used to estimate the degree of overall consensus or agreement for each round of the Delphi process. A two-way analysis of variance mixed model (for absolute agreement) was used for the estimation of the ICC. SPSS v11 was used to perform these two estimates. Only responses from participants who took part in both rounds of the Delphi process were included in the calculations of indices of agreement.

Section 3.3. Results

A total of 26 individuals were selected to receive invitations to form the expert panel and participate in the Delphi consultation process. These individuals were located in North America, United Kingdom and Europe. Twenty-two experts had responded by 8th November 2002 with a completed first round questionnaire - indicating a willingness to participate. Seventeen of the panel elected to participate in the second round using e-mail. Nineteen responses to the second round questionnaire had been received by 9th December 2002; no further questionnaires were received after this date. The whole process, from mailing of the first round to the end of the second round, took 55 days. Participants in the first round recommended eleven additional criteria to be included in the second round of the consultation, often the same suggestions were made by more than one individual.

The medians and inter-quartile ranges of participants' responses in the first and second rounds, for all criteria, are provided in appendices A3.5 to A3.8. Between the first and second round no median values changed but a considerable number of inter-quartile ranges narrowed. In the first round the mean maximum percentage of participants within 3 points of each other was 77.2% (standard deviation (s.d.) = 11.1; n = 55). This increased to a mean of 86.7% (s.d. = 9.9, n = 55); the mean difference being –9.6% (95% confidence interval (95%CI) = -11.4% to -7.7%) (figure 3.2). This suggested that there had been an increase in consensus between the two rounds.

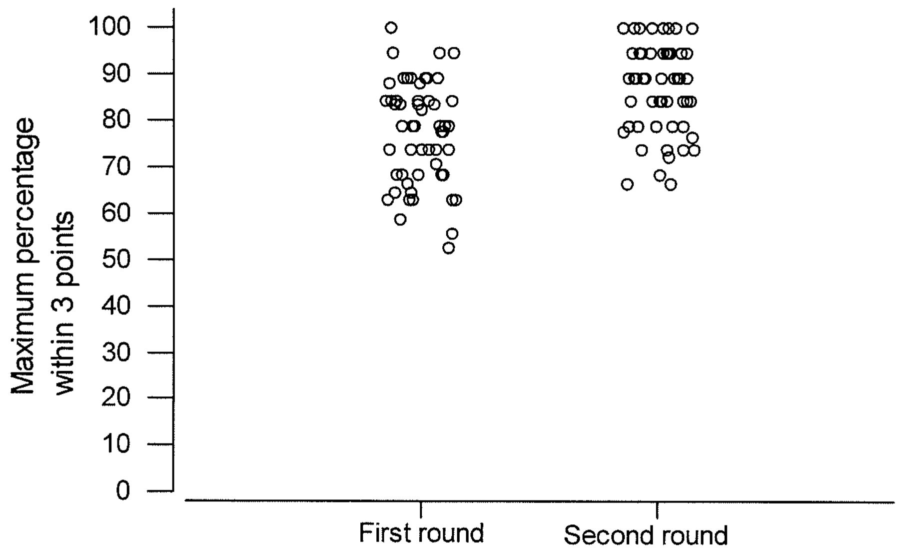

Figure 3.2 Scatter plot comparing the proportion of participants placing their responses for each item within 3 points of each other between the two rounds of the Delphi process.

Across all criteria the ICC in the first round was 0.94 (95%CI = 0.91 to 0.96) and in the second round agreement had increased to 0.97 (95%CI = 0.95 to 0.98). The ICC for each of the separate sections are provided in table 3.1, with agreement increasing from rounds one to two. Cronbach's α, for all the items, increased from 0.95 to 0.97 between the two rounds. There was a similar increase between rounds across the four sections taken in isolation (table 3.2).

Table 3.1. Table of the average measure intraclass correlation coefficients (ICC) for both rounds of the Delphi consultation for each individual section.

	ICC (95% confidence interval)	
	Round one	Round two
Historical information	0.94 (0.89 to 0.98)	0.97 (0.95 to 0.99)
Clinical signs (part 1) – associated with respiratory inflammation	0.94 (0.84 to 0.99)	0.95 (0.89 to 0.98)
Clinical signs (part 2) – associated with respiratory obstruction	0.85 (0.56 to 0.99)	0.95 (0.88 to 0.99)
Clinical signs (part 3) – associated with detailed clinical examination	0.95 (0.90 to 0.98)	0.98 (0.96 to 0.99)

Table 3.2. Table of the Cronbach's α indices for both rounds of the Delphi consultation for each individual section.

	Cronbach's α	
	Round one	Round two
Historical information	0.95	0.98
Clinical signs (part 1) – associated with respiratory inflammation	0.95	0.96
Clinical signs (part 2) – associated with respiratory obstruction	0.92	0.97
Clinical signs (part 3) – associated with detailed clinical examination	0.96	0.98

Consensus was achieved for many criteria and the median ranges are provided in tables 3.3 to 3.6. Consensus was only achieved in three of the eleven criteria introduced after the first round.

Table 3.3. Summary table of the final group responses for the items included in the historical information section of the Delphi consultation.
*Scale of importance **proportion of participants within 3 points, or less, of each other.

	Median* (range)	Consensus %**	Consensus Range
Age of horse	81 to 99	78.9	61 to 100
Breed of horse	0	89.5	0 to 20
Activity or use of horse	21 to 40	78.9	1 to 40
Genetic relative already diagnosed with RAO	1 to 20	89.5	0 to 40
Vaccination history	1 to 20	84.2	0 to 20
History of poor performance (subtle effect on athletic potential)	61 to 80	78.9	21 to 80
History of exercise intolerance (intolerance to low level of exercise)		No consensus	
History of viral infection (febrile respiratory disease)		No consensus	
History of recent viral infection (febrile respiratory disease)	21 to 40	78.9	1 to 60
Geographical location of horse (prevalent weather conditions)		No consensus	
Season in which disease first developed (owner reported)	61 to 80	78.9	41 to 99
Stable design – ventilation	81 to 99	89.5	61 to 100
Stable design – drainage	21 to 40	89.5	1 to 60
Type and quality of bedding provided in stable	100	84.2	81 to 100
Type and quality of forage provided	100	94.7	81 to 100
Length of time spent each day at pasture	81 to 99	78.9	61 to 100
Criteria from second round only			
Location of horse for feeding/mucking out/grooming		No consensus	
Storage of forage/bedding and location of muck heap in relation to stable/stall		No consensus	
History of exposure to donkeys		No consensus	
Duration of problem (Recurrent?)	81 to 99	84.2	61 to 100
Improvement when at pasture	81 to 99	84.2	81 to 100
Health of contemporaries, including others diagnosed with RAO		No consensus	
Alterations to normal routine exposing horse to high dust conditions e.g. transportation, stabling at competitions etc	61 to 80	78.9	41 to 99

Table 3.4a. Summary table of the final group responses for the items included in the clinical signs section (part 1; respiratory inflammation) of the Delphi consultation. *Proportion of cases diagnosed **proportion of participants within 3 points, or less, of each other.

	Median* (range)	Consensus %**	Consensus Range
A cough	81 to 99	89.5	61 to 100
Coughing more than four times in 24 hrs	61 to 80	78.9	61 to 100
Coughing at the start of exercise	81 to 99	78.9	61 to 99
Coughing during exercise	61 to 80	94.7	41 to 99
Cough more than four times during exercise		No consensus	
Coughing when eating		No consensus	
Coughing during an examination period	21 to 40	94.7	1 to 60
Coughing when squeeze larynx/trachea		No consensus	
A nasal discharge	41 to 60	84.2	1 to 60
A nasal discharge following exercise		No consensus	
Criteria from second round only			
Evidence of previous nasal discharge i.e. dried debris		No consensus	
Nasal discharge after sedation or when head lowered after travelling		No consensus	

Table 3.4b. Summary table of the final group responses for the items included in the clinical signs section (part 1; chronicity of cough) of the Delphi consultation. *Proportion of cases diagnosed **proportion of participants within 3 points, or less, of each other.

Time period of cough	Median* (range)	Consensus %**	Consensus Range
Two weeks	21 to 40	78.9	1 to 40
Four weeks	61 to 80	84.2	41 to 80
Three months	81 to 99	89.5	81 to 99

Table 3.5. Summary table of the final group responses for the items included in the clinical signs section (part 2; respiratory obstruction) of the Delphi consultation. *Proportion of cases diagnosed **proportion of participants within 3 points, or less, of each other.

	Median* (range)	Consensus %**	Consensus Range
Increased expiratory abdominal effort:			
• **at rest**	61 to 80	94.7	21 to 80
• **when stabled in high dust conditions**	81 to 99	89.5	61 to 100
• **when fed dry hay** (poor quality)	61 to 80	78.9	61 to 99
• **following/during mucking out**	No consensus		
Increased/prolonged expiratory abdominal effort post-exercise	61 to 80	78.9	61 to 100
A visible heave line	21 to 40	78.9	1 to 40
Movement of anus with respiration	1 to 20	100	1 to 40
Flaring of nostrils:			
• **at rest**	21 to 40	84.2	1 to 60
• **when stabled in high dust conditions**	61 to 80	77.8	41 to 99
• **when fed dry hay** (poor quality)	No consensus		
• **following/during mucking out**	No consensus		
Increased/prolonged flaring of nostrils post-exercise	No consensus		

Table 3.6. Summary table of the final group responses for the items included in the clinical signs section (part 3; detailed clinical examination) of the Delphi consultation. *Proportion of cases diagnosed **proportion of participants within 3 points, or less, of each other.

	Median* (range)	Consensus %**	Consensus Range
Increased respiratory rate	41 to 60	84.2	21 to 80
Increased heart rate	1 to 20	94.7	1 to 40
Wheezing audible at nostrils	1 to 20	84.2	1 to 20
Abnormal tracheal auscultation	61 to 80	84.2	21 to 80
Thoracic auscultation:			
• **Audible wheezes**	21 to 40	94.7	1 to 60
• **Audible crackles**	21 to 40	78.9	21 to 60
• **Silence** (regions of minimal air flow as a result of severe obstruction)	1 to 20	94.7	0 to 20
Thoracic auscultation, with rebreathing bag:			
• **Audible wheezes**	61 to 80	89.5	21 to 80
• **Audible crackles**	41 to 60	84.2	21 to 80
• **Silence** (regions of minimal air flow as a result of severe obstruction)	1 to 20	89.5	0 to 20
Weight loss	1 to 20	84.2	0 to 20
Exercise intolerance	81 to 99	89.5	41 to 99
Prolonged recovery from exercise	81 to 99	84.2	81 to 99
Exacerbations of clinical signs when challenged by hay/straw	81 to 99	89.5	61 to 100
Criteria from second round only			
Coughing following use of rebreathing bag or nasal occlusion	No consensus		
Increased area of auscultation	No consensus		

Section 3.4. Discussion

The Delphi method was developed and pioneered in the 1950s and 60s by the RAND Corporation as a method for predicting the consequences of nuclear war and forecasting other technological events (Linstone and Turoff, 1975). It was named after the Greek oracle at Delphi, who was believed to have the power to predict the future, although it was notorious for the ambiguity of its utterances. Since then, the technique has been widely used in the medical field for gaining information and consensus on topics where evidence is sparse (Kors *et al.*, 1990, Oddone *et al.*, 1994, Jones and Hunter, 1995, Baumann *et al.*, 2001). The technique uses written responses to questionnaires over a series of rounds, with the results of the previous round being fed back to the participants in the next. The process stops when consensus has been approached among respondents across the majority of criteria (Delbecq *et al.*, 1975); in this study this was deemed to have occurred after just two rounds. Generally, two or more rounds are likely to result in some convergence of individual judgements, although it is unclear whether this increases the accuracy of group decision (Murphy *et al.*, 1998). Thus, participants are given at least one chance to re-evaluate their responses based on the group's response. Often the first round consists of open-ended questions intended to generate criteria for the subsequent rounds where quantitative responses are utilised. However, this can generate a large amount of qualitative information that is hard to deal with and prolongs the consultation considerably. In many situations, as in this study, a modified Delhi method is used where pre-existing information is assimilated to form quantitative questions (Hasson *et al.*, 2000). A significant advantage to the qualitative Delphi process is that it provides statistical group responses and quantitative estimates as an output.

This study was able to consult experts from throughout the world, as the panel did not have to come together physically. It would have been prohibitively expensive to organise a meeting of this geographically disparate group. The response rate in a Delphi process is not critical - the most important factor is expertise. Experts can be defined as a group of informed individuals or as specialists in their field (Goodman, 1987, McKenna, 1994). It was hoped that by selecting clinicians and researchers who had published on the subject of RAO, we would select such individuals in an unbiased way. Care should be taken with the term 'experts' and some authors have expressed reservations about its use (Linstone and Turoff, 1975, Sackman, 1975). The 'sin of expertness' can lead to the perpetuation of an individual's opinions, not necessarily based on evidence, which can go unchallenged as a result of the esteem in which they are held (Sackett, 2000). The Delphi process, by maintaining anonymity ensures the opinions of respondents are equally weighted. This prevents domination of the process by assertive individuals. However, anonymity can be a seen as a double-edged sword with lack of accountability resulting in hasty, ill-considered judgements, as there will be no comeback for the participant (Goodman, 1987). The initial intention to invite 26 experts, and the final 19 actual participants, appears to have been a reasonable sized panel with groups larger than 12 obtaining improvements in reliability with diminishing returns (Murphy *et al.*, 1998). Certainly, with group sizes greater than 30 there appear to be no advantages (Delbecq *et al.*, 1975).

There is no universally agreed standard method for determining and communicating the degree of group consensus (Graham *et al.*, 2003). However, it is recognised that basic statistics describing both the central tendency and, in particular, the dispersal of judgements or opinions should be reported for each criterion as a means of indicating the degree of consensus. For this purpose, in general, the median and inter-quartile range is more robust than the mean and standard deviation (Murphy *et al.*, 1998). Between the two consultation rounds in the current study, there was a decrease in the size of the inter-quartile ranges for many of the items indicating that panellists were grouping more closely around the median, implying consensus.

Consensus or agreement can be thought of as a special form of association between the participants and should be reflected in decreases in the variance of the responses. Cronbach's α can be used to evaluate consensus if it is equated to homogeneity of opinion expressed by a group of individuals rating items on a scale (Bland and Altman, 1997, Graham *et al.*, 2003). It estimates the reliability of the sum of participants' responses. The smaller the variance between participants compared with the variance within each participant, the closer Cronbach's α will be to 1. Average measure ICC is the reliability of all the judges averaged together (Shrout and Fleiss, 1979). It compares the variability of different ratings of the same subject to the total variation across all ratings and all subjects. The two-way mixed model used in its estimation assumes that raters, who are the only judges of interest, evaluate each criterion; the judges are fixed effects while criteria ratings are random. The ICC is closely related to kappa and weighted kappa measures of agreement that are used when ratings are recorded on a nominal scale (Cohen, 1968, Armitage *et al.*, 2001). Excellent agreement is indicated by reliability coefficients, like Cronbach's α or ICC, having a value greater than 0.9 (Shrout and Fleiss, 1979, Bland and Altman, 1997). In this study agreement between the participants can be considered to have been satisfactory after the first round alone, but consensus increased even further after the second round. This suggests that the feedback to the group of the results of the first round may have influenced participants' responses in the second round. Alternatively, the increase in consensus may simply be a consequence of the iterative process of participants making their judgement again (Woundeberg, 1991). The increase in agreement appears to have occurred in all four of the separate sections. In particular, the greatest change in ICC and Cronbach's α occurring between the two rounds was for the clinical signs section related to respiratory obstruction (part 2).

The Delphi process does have its limitations, not least in the amount of time taken to complete the process. The time taken to complete this study was similar to the 45 days or more that most Delphi consultations take (Delbecq *et al.*, 1975). The use of electronic media appeared to be acceptable to the majority of participants, reducing costs for the study and, although not reported, did speed up response times. The design and administration of the questionnaires used in the process can greatly affect the final output of a Delphi study. It was aimed to minimise this bias by using more than one individual's opinion to select the items for consideration by the panel from the scientific literature and the previous survey of practitioners. The experts taking part in the process do need high motivation. The loss of the three participants between round 1 and round 2 is regrettable and, although their opinions did contribute to the feedback in the second round, their important information is not assimilated in the final output. The Delphi process should make the best use of available information but it does not create new knowledge - there is a danger of deriving collective ignorance from the process (Jones and Hunter, 1995). There are a number of alternatives to the Delphi method including informal meetings, the nominal group technique and consensus development conferences. These would all have required face-to-face meetings of participants, something that was not possible in this study. Studies have been hard pressed to suggest which is the best consensus method to use (Murphy *et al.*, 1998).

The outputs from this Delphi process largely concur with the information that is available from the scientific literature, but with the additional benefit of quantitative estimates for the importance or occurrence of these historical or clinical outputs. The experts placed great importance on historical information related to the stable design (ventilation) and the type of bedding and forage that a horse is exposed to when they are investigating cases of RAO. This belief is mirrored by that expressed in the survey of equine practitioners (chapter 2.2) that these three pieces of historical information were the most important. The involvement of respiratory viral infection in the pathogenesis of RAO is still to be fully explored and this is perhaps reflected by the lack of consensus on the subject by the experts. However, the suggestion that RAO is precipitated and/or exacerbated by a recent viral infection is given some support by the experts' opinions (Halliwell *et al.*, 1993, Robinson *et al.*, 1996). The recurrent and reversible nature of RAO is highlighted by the consensus achieved and the importance placed on the two items '*duration of problem – recurrent*' and '*improvement when at pasture*' following their introduction by panellists after the first round (Robinson, 2001b).

As would be expected from the literature, most experts stated that many cases of RAO that they had observed had coughing as a presenting clinical sign, with the likelihood of them having the disease increasing the longer the time period over which coughing had occurred (McPherson *et al.*, 1978, Littlejohn, 1980). Practitioners in the UK-based survey had indicated that they believed coughing in response to squeezing the larynx/trachea was associated with mild RAO. However, the lack of consensus amongst the experts perhaps suggests this diagnostic test's validity is controversial. Coughing in association with exercise is mentioned in passing in the literature (Mair and Derksen, 2000, Robinson, 2001d). Perhaps its importance should be further investigated, as experts appear to observe this in many of the cases of RAO that they examine. The presence of a nasal discharge was less frequently noted by experts, also reported by equine practitioners (chapter 2.2) and a case control study of 300 referred cases of pulmonary disease (Dixon *et al.*, 1995b).

The expert panel reported that they observed the majority of cases to have increased expiratory abdominal effort. This is a well-established clinical sign found in association with RAO; it has been reported in two different case series to occur in 43% of cases (Dixon *et al.*, 1995b) and 68% percent of cases (Aviza *et al.*, 2001). Nostril flare was recognised in chapter 2.2 by the majority of equine practitioners to be associated with severe disease and experts identified this clinical sign to be less common in the cases they observed, unless placed in a challenge environment. The importance of a rebreathing bag in auscultation (Beech, 1989a, Naylor *et al.*, 1992) is emphasised by the greater proportion of cases identified by experts to have wheezes and crackles following use of this aid. Unsurprisingly, exercise intolerance and poor recovery post-exercise were recognised by the consensus group to occur in almost all the cases they examined; this effect on performance is well-established in association with RAO (McPherson *et al.*, 1978).

The modified Delphi process used in this study proved to be both an efficient and productive way of consulting an expert panel to answer a question that could not be directly answered from available information. Many of the historical information and clinical sign items that reached consensus appear to be logical criteria for inclusion in a RSQ that has the purpose to discriminate between horses with and without RAO. Some of the criteria relating to detailed clinical examination would obviously not be applicable to a RSQ completed by a layperson with the other clinical signs being considered as 'symptoms'. The median ranges generated for each item on a scale of 1-100 can be interpreted as a likelihood or probability of a horse having RAO if it exhibits a particular clinical sign or is associated with an historical fact. Such probabilities will provide one solution for scoring the RSQ (chapter 4).

CHAPTER 4

CONSTRUCTION AND VALIDATION OF A RISK-SCREENING QUESTIONNAIRE FOR THE INVESTIGATION OF RECURRENT AIRWAY OBSTRUCTION IN EPIDEMIOLOGICAL STUDIES

Section 4.1. Introduction and Aims of Study

To date there is no practical epidemiological instrument for identifying horses with recurrent airway obstruction (RAO) in the UK horse population. The use of existing diagnostic tests for RAO, such as pulmonary function testing and respiratory cytology, is unfeasible because of costs and problems of consent. Similar problems have been encountered in human respiratory epidemiology and the principal solution since the 1960s has been questionnaires (Toren *et al.*, 1993). The desire to perform large-scale epidemiological surveys for the prevalence of asthma has led to the development of specific screening or symptom questionnaires (Burney *et al.*, 1994, Asher *et al.*, 1995). Parental completion of screening questionnaires has been utilised for younger children (Ponsonby *et al.*, 1996). An accurate screening questionnaire to identify RAO for completion by horse owners would be a useful and relatively inexpensive epidemiological tool.

The purpose of this study was to construct a risk-screening questionnaire (RSQ) for RAO able to discriminate between horses with and without the disease. Two methods of scoring the RSQ were assessed. The first, the Delphi score, utilised probabilities for clinical signs and criteria associated with RAO from the previous consultation with experts (chapter 3). The second, the homogeneity analysis by means of alternating least squares (HOMALS) score, was calculated using homogeneity analysis of actual response data obtained from the RSQs completed during the study. The validity of the RSQ was assessed by comparison with a reference standard of a clinical diagnosis of RAO that included the use of respiratory cytology.

Section 4.2. Materials and Methods

4.2.1. Construction of the risk-screening questionnaire

Information from the literature review, the survey of equine practitioners (chapter 2) and the modified Delphi consultation with international equine respiratory experts provided the guidance for the construction of the RSQ. The Delphi consultation provided the principal framework of the RSQ with topics reaching consensus (chapter 3) included in the list of possible criteria for inclusion in the RSQ. Questions were generated that would seek to obtain the information relating to these criteria. These questions were grouped and edited to produce a clear, concise questionnaire. This was partly driven by the prior decision to limit the RSQ to two sides of A4 paper. The questionnaire was designed, edited and pre-coded using a data capture software program, TELE*form* Elite v8.

4.2.2. Final format of the risk-screening questionnaire

The RSQ utilised 32 questions regarding the horse and its respiratory health over two sides of A4 (appendix A4.4). The questionnaire consisted of three sections of closed questions. The first requested information on whether or not the subject horse had ever demonstrated clinical signs that are commonly associated with RAO, both at any time and in the last 12 months. The second section (part A) contained questions related to these clinical signs, if present, such as exacerbating factors and the possibility of infection. If an owner indicated that their horse had never had any of these signs then they were filtered to the last section (part B). Part B contained questions relating to poor performance and direct questions regarding a veterinary diagnosis of RAO and summer pasture associated obstructive pulmonary disease (SPAOPD). For the purposes of the RSQ validation, a short section that sought to obtain general information about the horse (age, breed etc.) was inserted at the beginning of the questionnaire. For the validation, the RSQ was administered stapled to a covering letter, on the back of which was a general explanation of the clinical signs that can be associated with respiratory disease. This explanation included a graphical depiction of nostril flare and abdominal respiratory involvement (appendix A4.3). Finally the RSQ was tested on ten clients attending the clinics of the Weipers Centre for Equine Welfare; none of these horses had been referred for respiratory problems. The horse owners were able to complete fully the appropriate sections of the questionnaire and did not report any difficulties associated with the process.

4.2.3. Scoring of the risk-screening questionnaire

a. The Delphi score

The median responses to the Delphi process provided point probabilities for the likelihood of RAO given a certain historical criterion or clinical sign. The lowest point of the median range was selected for use in the scoring system. These point probabilities were assigned to the corresponding response of the RSQ (appendix A4.7). All responses were included in the calculation of the score except for the month(s) with which the clinical signs were associated, whether or not two or more 'symptoms' occurred at the same time, pasture exacerbating signs and nostril flare at any time in the past. Point probabilities were converted into odds before multiplication together to form a total odds score for the response recorded on each RSQ. This multiplication included a correction factor for when a horse was indicated to have had clinical signs associated with an increased temperature or respiratory infection. This odds score was then converted into a final probability for the likelihood of RAO for the horse that was the subject of the RSQ (the Delphi score). The calculation of the Delphi score was executed using a Microsoft Excel 2000. The responses from an RSQ validation could be imported directly into the spreadsheet to calculate a score.

b. The HOMALS score

An alternative means of scoring the RSQ was investigated, following evaluation of its dimensionality, using homogeneity analysis of the actual responses collected by the RSQ during the study. This followed the method described by Grassi *et al.* (2003). Response categories from the RSQ were collapsed down to form only 12 items with dichotomous responses (Yes/No) for use in this score construction (appendix A4.17).

4.2.4. Estimate of sample size for sensitivity and specificity

The estimated performance of RSQ for both sensitivity and specificity was 0.8 and the desired confidence interval for both proportions was 0.7 to 0.9. Based on these assumptions the standard error was calculated.

$$\text{Where: } SE = \frac{CI - p}{1.96} = \frac{0.1}{1.96} = 0.05102$$

$$\text{and } CI = p \pm 1.96SE$$

(SE = standard error, CI = confidence interval, p = proportion, either sensitivity or specificity). The required sample size (n), given the estimates of the proportion and desired standard error, was calculated as

$$n = \frac{p(1-p)}{SE^2}$$

Hence, given these assumptions, the sample size required for estimating sensitivity (i.e. total number of cases or positive animals) was 62. Similarly 62 horses (total number of controls or negative animals) were required for estimating specificity.

4.2.5. Validation of the risk-screening questionnaire

There were two phases of RSQ validation, one over the winter/spring of 2003 and the second over the winter/spring of 2004. The first phase commenced on 19th February 2003 when a subsection of veterinary surgeons who had responded to the survey of equine practitioners were mailed an invitation to assist in the validation of the RSQ. These practitioners had indicated that they would be willing to participate in a future survey and that they used cytological analyses of tracheal washes (TW) and/or bronchoalveolar lavage (BAL) *sometimes*, *frequently* or *always*. This invitation was extended to participants in the Delphi consultation of experts who practised in the United Kingdom. It was requested that when they were proposing to perform a TW and/or a BAL (for whatever reason) on a horse that they obtain an owner completed RSQ (case). A form was provided for recording the results of the respiratory cytology, endoscopic observations, the clinical signs identified and the final diagnosis for the case. A control was obtained by asking the practitioner to acquire an owner completed RSQ for the next horse, without obvious or known respiratory problems, that the practitioner encountered in their working day.

One hundred and seventy veterinary surgeons were mailed a personalised invitation letter explaining the study that had been signed by the author and the Head of Division of Equine Clinical Studies (Professor Sandy Love) (appendix A4.1). Included with the letter were two RSQ validation "packets" that consisted of:

1. A record sheet for the case, intended for completion by the veterinary surgeon; printed on blue paper. This sheet also had instructions for administration of the RSQ validation (appendix A4.2).
2. An RSQ (appendix A4.4), printed on yellow paper, for completion by the owner of the case complete with a cover letter (appendix A4.3).
3. An identical RSQ and information/cover letter except without a title and printed on green paper, for owners of control horses.
4. A prepaid return envelope (appendix A2.1.2) folded around the above documents and secured by a paper clip to form a "packet".

Approximately one month after this mailing (24th March 2003), a postcard reminder was mailed to all the veterinary surgeons encouraging participation in the validation study (appendix A4.5).

In the second phase of validation in late November 2003, thirty veterinary surgeons who had either already contributed or who held certificates in equine medicine and equine practice from the original mailing, were again invited to participate (appendix A4.6); two RSQ packets were enclosed with each invitation. The cover letter summarised limited results of the RSQ validation up to that time. Those veterinary surgeons that had already participated were sent an amended letter that thanked them for their contribution so far. An additional incentive was provided by the promise of a £5 voucher (redeemable at a national wine retailer) in return for every completed RSQ packet. The collection of validation RSQs was continued until the end of May 2004.

The contents of each returned RSQ packet (the veterinary surgeon record sheet, the case RSQ and the control RSQ) were scanned separately using a fujitsu fi-4110cu image scanner. The resultant images were then imported into TELE*form* Elite v8 data capture software. The software processed the images automatically extracting the data from the completed fields before verification and correction by the operator. The actual scanned image of each page was visually compared with the image generated by the software (with its interpretation of the responses) before the data were exported separately into Microsoft Excel 2000. Calculation of the RSQ score from the responses provided in the questionnaires was independent of any knowledge of the reference standard results. Similarly, the reference standard result was established by the veterinary surgeons concerned, without knowing the RSQ outcome. All cases that were diagnosed as having RAO by the participating veterinary surgeons were included in the RAO group. The remaining cases that had alternative diagnoses (not RAO) and all the controls were included in the non-RAO group.

4.2.6. Analysis

Comparisons between groups were made using Mantel-Haenszel chi-square analysis for categorical data (Epi Info v6.04d). Fisher's exact test was used when expected cell counts were less than 5. If the variable was ordinal, trend chi-square analysis was also performed. Comparisons between groups for continuous data utilised the Mann-Whitney test (U) (two-sample rank test) for two populations (Minitab v12.21). Significance for all analyses was taken as $p < 0.05$.

In preparation of the HOMALS score, factor analysis and homogeneity analysis (HOMALS procedure) were used to assess the dimensionality of the RSQ (SPSS v11). Dimensionality indices (Cronbach's α and Divgi index), based on eigenvalues, were calculated as well as the proportion of data variability explained by each dimension (Grassi *et al.*, 2003). The score was constructed through the HOMALS procedure by replacing dichotomous responses with category quantifications. The category quantifications were used to create a scaled score for each horse with a possible minimum equal to zero and a possible maximum equal to ten.

Non-parametric (empirical) receiver operating characteristic (ROC) curves were plotted using the observed measurement points as bin limits. Cut-off (threshold) values were chosen by selecting the point on the ROC curve closest to the upper left corner of the unit square. The cut-off value was included for positive classification. The area under the curve (AUC) was estimated using the Wilcoxon/Mann-Whitney method as a summary statistic for test performance (SPSS v11)(Hanley and McNeil, 1982). Other single indicators of diagnostic performance were calculated, namely Youden's index (Youden, 1950) and the diagnostic odds ratio (Glas *et al.*, 2003). Sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios for a positive or negative result were also calculated (Sackett *et al.*, 1991). Confidence intervals were calculated using binomial methods rather than large sample methods where appropriate (Bland, 2000).

Section 4.3. Results

4.3.1. Description of case and control groups

A total of 40 cases and 40 controls were provided by the veterinary surgeons that participated in the study (appendices A4.8 & A4.13). Half of these cases and controls were obtained during the first phase of the validation. Twenty-two of the case/controls were supplied by veterinary surgeons in private practice. There was no significant difference between the case and control groups regarding age (U = 1775, p = 0.1), length of ownership (U = 1609, p= 0.9), gender (χ^2_2 = 4.8, p = 0.09) or breed (χ^2_2 = 0.3, p = 0.9)(table 4.1).

Table 4.1. Summary table of descriptive information for case and control groups included in the validation study.
IQR = inter-quartile range

	Case group (n = 40)	**Control group** (n = 40)
Median age (IQR)	10y (8y10m to 13y)	9y 3m (7y2m to 11y3m)
Median length of ownership (IQR)	2y 8m (1y to 5y8m)	2y 4m (1y to 6y)
Number of mares	12	20
Number of geldings	26	20
Number of stallions	2	0
Number of thoroughbreds	11	12
Number of part thoroughbreds	10	8
Number of non-thoroughbreds	19	20

Eighteen of the cases were diagnosed as having RAO by the participating veterinary surgeons; the remainder of cases had a range of diagnoses including normal respiratory tract (table 4.2; appendix A4.14).

Table 4.2. Summary table of diagnoses for the case group included in the validation study.
EIPH=Exercise induced pulmonary haemorrhage

Diagnosis	**Number of cases**
Recurrent airway obstruction	18
Inflammatory airway disease	3
Infectious respiratory disease	6
Other	5
EIPH, Upper airway inflammation, Idiopathic eosinophilia, Pulmonary granular cell tumour & Allergic airway disease (upper and lower)	
Normal respiratory tract	8

In the case group comparison of the RAO and non-RAO sub-groups there was no significant difference regarding age (U = 438, p = 0.06), length of ownership (U = 399, p = 0.4), gender (χ^2_2 = 4.7, p = 0.09) or breed (χ^2_2 = 1.9, p = 0.4)(table 4.3). Figures 4.1 and 4.2 summarise the clinical findings recorded by veterinary surgeons associated with the cases group (appendices A4.9 & A4.10). The RAO group had a greater proportion of cases with a nasal discharge (χ^2_1 = 4.4, p = 0.04), increased respiratory rate (χ^2_1 = 7.6, p = 0.006), exaggerated abdominal effort ($\chi^2_1 = 11.91$, $p = 0.0006$) and nostril flare (Fisher's exact $p = 0.005$). There was not a statistically significant difference between the groups regarding the remaining clinical signs. Cases of RAO tended to have increased quantities of mucus in the trachea identified on endoscopy, although this was not statistically significant (χ^2 for linear trend = 1.9, $p = 0.2$).

Table 4.3. Summary table of descriptive information for the RAO and non-RAO groups in the validation study.

	RAO group (n = 18)	Non-RAO group (n = 22)
Median age (IQR)	11y (9y10m to 14y6m)	9y (8y to 11y9m)
Median length of ownership (IQR)	3y (2y to 5y10m)	1y 9m (1y to 5y2m)
Number of mares	8	4
Number of geldings	10	16
Number of stallions	0	2
Number of thoroughbreds	3	8
Number of part thoroughbreds	5	5
Number of non-thoroughbreds	10	9

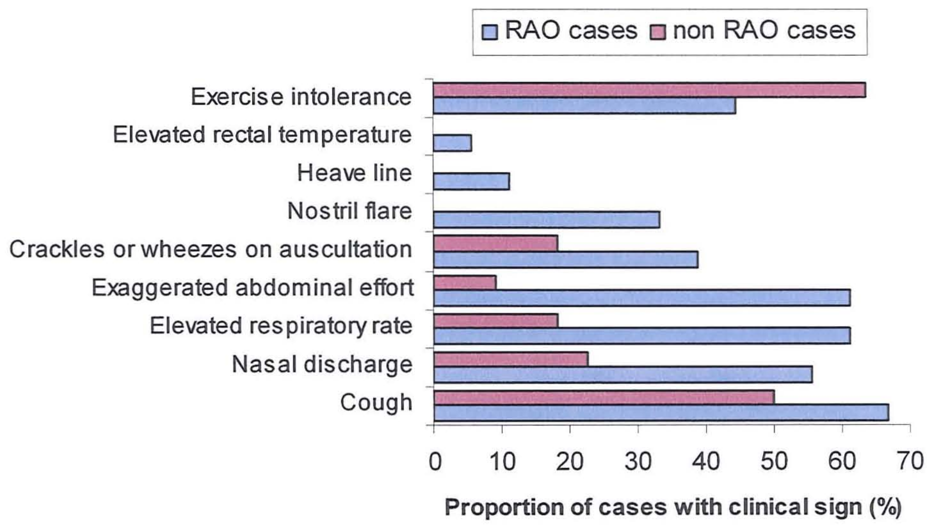

Figure 4.1. Comparison of clinical signs identified by veterinary surgeons between the RAO and non-RAO cases (from the cases group).

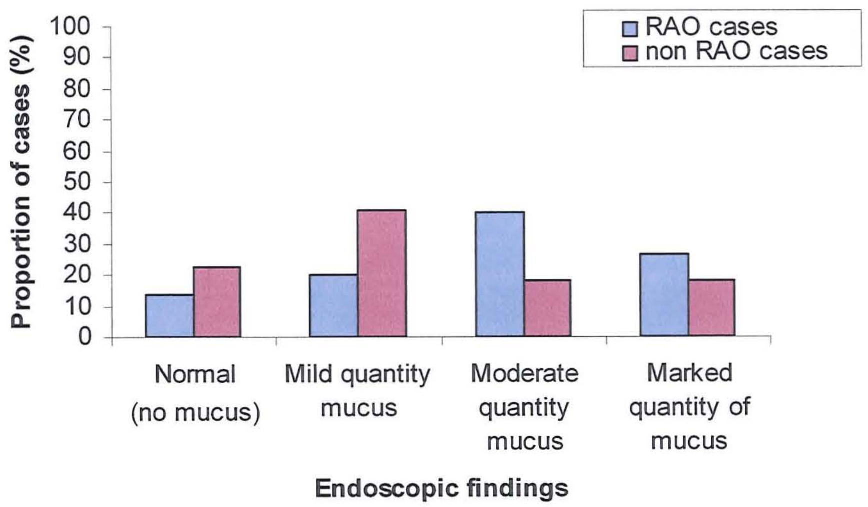

Figure 4.2 Comparison between the quantities of mucus identified in the trachea on endoscopy of horses with and without RAO in the cases group.

Veterinary surgeons used both TW and BAL techniques for obtaining samples for submission for respiratory cytology analysis (figure 4.3) (appendices A4.11 & 4.12). Both techniques were utilised in some cases. The format of the results obtained from analyses of the samples depended on the laboratory the participating veterinary surgeons used. Some of the cytology results were in the subjective format, where the following scale was used to represent the proportion of neutrophils (and other cells) visible on smear examination: - (none seen), +/- (occasional), 1+ (few), 2+ (moderate) and 3+ (numerous). The remainder of the results were provided in the form of proportional counts (percentage). This made it hard to compare cases directly. Cases that were diagnosed as having RAO by participating veterinary surgeons tended to have greater proportions of neutrophils than non-RAO cases, although this was only significant for cases that had a BAL performed ($U = 119$, $p = 0.0015$).

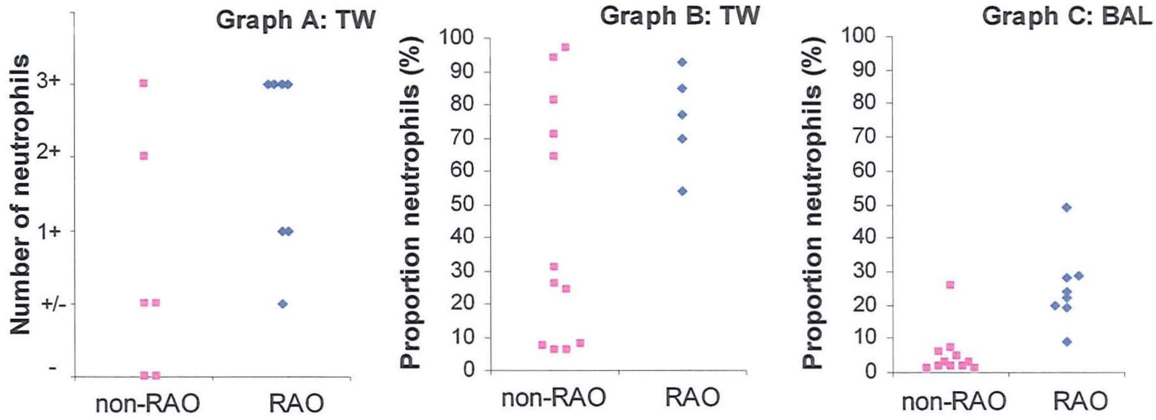

Figure 4.3. Number/proportion of neutrophils identified in respiratory samples obtained from all the cases in the validation study.
TW = tracheal wash. BAL = Bronchoalveolar lavage. Graph A: scale – (none seen), +/- (occasional), 1+ (few), 2+ (moderate) and 3+ (numerous).

4.3.2. Selection of the cut-off for, and the diagnostic performance of, the RSQ

a. The Delphi score

The median Delphi score for the non-RAO horses was significantly lower (0.25, inter-quartile range (IQR) = 0.23 to 0.66) than that for horses diagnosed as having RAO (0.96, IQR = 0.88 to 0.99; $U = 1194$, $p < 0.0001$)(figure 4.4 & 4.5; appendices A4.14 & A4.15).

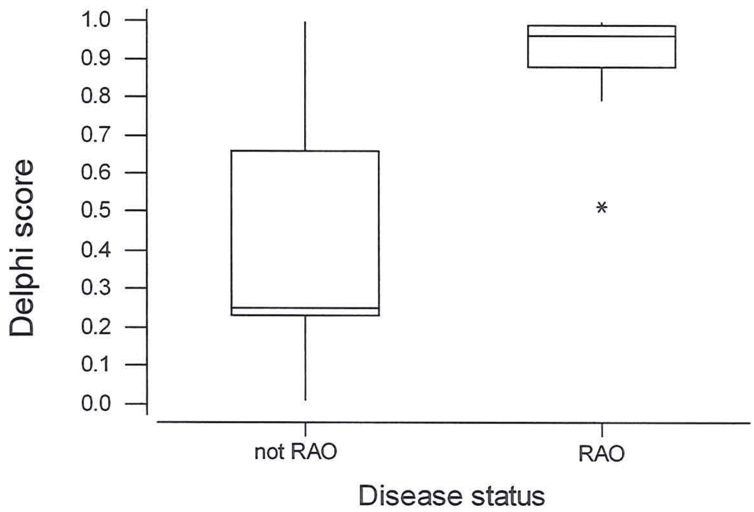

Figure 4.4. Box plots of the Delphi scores for the RAO and non-RAO group in the validation study.

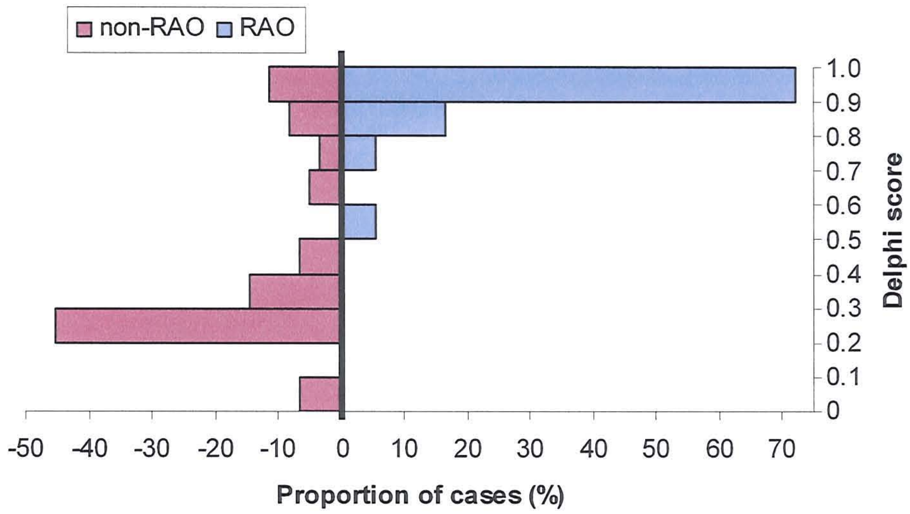

Figure 4.5. Bar chart of Delphi score for horses diagnosed with and without RAO in the validation study.

The non-parametric ROC curve for the Delphi scored RSQ is plotted in figure 4.9; its coordinates are provided in appendix A4.16. A cut-off of a RSQ score greater than or equal to 0.8696 for a positive result was selected from the ROC curve. The diagnostic performance of the RSQ at this threshold is summarised in table 4.4 and in figure 4.6.

Table 4.4. Summary table detailing the diagnostic performance of the Delphi scoring system for the RSQ for RAO.
*Binomial confidence interval

		95% Confidence Interval
Sensitivity	0.83	0.59 to 0.96*
Specificity	0.85	0.74 to 0.93*
Youden's index	0.69	0.49 to 0.89
Positive predictive value	0.63	0.41 to 0.81*
Negative predictive value	0.95	0.85 to 0.99*
Likelihood ratio for a positive result	5.7	3.0 to 10.9
Likelihood ratio for a negative result	0.19	0.07 to 0.55
Diagnostic Odds Ratio	29.4	7.1 to 122.7

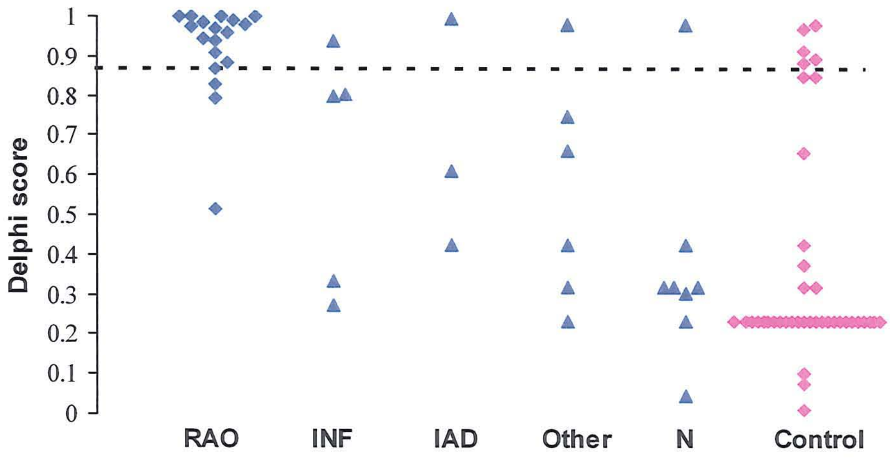

Figure 4.6. Scatter plot of cases and controls versus their Delphi scores according to the RSQ.
Dashed line = cut off point for a positive diagnosis of RAO. Cases coded as RAO = recurrent airway obstruction, INF = infectious respiratory disease, IAD = inflammatory airway disease and N = normal respiratory tract.

b. The HOMALS score

The correlation matrix between questionnaire items contained many Pearson's correlation coefficients > 0.2 (appendix A4.18) suggesting that it could be meaningfully factored. Eigenvalues, descriptive indices and percentage of explained data variability, obtained through factor analysis and homogeneity analysis, are displayed in table 4.5. Three factors/dimensions had eigenvalues greater than one and, in factor analysis, they explained 63.6% of the data variation. In homogeneity analysis the first dimension alone explained 84.8% of the cumulative variation, this suggested the RSQ had only one dimension. This belief was supported by the descriptive indices with the first factor/dimension having a Cronbach index greater than 0.7 and a maximum Divgi index with respect to the other factors/dimensions.

Table 4.5. Descriptive and Goodness of fit dimensionality indices from factor analysis/homogeneity analysis of the RSQ for RAO items ($p = 12$, $n = 80$) using factors/dimensions with eigenvalue > 1.

Dimension/ factor	Descriptive indices			Factor analysis		Homogeneity analysis	
	Eigenvalue	Cronbach	Divgi	Proportion of explained variability (%)	Cumulative explained variability (%)	Proportion of explained variability (%)	Cumulative explained variability (%)
1	5.041	0.87	5.98	42.0	42.0	84.8	84.8
2	1.587	0.40	3.44	13.2	55.2	1.8	86.6
3	1.010	0.01	2.06	8.4	63.6	0.0005	86.6

The HOMALS category quantifications (for one dimension only) are reported in appendix A4.19 and the scaled category quantification scores for each RSQ variable's dichotomous response are provided in table 4.6. A HOMALS score for each horse was calculated by summing the scaled scores dictated by the response pattern in the corresponding RSQ (appendices A4.14 & A4.15).

Table 4.6. Scaled HOMALS category quantifications utilised in the HOMALS score.

	Cough	Nasal discharge	Exaggerated abdominal effort	Nostril flare	Symptoms coincide	Improve at pasture	Worse with risk factor	Recurrent	Coughed for >1 month	Frequent cough	Cough when exercise	Poor performance	Total
Yes	0.97	0.93	0.79	0.97	0.89	0.56	0.82	0.91	0.98	0.99	0.83	0.36	10
No	0	0	0	0	0	0	0	0	0	0	0	0	0

The ROC curve for the HOMALS scored RSQ is provided in figure 4.9 (coordinates in appendix A4.20). The cut-off selected from the curve was 4.74 and summaries of the diagnostic performance at this threshold are in table 4.7 and figure 4.7.

Table 4.7. Summary table detailing the diagnostic performance of the HOMALS scoring system for the RSQ for RAO.
*Binomial confidence interval

		95% Confidence Interval
Sensitivity	0.83	0.59 to 0.96*
Specificity	0.81	0.69 to 0.90*
Youden's index	0.64	0.44 to 0.84
Positive predictive value	0.56	0.35 to 0.74*
Negative predictive value	0.94	0.84 to 0.99*
Likelihood ratio for a positive result	4.30	2.5 to 7.5
Likelihood ratio for a negative result	0.21	0.07 to 0.58
Diagnostic Odds Ratio	20.8	5.2 to 83.7

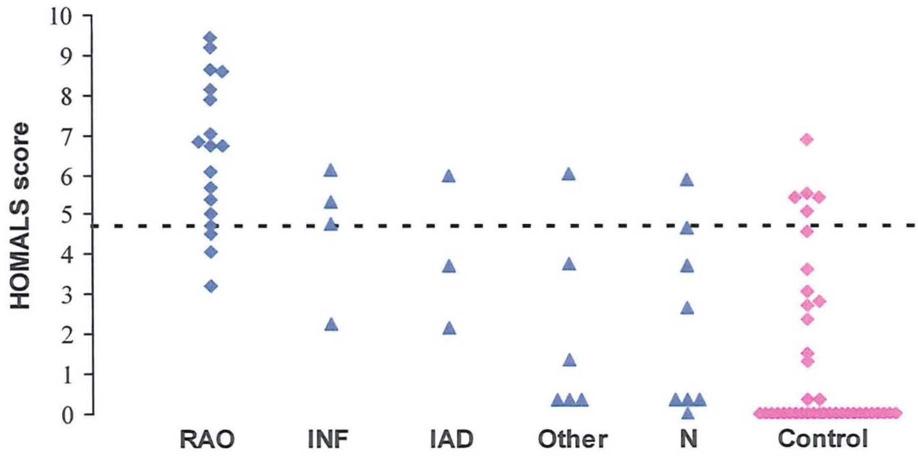

Figure 4.7. Scatter plot of cases and controls versus their HOMALS scores. Dashed line = cut off point for a positive diagnosis of RAO. Cases coded as RAO = recurrent airway obstruction, INF = infectious respiratory disease, IAD = inflammatory airway disease and N = normal respiratory tract.

4.3.3. Comparison of the Delphi and HOMALS scoring systems

The scatter plot in figure 4.8 demonstrates that the majority of horses were classified into the same groups (RAO or non-RAO) by the two scoring systems. However, it is clear that the two scoring systems did not always agree. The AUC of both scoring systems from the non-parametric ROC curves implied they had similar diagnostic performance, AUC_{Delphi} = 0.92 (95%CI = 0.86 to 0.98) and AUC_{HOMALS} = 0.93 (95%CI = 0.88 to 0.99) (figure 4.9).

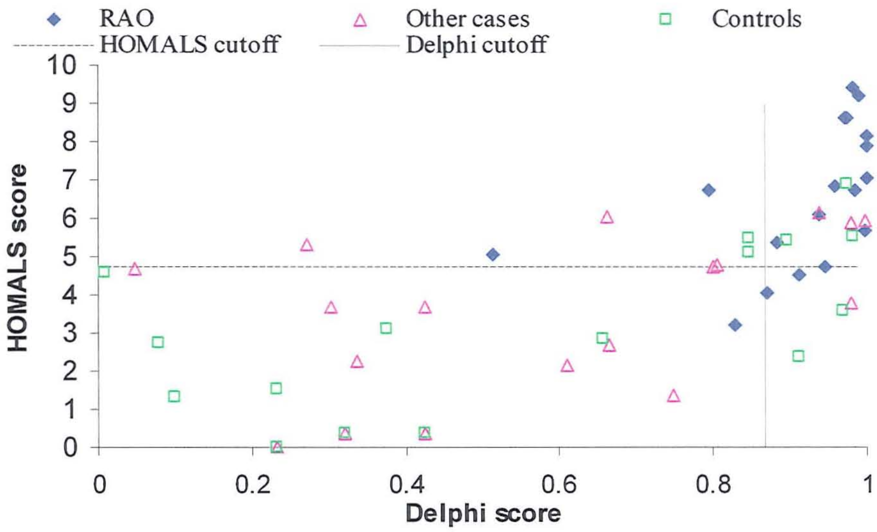

Figure 4.8. Scatter plot of Delphi scores versus HOMALS scores for each individual horse included in the validation study.
Each marker may represent more than one horse, n = 80.

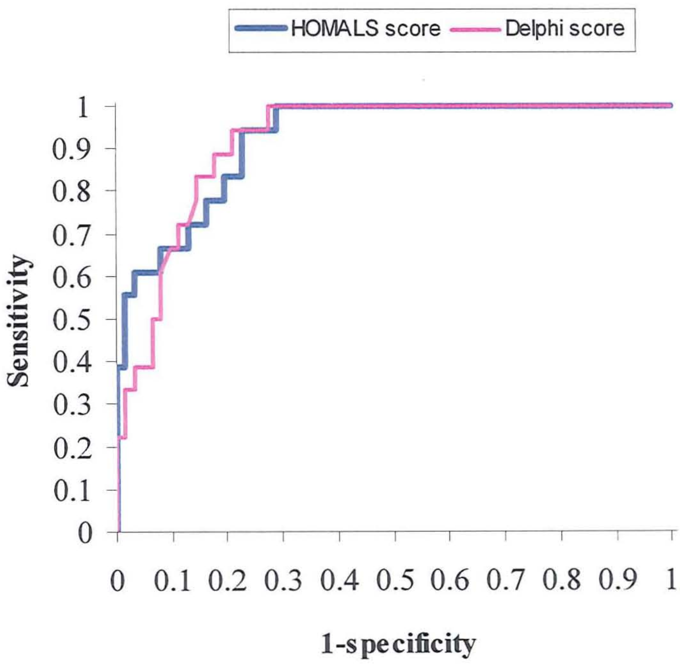

Figure 4.9. Non-parametric receiver operating characteristic curves for the Delphi and HOMALS scoring of the RSQ for RAO.
(Coordinates are provided in appendices A4.16 & A4.20)

Section 4.4. Discussion

This is the first attempt to create and validate a RSQ for identifying RAO in the UK horse population. Such an instrument is essential for the investigation of the epidemiology of RAO. A precedent for this type of tool has been provided from human asthma research where standardized questionnaires have been used in worldwide epidemiological surveys and include both sensitive and specific indicators of asthma (Burney *et al.*, 1989, Toren *et al.*, 1993). Episodic wheezing, cough and shortness of breath are the cardinal symptoms of asthma and are, in principle, easy to measure either by interview or questionnaire. Examples of questionnaires include the International Union against Tuberculosis and Lung Disease (IUATLD) bronchial symptoms questionnaire in adults (Burney *et al.*, 1989) and, more recently, the International Study of Asthma and Allergies in Childhood (ISAAC) in children (Asher *et al.*, 1995) and the European Community Respiratory Health Survey (ECRHS) for adults (Burney *et al.*, 1994). The ISAAC study focuses on two age groups of children, 13 to 14 year olds who answer questions themselves, and 6 to 7 year olds whose parents answer the questions on their behalf (Ponsonby *et al.*, 1996). Both ISAAC and ECRHS utilise questions from pre-existing questionnaires that have found differences between populations, and where validity has been assessed.

No pre-existing questionnaires were available from which questions could be utilised in the creation of the RSQ. Therefore, the construction of the RSQ was guided by information obtained from review of the scientific literature (chapter 1), the survey of veterinary practitioners (chapter 2) and the modified Delphi consultation with international experts (chapter 3). For example, all three processes identified the importance of the presence or absence of a cough, and its duration, in the diagnosis of RAO. The Delphi consultation provided not only the principal framework but also the probabilities for the Delphi scoring system for the RSQ. Studies conducted on asthma screening questionnaires (such as the ECRHS and ISAAC) tend to report the prevalence of symptoms corresponding to individual items without considering the questionnaire as a whole, and thus without computing a score. In this study, a score for combining all the items of the RSQ was thought more appropriate as clinical signs of RAO were felt to be less specific and harder to define than those in asthma (for example wheeze) and this method permitted incorporation of factors known to exacerbate or induce the disease. Scoring has been performed in studies involving the ECRHS screening questionnaire using homogeneity analysis (Grassi *et al.*, 2003). This method was utilised to create the HOMALS system of scoring the RSQ. It was important that the RSQ was determined to have only one dimension if the category quantifications were to be used to create a total score. This single, unobserved, dimension provided the association between the observed variables. This unobserved dimension could be termed *the likelihood of RAO.*

Validation of asthma questionnaires is problematic because the definition and classification of asthma remains controversial (Sears, 1997a, Britton, 1998). Asthma diagnosis is not straightforward and tests sometimes considered to be gold standards, such as bronchial responsiveness, may actually have lower validity than symptom questionnaires (Pekkanen and Pearce, 1999). Diagnosis involves an overall assessment of the patient's medical history, physical examination, and laboratory tests; in fact often history alone is used (Burr, 1992, Jenkins *et al.*, 1996). A clinical diagnosis of asthma has been considered to be a superior reference standard and has been utilised in the validation of questionnaires proposed for diagnosing asthma (Jenkins *et al.*, 1996, Fuso *et al.*, 2000, Grassi *et al.*, 2003). A brief paediatric asthma screen for completion by parents has been validated against a medical assessment consisting of medical history, physical examination and pulmonary function tests (Wolf *et al.*, 1999).

The reference standard chosen was that of a clinical diagnosis by a veterinary surgeon that included the use of respiratory cytology. Criteria for defining the RAO phenotype have been published (Robinson, 2001b). These criteria dictate demonstration of reversible airway obstruction and an increase in the proportion of neutrophils in BAL fluid during environmental challenge. In the clinical situation, these criteria are difficult to assess, as it is not usually acceptable to horse owners to allow their horse to undergo an environmental challenge test. In addition, pulmonary function testing is not widely available and the majority of veterinary surgeons in the survey in chapter 2 did not utilise this test. A clinical score for obstruction has been suggested that requires repeated observations (Robinson *et al.*, 2000). However, it does not appear to have been validated and the author proposing the system originally suggested it was not useful (Robinson *et al.*, 1994b). Therefore, it was not felt practical to include a measurement of obstruction in the criteria for the clinical diagnosis. However, veterinary surgeons were asked to record clinical signs associated with respiratory obstruction, and its presence is implicated by a diagnosis of RAO. As environmental challenge tests were not possible, it was likely that some horses with RAO included in the study would have been in remission when examined and respiratory samples were taken. The overall diagnosis made by the veterinary surgeon was able to adjust for such problems, as they had access to all historical information, all clinical examinations (including those done previously) and respiratory cytology analyses.

The clinical diagnostic process that veterinary surgeons were required to perform was not fully standardised; it was felt that this would not have been possible within the format of the study. For example, to dictate that all cases must have a BAL performed would have eliminated many cases from the study, and to have individually visited all veterinary surgeons, who potentially might contribute cases, to standardize their approach to diagnosing cases of RAO was not possible. This lack of standardisation does indicate there might have been scope for both inter- and intra-observer variability when veterinary surgeons were establishing the reference standard result. An improvement would have been to have provided formal criteria on the case recording form that had to be satisfied before a diagnosis of RAO could be established. Alternatively, an independent expert panel could have been utilised to establish a final diagnosis for each horse based on the available clinical information (Knottnerus and Muris, 2003). Additional error may have been introduced by the way cases were recruited, as it implies a form of convenience sampling; it would not necessarily be possible to reproduce the spectrum of submissions that occurred during this validation study (Greiner and Gardner, 2000b).

The validation of the RSQ proved to be a difficult task with fewer cases being obtained than was desired from the sample size calculations. It had been hoped that at least one third of invitees would return just one RSQ validation packet, providing sufficient power. The poor response may reflect a number of factors. Firstly, veterinary surgeons may have been deterred from participating as a result of the apparent complex nature of the validation process (three separate forms requiring completion) although actual participants reported that the process was not troublesome. Participation was also not instantly possible, suitable cases had to be recruited prospectively and the RSQ validation packet had to be remembered by a veterinary surgeon, sometimes weeks after the original mailing. The use of the postcard reminder was intended to maintain awareness of the survey. It may also be that the use of respiratory cytology for investigating cases, for whatever reason, is a relatively infrequent occurrence outside of the racing industry. In the second round of the validation, attention was focused on a smaller group of veterinary surgeons in the hope that they would be more motivated to participate. It is unclear if the use of an incentive in this round had a beneficial effect.

The decision to utilise veterinary practitioners from the previous survey who had indicated that they used respiratory cytology in the diagnosis of RAO, was made as it was hoped that the horses they would be investigating would reflect the UK horse population, the intended target population. In addition, by selecting cases as any undergoing respiratory cytological sampling it was hoped to include other respiratory diseases that may be differentials for RAO, rather than just comparing RAO cases to healthy controls. Direct and formal communication to establish a relationship with all the veterinary surgeons invited to participate might have improved the response but was not feasible in the time period or budget available. It might have been easier to obtain sufficient numbers for validation using formal arrangements with a fewer number of clinics. However, these clinics would most likely have had a large referred caseload and validation of the RSQ in this population would be irrelevant to the questionnaire's final use. Unfortunately, it was not possible to subject the controls in this validation to the reference standard of clinical diagnosis that included respiratory cytology, principally because of the problems of ethics and owner consent. In further validations of this RSQ, it would be extremely beneficial if this barrier could be overcome, perhaps by the use of a less invasive reference standard such as breath condensate collection (Wyse *et al.*, 2004b). Implementation of the RSQ in a horse population followed by blinded use of a reference standard in a proportion, or all, of the horses would provide a better estimation of the performance of this new epidemiological instrument.

The case and control groups were similar regarding age, gender, breed and length of ownership. In addition, within the case group, there were no significant differences across these same variables between horses depending on diagnosis of RAO. Horses diagnosed as having RAO by the participating veterinary surgeons were recorded as demonstrating four clinical signs (nasal discharge, increased respiratory rate, exaggerated abdominal effort and nostril flare) more frequently than the other cases (the latter two being indicators of respiratory obstruction). The increased proportion demonstrating nasal discharge may be related to the trend for increased quantities of mucus in the trachea, identified by endoscopy, associated with the RAO horses. As would be expected, cases diagnosed with RAO tended to have greater proportions of neutrophils identified in analyses of TW or BAL samples (McGorum *et al.*, 1993d).

The RSQ, using either the Delphi or the HOMALS scoring system, appeared to be able to discriminate between horses with and without RAO. ROC curves were used both to select the optimal cut off value for the RSQ and to provide a summary of the performance of the scoring systems. Non-parametric (empirical) ROC curves were utilised, as no assumptions were required regarding distributions of the test data (Zweig and Campbell, 1993). Importantly, the ROC curve is independent of prevalence. The two diagonal segments of the Delphi score system ROC curve reflect tied data, a non-RAO horse having the same score as an RAO horse. The estimated AUC for both non-parametric ROC plots provide a summary statistic of the overall diagnostic performance of the respective scoring systems. These were estimated using the Wilcoxon/Mann-Whitney method that tends to underestimate the AUC (Hajian-Tilaki *et al.*, 1997). The HOMALS score area was estimated to be slightly larger than the Delphi score area, although this was marginal and in essence the two areas were very similar, with the 95% confidence intervals for the two areas overlapping. The high AUC for both plots suggest a good diagnostic performance for the RSQ using either scoring system. It is worth noting that despite the two scoring systems being calculated by different methods from different origins, the Delphi score being heavily influenced by prior probabilities from experts and the HOMALS score using field data alone, they are approximately equally effective in discriminating between horses regarding the presence of RAO. Hence, it appears that the questions contained in the RSQ instrument may have an underlying 'link' to the disease.

Selecting the cut-off values from the point of the ROC curve closest to the upper left corner assumes a prevalence of 50% in the target population. It maximises the Youden index, which is independent of prevalence (Greiner *et al.*, 2000). Threshold points chosen from the non-parametric ROC curve provided an unbiased estimate of sensitivity and specificity for each scoring system (Zweig and Campbell, 1993). The cut-off selected for the Delphi score for a positive result provided satisfactory sensitivity and specificity for the RSQ. The wide confidence interval around the sensitivity is regrettable and reflects the paucity of RAO cases, something that can hopefully be rectified in the future studies. Importantly, the ROC curve for the Delphi score was superior to that of the HOMALS score over the critical region adjacent to the upper left corner of the unit square. The HOMALS scoring system for the RSQ had a similar sensitivity but was less able correctly to identify negative cases (specificity). Of particular concern was its inability to distinguish between horses with infectious respiratory disease and horses with RAO. The diagnostic odds ratio and the Youden index also summarised that the Delphi scored RSQ had the potential to be a good discriminatory diagnostic instrument. Based on this it is recommended that the Delphi scored RSQ be utilised in investigation of RAO in horse populations.

The Delphi scored RSQ appears to have validity as a discriminatory instrument. However, in its method of acquisition of information from an owner there is opportunity for the introduction of bias. The RSQ relies on the horse owner being able to answer the questions and this introduces recall and reporting bias; they may not be able reliably to recall events that happened in a horse's past. Indeed, many owners will not have owned a horse for all its life. It has been demonstrated that riders of horses in Eventing do exhibit memory decay and reporting bias regarding performance in a study that utilised telephone questionnaires (Murray *et al.*, 2004). Owners of horses with respiratory problems may be able to recall related events more effectively, or over/under report certain factors, than other owners. The time of year at which the RSQ was completed may also introduce recall bias as RAO is thought principally to become apparent during winter. An owner completing an RSQ in summer may have recall that is biased towards recent months, when clinical signs of RAO may be less apparent. A very limited effect of season-of-response to ISAAC questions about asthma has been identified in one study (Stewart *et al.*, 1997). Questionnaire bias (due to the way questions were asked) may have influenced the replies. It was hoped that this bias was minimised by attempting to ensure that the questions were straightforward, unambiguous and in language the respondents would understand. Further studies will be required to address these concerns, principally by addressing the reliability (repeatability) of the questionnaire.

The Delphi scored RSQ constructed in this study for the epidemiological investigation of RAO has been shown to be an effective instrument for discriminating between horses with and without the disease. Knowledge of the sensitivity and specificity of the questionnaire will assist in estimates of the true prevalence when the RSQ is implemented in a larger survey of the UK horse population.

CHAPTER 5

A SURVEY OF HORSE OWNERS IN GREAT BRITAIN REGARDING THE HEALTH OF HORSES IN THEIR CARE, INCORPORATING A RISK-SCREENING QUESTIONNAIRE FOR RECURRENT AIRWAY OBSTRUCTION

Section 5.1. Introduction and Aims of Study

The prevalence of recurrent airway obstruction (RAO) in the horse population of Great Britain is unknown. The suggestion that the prevalence is increasing is a matter for concern (Mair, 1995). If there is a high prevalence of this disease in the horse population it has implications for equine welfare, particularly as it is a disease that can largely be controlled by altering a horse's immediate environment. With the increasing application of evidence-based medicine in veterinary science it is important that the prevalence of the disease in the population is known so that the results of diagnostic tests can be properly interpreted (Sackett *et al.*, 1991). In order to estimate the prevalence of RAO, a survey of horse owners was undertaken using a mailed questionnaire. These owners were randomly selected, following geographical stratification, using two-stage cluster sampling of veterinary practices and then their clients. The questionnaire, administered using a modified total design method (TDM) (Dillman, 1999), included the risk-screening questionnaire (RSQ) for RAO described in chapter 4 as well as questions related to the characteristics and management of each horse. The aim of this study was to estimate the prevalence of RAO in the horse population of Great Britain utilising the RSQ for RAO and generate data for later analyses of associated factors.

Section 5.2. Materials and Methods

5.2.1. Selection of study sample (survey population)

The survey target population was horse owners that kept their horses on mainland Britain. The study population was considered to be horse owners and their horses that were attended by veterinary surgeons. The Royal College of Veterinary Surgeons (RCVS) database of practices for 2001 was searched to identify practices that provided care for horses; all branch practices were excluded (Anon, 2001b). The final database was managed using Microsoft Excel 2000. The identified practices were divided into eight geographic regions, defined by their postcodes, Scotland, North West, North East, Wales, Midlands, Eastern England, South West and South and South East. Within each region each practice was assigned a number. The required sample size (n) for the survey was estimated using:

$$n = \frac{1.96^2 P_{\exp}(1 - P_{\exp})}{d^2}$$

Where $P_{\exp}$ = expected prevalence, estimated at between 10 and 25%, and d = desired absolute precision set at 3%. The required sample size was estimated to be between 384 and 800 horses. It was elected to select two practices from each region with each practice supplying 110 clients, except for the Midlands and South and South East regions from which three and four practices were selected respectively. This would equal 2090 horses assuming each horse owner would complete only one questionnaire. Response rates between 60 and 70% to the questionnaire would result in an estimate of between 1254 and 1463 completed questionnaires being returned.

Practices were selected from each region using random numbers generated using Microsoft Excel 2000. The principal partner of each practice was invited to participate in the survey by supplying their client list. An invitation letter outlined the study and who was conducting and funding the survey (appendix A5.1). It emphasised the confidentiality of the study and that the client names and addresses supplied would be only used for the purposes of the study and that they would be destroyed at its conclusion. The letter was printed black on white A4 Division of Equine Clinical Studies, University of Glasgow Veterinary School headed notepaper, with the colour crest alongside the Home of Rest for Horses (HORFH) logo. Included with each mailing was a one sided form (with code number) for recipients to indicate their willingness to participate in the survey, the total number of horse clients using the practice, the name of the appropriate person to contact and the best time of day to telephone (appendix A5.2) as well as a return envelope. The invitation was enclosed in a white address windowed envelope (A6) so that the individual's address was visible; the return address was pre-printed on the reverse. Individual addresses and salutations were created using the mail merge option of Microsoft Word 2000 linked to the database of address contacts. The author and the Head of the Division of Equine Clinical Studies (Professor Sandy Love) individually signed each letter in blue pen. First class stamps were used throughout this recruitment, including for return envelopes.

Invitation letters to the first group of selected practices were mailed on 6th August 2003. When practices declined to participate, another practice from the appropriate region was randomly selected to receive an invitation. If no response was obtained two weeks after the letter had been posted, one attempt was made to contact the practice by telephone; if the addressee of the original invitation was unavailable a message was left encouraging the return of the response form even if participation was to be declined. If no response was obtained after 3 weeks, then the practice was replaced. This process continued until 30th January 2004.

Practices that agreed to participate in the survey were sent by return a letter thanking them for their positive response and providing further information regarding the study (appendix A5.3), a copy of the horse owner questionnaire (appendix A5.4) and a pre-paid envelope (appendix A2.1.2) to assist return of their client list. This information pack was followed up by a telephone call 2 or 3 days later so that the practice's participation could be discussed in detail. When a list of horse clients had been returned, each client was assigned a number and 110 were selected using random numbers generated using Microsoft Excel

2000. This list was returned to the practice concerned for their final approval, where they were given the option to remove any clients they did not wish to be surveyed. In anticipation, five extra clients had been selected as replacements. The final list of clients was manually entered into a separate Microsoft Excel spreadsheet for each practice. Where possible the addresses were verified and postcodes obtained, if required, using the Royal Mail website or QuickAddress Batch v3 mailing software.

5.2.2. Construction of questionnaire

A modified TDM was adhered to throughout the construction and administration of this survey (Dillman, 1999). A list of topics that encompassed all possible information that the proposed questionnaire could obtain from a horse owner concerning their horse was created including horse details, early life of the horse, management practices and details of the premises the horse was kept on. Questions were then phrased that would elicit this information using self-administration. It was predetermined that the RSQ (chapter 4) would occupy two pages of the questionnaire. The format and final content of the questionnaire was designed, edited and pre-coded using data capture software, TELE*form* Elite v8.

The questionnaire was limited to an A4 8-page booklet (appendix A5.4). The cover page aimed to create a strong identity, emphasising the study affiliations and title of the questionnaire (Confidential Horse Health Questionnaire). An enlarged version of the HORFH logo occupied the majority of the cover page, which also contained a contact address, instructions for completion of the questionnaire and a unique code number to identify the respondent. It was emphasised that the questionnaire was about the recipient's horse, which they might own, part own or have on loan. The recipient was requested to complete the questionnaire for the horse whose name came first in the alphabet, if they owned more than one. Apart from titles, Arial size 11-type font was used throughout the questionnaire. The back cover page finished with a closed-ended question asking if the participant would be willing to take part in a further study (a proposed educational study) and with instructions on returning the questionnaire and the extension of gratitude to the respondent.

The questionnaire comprised of five sections namely 'About Your Horse' (page 2), 'Your Horse's Early Life' (page 3), 'The Following Questions are About Your Horse's Respiratory Health and Breathing' (pages 4 & 5), 'About Your Horse's Management' (page 6), 'About Your Horse's Stable' (page 7) and 'About The Premises Where You Keep Your Horse' (page 7 & 8). Where appropriate, boxes were provided for participants to place a tick or cross in to indicate their responses. Some of the questions had an empty "other" field as the last option. This gave the opportunity for respondents to provide additional information with a space for a written explanation (known as other/specify). Faint shading was used to help guide the completion of the form. The open-ended questions provided either constrained print comb style boxes for insertion of one letter or number at a time or open boxes that permitted free written text. Horse owners who acquired or started looking after their horse after it was five years of age were directed to skip the early life section and proceed to the next page (page 4; the RSQ).

5.2.3. Pre-testing of questionnaire

The questionnaire was tested on 8 horse owners (no veterinary qualifications) who were either working at, or were relatives of people working at, the University of Glasgow or University of Liverpool veterinary schools. None of the test participants had previously read the questionnaire. All participants took less than 15 minutes to complete the questionnaire and item omission was minimal. Participants did not find the layout of the questionnaire difficult to follow or too tedious. These horse owners did make some valuable suggestions for rephrasing of questions or alterations to encompass all possible situations.

5.2.4. Distribution of the questionnaire

The questionnaire was professionally printed black on white 80g A3 paper and collated, folded and wire stitched to form an A4 8-page booklet. The front covers were pre-numbered from 1 to 2000. A cover letter was written inviting recipients of the questionnaire to participate in the survey (appendix A5.5). The letter explained the nature of the questionnaire and who was conducting and funding the survey. The study was described to be a collaboration with their veterinary practice and that this was how they had been contacted. The importance of responding to the survey and its confidentiality was emphasised. Details of the proposed educational study were provided. The inclusion of the pre-paid return envelope was highlighted and a deadline (14 days after mailing) for return of the completed questionnaire was given. This letter and all subsequent reminders included the logo of the HORFH and the University crest. They were printed black on white A4 paper using size 12 Arial font, included the postal date and were signed in blue ink by the author. Postcards were an exception and were printed on white card (105 mm x 148 mm) using size 11 Arial font. All envelopes were pre-printed with the return address. Mailings utilised first class mail apart from the reminder postcards (second class).

The survey was mailed to owners when the client list for a particular practice became available. Initial mailings included a cover letter individually addressed to the recipient, one pre-paid return envelope (appendix A2.1.2, utilising a Royal Mail Freepost response service) and a questionnaire printed with the code number corresponding to that person.

This survey pack was placed in a C4 white address windowed envelope so that the individual's address was visible. Individual addresses and salutations were created using the mail merge option of Microsoft Word 2000 linked to the database of address contacts. After two weeks, all non-responders were sent a personalized postcard encouraging return of a completed questionnaire (appendix A5.6). Four weeks after the initial mailing a second, identical, questionnaire (with hand written code numbers on front cover) was sent to all non-responders. A cover letter with similar layout and content to the first one was included (appendix A5.7), as well as a pre-paid return envelope. It was reported that, to date, there had been an excellent response to the survey. No deadline for return was given.

5.2.5. Processing of returned questionnaires

Returned questionnaires were stamped with the date of arrival and the spine of the booklet was removed to separate the pages. The questionnaires were scanned using a fujitsu fi-4110cu image scanner. The resultant images were imported into the TELE*form* Elite v8 data capture software. The software processed the images extracting the data from the completed fields before verification and correction by the operator. This included manual entry of free text information. The actual scanned image of each page was visually compared with the image generated by the software (with its interpretation of the responses) before the data were exported into Microsoft Excel 2000.

5.2.6. Analysis

Responses were considered useable if at least one question was answered. The overall response rates (return proportion and response proportion) were calculated using all responses. This included those who were unable to contribute, namely those who no longer owned horses and 'addressee has gone away'. The completion proportion (number of completed questionnaires) was also calculated. These response patterns were calculated overall and for the individual practices. For the completed responses from all 14 practices, the time taken to respond (stage of response) was assessed using information provided by the responders regarding their horse. The stage of response was defined as early (if response had occurred before the mailing of the second questionnaire) or late (if it occurred after this mailing). Comparisons between these groups were made using Mantel-Haenszel chi-square analysis. Trend chi-square analysis was performed for assessment of the association between the number of interventions before response and the proportion of recipients who were unable to contribute. Epi Info v6.04d was used to conduct these statistical analyses. Where the information provided by respondents was continuous, the difference in the stage of response was assessed using Mann-Whitney test (U)(two-sample rank test) for two populations (Minitab v12.21). The critical probability for all analyses was taken as 0.05. Grid references for horse locations were obtained from address/postcode information using QuickAddress Batch software and plotted using R v1.9.1 (R Development Core Team, 2004).

True prevalence was estimated using the formula:

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1}$$

TP = true prevalence, AP = Apparent prevalence, Sp = Specificity and Se = Sensitivity (Rogan and Gladen, 1978). The variance of TP (var(TP)), and hence confidence interval, was estimated using the formula below in which n = total sample size and J = Youden index:

$$\mathrm{var}(TP) = \frac{AP(1 - AP)}{nJ^2}$$

Section 5.3. Results

5.3.1. Description of study population

A total of fourteen practices agreed to participate in the survey and supplied their client lists. A total of 94 practices were eventually invited to participate with 22 expressing an interest to help, 25 declined to participate and 47 did not reply. After one month of the invitation process the recruitment target for the number of practices was revised to 16, with 2 practices per region. The regional distribution of the practices and the number of horse clients included in the final study sample database is summarised in table 5.1. The entire client list for two of the practices was used due to their small size. It was not possible to validate a limited number of selected addresses and, consequently, just fewer than 110 clients were surveyed in some practices. A total of 1431 horse owners were sent questionnaires, as part of the first mailing, over the survey period that extended from 7th October 2003 to 16th April 2004 (table 5.2). Between 30th October and 17th November 2003 a four-day postal strike (and the back log it created) disrupted the distribution of questionnaires and their return; some mailings were postponed as a consequence. This affected practices 3 to 7, 9 to 11 and 14.

Table 5.1. Summary of numbers, and proportions, of horse owners mailed questionnaires by region and practice.

Region	Number of horse owners	Proportion of horse owners (%)	Practice	Number of horse owners	Proportion of horse owners (%)
Scotland	220	15.4	1	110	7.7
			2	110	7.7
North-West	220	15.4	3	110	7.7
			4	110	7.7
North-East	174	12.2	5	105	7.3
			6	69	4.8
Wales	213	14.9	7	103	7.2
			8	110	7.7
Midlands	114	8.0	9	114	8.0
Eastern England	164	11.5	10	54	3.8
			11	111	7.8
South-West	110	7.7	12	110	7.7
South and South-East	216	15.1	13	110	7.7
			14	106	7.4
Total	**1431**	100		**1431**	**100**

Table 5.2. Practice mailing dates and deadlines for each stage of the horse owner survey.
Including days from first mailing.

	First mailing		**Deadline**		**Postcard**		**Second mailing**	
Practice	Date	Day	Date	Day	Date	Day	Date	Day
1	13/01/04	0	30/01/04	17	02/02/04	20	16/02/04	34
2	16/02/04	0	27/02/04	11	01/03/04	14	15/03/04	28
3	30/10/03	0	13/11/03	14	17/11/03	18	02/12/03	33
4	16/10/03	0	31/10/03	15	31/10/03	15	17/11/03	32
5	07/10/03	0	21/10/03	14	21/10/03	14	07/11/03	31
6	30/10/03	0	13/11/03	14	17/11/03	18	02/12/03	33
7	23/10/03	0	07/11/03	15	07/11/03	15	24/11/03	32
8	16/02/04	0	27/02/04	11	01/03/04	14	15/03/04	28
9	16/10/03	0	31/10/03	15	31/10/03	15	17/11/03	32
10	07/10/03	0	21/10/03	14	21/10/03	14	07/11/03	31
11	06/11/03	0	21/11/03	15	24/11/03	18	08/12/03	32
12	14/01/04	0	30/01/04	16	02/02/04	19	16/02/04	33
13	07/10/03	0	21/10/03	14	21/10/03	14	07/11/03	31
14	13/10/03	0	24/10/03	11	24/10/03	11	12/11/03	30
Median				14		15		32

5.3.2. Description of response rate

The overall response rate was 68.2% and is summarised in table 5.3 and figure 5.1. Prior to the mailing of the reminder postcard (on or just after the return deadline) 38.4% (550/1431) of the recipients had returned their questionnaire (this included addressee unknown and no longer ownership of horses). By the second mailing of the questionnaire, a further 13.8% (198/1431) of the horse owners had responded. After the second mailing of the questionnaire and by the conclusion of the study period, 31.8% (455/1432) of the recipients had not responded.

Table 5.3. Response rate to each stage of the horse owner survey.

	Cumulative frequency	Percentage (%)	95% confidence interval (%)
Response by first mailing deadline	532	37.2	34.7 to 39.7
Response prior to postcard	550	38.4	35.9.4 to 41.0
Response prior to second mailing	748	52.3	49.7 to 54.9
Total response	976	68.2	65.8 to 70.6

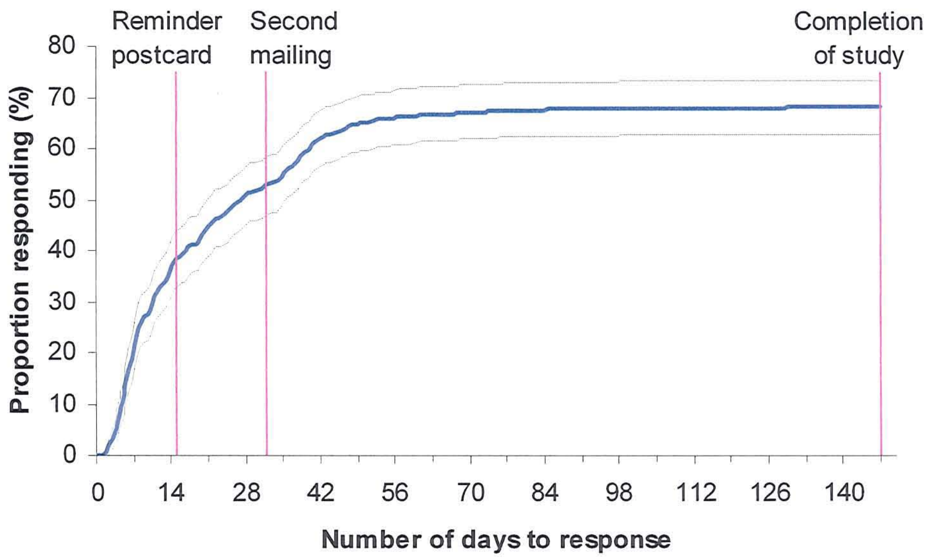

Figure 5.1. The cumulative return rate over time for the horse owner survey. The pink lines represent the reminder postcard (median day = 15), second mailing (median day= 32) and completion of study (day = 147). Black lines represent 95% confidence interval.

The proportion of completed questionnaires (useable) returned from the 1431 horse owners surveyed was 61.0% (table 5.4; figure 5.2). Eleven horse owners contacted the study on receiving a reminder postcard to request a questionnaire, as they had not received the first mailing. A total of 103 individuals who were sent a questionnaire either no longer owned a horse or had moved away. These can be considered to not be valid members of the sample population resulting in a corrected total mailing of 1328 and a corrected response proportion for completed questionnaires of 65.7% (95% confidence interval (95%CI) = 63.2 to 68.3%). The geographic distribution of horses for which completed questionnaires were returned is provided in figure 5.3. The response patterns for the fourteen individual practices are provided in appendices A5.8 and A5.9.

Table 5.4. Mailing numbers, return categories and observed return, response and completion proportions of the horse owner survey.
95%CI = 95% confidence interval

Return Category	Returned (of 1431)	
	Number	Percentage (%) (95%CI)
First Mailing	782	54.6 (52.1.4 to 57.2)
Second Mailing	194	13.6 (11.8 to 15.3)
No longer own a horse	61	4.3 (3.2 to 5.3)
Addressee has gone away	42	2.9 (2.1 to 3.8)
Returned proportion (also response proportion)	976	68.2 (65.8 to 70.6)
No return by end of study period	455	31.8 (29.4 to 34.2)
Completion proportion (usable surveys)	873	61.0 (58.5 to 63.5)

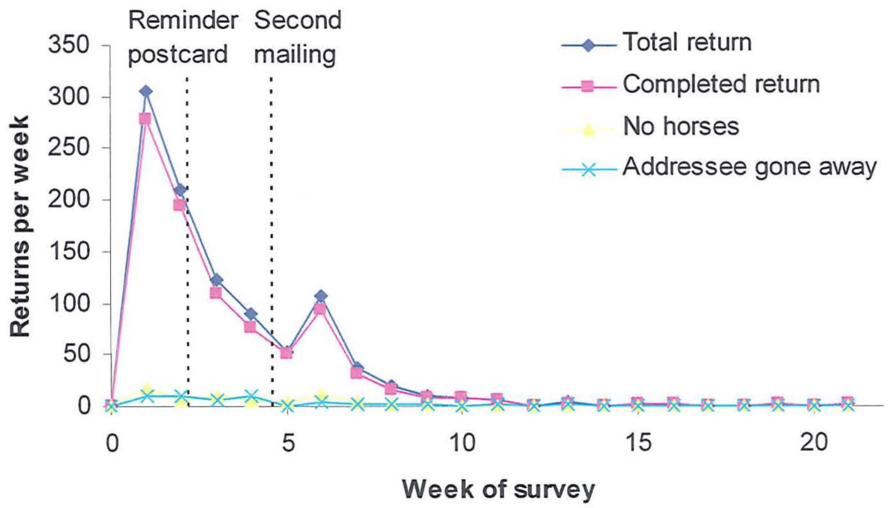

Figure 5.2. Overall return frequencies over time for the horse owner survey.
Dashed lines indicate follow-up mailings. Total return: total number of returns regardless of reason; Completed return = useable questionnaires, No horses = no longer own a horse.

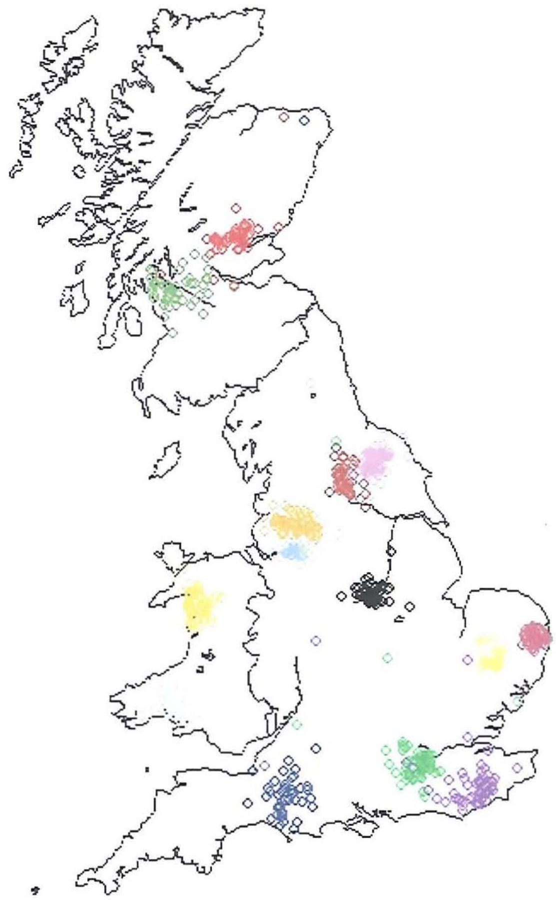

Figure 5.3. Geographic distribution of horses in Great Britain for which completed questionnaires were returned.
One diamond = one horse, n = 850 (23 locations not provided). Grid references derived from owners' responses as to the location of the premises on which their horse was kept.

Overall, the median time to respond was 13 days (range 2 to 147) and more than 90% of responses had been received by the end of the sixth week of the mailing periods. The first mailing elicited 75/782 (9.6%) no longer own horses/addressee gone away compared to 28/194 (14.4%) in the second mailing. As the number of interventions required to obtain a response increased, there was a trend for the proportion of respondents unable to contribute also to increase (χ^2 for linear trend = 7.0, p = 0.008)(table 5.5).

Table 5.5. Table comparing the number of questionnaires returned completed per intervention to the number of responders that were unable to contribute – either no longer owner of a horse or addressee had gone away.

	Completed questionnaires	Unable to contribute	Proportion declined (%)	Odds ratio
First mailing	474	42	8.2	1
Reminder postcard	233	33	12.4	1.6
Second mailing	166	28	14.4	1.9

5.3.3. Responder provided factors associated with time to respond

The following information, supplied by the 873 horse owners that returned a completed questionnaires, was assessed regarding the proportion that had been returned early (before the second questionnaire) and the proportion that had been returned late (after second questionnaire): age of horse, length of ownership, gender of horse, breed of horse, use of horse, height of horse, time spent per week with horse by owner, amount of exercise per week, number of horses owned, a positive result as determined by the RSQ for RAO, a cough in the last 12 months and a diagnosis of RAO by a veterinary surgeon. There was a difference between early and late response by horse owners for only two of these criteria, namely gender and use of horse (figure 5.4). Owners of male horses were 1.45 (95%CI = 1.04 to 2.05) times more likely to respond late than owners of mares ($\chi^2_1 = 5.04$, $p = 0.025$). Owners of horses used for performance activities were 1.78 (95%CI = 1.14 to 2.19) times more likely to respond late than owners of horses put to other uses ($\chi^2_1 = 7.62$, $p = 0.006$).

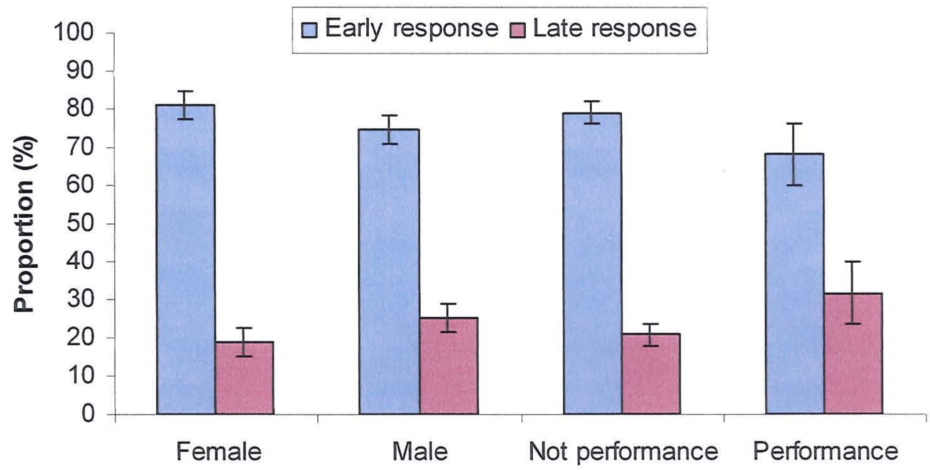

Figure 5.4. Proportion of early and late responses by horse owners according to the gender and use of the horse that was the subject of the returned questionnaire. Error bars = 95% confidence intervals.

5.3.4. Cost of survey and item omission

The cost of the survey per completed questionnaire returned was £2.40 for all consumables and postage (appendix A5.10). Many of the questions contained in the questionnaire did not require horse owners to provide a response unless applicable to them. Therefore, assessment of item omission was limited. Most of the questions on page two and the last few questions on pages seven and eight were applicable to all respondents and the omission rate for these questions are provided in table 5.6. Omissions were relatively rare overall; the most frequently omitted questions concerned number of hours exercised per week (43.9% and 15.9% of these omissions related to retired horses and horses used for breeding respectively), vaccination status and size of premises. The RSQ component (pages 4 and 5) within the questionnaire was sufficiently completed to permit calculation of a RSQ score for all horses.

Table 5.6. Table of item omissions from the horse owner questionnaire.

Question subject	Percentage omission rate (n = 873)
Page 2 (about horse)	
Age	0.5
Length of ownership	1.5
Breed	2.5
Gender	0.2
Height (> or < 15 hands)	1.3
Use (performance, pleasure etc)	0.7
Exercise per week	9.4
Vaccination status	9.2
Deworming	1.3
Time spent with horse	0.0
Carer of horse	0.3
Allergic disease	3.4
Number of horses owned	0.6
Page 7 and 8	
Type of premises	2.1
Size of premises	7.7
Number of other horses on premises	2.5
Land around the premises	0.8
Local area	0.8
Time on premises	0.5
Address of premises	2.3

5.3.5. Results of survey

5.3.5.1. Horse description

The average age of the sample population was 12 years and 5 months (standard deviation (s.d.) = 6 years 9 months). Mares comprised 43.5% (95%CI = 40.2 to 46.8%), geldings 53.8% (95%CI = 50.5 to 57.1%) and stallions 2.4% (95%CI = 1.3 to 3.4%) of the horses for which questionnaires were returned (figure 5.5). The median length of ownership was 4 years and 11 months (inter-quartile range (IQR) = 2 years to 9 years 6 months). The majority (61.1%; 95%CI = 58.4 to 64.9%) of the horses were over 15 hands in height. Thoroughbred or thoroughbred-cross animals were most numerous, and constituted 36.2% (95%CI = 33.0 to 39.4%) of the study population. Some of the other breeds in the population included Welsh or Welsh-cross animals (15.2%; 95%CI = 12.9 to 17.6%), Arab or Arab-cross (7.3%; 95%CI = 5.6 to 9.1%) and Irish draught or Irish-cross (6.2%; 95%CI = 4.4 to 7.8%). The full composition of the population is summarised in table 5.7 and appendix A5.11.

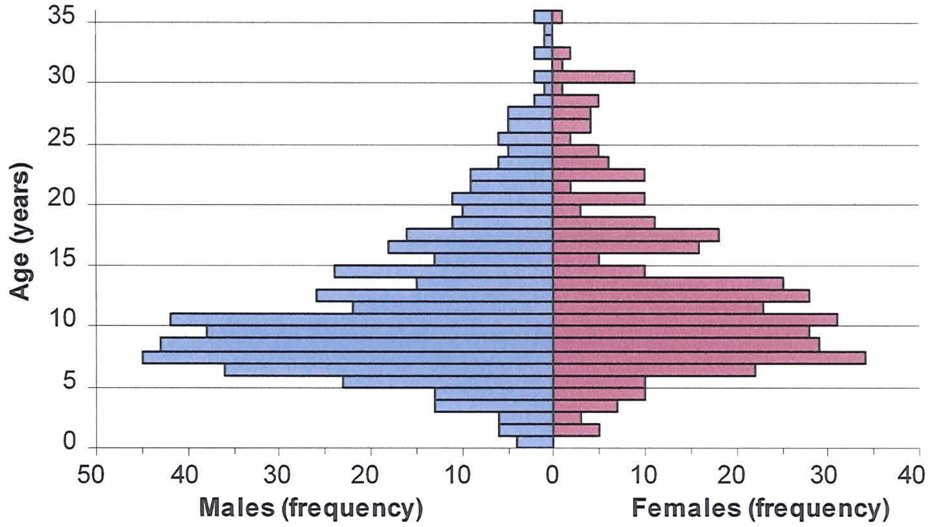

Figure 5.5. Population pyramid for the sample horse population in the horse owner survey.

The greatest proportion of respondents owned just one horse (mode = 1; median = 2; IQR = 1 to 4)(figure 5.6). The activity for which owners used their horse is summarised in table 5.7, and the median time these horses were exercised per week was 6 hours (IQR = 3 to 8 hours). The majority of horse owners looked after their own horse (table 5.7) with all owners spending a median of 14 hours per week with their horse (IQR = 12 hours to 14 hour 45 minutes). Just over 90% of the horses were reported by their owners to be vaccinated against at least one of the following diseases: influenza, tetanus and equine herpes; most were dewormed regularly (table 5.7). Owners reported the prevalence of sweet itch, in this sample of horses, to be 7.3% (table 5.7). The prevalence for horses less than 15 hands in height was 11.1% (95%CI = 7.7 to 14.4%) and these were 2.2 (95%CI = 1.3 to 3.8) times more likely to have the disease than larger horses ($\chi^2_1 = 9.6$, p = 0.002).

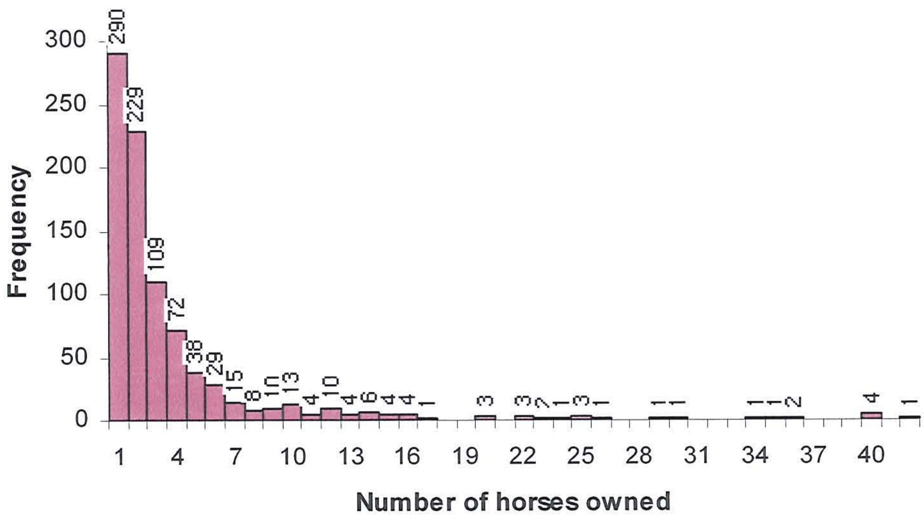

Figure 5.6. Bar chart of the total number of horses owned by each respondent

Table 5.7. Summary of descriptive details provided by responding horse owners regarding their horse.

	Proportion (%)	**95% confidence interval (%)**
Breeds		
Thoroughbred	11.5	9.3 to 13.6
Part Thoroughbred	24.7	21.9 to 27.6
Native	37.8	34.6 to 41.0
Arab	8.1	6.3 to 9.9
Warmblood	5.8	4.3 to 7.3
Unknown	3.6	2.3 to 4.8
Foreign	8.5	6.6 to 10.3
Use		
Performance	11.7	9.6 to 13.8
Performance and pleasure	2.4	1.4 to 3.4
Pleasure	56.7	53.4 to 60.0
Pleasure and show	7.1	5.4 to 8.8
Pleasure and stud	1.0	0.4 to 1.7
Show	3.1	1.9 to 4.2
Show and stud	1.3	0.5 to 2.0
Stud	3.2	2.0 to 4.4
Retired	12.8	10.6 to 15.0
Not reported	0.7	0.1 to 1.2
Vaccination		
Not vaccinated/reported	9.2	7.2 to 11.1
Influenza and tetanus	55.8	52.5 to 59.1
Influenza, tetanus and herpes	19.5	16.8 to 22.1
Tetanus	11.2	9.1 to 13.3
Influenza	3.2	2.0 to 4.4
Tetanus and herpes	0.8	0.2 to 1.4
Influenza and herpes	0.2	-
Herpes	0.1	-
Deworming		
Not reported	1.3	0.5 to 2.0
Never	0.2	-
Annually	1.7	0.9 to 2.6
6 monthly	18.9	16.3 to 21.5
3 monthly	50.6	47.3 to 53.9
2 monthly	21.8	19.0 to 24.5
More often than every 2 months	3.7	2.4 to 4.9
Deworm depending on faecal worm egg counts	1.8	0.9 to 2.7
Carer		
Not reported	0.3	0.0 to 0.7
Livery staff (full time)	9.5	7.6 to 11.5
Livery staff (part time)	6.2	4.6 to 7.8
Private staff (full time)	2.1	1.1 to 3.0
Private staff (part time)	6.2	4.6 to 7.8
Horse owner (full time)	75.7	72.9 to 78.6
Allergy		
Not reported	3.4	2.2 to 4.6
Sweet itch	7.3	5.6 to 9.1
Food allergy	1.7	0.9 to 2.6
Both allergies	0.2	-
Neither allergy	87.4	85.2 to 89.6

5.3.5.2. Early life of horse

Three hundred and eighty horse owners were able to provide information on their horse's early life, as they had owned it since before it was five years of age. Owners had bred 27.9% (95%CI = 23.4 to 32.4%) of these horses and the median age at which the remainder were acquired was 3 years (IQR = 1 to 4 years). According to respondents, 58.0% (95%CI = 53.1 to 63.0%) of these horses had commenced vaccinations for protection against tetanus before the age of two years; the proportion for influenza vaccination by this age was 49.2% (95%CI = 44.3 to 54.4%) (figure 5.7). Most horses in this early life group, 67.5% (95%CI = 62.8 to 72.3%), had had some exposure to straw bedding before the age of 5 years (figure 5.8) and 80.0% (95%CI = 76.2 to 84.2%) had been exposed by their owners to hay in some form or other (table 5.8).

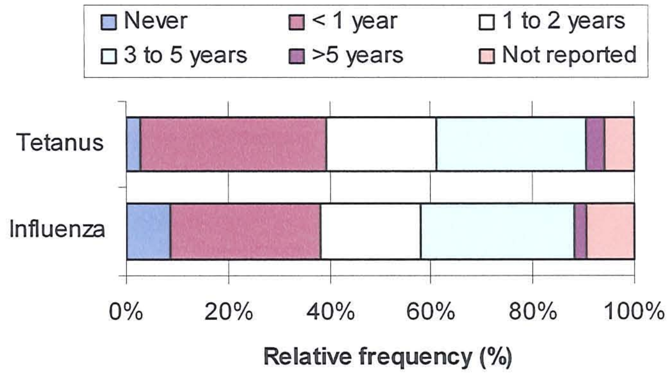

Figure 5.7. Age at which owners reported that horses (that had been in their care since before the age of five years) commenced vaccinations for protection against influenza and tetanus (n = 380).

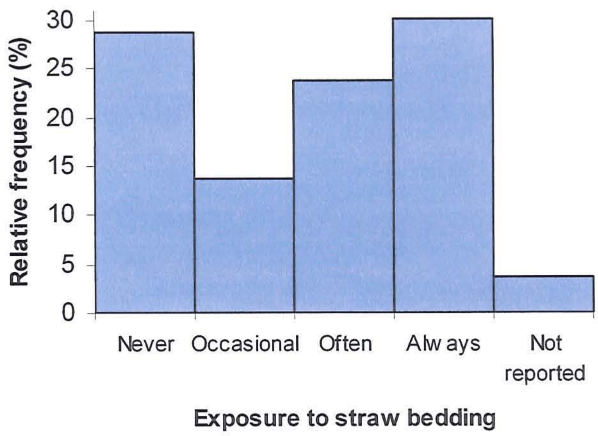

Figure 5.8. Bar chart of how frequently horses under the age of five were exposed to straw bedding (n = 380).

Table 5.8. Proportions of animals under the age of five that were exposed to different types of forage (n = 380).

Type of forage	Proportion (%)	95% confidence interval (%)
Dry hay	52.2	47.2 to 57.3
Wet hay	8.4	5.6 to 11.2
Haylage	16.1	12.4 to 19.8
Wet and dry hay	5.0	2.8 to 7.2
Hay and haylage	14.8	11.2 to 18.3
Not reported	3.7	1.8 to 5.6

Owners reported that 14.5% (95%CI = 10.9 to 18.0%) of the horses had suffered a respiratory infection that had lasted for more than a few days before the age of five years (figure 5.9). A slightly smaller proportion, 10.3% (95%CI = 7.2 to 13.3%), reported that their horse had required treatment by a veterinary surgeon. In 64.1% of these cases, the veterinary surgeon had diagnosed bacterial involvement, with *Streptococcus equi* being most frequently implicated (table 5.9).

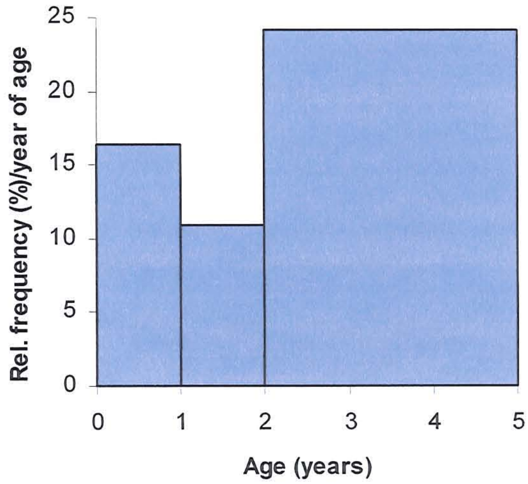

Figure 5.9. Relative frequency density histogram of the age distribution of horses that were reported, by their owners, to suffer from a respiratory infection before the age of five years (n = 55).

Table 5.9. Diagnoses made by veterinary surgeons for horses that had required veterinary treatment before the age of five years for a respiratory infection (n = 39).

Type of infection	Number of horses	Proportion (%)
Viral	14	35.9
Viral and bacterial	6	15.4
Strangles	14	35.9
Other bacterial	5	12.8

5.3.5.3. Respiratory health and risk screening questionnaire

A cough, either in the last 12 months or at any time in the past, was the most commonly reported clinical sign exhibited by an owner's horse (figure 5.10). According to the horse owners, 8.4% (95%CI = 6.4 to 10.2%) of horses had been diagnosed, by a veterinary surgeon, as having RAO. The median age at which this diagnosis was made was 10 years 9 months (IQR = 7 years 4 months, to 16 years). Only eleven horse owners reported that a relative of their horse had RAO, the remainder either did not know (50.4%; 95%CI = 47.1 to 53.7) or indicated that no relatives suffered from the disease (40.9%; 95%CI = 37.6 to 44.2%). Summer pasture associated obstructive pulmonary disease was reported to have been diagnosed, by a vet, in only 1.6% (95%CI = 0.8 to 2.4%) of horses. These horses had been diagnosed at the median age of 12 years (IQR = 9 years 6 months to 15 years 2 months).

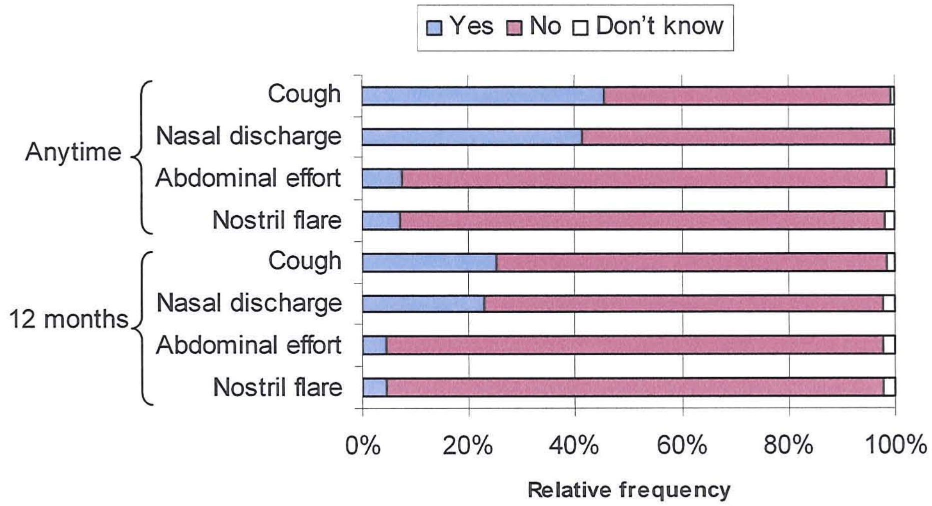

Figure 5.10. Owner-reported respiratory clinical signs exhibited by their horse either anytime in past or in the last 12 months.

The apparent prevalence of RAO using the RSQ was 24.2% (95%CI = 21.3 to 27.0%). The sensitivity and specificity of the RSQ, using a positive cut-off score of 0.87, were 83.3% and 85.5 % respectively (chapter 4). Therefore, the estimated true prevalence of RAO in this population, according to the RSQ, was 14.0% (95%CI = 10.7 to 17.4%). The overall percent agreement between the RSQ and owner-reported veterinary diagnosis of RAO was 80.5% (table 5.10). Nine of the owner reported RAO horses not identified by the RSQ had been diagnosed by a vet as having the disease over 5 years previously. Horses with a positive RSQ for RAO were 1.95 (95%CI = 1.27 to 3.01) times more likely to have exhibited respiratory clinical signs in autumn and winter months ($\chi^2_1 = 10.2$, $p = 0.001$) (figure 5.11).

Table 5.10. Two-by-two table summarising the agreement between owner reporting of a veterinary diagnosis of RAO for their horse and the RSQ classification.

		RSQ RAO		Total
		Yes	No	
Owner reported RAO	Yes	57	16	73
	No	154	646	800
Total		211	662	873

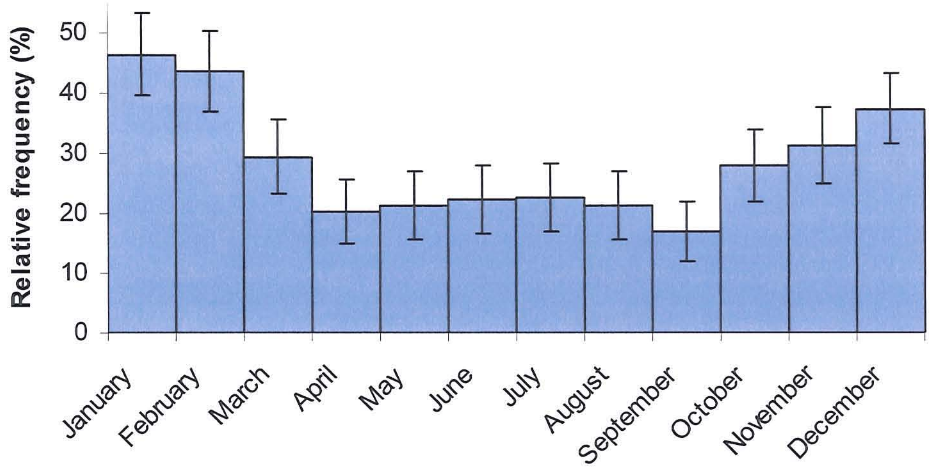

Figure 5.11. Months in which respiratory clinical signs were reported to occur in horses identified as RSQ positive for RAO (n = 211).
Error bars = 95% confidence intervals.

5.3.5.4. Management of horse

Most owners reported that their horses spent at least some time at pasture (figure 5.12) spending a mean of 17.5 ± 7.8 hours turned out in the summer and 8.9 ± 7.0 hours in the winter. During the summer, 48.1% (95%CI = 44.8 to 51.4%) of horses spent 24 hours at pasture and 74.3% (95%CI = 71.4 to 77.2%) were stabled for part of the day in the winter. Of horses that spent any time stabled, approximately equal proportions were exposed to straw bedding (44.3%; 95%CI = 41.0 to 47.6%) or shavings bedding (43.6%; 95%CI = 40.4 to 46.9%), whether on its own or in combination with other types of bedding (table 5.11). A deep litter system was operated by 27.6% (24.6 to 30.6%) of owners for their horses. Only 13.4% (95%CI = 11.5 to 16.0%) of horses were in their stables when mucked out, whilst 33.7% (95%CI = 30.5 to 36.8%) were groomed indoors.

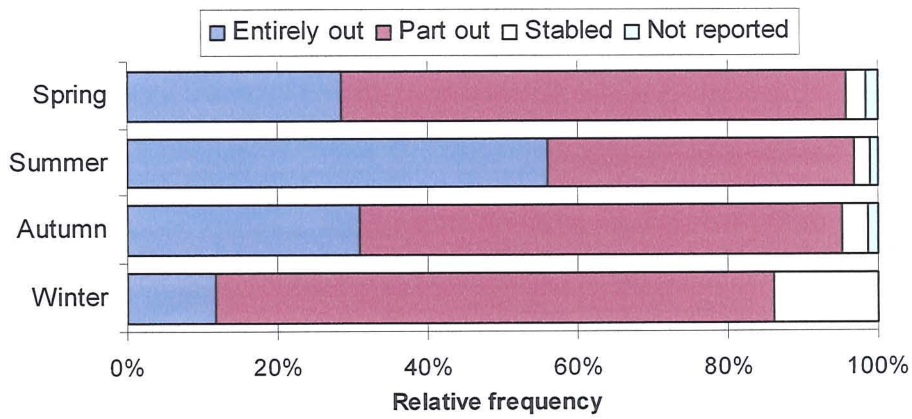

Figure 5.12. Owner-reported seasonal exposure of horses to pasture (n = 873).

Table 5.11. Table of the different types of bedding and combinations of bedding that horses were exposed to, and the frequency of their use, as reported by owners. Figures in brackets = proportion of 873 horses (%). *13 horses were kept on straw, shavings and rubber, 2 on straw, hemp and rubber and 1 on shavings, paper and hemp.

	Never	Straw	Shavings	Fibre pulp	Paper	Hemp	Rubber	Other combinations	Not reported
Never	31 (3.6)								
Straw		298 (34.1)							
Shavings		41 (4.7)	272 (31.2)						
Fibre pulp			2 (0.2)	4 (0.5)					
Paper		3 (0.3)	1 (0.1)		5 (0.6)				
Hemp			3 (0.3)			11 (1.3)			
Rubber		30 (3.4)	103 (11.8)	7 (0.8)	3 (0.3)	2 (0.2)	10 (1.1)		
Other combinations								10 (1.1)	
Not reported									21 (2.4)
Totals	31	372 (+15)*	381 (+1)*	11	8	13	10	10	21

The majority of horses did have exposure to grass (i.e. grazing) during the year and hay was the most commonly fed forage with, 69.3% (95%CI = 66.2 to 72.4%) of horses being exposed at some point during the year. Most frequently, hay was unsoaked (figure 5.13). A smaller proportion of horses were exposed to haylage (44.7%; 95%CI = 41.5 to 48.1%). Of the 246 horse owners that fed wet hay at some point in the year, 26.8% (95%CI = 21.9 to 33.3%) declared that they did not fully immerse hay when soaking it, with the remainder soaking it for a median of 30 minutes (IQR = 10 to 60 minutes). Damping down of bucket feeds was a relatively common practice, with 61.5% (95%CI = 58.3 to 64.7%) of respondents doing so.

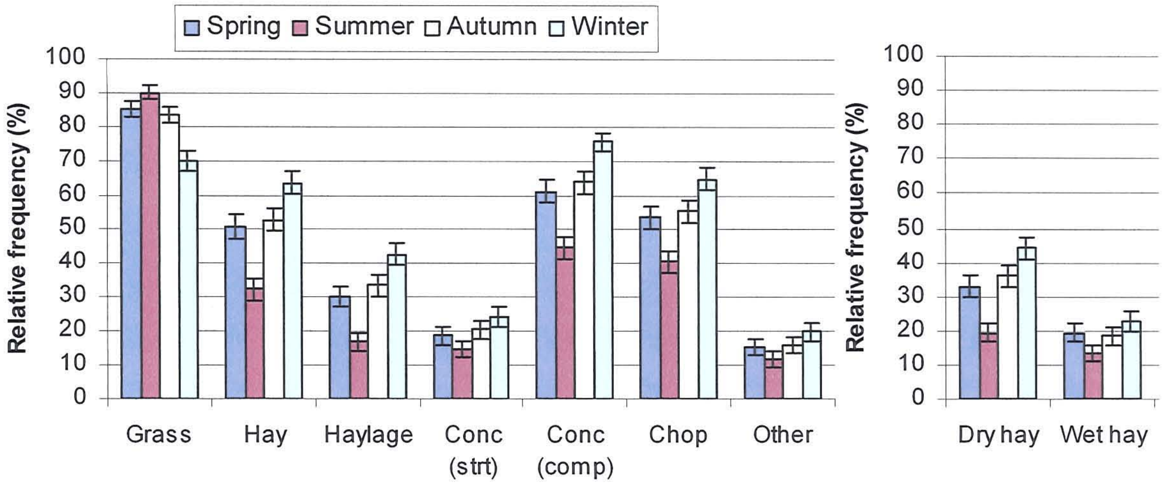

Figure 5.13. Seasonal feeding of horses (n = 873). The chart on the right represents the seasonal feeding of hay broken down into either dry or wet (n = 873).
Conc (strt) = Straight concentrates, Conc (comp) = compound concentrates. Error bars indicate 95% confidence limits.

5.3.5.5. Stabling of horse

Most of the horses (67.2%; 95%CI = 64.1 to 70.4%) for which owners completed a questionnaire were housed in a loose box/stable type building (table 5.12); the owner-reported ventilation for these stables was very variable with many stables (48.2%; 95%CI = 44.2 to 52.3%) having no form of roof ventilation (figure 5.14). Whatever the roof ventilation provided the most common ventilation configuration included just one wall ventilation point (or window) in addition to the open top door of the stable (37.3%; 95%CI = 33.4 to 41.2%).

Table 5.12. Type of housing provided by owners for their horse.

Type of housing	Proportion (%)	95% Confidence interval (%)
Stable (loose box)	67.2	64.1 to 70.4
American barn	21.2	18.5 to 23.9
Field shelter	5.6	4.1 to 7.1
Not stabled	5.2	3.7 to 6.6
Not reported	0.8	0.2 to 1.4

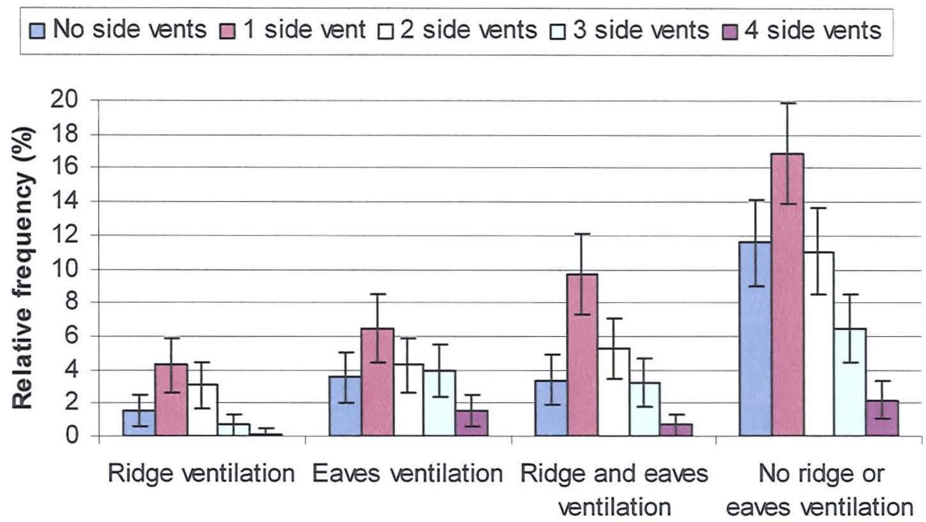

Figure 5.14. Reported provision of ventilation in stables/loose boxes (n = 587).
Side vent = the number of windows or ventilation points in the walls of the stable in addition to the open top door.

Respondents indicated that 15.7% (95%CI = 13.3 to 18.1%) of horses were stabled within 6 metres of a muckheap and 23.4% (95%CI = 20.6 to 26.2%) were housed in a common air space with a hay or straw store. A total of 738 of the horses were stabled with other horses in adjacent stables. If a horse had neighbouring stables, the adjacent horses were most likely to have straw bedding (including straw and shavings) and to be fed dry hay (table 5.13).

Table 5.13. Bedding type and forage type of horses in neighbouring stables to that of the subject horses (n = 873).

Neighbours management	Proportion (%)	95% confidence interval (%)
No neighbour	15.8	13.4 to 18.2
Bedding		
Straw	29.6	26.5 to 32.6
Straw and shavings	15.7	13.3 to 18.1
Shavings	33.8	30.7 to 36.9
Rubber	1.5	0.7 to 2.3
Hemp	1.3	0.5 to 2.0
Fibre pulp	0.7	0.1 to 1.2
Paper	1.7	0.9 to 2.6
Forage		
Dry hay	44.0	40.7 to 47.3
Wet hay	15.0	12.6 to 17.4
Haylage	25.2	22.3 to 28.1

5.3.5.6. Premises horse kept on

The median size of the premises on which horse owners kept their horse was 20 acres (IQR = 7 to 55.8 acres) and a median of 5 (IQR = 2 to 17) other horses were kept there (in addition to the subject horse). Most commonly, horses were kept on the respondent's own premises (table 5.14). In most instances, horses had only been kept on premises for a relatively short period of time; the median was 3 years (IQR = 1 year 2 months to 6 years 10 months). The surrounding environment of the premises, as reported by owners, is summarised in figure 5.15.

Table 5.14. Types of premises where owners reported they kept their horses.

Premises	Proportion (%)	95% confidence interval (%)
Stables on own premises	37.2	34.0 to 40.4
Livery	16.2	13.7 to 18.6
Livestock	21.4	18.7 to 24.1
Private yard	15.2	12.9 to 17.6
Riding school	3.3	2.1 to 4.5
Rented pasture	2.5	1.5 to 3.6
Arable farm	2.9	1.8 to 4.0
Racing yard	0.6	0.1 to 1.1
Not reported	0.7	0.1 to 1.2

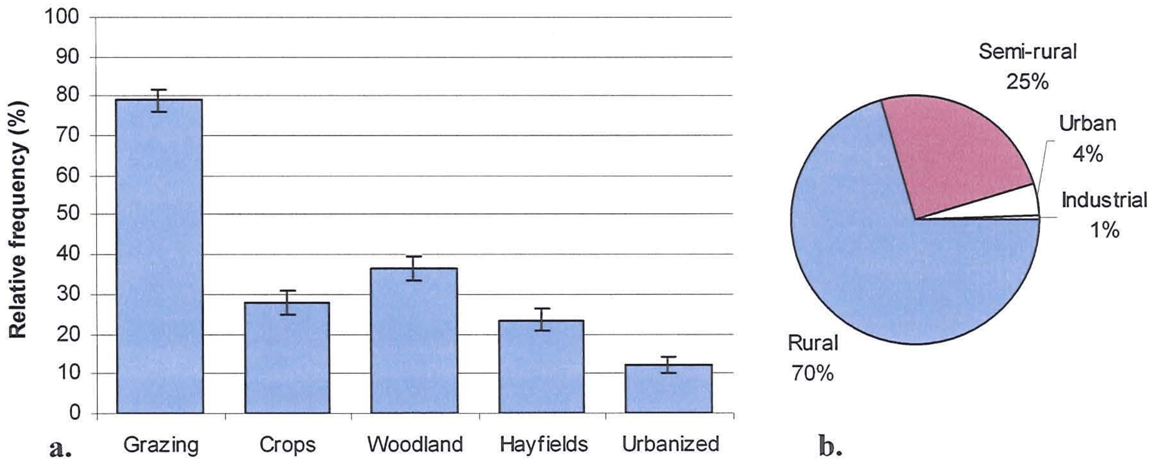

Figure 5.15a. Type of land reported by owners to surround their horse's pasture. Urbanized was described as housing, roads, industry etc. Error bars = 95% confidence intervals.

Figure 5.15b. Type of environment surrounding the premises on which responding owners kept their horses.

Section 5.4. Discussion

The main aim of this study was to investigate the prevalence of RAO in the horse population of Great Britain using a mailed questionnaire survey of owners. No database representative of all horse owners was available at the time of this study. Perhaps this obstacle will be overcome in the future as a result of the legal requirement for all horse owners to register their horses with an appropriate body in order to obtain a passport (Great Britain. Parliament, 2004). The target population for this study was the horses owners, and by proxy the horses, of Great Britain (England, Scotland and Wales). In the absence of a complete database, this population could be approached via a number of routes including veterinary surgeons, farriers, horse breed societies and horse activity societies. Mellor *et al.* (1999) utilised sentinel veterinary practices to enable him to contact horse owners in order to investigate the demographics and health of the horse population of Scotland and Northern England (Mellor *et al.*, 1999). The aforementioned author did suggest the use of farriers (and their clients) but later cast some doubt on their usefulness (Mellor *et al.*, 2001). Breed and activity based societies do hold the names and addresses of many horse owners but, in 2001, it was estimated that up to 70% of the horse population was not registered with any organisation (Leckie, 2001). These societies can be used for investigating problems specific to a breed (Brostrom *et al.*, 1987, Halldorsdottir and Larsen, 1991, Littlewood, 1998). The British Equestrian Trades Association (BETA) has conducted surveys of the general population followed by more focused questionnaires of horse owning individuals and businesses (Anon, 1999). Surveys investigating the deworming practices of horse owners have tended to focus on equine establishments but usually do not clearly describe how these addresses were obtained (Lloyd *et al.*, 2000, O'Meara and Mulcahy, 2002).

Questionnaires have been widely used to consult animal owners/managers (Vaillancourt *et al.*, 1991). Questionnaire surveys investigating specific disease prevalence in the UK have been conducted in other species, for example sheep (Morgan *et al.*, 1990, French *et al.*, 1992). Direct investigations of disease prevalence in horses using this format have been scarce. Several studies, mostly outside of the UK, have investigated the prevalence of sweet itch, usually in specific breeds of horses (Braverman *et al.*, 1983, Brostrom *et al.*, 1987, Halldorsdottir and Larsen, 1991, Littlewood, 1998). The prevalence of behavioural disorders in horses has been studied using questionnaires, surveying registered equine establishments (Bachmann *et al.*, 2003). RAO has been predominately investigated at the experimental level, focusing on disease processes, and clinical reports have almost exclusively concerned case studies of referred cases to clinics (Naylor *et al.*, 1992, Dixon *et al.*, 1995a). Although lower respiratory disease in Thoroughbred racehorses has been extensively investigated using diagnostic tools, such as endoscopy and sampling of airway fluid lining, often utilising case control studies (Burrell *et al.*, 1996, Chapman *et al.*, 2000, Christley *et al.*, 2001, Newton *et al.*, 2003), the general horse population has been largely ignored. One researcher targeted members of a Pony Club regarding respiratory disease using a questionnaire (Wheeler *et al.*, 2002). These riders reported, for instance, that 16.9% of the horses coughed during exercise and that 3.2% had been diagnosed by a vet as having RAO.

Mailed questionnaires have been found to be effective for collecting horse demographic, management and environmental data for a case control study on *Corynebacterium pseudotuberculosis* infection (Doherr *et al.*, 1998). The ability of horse owners to provide repeatable information concerning horse health and management data has been demonstrated, however, this was obtained via telephone interview (Reeves *et al.*, 1996). The Michigan equine monitoring system undertook the arduous task of generating a list-sampling frame for the horse population in the state (Kaneene *et al.*, 1997c). This monitoring and surveillance system operated for two years recording health and management information; the reported incidence of respiratory diseases was 6 per 100 horse years (Kaneene *et al.*, 1997a, Kaneene *et al.*, 1997b). The human population of Michigan State is approximately 10,000,000 compared to the UK population of 60,000,000. To generate a sampling frame using their methods would verge on the impossible; the advent of the horse passports and a National Equine Database may circumnavigate this, if owners comply with the law (Great Britain. Parliment, 2004).

In this study, we opted to follow an approach similar to that pursued by Mellor *et al.* (1999) although a more direct and 'aggressive' mailing procedure was adopted. Mellor *et al.* (1999) conducted a census of all practices in the study area that offered equine services before randomly selecting 25 of the responders as sentinels. The clients of the sentinel practice were mailed a short questionnaire concerning the numbers of horses owned (for example) and were invited to participate in a later, more detailed survey concerning the health and management of all their horses. In the current study, management details were required for each individual horse; they could not be generalized as in Mellor's study. This resulted in a relatively long questionnaire requiring completion for one horse. It was felt that an owner of more than one horse, when confronted by multiple questionnaires, would be less likely to return useable information (if at all).

Not all horses are attended to by a veterinary surgeon and many owners will not be registered with a practice. Therefore, some bias will be introduced by using this approach. It was hoped that this veterinary-served population would reflect the majority of the general population and it provided the most practical solution to the problem. In the circumstances of a non-infectious disease such as RAO, there is no need to worry about sections of the population being unrecognised and acting as a pool of infection. Furthermore, the post-test probability of disease depends on the sensitivity and specificity of the diagnostic test in question and the prevalence of the disease in the population in which the test is used. The limited number of practices that consented to our survey was disappointing. The reluctance to participate may be a reflection of the perceived problems of data protection in relation to client records. By making the survey a collaboration with the practice, it was hoped to avoid this pitfall; this may have an advantage as in the human field a better response has been obtained when general practitioners, rather than a research unit, approach patients (Smith *et al.*, 1985). A similar approach to selecting a study population of dog owners met with better success in recruiting practices with 61% of those contacted agreeing to participate (Edwards *et al.*, 2004). Practices may have been more highly motivated, as the topic of that study was central to their livelihood; the general health of dogs following vaccination was under media scrutiny at the time. The loss of eight practices that initially agreed to help may have been avoided if personal visits had been made to practices, as employed in the dog owner survey. The owner response rate in the study conducted by Edwards *et al.* (2004) was 44% with the use of only a postcard reminder after 3 weeks. In the study performed by Mellor *et al.* (1999) the first client survey achieved a response rate of 40% and the second more detailed survey (of recruited clients) achieved a response of 71%, both with just one mailing and no follow-ups. The response rate obtained in the current study again emphasises the effectiveness of the total design method discussed in chapter 2.1 (Dillman, 1999). This method attempts to minimise non-response bias, using careful design of the questionnaire and timed follow-ups. As already discussed in chapter 2.1, there is great debate and conflicting reports as to the relative importance of non-response.

The opportunity to assess non-response in this survey was limited as no existing information was available concerning the horse owners or their horses. In the survey of companion animal owners, Edwards *et al.* (2004) were able to compare a limited number of responders and non-responders and did not identify any systematic bias. The comparison of early and late responders gave the only opportunity for assessing the potential for non-response bias (Sheikh and Mattingly, 1981). The number of males may be under-represented in this survey, by extrapolation to non-responders, because their owners were more likely to respond late to the survey. This is particularly interesting considering that males predominated in the sample of 873 horses. Performance horses were also underrepresented. Otherwise, there was little difference, suggesting that non-responders' horses might be little different from those of responders. The lack of difference between early and late responders concerning respiratory disease of their horses is encouraging. In questionnaire surveys regarding human health, non-response may be affected by a subject's interest in the matter depending on their own health status (Criqui *et al.*, 1978, Bakke *et al.*, 1990). The number of non-responders will undoubtedly include further numbers of people who had moved away, people who no longer owned horses and mail that had simply been lost; 14.4 million letters are lost by the Royal Mail per year (source: Postwatch, the watchdog for postal services). Certainly, some horse owners did not receive the first mailing but responded to the postcard. There may have been other similar cases where no effort was made to inform this study. The mail strike that affected the majority of practices may have had a part to play in lost mail.

The questionnaire collated information regarding the demographic characteristics and management of horses in this study population. Information of this type regarding the horse population of the UK is limited; a review was published in 1999 that predominately sourced surveys by BETA and the study by Mellor (1997) (Harris, 1999). It is possible to make comparisons between the findings of Mellor *et al.* (1999) and those from this survey. There are many similarities with the aforementioned author's study; the mean age of the population was 11 years, the mean length of ownership was 5.6 years and there were approximately equal proportions of male and females. Thoroughbreds and Thoroughbred crosses constituted 30% of his study population with Welsh breeds and Welsh crosses (12%), Arabs and Arab crosses (6%) and Warmbloods (4%) being representatives of some of the other breeds- a very similar picture to that detailed in the current study. The population pyramid indicates that horses below the age of 5 years were probably under-represented in this study; this is not necessarily a concern because RAO is believed to be a disease of older horses (McPherson *et al.*, 1979b). It was anticipated that most horses registered with a practice would be vaccinated, although the high proportion of owners reporting that their horses were vaccinated for equine herpes virus is puzzling. This vaccination is not widely used in the general horse population, being predominately reserved for brood mares. This highlights that horse owners may not be fully informed or aware of all aspects of their horse's management. Most horses were dewormed four times a year; this is in contrast to many studies where deworming, on average, has been six to seven times a year (Lloyd *et al.*, 2000, Mellor *et al.*, 2001, O'Meara and Mulcahy, 2002). This shift is probably as a result of the introduction of Moxidectin (dosing interval 13 weeks) to the British market in 1999 (all the listed studies were carried out prior to this). The owner-reported prevalence of sweet itch in this study was higher than the 2% reported by Mellor *et al.* (2001). The relatively low proportion of horses having a respiratory infection early in life may well reflect that many owners had not possessed their horse since birth.

The majority of horse owners kept their horses on their own premises, although it was a smaller proportion, 57%, than that identified by Mellor *et al.* (2001). The study area for that survey was less densely populated, where it may be easier to obtain properties with facilities for horses, than some of the regions surveyed in the current study. Regional variation in how horses are kept in the UK certainly warrants further investigation and, to a certain extent, these data are available from the current study. In the study conducted by Mellor *et al.* (2001), 58% of horses were turned out 24 hours per day and 72% were stabled part of the time in winter, similar to the current findings. It is reassuring to note that the majority of horses were turned out 24 hours per day during the summer, hopefully providing ideal conditions for a healthy respiratory tract. However, over winter the vast majority were stabled for at least part of the day, potentially exposing the horse to respiratory challenge, particularly as many were bedded on straw and/or feed dry or soaked hay and often in a stable with limited ventilation in addition to the top door. In the survey of Scotland and Northern England, performed in 1996, 87% of horses were fed hay and 18% were fed haylage (Mellor *et al.*, 1999). It would appear likely that since that time the use of haylage as forage has dramatically increased. In recent years, since this survey was performed, there has been a dramatic increase in the use of haylage at the expense of hay (Harris, 1999). The suitability of American barns as accommodation for horses has been questioned because of the difficulty of maintaining adequate ventilation throughout the structure (Slater, 1997). It is therefore concerning that a significant proportion of horses are kept in such buildings. The soaking of hay was carried out poorly by at least a quarter of respondents, thus minimally reducing the challenge to their horse. However, the majority of those that did fully immerse hay did soak it for at least the time period recommended by many sources (Blackman and Moore-Colyer, 1998, Swain, 2004).

Over one fifth of the study population had exhibited coughing and nasal discharge in the last 12 months, suggesting that respiratory disease is a significant health issue for horses in the general population. The owner reported prevalence of RAO was higher than the 4% (3 to 5%) identified by Mellor *et al.* (2001). However, the difference is relatively small, especially when confidence intervals are taken into account. There may have also been an impact of the wording of the questions, and the context in which they were asked, in the two studies. The question concerning RAO in the questionnaire used in this study was at the end of a two-page section devoted to respiratory health.

There are no similar studies of RAO prevalence to which this study can be compared, either in this country or in the rest of the world. The much-quoted incidence of 54% in a random population of Swiss horses identified by clinical investigation is not comparable (Bracher *et al.*, 1991). There are problems of disease definition in this study, many of the horses may have had just had lower respiratory inflammation rather than RAO and the horses were not randomly selected from a general population. In fact, approximately half were horses referred (for non-respiratory problems) to a University clinic and the remainder appear to be a convenience sample of military horses. The variance (and confidence interval) for the true prevalence in the current study was estimated following the method described by Rogan and Gladen (1978). This adjusted for misclassification by including the Youden index. It was not possible in this study to include the additional uncertainty related to the unknown true values of sensitivity and specificity for the RSQ for RAO. This uncertainty is expressed by including sample estimates based on the validation-study sample sizes using a Taylor's series expansion in the estimation of the variance (Rogan and Gladen, 1978). The confidence interval is then estimated using the normal approximation. The normal approximation was not appropriate in this study as true prevalence plus or minus 3 times the square root of the variance of the true prevalence included 0 (Greiner and Gardner, 2000a).

The high prevalence of RAO, as estimated by the RSQ, compared to the owner reported veterinary diagnosed prevalence is potentially as a result of owners' intervention without seeking veterinary advice. For example, owners may respond when their horse has mild respiratory signs in association with, perhaps, dry hay or straw bedding, by changing to a minimal dust environment, without consulting a veterinary surgeon, in effect making a self-diagnosis of RAO. Other owners may be unconcerned by their horse occasionally exhibiting respiratory signs, perhaps accepting that their horse has a 'stable cough', and never consulting a health professional. Not all horses reported by their owners to have been diagnosed as having RAO were identified as positive by the RSQ, although the number was relatively small. This is perhaps not surprising considering that the clinical signs of RAO, and their severity, depend on the horse being exposed to challenge. For example, an owner of a horse that was diagnosed as having RAO in the past, and has kept the horse in minimal challenge conditions since, may have minimal recall of the problems encountered when the diagnosis was first made. There is also no guarantee that the original veterinary diagnosis was correct. Horses with a positive result for the RSQ were more likely to have exhibited respiratory signs in the periods of the year when horses are more frequently housed (autumn and winter) and therefore when respiratory challenge is greatest. A North American study of horses examined for RAO at referral centres identified that this was most likely to occur during winter and spring; the difference may be geographical or because of a lag phase from a problem occurring and referral (Couetil and Ward, 2003).

If the estimated true prevalence of RAO determined by this survey is representative, then this disease is a significant health problem in the general horse population. This is of particular concern considering that simple management steps can minimise the impact of this disease. Only surveying further population samples, combined with the use of alternative screening tools, will corroborate or refute the prevalence determined in this study.

CHAPTER 6

A STUDY OF THE RISK FACTORS FOR A POSITIVE RISK-SCREENING QUESTIONNAIRE FOR RECURRENT AIRWAY OBSTRUCTION IN A SAMPLE OF THE GENERAL HORSE POPULATION IN GREAT BRITAIN

Section 6.1. Introduction and Aims of Study

To date, there have been no published epidemiological studies of a randomly selected horse population (attended by veterinary surgeons) that were designed to determine risk factors for recurrent airway obstruction (RAO) in horses in Great Britain. Although there are many putative risk factors for RAO, many are based on anecdotal experiences, reports of case series (principally of referred populations) and experimental research herds. Therefore, an investigation of the disease and associated factors in a general horse population would be beneficial for guiding future research, perhaps exposing as yet unconsidered risk factors, and highlight factors that may be pertinent to the diagnosis and treatment/management of RAO. The management of individual horses varies enormously and the interaction of stable management and feeding (high or low respiratory challenge) with the duration of exposure (i.e. time stabled) to these factors may have a significant effect on a horse's likelihood of having RAO. For example, a horse kept in a high respiratory challenge environment for only brief periods may be at greater or lesser risk than a horse kept for longer periods in medium respiratory challenge environment.

Multivariable analysis of information of how horses from a general population are kept may provide an insight into these complex scenarios. The construction of two multilevel, multivariable logistic regression models was undertaken to investigate risk factors associated with an outcome of a positive risk-screening questionnaire (RSQ) for RAO. The first related to the general management of a horse and its likelihood of having RAO (host and environmental factors). The second was created in an attempt to identify the effect of factors in early life on the likelihood of a horse having RAO.

Section 6.2. Materials and Methods

6.2.1. Data collection

Information on individual horses regarding their management and respiratory health was available from the survey outlined in chapter five. In brief, a survey of horse owners in Great Britain that used the services of veterinary surgeons was conducted, between 7th October 2003 and 16th April 2004. The study population was selected by two-stage cluster sampling. Following geographic regional stratification, veterinary practices (that provided care for horses) were randomly selected and invited to supply their client lists. Horse owners were then randomly selected from these lists to receive a questionnaire (appendix A5.4). The questionnaire was designed to collect information on a horse in the recipient's possession including: horse descriptive details, early life (before the age of five years), current management, premises and the surrounding area of the premises. The questionnaire also included the RSQ for RAO as detailed in chapter 4. When completed by an owner, regarding their horse, scoring of the responses gave a likelihood of that horse having RAO. Use of a positive cut-off, selected to maximise the sensitivity and specificity of the RSQ, gave a binary variable where a positive result deemed a horse to have RAO and a negative result not to have RAO. The questionnaire was followed by two interventions (postcard reminder and second mailing of questionnaire) to encourage non-responders to return a completed questionnaire.

A total of fourteen practices participated and 1431 horse owners were mailed a questionnaire. A total of 873 completed questionnaires were returned and, after adjusting for people who had moved away and no longer owned a horse, the response rate was 65.7% (95%CI = 63.2 to 68.3%). Considerable information from all horses was available as possible explanatory variables for modelling the outcome of a positive RSQ for RAO (model one: host and environmental factors). These data covered descriptive information for the horse as well as its use, care, access to pasture, feeding regimen, stable environment and premises. Variables were predominately categorical, a limited number were continuous. To investigate risk factors associated with management in early life (prior to the age of five years) on the outcome of a positive RSQ for RAO, data were also available for building a separate model (model two: early life). Responses were only considered for inclusion in this second model if the horse in question was over the age of five years at the time the questionnaire was completed. All variables were categorical; except for the age at which the horse was acquired (if not bred by owner).

6.2.2. Statistical analysis

The outcome was a binary variable designated by the result of the RSQ for RAO, either positive or negative. The fixed effect (explanatory) variables were screened initially using χ^2 test for categorical variables and Mann-Whitney test (U)(two-sample rank test) for continuous variables. The functional forms of the relationship between the binary outcome (RSQ for RAO) and the continuous variables identified as potential risk factors were explored using generalised additive models (GAM) using spline smoothing functions. The functional form of the continuous variable was then used to inform the polynomial fits in the subsequent multivariable logistic regression models. Prior to development of polynomial terms, continuous variables were centred to reduce multicollinearity. Fixed effect independent variables with a P value of < 0.3 were considered for inclusion in multilevel, multivariable logistic regression models that were fitted using a generalised linear mixed model (GLMM) via penalised quasi-likelihood estimates (PQL). Models were built using backward/forwards elimination of non-significant variables. The critical probability of Wald χ^2 was taken to be 0.05 within the models. Veterinary practice was set as a random effect in order to account for potential clustering within practice area. Variables were maintained in the model if their presence significantly improved the fit of the model. The fit was assessed using the Akaike information criterion (AIC). The aim was to achieve the most parsimonious model with the lowest AIC. Variables also remained if removal caused substantial alteration in the coefficients of other variables in the model (by 10% or more). All biologically plausible interactions between variables were tested. Partitioning of variation between the two levels (practice and horse) was assessed using an intercept-only random effects model (GLMM via PQL). The latent-variable approach was utilised that assumes the variance at level one is approximately equal to $\pi^2/3$, where π = 3.1416 (Goldstein *et al.*, 2002). The selected explanatory variables included in the final multivariable models were run separately in the multilevel (GLMM via PQL) model for comparison.

Statistical analyses were performed using the software packages SPSS v11 (data exploration) and R v1.9.1 (R Development Core Team, 2004) (GAM from package mgcv, GLMM via PQL from package lme4).

Section 6.3. Results

6.3.1. Host and environmental factors

6.3.1.1 Descriptive statistics

The median age of the horses that were identified as having RAO, by the RSQ, was 13 years (inter-quartile range (IQR) = 9 yrs 6 mths to 20 yrs 6 mths) compared to 10 years (IQR = 7 yrs to 14 yrs 6 mths) for negative horses, this was significantly different ($U = 46237$, $p < 0.001$) (figure 6.1).

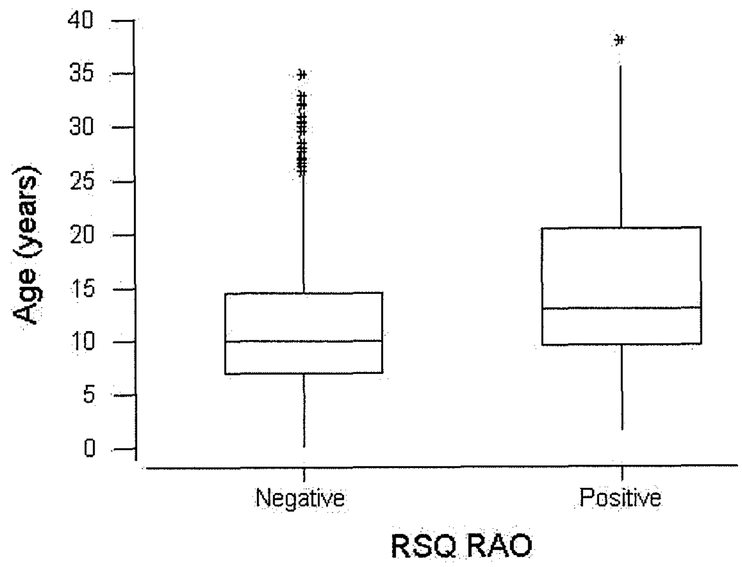

Figure 6.1. Box plots summarising the ages of horses with and without RAO according to the RSQ.
Whiskers represent lower limit (Q1-1.5(Q3-Q1)) and upper limit (Q3+1.5(Q3-Q1), where first quartile =Q1 and third quartile = Q3. Outliers are plotted with asterisks (*).

There was no significant difference between the different breeds of horse as to the proportion with a positive result for the RSQ for RAO (table 6.1). Similarly, there was no difference in proportions according to gender or height of the horse. Horses used for performance activities were less likely to be RSQ positive for RAO; likewise for horses used for studwork (table 6.1). Retired horses were more likely to have a positive result for the RSQ.

Table 6.1. Descriptive statistics and χ^2 test results of selected categorical variables for association with a positive result for the RSQ for RAO (case) in a survey of horses in Great Britain.

	Proportion of controls % (n)	Proportion of cases % (n)	
Breed			$\chi^2_6 = 11$, $p = 0.09$
Thoroughbred	78 (78)	22 (22)	
Part Thoroughbred	71 (154)	29 (62)	
Native	78 (259)	22 (71)	
Arab	65 (46)	35 (25)	
Warmblood	82 (42)	18 (29)	
Unknown	74 (23)	26 (8)	
Foreign	81 (60)	19 (14)	
Gender			$\chi^2_2 = 0.4$, $p = 0.8$
Mare/filly	77 (292)	23 (88)	
Stallion/colt	76 (16)	24 (5)	
Gelding	75 (352)	25 (118)	
Height			$\chi^2_1 = 0.8$, $p = 0.4$
Less than 15 hands	74 (247)	26 (86)	
Greater than 15 hands	77 (413)	23 (125)	
Activity horse used for			
Performance	84 (111)	16 (21)	$\chi^2_1 = 5.8$, $p = 0.02$
Pleasure	75 (451)	25 (153)	$\chi^2_1 = 1.4$, $p = 0.2$
Show	77 (82)	23 (24)	$\chi^2_1 = 0.2$, $p = 0.7$
Stud (breeding)	91 (49)	9 (5)	$\chi^2_1 = 7.0$, $p = 0.08$
Retired	62 (69)	38 (43)	$\chi^2_1 = 14$, $p < 0.001$

6.3.1.2. Multilevel, multivariable model.

The explanatory variables selected by univariable screening ($p < 0.3$) for consideration in creating the multivariable model are listed in table 6.2. In the two-level intercept-only model, there was very little variation at the practice level ($n = 14$) with variance = 0.07 (standard deviation (s.d.) = 0.27). This gave an estimate for the proportion of variation at the practice level = 2.1%. The GAM suggested that the relationship between a positive RSQ for RAO and time spent at pasture in winter was best described by a quadratic relationship (for time at pasture in winter = 0.04; for time at pasture in winter squared = -0.006). This is illustrated in figure 6.2. The relationship between age and a positive RSQ for RAO was non linear as shown in the GAM plot in figure 6.3. This variable was subsequently changed to a categorical variable as in figure 6.4. All remaining continuous variables were considered to have an approximately linear relationship with the log odds of a positive RSQ for RAO.

Table 6.2. Independent or explanatory variables from univariable screening (p < 0.3) considered for inclusion in the multivariable model for the association between host and environmental factors with a positive RSQ for RAO.

Independent variables	Description
Descriptive information	
Age of horse	Months
Duration of ownership	Months
Use of horse	Activity level: performance, pleasure, show, stud, retired
Breed	TB, PTB, native, arab, warmblood, foreign, unknown
Carer	Full livery, half livery, full private, half private, personal
Exercise per week	hours
Number of horses owned	Including questionnaire subject
Management	
Forage	Dry hay, wet hay (including soak time), haylage
Bucket feed	Type of concentrate, chaff/chop, damping down
Time under current management	months
Type of stable	Not stabled, field shelter, loose box (with degree of ventilation), american barn
Location of haystore	Shared airspace with stable – yes, no
Location of muck heap	Less than 6m or greater than 6m from stable
Turn out to pasture	
Seasonal turn out	Per season: entirely out, part out, entirely stabled
Time at pasture in winter	Time per day - hours
Access to grass	Number of seasons
Neighbour's management	
Forage	No neighbour, haylage, wet hay, dry hay
Bedding	No neighbour, hemp, paper, woodpulp, shavings, straw
Premises	
Area around pasture	Grazing, hay fields, crops, woodland, urbanised (housing, roads, industry etc)
Area around premises	Urban, semi-rural, rural, industrial
Time on premises	Months
Number of horses on premises	In addition to questionnaire subject

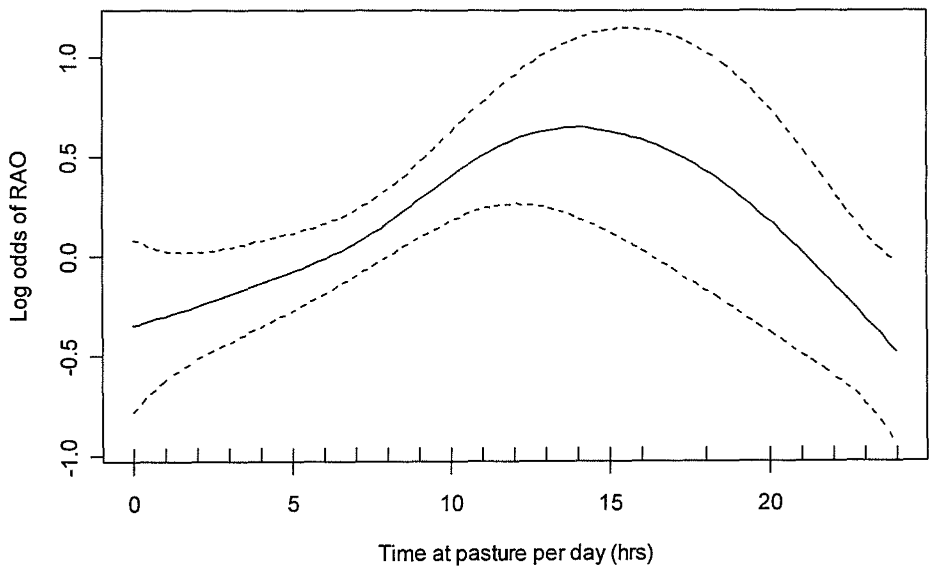

Figure 6.2. Functional form of the relationship between time spent at pasture in winter and the log odds of having a positive RSQ for RAO using a generalised additive model. The plot shows the fitted curve with 95% confidence intervals and a rug plot on the x-axis representing aggregated data points.

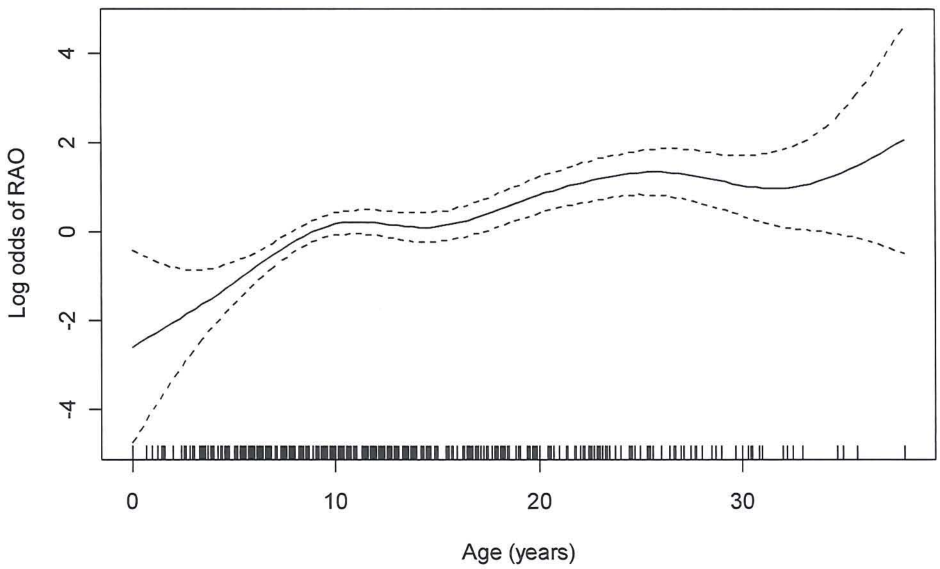

Figure 6.3. Functional form of the relationship between age of horse and the log odds of having a positive RSQ for RAO using a generalised additive model. The plot shows the fitted curve with 95% confidence intervals and a rug plot on the x-axis representing the number of data points.

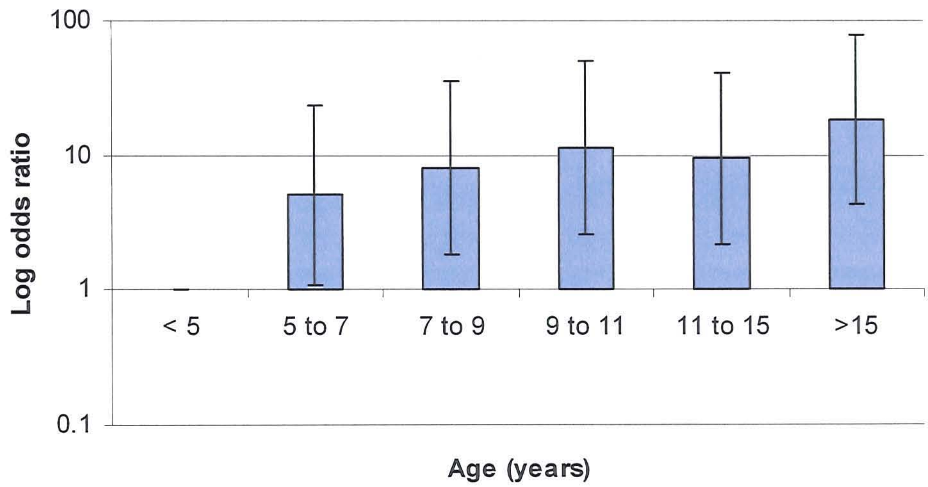

Figure 6.4. Relationship between age of horse (categorised) and the likelihood of having a positive RSQ for RAO.
Error bars = 95% confidence intervals.

The final multilevel, multivariable model of risk factors associated with a positive RSQ for RAO is shown in table 6.3. The descriptive data and the results of multilevel, univariable models (GLMM via PQL) for the explanatory variables are provided in appendix A6.1. The proportion of the total variation occurring at the practice level was 0.29% (variance estimate = 0.01, s.d. = 0.10) after allowing for the fixed effects. Increasing age was associated with an increased risk of having a positive result for RAO. Horses that were used for breeding had a decreased risk, as did horses that had none or only one season of exposure to grass pasture, after allowing for other factors. Initially, increasing duration of turn out to pasture in winter was associated with increased risk but with over 15 hours of turn out the risk began to decrease. The feeding of dry hay for 3 or more seasons was associated with a decreased risk. Conversely, the provision of wet or soaked hay was associated with an increased risk of a positive RSQ for RAO outcome.

Table 6.3. A multilevel, multivariable logistic regression model of host and environmental risk factors associated with a positive RSQ for RAO in a randomly selected population of horses in Great Britain.
Random effect variance estimate = 0.01 (0.1). s.e. = standard error, 95%CI = 95% confidence interval.

	β coefficients	s.e.	P value	Odds ratio (95%CI)	
Age					
Less than five years	Ref.			Ref.	
Five to seven years	1.63	0.78	0.04	**5.08**	(1.10 to 23.43)
Seven to nine years	2.10	0.75	0.005	**8.13**	(1.85 to 35.68)
Nine to eleven years	2.43	0.75	0.001	**11.38**	(2.60 to 49.82)
Eleven to fifteen years	2.25	0.75	0.003	**9.45**	(2.18 to 40.98)
Greater than fifteen years	2.91	0.74	<0.001	**18.30**	(4.31 to 77.66)
Breeding					
Not used for breeding	Ref.			Ref.	
Used for breeding	-0.99	0.50	0.045	**0.37**	(0.14 to 0.98)
Urbanisation					
Rural	Ref.			Ref.	
Semi-urban, urban	0.79	0.25	0.002	**2.20**	(1.34 to 3.61)
Forage					
2 seasons or less exposure to dry hay	Ref.			Ref.	
3 seasons or more exposure to dry hay	-0.52	0.21	0.02	**0.60**	(0.39 to 0.91)
1 season or less exposure to wet hay	Ref.			Ref.	
2 seasons or more exposure to wet hay	0.63	0.22	0.004	**1.88**	(1.22 to 2.90)
Access to grass					
Grass for 2 seasons or more	Ref.			Ref.	
None or only one season	-0.69	0.33	0.04	**0.50**	(0.26 to 0.95)
Time at pasture in winter					
Centred (hours/day)	0.04	0.02	0.03		
Centred squared	-0.01	0.002	0.001		

6.3.2. Early life factors (before the age of 5 years)

6.3.2.1 Descriptive statistics.

A total of 316 horse owners, who owned horses over the age of 5 years, were able to provide information regarding their horse's life prior to five years of age. The median age of the sub-group for which early life information was available was 10 years (IQR = 7 yrs 6 mths to 14 yrs 1 mth). Mares constituted 46.2% (95% confidence interval (95%CI) = 40.7 to 51.7%) of this population, stallions 3.5% (95%CI = 1.5 to 5.5%) and geldings 50.3% (95%CI = 44.8 to 55.8%). The most common breed was native (table 6.4). The apparent prevalence of RAO using the RSQ in this population was 21.9%, after correction for the sensitivity and specificity of the test the true prevalence estimate was 10.6%.

Table 6.4. Breeds of horses included in the data set for investigation of the association between early life factors and a positive RSQ for RAO.

	Proportion (%)	95% confidence interval (%)
Thoroughbred	10.8	7.3 to 14.2
Part Thoroughbred	27.2	22.3 to 32.1
Native	38.0	32.6 to 43.3
Arab	8.9	5.7 to 12.0
Warmblood	5.1	2.6 to 7.5
Unknown	2.2	0.6 to 3.8
Foreign	7.9	4.9 to 10.9

6.3.2.2. Multilevel, multivariable model.

Univariable screening identified eight independent variables with a p value of less than 0.3 for consideration in construction of the multivariable model; these are listed in table 6.5. There was little variation at the practice level with 3.5% of the proportion explained by this level (variance = 0.12, s.d. = 0.34). The continuous variable, the age at which an owner had acquired a horse, had a non-linear relationship with the log odds of a positive RSQ for RAO according to GAM analysis (p = 0.019). This variable was converted into a binary variable; homebred or acquired before 2 years of age and acquired after 2 years of age.

Table 6.5. Independent or explanatory variables from univariable screening ($p < 0.3$) considered for inclusion in the multivariable model for the association between early life factors with a positive RSQ for RAO.

Independent variables	Description
Age horse acquired	Months
Ownership since birth	Owned since birth – yes, no
Early forage	
Exposure to hay	Hay - subdivided into wet hay and dry hay
Exposure to haylage	Yes, no
Early respiratory infection	
Respiratory infection	Owner observed – yes, no (included treated)
Treated respiratory infection	Treatment by a vet – Yes, no - subdivided bacterial, viral

The final model is summarised in table 6.6 and descriptive data and the results of multilevel, univariable models (GLMM via PQL) for the explanatory variables are provided in appendix A6.2. The proportion of total variation occurring at the practice level was 3.7% (variance estimate = 0.13, s.d. = 0.36). Acquisition of a horse after the age of 2 years was associated with an increased risk of a positive RSQ for RAO. There was also an increased risk in association with exposure during early life to hay rather than no forage or haylage and an increased risk with owner-reported occurrence of a respiratory infection before the age of five years.

Table 6.6. A multilevel, multivariable logistic regression model of early life factors (before the age of 5 years) associated with a positive RSQ for RAO in a randomly selected population of horses in Great Britain.
Random effect variance estimate = 0.13 (0.36). s.e. = standard error, 95%CI = 95% confidence interval.

	β coefficients	s.e.	P value	Odds ratio (95%CI)	
Age acquired					
Bred as foal or acquired before 2 years	Ref.			Ref.	
Acquired after 2 years	0.59	0.30	0.045	**1.81**	(1.01 to 3.25)
Early exposure to hay					
No	Ref.			Ref.	
Yes	1.37	0.52	0.009	**3.92**	(1.41 to 10.95)
Early respiratory infection					
No	Ref.			Ref.	
Yes	1.53	0.37	<0.001	**4.62**	(2.23 to 9.55)

Section 6.4. Discussion

This is the first attempt to identify risk factors for RAO in the general horse population of Great Britain. The data set was collected from the owners of horses from throughout the mainland, clustered according to the veterinary practice they employed to provide care for their animal. This clustering needed to be accounted for using multilevel modelling as, not only could a certain type of client use a particular kind of practice, but also practices, by their nature, are limited to a specific geographical location. Horses kept in different areas are probably not kept and managed in an identical manner. The management regimens adopted are likely to be dictated by, amongst other factors, the local climate, traditional management practices in an area, availability of local types of bedding or forage, guidance of local veterinary surgeons, density and use of the local horse population plus the density, demographic and socio-economic distribution of the human population. It is perhaps not unreasonable to suspect that horses kept in the environs of a former industrial city in Lancashire are likely to be kept under different management than a horse kept in rural Norfolk. Despite this, it is of note that the bulk of the total variation in the risk of a positive RSQ for RAO occurred at the horse level in both final models. It is important to highlight that the latent-variable approach for estimating the partitioning of variation in these models can be subject to error (Goldstein *et al.*, 2002). Therefore, the results should be used qualitatively rather than quantitatively, but do suggest that horse-level factors account for most of the variation between individuals. In the host and environmental multivariable model, there was a large reduction in the proportion of variation at the practice level when compared to the intercept only model (change from 2.9% to 0.29%). This suggests that the fixed effects in the multivariable model explain most of the limited variation present at the practice level. There was little difference between the null and full models for the early life regression model.

The first regression analysis identified a number of explanatory variables, related to host or environmental factors, which fitted a parsimonious model. RAO has long been associated with the more mature horse (McPherson *et al.*, 1979b, Dixon *et al.*, 1995b) and this is supported in the model by the increasing odds of having the disease with increasing age. The slight decrease in odds associated with the age category of eleven to fifteen years is not readily explainable; although taking into account the confidence intervals associated with the adjoining age categories the effect was minimal. Increasing age was also found to be a risk factor for RAO in the analysis of 1,444 referred cases in North America (Couetil and Ward, 2003). This relationship between age and RAO could suggest dose dependency, with years of exposure to the allergens associated with housing being required before the disease is expressed; a period of sensitisation. It has been proposed that younger horses do have RAO, with associated respiratory inflammation, but it takes a lag period of development, or cycles of exposure, before the disease changes from being sub-clinical to clinical, when it becomes apparent to the owner (Viel, 1997). However, evidence from young racehorses demonstrates that the risk of lower respiratory tract inflammation deceases with increasing age (Burrell *et al.*, 1996, Chapman *et al.*, 2000). At present, there is no evidence to link inflammatory airway disease in young racehorses and RAO (Robinson, 2003).

Generally, RAO is considered to have no gender or breed predispositions (McPherson *et al.*, 1979a). Certainly, neither factor appeared to have an influence on the likelihood of a horse having the disease in this study. The recent retrospective study of referred cases in North America did identify a tentative association between the female gender and the disease (Couetil and Ward, 2003). There has been some evidence to support a genetic component to the disease (Gerber, 1989, Marti *et al.*, 1991, Robinson, 2001b). Some authors have suspected ponies might be more commonly affected as a result of exposure to poorer fodder due to their lower economic value, rather than as a result of genetic predisposition (McPherson *et al.*, 1979a, Derksen, 1991b). It could be conjectured that ponies in this study were perhaps likely to be looked after by responsible horse owners, as they were registered with a veterinary surgeon and took the time to complete a questionnaire. It is perhaps surprising that the study in veterinary teaching hospitals in North America identified a link with the Thoroughbred breed. Anecdotally, Cook (1963) remarked that he particularly associated the disease with thoroughbred brood mares when stabled overnight during the summer (Cook and Rossdale, 1963). In the current study, horses that were used for studwork (not necessarily exclusively) were less likely to have the RAO. This could reflect a desire to breed from 'healthy' animals. Alternatively, these horses may be actively exercised to a lesser degree than their counterparts. The categories in the question relating to the horse's use was intended partly as a measure of athletic activity, and the owner may be less likely to observe a mild respiratory problem if the horse is not exercised.

There was an increase in the odds of a positive RSQ for RAO for horses located in urbanised areas. This variable was not significant in the multilevel, univariable model but its significance changed when included in the multivariable model. This association may be related to the increased exposure to airborne pollution as a consequence of modern urban living. One author has already speculated that air pollution, particularly in urban and semi-urban areas, may have an influence on the prevalence of RAO (Mair, 1995). Airway reactivity differences have been identified between horses that live in urban areas and rural areas (Mazan *et al.*, 2001). Pollution and climate change have both been heavily implicated in the inception and morbidity of asthma in humans, particularly in the western industrial world (Brauer *et al.*, 2002, D'Amato *et al.*, 2002, Beggs, 2004, Wong and Lai, 2004). The association between urbanisation and the increased risk of RAO may also be a consequence of different management practices between rural and semi-urban areas. In the sentinel practice-based survey that was conducted by Mellor *et al.* (2001), horse owners in more densely human populated areas were more likely to keep their horses away from home and were less likely to turn their horses out day and night during summer and winter. Often, horses kept away from home will be stabled at livery yards or on shared premises. In the survey of northern Britain, by Mellor (1997), a greater proportion of horses kept in the central belt of Scotland, where the human population is concentrated, were kept on shared premises. These establishments may keep horses at higher 'stocking rates' both in buildings and at pasture (with consequent limit on turn out). Socio-economic constraints may also dictate that horses are perhaps kept in poorer buildings, vis-à-vis ventilation, and fed poorer quality forage in semi-urban areas. However, the effect of urbanisation in the multivariable model allowed for access to pasture and forage type. The potential effect of urbanisation on the prevalence of RAO is worthy of further investigation. Existing, contemporaneous external data (perhaps from Governmental sources) on human demographics and pollution could be used in combination with the results of this study for this purpose.

The effect of the type of hay was the antithesis of what would be expected from what is known about the aetiology of RAO. It is probably not unreasonable to suspect that if a horse has a cough in association with the feeding of dry hay that one of the first reactions of many owners would be to soak the hay or provide alternative forage. In effect, they are making a self-diagnosis of their horse having a 'dust allergy' or 'stable cough'; in all likelihood their horse may have RAO. If the horse responds to this change and a veterinary surgeon is not consulted, then the condition may then go undiagnosed. The RSQ for RAO hopefully identified these horses and therefore the feeding of wet hay would be associated with an increased risk of the horse having the disease. The converse was true of horses fed dry hay. These horses could be tolerant of the allergens in hay (likely not to have RAO) and consequently do not express any clinical signs, providing no stimulus for owners to alter management. However, these horses could have sub-clinical lower respiratory tract inflammation that is unrecognised. This tolerance of a high respiratory challenge environment (horse likely not to have RAO) may well be influencing the decreased risk of a positive RSQ for RAO in association with horses that had none, or only one, season of access to grass pasture, i.e. implying that these horses were stabled for a large proportion of the year. Again owners were perhaps not being motivated to alter management or it may be the case that stables, in which horses spent prolonged periods, were already maintained under minimal challenge regimens.

Winter is the time of year when most horses in Great Britain are stabled for at least part of the day. Thus, this is the season when horses are most likely to be exposed to a high challenge environment. The GAM plot of the relationship between time spent at pasture in winter and the risk of a positive RSQ for RAO suggested that initially horses spending longer at pasture did not appear to accrue any benefit, with the risk of RAO increasing. This could represent owners responding to their horses having respiratory clinical signs by turning their horse out to pasture for longer periods. However, it would seem that there is only a benefit, a decrease in the likelihood of RAO, if this time period is over 15 hours. A major factor in the effect of the amount of time spent at pasture on RAO is the actual quality of stable environment (relating to bedding and ventilation) that a horse spends the remainder of its time in. It is perhaps, therefore, surprising that this analysis did not elicit any effect of type of stabling or bedding. However, it is difficult to classify adequately and quantify the myriad of stable types and management regimens that horse owners utilise. A logistic regression model utilising data from just stabled horses did not identify any risk in association with, what could be considered, stables of a poor standard (not presented).

It is possible that the environment a horse is exposed to in early life will influence the development of RAO in later years. Exposure to certain environmental risk factors, both pre- and post- natal, have been associated with asthma in later life (Sears, 1997b). For example, exposure to allergens such as housedust mite or parental smoking during early infancy increases the risk of asthma. Early exposure to allergens, for example *Micropolyspora faeni* and *Aspergillus fumigatus,* contained in the hay that horses are exposed to in early life may result in later development of RAO. This is implicated in the increased risk of a positive RSQ for RAO associated with feeding hay in the early life multilevel, multivariable model. The significance of this independent variable was increased by inclusion of the other variables in the multivariable model. Pulmonary infection (in particular viral) has been implicated as a trigger factor in the induction of RAO (Gerber, 1973). In asthma research, respiratory viral infections have been implicated in its inception (Lemanske, 2003). It has been suggested that the immune response to a viral infection may influence the pattern of response to subsequent allergic stimulation (Sears, 1997b). Horses that were reported by their owners to have had a respiratory infection early in life were at a greater risk of having a positive RSQ for RAO. Respiratory infection may also play a role in subsequent development of the disease. However, owners with a horse with current or ongoing respiratory problems may have superior recall of previous episodes. Alternatively, because these owners dealt with a respiratory problem in the past they are sensitised to, and better able to recognise, clinical signs however minor. A hypothesis for the increased risk of RAO in association with the horse being acquired after the age of two years is not immediately apparent. It may be that horses bought later in life may have had a more uncertain past, been kept in poorer conditions and been exposed to a greater variety of respiratory challenge (both environmental and infectious), for example through dealers or large stud farms. On the other hand, owners may be more likely to find fault with a horse that they did not breed themselves or own since a foal or yearling.

The explanatory variables put forward by these models, although not definitive, are certainly thought provoking. It was not possible to corroborate the information provided by horse owners, and this could be a source of error or bias. The use of external data from other sources, including directly observed independently collected data, would perhaps be beneficial for further investigation of some of these risk factors. In addition, the true disease status of these horses was not known. It is recognised that the RSQ for RAO does not have 100% sensitivity and specificity and therefore some of the horses will have been classified incorrectly. However, provided this misclassification bias was non-differential (as would be anticipated), such bias would tend to decrease the measures of effect toward the null, rather than falsely increasing apparent effects. Despite these reservations, the analyses in this chapter highlight that this form of epidemiological investigation can generate a valuable knowledge of the disease RAO; particularly in relation to the general horse population and the way they are kept. It brings to our attention risk factors that warrant investigation and research in the future.

CHAPTER 7

IMPACT OF WRITTTEN EDUCATIONAL MATERIAL, CONCERNING HORSE HEALTH, ON A POPULATION OF HORSE OWNERS

Section 7.1. Introduction and Aims of Study

Keeping and providing appropriate care for a horse is a complex task requiring a high level of skill and knowledge, especially when compared to the care of other companion animals. Many management practices directly affect the health of a horse. In relation to respiratory health of horses, it is concerning that in the survey reported in chapter 5, many owners appear to use practices or management (for example poor provision of ventilation) that could have an adverse affect. This was particularly alarming as many of the horses (true prevalence = 14%) had a positive result for the risk-screening questionnaire (RSQ) for recurrent airway obstruction (RAO).

It is unknown how British horse owners acquire information related to the management of horses. There are many publications on the subject available for reference including horse-related weekly or monthly journals. Information and education is available from further education establishments as well as organisations such as the Home of Rest for Horses, the British Equine Veterinary Association (for example 'the best of care pamphlet' (Anon, 2004)), the British Horse Society and the Pony Club. Other sources, including the Internet and fellow horse owners, are also available for consultation, although the reliability of such sources may vary. Veterinary practices often produce newsletters, have evening meetings or provide information on their website for their clients, particularly with regard to the prevention of disease, which could conversely be considered to be the promotion of health.

Written material is central to many of these mediums. However, the effectiveness of such methods for disseminating knowledge to owners, as to the best practice for caring for their horse, has not been investigated. In this study, two educational booklets were created, one on ragwort poisoning and one on respiratory health. These were designed to improve the knowledge of horse owners with regard to these conditions, and to suggest preventative control measures. The aim of this study was to assess the impact of these pamphlets on the knowledge, and attitude, of a sample of horse owners.

Section 7.2. Materials and Methods

7.2.1. Study population

The study population was randomly selected from the responders to the survey in chapter 5 who had agreed to participate in a further study. A total of 873 horse owners returned completed questionnaires, of which 651 (74.6%, 71.7 to 77.5%) indicated a willingness to participate in this study. Using random numbers generated using a function of Microsoft Excel 2000, four hundred of the responders were selected with 200 assigned to group A and the remainder to group B.

7.2.2. Construction of educational booklets and questionnaire

Two educational booklets were created on the topics of 'Horse respiratory health' and 'Ragwort: a danger to horse health'. The information included in the booklets was obtained from the available scientific literature and as far as possible represented best practice. Each pamphlet was designed to follow a similar format and style (appendices A7.1 & A7.2). Information was provided about what the health problem was, the normal function of the organ system affected (hepatic or respiratory), how this function was affected by disease, how horses were exposed and finally management changes that could be practised to minimise disease. The active tense was principally used and the pamphlets were as simple as possible, included colour illustrations, and were not intended to be controversial. The readability of the written text was scored, using the Fog index, as 27 and 24 for the ragwort and respiratory booklets respectively (Gunning, 1969). The A5 booklets, 12 pages each in total, were professionally printed; text was principally Arial size 12-type black font.

A short questionnaire for self-administration was created to investigate booklet recipients' attitudes to the type and style of information provided (appendix A7.3). The second side of the questionnaire had six questions designed to ascertain the knowledge of the owners regarding the two topics. A modified total design method (TDM) was followed throughout the construction and administration of this questionnaire (Dillman, 1999). It was designed, edited and pre-coded using data capture software, TELE*form* Elite v8. Apart from titles, an Arial size 11-type font was used throughout the questionnaire, which was printed in-house black on white A4 paper.

7.2.3. Administration of educational intervention and questionnaire

The educational booklets were mailed, with a cover letter (appendix A7.4), to the selected horse owners on 7th April 2004; Group A was sent ragwort booklets and Group B respiratory booklets. Two weeks later (21st April 2004) the questionnaire was mailed together with a cover letter (appendix A7.5) and a Freepost return envelope (appendix A2.1.2) enclosed in a C4 address windowed envelope. The cover letter informed the recipient that if they returned a completed questionnaire they would receive a second booklet and dictated a return deadline of 7th May 2004. All non-responders by 5th May 2004 were sent a reminder postcard (appendix A7.6). If no response had been received by 25th May 2004, a second copy of the questionnaire was mailed with a further cover letter (appendix A7.7). All letters and postcards were signed in blue ink by the author. All mailed envelopes were pre-printed with the return address and utilised first class stamps. The reminder postcards and the Freepost returns used second-class postage. A final deadline was set at 13th August 2004.

7.2.4. Processing of returned questionnaires

All respondents were mailed the booklet they had not originally received (appendix A7.8). Returned questionnaires were stamped with the date of arrival and then scanned using a fujitsu fi-4110cu image scanner. The resultant images were then imported into the TELE*form* Elite v8 data capture software. The software processed the images automatically, extracting the data from the completed fields before verification and correction by the operator. This included manual entry of free text information. The actual scanned image of each page was visually compared with the image generated by the software (with its interpretation of the responses) before the data were exported into Microsoft Excel 2000.

7.2.5. Analysis

The 400 selected horse owners were compared to all the responders to the survey in chapter five using information that had been collected in that study. Similarly, group A was compared to group B using this information. Responses were considered useable if at least one question was answered. Non-responders were compared to responders using information that had been provided in the horse health questionnaire (chapter 5). This information was also used to compare responders according to the time taken to respond (stage of response). The stage of response was defined as early if response had occurred before the mailing of the second questionnaire and late if it occurred after this mailing. The responses given in the questionnaires by the horse owners were compared according to their membership of group A or group B. Responses were also investigated according to the result of the RSQ for RAO for each horse. Comparisons between groups were made using Mantel-Haenszel chi-square analysis apart from when expected cell counts were low when Fisher's exact test was used (Epi Info v6.04d). Where data were continuous, the Mann-Whitney test (U) (two-sample rank test) for two populations was utilised (SPSS v11). The critical probability for all analyses was taken as 0.05.

Section 7.3. Results

7.3.1. Description of study population

The 400 horse owners randomly selected for this educational intervention study owned horses that were representative of the horse population described in detail in chapter 5. There were also no significant differences between the two groups with respect to the information previously provided in the horse health survey (chapter 5). The average age of the 400 horses was 12 years and 4 months with mares comprising 44.7% (95% confidence interval (95%CI) = 39.8 to 49.6%), stallions 2.5 (95%CI = 1 to 4.1%) and geldings 52.5% (95%CI = 47.9 to 57.7%) of the sample. Following mailing of the educational booklets, one was returned from group A as 'addressee has gone away' and three were returned in a similar manner from group B, this gave a revised sample sizes for the subsequent survey of 199 and 197 respectively (total = 396).

7.3.2. Description of response rate

The overall response rate was 73.7%, with no statistical difference in the proportions responding between the two groups. The response patterns are summarised in table 7.1 and figure 7.1. Only completed questionnaires were returned. The median time to respond was 14.5 days (inter-quartile range (IQR) = 7.0 to 29.0) and overall 78.4% of the owners had responded early, i.e. by the time of the second mailing. Owners receiving the ragwort booklet responded in a median of 13 days (IQR = 6 to 23 days), significantly quicker than those receiving a respiratory booklet that responded in a median of 19 days (IQR = 7 to 34 days)(U = 89981, p = 0.02).

Table 7.1. Response rate to each stage of the educational questionnaire.

	Cumulative Frequency	Percentage (%)	95% confidence Interval (%)
Overall (n = 396)			
Response prior to postcard	145	36.6	30.5 to 42.8
Response prior to second mailing	228	57.6	52.7 to 62.4
Total response	292	73.5	69.4 to 78.1
Group A (n = 199)			
Response prior to postcard	83	41.73	34.9 to 48.6
Response prior to second mailing	115	57.8	50.9 to 64.7
Total response	141	70.9	64.5 to 77.2
Group B (n = 197)			
Response prior to postcard	63	32.0	25.5 to 38.5
Response prior to second mailing	114	57.9	51.0 to 64.8
Total response	151	76.6	70.7 to 82.6

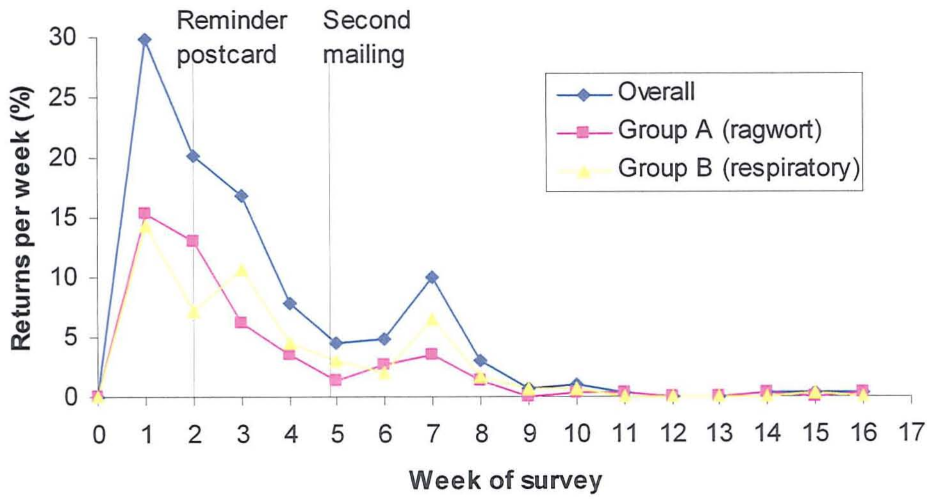

Figure 7.1. Return frequencies over time relative to total response (n = 291) for the educational survey; overall and separately for group A and group B.

7.3.3. Factors associated with response

The only statistically significant differences between non-responders and responders are listed in table 7.2. Non-responders owned younger horses, had owned them for shorter periods of time and had kept their horse on the current premises for less time. This finding was also identified when group A and group B were investigated separately. Group B owners (recipients of the respiratory booklet) that groomed their horse inside a stable were 2.3 (95%CI = 1.0 to 5 .7) times more likely to respond than those that groomed outside ($\chi^2_1 = 4.5$, p = 0.03). The comparison between early and late responders did not identify any significant differences apart from owners who had reported that their horse had coughed in the last 12 months. These owners returned their questionnaire earlier in the survey than other owners ($\chi^2_1 = 7.6$, p = 0.006). When the two groups were considered separately, this was only significant for group B. Recipients of the respiratory booklet who reported that their horse had had a cough in the last 12 months were 7.4 (95%CI = 1.7 to 66.7) times more likely to respond early than owners of horses without this clinical sign ($\chi^2_1 = 9.1$, p = 0.003).

Table 7.2. Statistically significant differences between responders and non-responders in the educational survey.

	Cumulative Frequency	Median (Inter-quartile range)	Mann-Whitney U	p
Age of horse	Non-respondent	9 yrs 6mths (7 yrs 2mths to 13 yrs 11 mths)	12823	0.018
	Respondent	11 yrs (8 yrs to 16yrs 7mths)		
Length of time in ownership	Non-respondent	3 yrs 11mths (2 yrs to 6 yrs 1 mth)	12878	0.024
	Respondent	5 yrs (2 yrs 2 mths to 9 yrs 3 mths)		
Length of time on current premises	Non-respondent	2 yrs 1 mth (9 mths to 4 years 6 mths)	12079	0.002
	Respondent	3 yrs 1 mth (1 yr 2 mths to 7 yr 7 mths)		

7.3.4. Cost of study and questionnaire item omission

The overall cost of this study was £1384.99, with a cost per useable return from the survey (including the booklets) of £4.74 (appendix A7.9). The omission rate over all the questions was 1.4 %, with the highest rate occurring in relation to questions concerning information in the booklet that the respondent was not already aware of and disagreement with the content (appendix A7.10). There was no difference in the omission rate between the two groups.

7.3.5. Questionnaire results

The booklets were generally well received by the horse owners, with most describing the overall presentation to be very good or excellent, being moderately to extremely interested and believing the degree of detail to be about right (figure 7.2). There was no difference between the two groups in their appraisal of the booklets apart from that almost twice as many in group A than in group B (odds ratio (OR) = 2.1; 95%CI = 1.1 to 4.2) considered the ragwort booklet they received to have excellent presentation ($\chi^2_1 = 5.3$, p = 0.02).

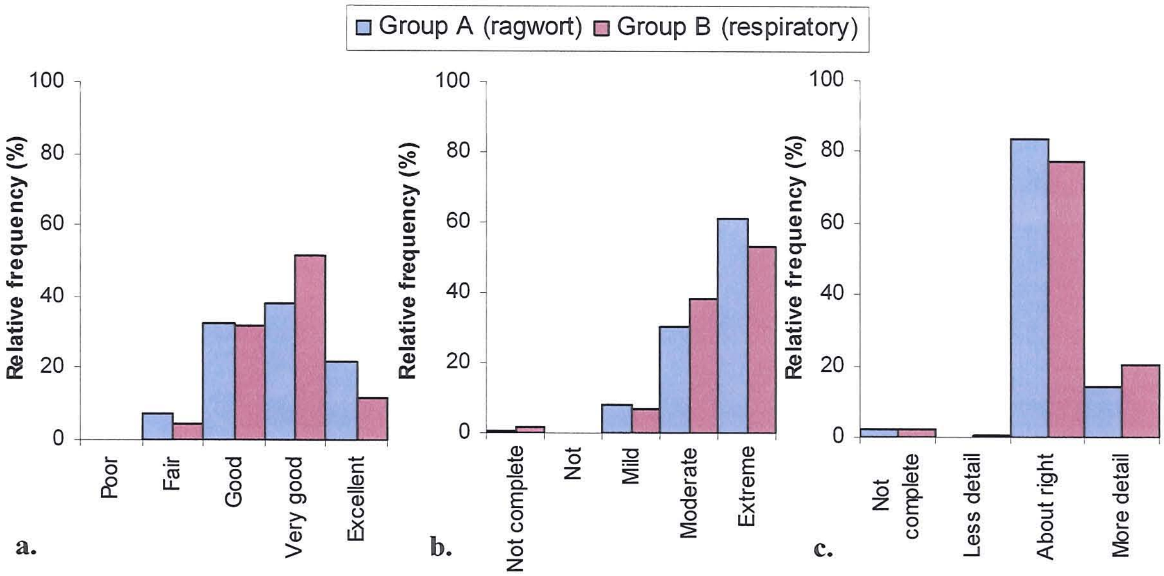

Figure 7.2. Responses by the two groups to the first three questions, concerning the educational booklet, relating to: a. presentation, b. degree of interest in content and c. degree of detail.

A greater proportion of group A (49.6%) stated that the ragwort booklet contained new information compared to a minority in group B, who gained new knowledge from the respiratory booklet (33.8%) ($\chi^2_1 = 8.9$, p = 0.003). In group B, 52.5% of owners whose horses had been identified as RSQ positive for RAO indicated that the respiratory booklet had contained new information compared to only 29.7% of owners with RSQ negative horses ($\chi^2_1 = 6.5$, p = 0.01) (table 7.3). A total of 120 respondents actually reported the information contained in the booklets that had impressed them (table 7.4).

Table 7.3. Two by two tables of owners' responses concerning information contained in the educational booklets divided according to the RSQ for RAO status of their horse.

	Group A (ragwort)		Group B (respiratory)	
	New information	No new information	New information	No new information
RSQ RAO positive	14	14	21	19
RSQ RAO negative	56	45	30	71

Table 7.4. Information contained in the educational booklet that respondents were willing to report had impressed them.

Information that created an impression	Number of respondents	Proportion (%)
Group A (Ragwort; n = 69)		
Clinical signs of poisoning	10	14.5
Liver function and damage	25	36.2
Control of ragwort (on own premises)	11	15.9
Control of ragwort (authorities to contact)	19	27.5
Booklet - praise	4	5.8
Group B (Respiratory; n = 51)		
Respiratory system function	9	17.6
Recurrent airway obstruction	10	19.6
Ventilation	15	29.4
Grooming outdoors	5	9.8
Minimal dust management	9	17.6
Booklet - criticism	1	2.0
Booklet - praise	2	3.9

Respondents had informed themselves previously regarding ragwort and respiratory health from the horse press and fellow horse owners (table 7.5). A greater proportion (OR = 3.8; 1.6 to 9.4) of respondents in group A had obtained information about ragwort from the general media compared to those in group B regarding respiratory health ($\chi^2_1 = 11.7$, p = 0.0006). The reverse was true of the Internet, with almost twice as many members of group B (OR = 1.9; 1.0 to 3.8) utilising this resource for investigating respiratory health compared to those in group A looking up about ragwort ($\chi^2_1 = 4.2$, p = 0.04).

Table 7.5. Responses to questions concerning where information in the educational booklets had been sourced previously and whether or not respondents would adopt any of the management practices detailed in the booklets.

Question	Response (%)	
	Group A (ragwort)	Group B (respiratory)
Previous sources of information		
Media	19.2	6.0
Horse press	90.8	87.4
Societies	25.5	17.9
Internet (world wide web)	12.8	21.9
Friends	72.3	72.8
Courses	25.5	28.4
Adoption of management practices in booklet		
Definitely not	0	0.7
Possibly	13.5	15.6
Definitely	23.4	20.6
Already use	61.7	68.1
Not complete	1.4	2.1

Overall, nine respondents did not agree with one or two items in the booklet, a further three did not agree with a large proportion of items; there was no difference between the groups. The respondents who had received a ragwort booklet cited their grievance as: a. maintaining good quality pasture can result in laminitis, b. criticised booklet and picture quality, c. authorities, when contacted, were useless, d. sheep do clear ragwort and e. approaching landowners can be difficult. Criticisms of the respiratory booklet included: a. booklet was condescending, b. difficult to implement, particularly in livery stables (2 people), c. should have included feeding off the floor, d. wanted guidance on mucus colouring and e. omitted problem of summer pasture associated obstructive pulmonary disease. The majority of respondents already practised many of the management suggestions made in the booklets and there was no difference between the two groups (table 7.5). Over 20% of the respondents in each group indicated that they would definitely adopt some of the booklets' suggestions, one person in group B would definitely not be following any.

The proportion of correct answers, as opposed to incorrect or incomplete answers, was broadly similar between the two groups, with most respondents able to identify the right response (table 7.6). The only exception was question one, which asked whether or not healthy horses could have an inflamed respiratory tract because of stabling. Just over twice as many of the respondents not receiving the respiratory booklet were likely to give the wrong answer (OR = 2.1; 0.96 to 4.95)(χ^2_1 = 4.1, p = 0.04). A greater proportion of respondents who had received a ragwort booklet were able correctly to identify the description of the weed (question four) (Fisher's exact (one-tailed) p = 0.048).

Table 7.6. Horse owners' responses to questions on their knowledge of information contained in the educational booklets.
Response in red = correct answer

Question	Response (%) Group A (ragwort)	Response (%) Group B (respiratory)
Question 1. Is it possible for healthy horses to have an inflamed respiratory system just because they are being kept in a stable?		
False	13.5	7.9
True	84.4	92.1
Not complete	2.1	0
Question 2. If the weather turns very cold and your horse needs extra warmth whilst it is stabled, it is best to do which of the following?		
Close windows or vents	0.7	0.7
Put an extra rug on your horse	97.2	96.7
Both of the above	2.1	1.3
Close the top door	0	1.3
Not complete	0	0
Question 3. Recurrent airway obstruction (also known as COPD or heaves) is a respiratory disease caused by which of the following?		
A viral infection	3.5	2.6
An allergy to fungal spores etc	96.4	96.7
Excessive exercise	0	0
Poor worming	0	0
Not complete	0	0.7
Question 4. When the weed, ragwort, is fully grown and flowering what does it look like?		
Low to the ground with yellow flowers	0	4.0
Tall with many small white flowers	0.7	0
Low to the ground with white flowers	0	0
Tall with many small yellow flowers	99.3	95.4
Not complete	0	0.7
Question 5. Is it true that toxins contained in ragwort damage the liver of horses that eat it?		
False	0.7	0
True	99.3	99.3
Not complete	0	0.7
Question 6. One way to get rid of ragwort is to pull it up. What should be done with ragwort after pulling it up?		
Allow it to dry out	0	0
Place it on the muck heap	0	0
It can be left in the field to rot	0	0
It should be taken away and burnt	100	100
Not complete	0	0

Section 7.4. Discussion

The educational booklets created for this study appear to have been well received by the recipient horse owners. They also appear to have had some, if marginal, impact on their knowledge and attitudes to management practices. The owners in this recruited population were highly motivated and this is reflected in the high response rate. The good response, as well as a result of prior agreement to participate, may also be a consequence of the booklets being of interest, the shortness of the questionnaire and some incentive may have been accrued from the reward of a second booklet. As discussed in previous chapters, the attentions to detail and the timed follow-up reminders dictated by TDM appear to have been beneficial. Recipients of the ragwort booklet tended to respond quicker than the other group; this may indicate a greater interest in, or awareness of, the topic. There may also have been some inherent property of this particular booklet that resulted in it having a greater impact, as suggested by those who first received it.

It was encouraging that there was minimal difference between responders and non-responders according to the information they had supplied regarding their horse in the previous horse health survey. In particular, the reporting of respiratory clinical signs, the status of their horse according to the RSQ for RAO and management practices did not appear to have any influence. Non-responders did own younger horses, with length of time owned and time on premises probably being related. The loss of information from these owners is to be regretted. An explanation could be that owners of older horses are more likely to be concerned about their horse's health, and thus be involved in this educational study, as they are may be more aware of, or have encountered, problems associated with the well-being of their horse. The increased response from owners who groomed their horses indoors may be a direct impact of the inclusion of this topic in the respiratory booklet, with attention drawn to it by a photograph. Much of the content of the booklet included management practices related to feeding and bedding that are perhaps widely known. It may be that the issue of where to groom your horse was a new concept to this particular group of owners. It certainly was to five members of group B. Normally the comparison of early and late responders is done using information provided in the actual questionnaire returned (Sheikh and Mattingly, 1981). In this investigation, data already known about the respondents were used to ascertain if this method would identify the same factors as comparing non-responders and responders. The comparison of early and late responders did not predict non-responders' characteristics in this study. It did suggest that group B owners, who had previously reported that their horses had had a cough in the last 12 months, responded quickly to the survey, possibly spurred on by their recent experience of respiratory disease.

The use of previously recruited horse owners, although beneficial for response rate, will have introduced some selection bias that should be taken into account when applying any findings to the general horse owner population. These owners had already completed a fairly complex questionnaire that required literacy. Non-responders could have been less literate and therefore the written material provided less of an effect. In addition, the participants in this study had indicated that they were willing to take part in a further study that included the provision of information. That is, these owners were perhaps willing to be educated, more intransient owners may not have been included in the sample. The pre-warning of pamphlet recipients that they would be receiving a questionnaire was essential in obtaining consent to participate. However, this may have resulted in the subjects performing differently because they knew they were being 'observed' (part of a study), commonly known as the Hawthorne effect (Holden, 2001).

The study aimed to disseminate knowledge to horse owners regarding the health of their horse and perhaps encourage voluntary change in their attitude towards, or even their behaviour as to, how they care for them by suggesting good management practices that could improve the health and welfare of their animal. This is analogous to the field of health promotion in the human medicine. The World Health Organisations' definition is: "health promotion is the process of enabling people to increase control over, and improve, their health" (World Health Organisation, 1986). Many varied methods are used for promoting health from awareness campaigns to community interventions (Speller *et al.*, 1997). This study disseminated information in the form of written material delivered in a unidirectional message (vertical teaching). This information only approach is widely used for patient education; despite this its usefulness is rarely assessed (Arthur, 1995). It has been demonstrated to be effective in educating adults with asthma and ulcerative colitis (Eaden *et al.*, 2002, Gibson *et al.*, 2002). There are better ways of educating adults, including interactive methods and problem based learning; as often practiced in human health promotion and usually delivered by health professionals (Sudre *et al.*, 1999, Mazor and Billings-Gagliardi, 2003). This approach is one utilised, for example, in veterinary practice meetings for their clients. However, these methods were outside the scope of this limited study.

Material for health promotion should be written, as far as possible, in the active voice, paragraphs should be used to break up text and clarity should be ensured using a reasonable print size with colour images (Ewles and Simnett, 1999). It is hoped that the booklets produced in this study came close to emulating these guidelines. Readability includes the use of plain English with simple words and short sentences. It can be assessed using scoring systems such as the Fog index that take into account sentence length and the number of words with more than three syllables, with lower scores indicating better readability (Gunning, 1969). These pamphlets were equivalent to The Sun newspaper in readability and lower than The Times (Ewles and Simnett, 1999). Readability formulae should not be entirely relied on as, at the end of the day, it is the ease with which the intended audience reads them that is paramount and no scoring system can emulate this (Mazor and Billings-Gagliardi, 2003).

Most horse owners thought the educational booklets were well presented and interesting. The ragwort booklet was deemed to be superior by its recipients and was believed to contain new information for almost half of them. This may be because the subject matter was simpler; there was less information to get across and the methods of control quite straightforward. Ragwort was relatively topical during the period of this study, with media coverage because of campaigns by the British Horse Society and the introduction of the Ragwort Control Act 2003 (Great Britain. Parliament, 2003). This increased awareness was supported by the greater proportion of group A that had obtained information about ragwort from the media. Ragwort poisoning is a potentially fatal disease and therefore may attract, or hold, a reader's attention. The broad nature of the respiratory booklet, rather than focusing purely on the disease of RAO, combined with the multiple management practices for control made it inherently difficult to convey a succinct and simple message. The aim of the educational booklets was not only to provide recommendations for management to prevent disease but also to provide owners with more detailed information about the disease that would help them recognise why these measures are necessary. Therefore, it is rewarding that the information about the normal function of the respective organ systems and the disease processes involved when things go wrong made an impression on so many of the respondents.

It is perhaps of particular note that, of the owners that received a respiratory booklet, a greater proportion possessing a horse designated as RSQ positive for RAO reported that the booklet had contained new information. It could be that many of these owners were not fully aware, or did not practise, low respiratory challenge environments for their horses. Therefore, their horses were more likely to exhibit respiratory clinical signs associated with housing and consequently score highly on the RSQ for RAO. It would be encouraging to believe that these owners may now use this new information to guide their choices in managing their horse, improving its respiratory health and welfare.

The horse press was widely used for obtaining information by horse owners and is already a well-recognised conduit for imparting veterinary advice as well as more general information. Fellow horse owners (friends) also played a large role in providing advice. These two sources were also identified as being important in a study investigating deworming practices (Lloyd *et al.*, 2000). In this study, veterinary surgeons were frequently turned to for deworming regimens and it was an oversight not to include them in this study as previous sources of information. The Internet was not used as much as I would have expected, considering its wide availability, but was used more often for finding out information about respiratory disease than ragwort. Perhaps it is utilised for investigating specific problems being encountered by an owner and respiratory disease is far more likely to occur than hepatic encephalopathy.

The dissemination of information in pamphlet form was intended as a step towards changing horse owners' behaviour as to how they care for their horses. It was not possible to ascertain within this small study if these interventions would actually change behaviour and the way horses were kept. It was only feasible to investigate if the educational material could firstly alter people's attitudes (i.e. likelihood of adopting management) and secondly, if it had increased their knowledge on the topics. A person's attitude indicates their settled mode of thinking on a topic, from which it can be possible to predict, but not always, their behaviour. Our intervention can be interpreted in the context of the transtheoretical model of behaviour change (Prochaska *et al.*, 1992). This model has been a useful concept for studying many health behaviours including smoking cessation, diet and antibiotic usage in children (Ma *et al.*, 2002, Spencer *et al.*, 2002, Taylor *et al.*, 2003). This model attempts to outline the stages of change that occur when new behaviours are adopted and old ones discarded. It is a cycle that passes from pre-contemplation (no awareness of need to change) to contemplation (thinking about change) to commitment (ready to change) to action (making changes) to maintenance (maintaining change), from which relapse can occur and the process start again. In the intervention in this study, our aim was to move owners from pre-contemplation to contemplation. Hopefully, this would then stimulate those horse owners, for whom an alteration in their horse's management was appropriate, to progress towards action and maintenance of changed behaviour and/or management practices.

The majority of the respondents claimed already to be adopting many of the management practices. It would be interesting actually to put this to the test, particularly in terms of provision of effective ventilation. However, I believe there would be little to achieve from finding out how many owners are potentially deceiving themselves. Over 20% of the respondents to the questionnaire indicated that they would definitely be implementing some of the preventative management practices suggested by the booklets and this can only be applauded. To attempt to assess the knowledge of the recipients of the educational booklets regarding the two topics in just three questions for each was difficult. However, a lengthy multiple-choice exam had to be avoided to ensure a good response rate. Hence, the extent of any transfer of knowledge by these educational booklets could not be properly assessed. Indeed, the pamphlets may have been used as 'study aids' for help in answering the questions. However, they did appear to have some impact on the owners. Although not striking, there was an increased proportion of those that received a ragwort pamphlet being able correctly to identify the weed. A one-tailed test for significance was used as it had been hypothesised that a correct answer would be positively related to knowledge. A greater proportion recognised that healthy horses can have an inflamed respiratory system associated with poor management, subsequent to receiving the respiratory booklet. In this author's opinion, this concept is fundamental to understanding how the way horses are kept in this country can directly affect their health and welfare. The education of owners as to the 'best practice' for caring for horses should be a priority for the prevention of disease and promotion of welfare. The appropriate method for transferring this information undoubtedly requires further investigation. This study indicates that written material may well have a role to play in this important undertaking.

CHAPTER 8

GENERAL DISCUSSION

The studies outlined in this thesis constitute the first major epidemiological investigation of recurrent airway obstruction (RAO) in Great Britain. There is a paucity of data regarding RAO in the general horse population where the disease has the potential for significant impact on the welfare of horses. To date, studies of RAO have tended, necessarily, to be performed on small numbers of horses, often from 'research herds', that may be unrepresentative of the general horse population. These current studies not only have produced an epidemiological instrument for investigating RAO but also have increased our knowledge of how veterinary professionals diagnose this condition. The main survey presented in this thesis was aimed at identifying the prevalence of RAO in the general horse population of Great Britain and eliciting data to aid the investigation of risk factors. These general data on horses and how they are kept and managed by their owners provides useful insights; the availability of such information is limited (Harris, 1999). Understanding of the risk factors for RAO, and the appropriate management alterations, within the veterinary profession is devalued if we cannot convey this knowledge to horse owners, especially in terms of prevention. The small educational study included in this thesis goes some way towards conveying our message.

Self-administered, mailed questionnaires were used three times in cross-sectional surveys in this project. In addition, the modified Delphi consultation included many of the features of a self-administered questionnaire. The personal interview is often believed to be the only reliable method of eliciting information, although it can introduce error caused by the interviewer in addition to that contributed by the respondent (Abramson and Abramson, 1999). Face-to-face interviewing is labour intensive, time consuming and expensive, especially when (as in this study) the survey covers a large geographic area (Mandel *et al.*, 2000). Mailed questionnaires have been widely and successfully used, as discussed in chapters 2 and 5. Mailed surveys with vigorous follow-up procedures to non-respondents, as advocated in the total design method (Dillman, 1983), can provide good response rates, as achieved in these studies. Non-response, and the bias it can introduce, will always be an issue but, provided attempts are made to investigate it, and results interpreted accordingly, then erroneous conclusions can be avoided. These investigations have provided strong support for the use of questionnaires to collect data regarding animal populations. Assessing the reliability of the questionnaires used in these surveys for obtaining data in a repeatable manner would be a further refinement; this includes the risk-screening questionnaire (RSQ).

As stated, all the surveys achieved good response rates and were conducted by a single individual for very little cost. This was partly attributable to the employment of the

TELE*form* Elite v8 data capture software. This software enabled the design of clear and attractive questionnaires that were used throughout this study; essential for encouraging a response. Data entry was fast and efficient using scanned images of completed forms before processing by the recognition component of the software. The software permitted operator validation of any ambiguous responses as well as visual comparison of the actual responses on scanned images with the computer-generated responses before data were committed to a computer spreadsheet for storage and later analysis. Few errors were identified during these checks. Even though the error rate was not investigated in these studies, it was felt that the double validation before committing the data was sufficient. The suppliers of this software (Verity Inc., USA) claim an accuracy approaching 100% and we have had no occasion to doubt this. An independent study that utilised the same computer workstation used in this project identified an error rate of 0.1% (Pinchbeck *et al.*, 2004). Data capture software has the potential to be a valuable tool in veterinary epidemiological studies, improving the accuracy and speed of data entry as well as reducing costs. As a consequence of its demonstrated use in this project, this type of software has been acquired by at least two other veterinary institutions in the UK.

Prior to undertaking the main survey of the general horse population of Great Britain, an instrument or tool for identifying horses with RAO was required. Existing clinical diagnostic tools are invasive and too costly for use in an epidemiological survey, especially when it is taken into account how geographically widely the horses were dispersed in the study described in chapter 5. Inspired by the use of such instruments in the investigation of asthma in human populations, it was elected to create a RSQ (Burney *et al.*, 1994, Asher *et al.*, 1995). A particular problem presented by RAO is that it is an incurable, potentially recurrent, disease that varies greatly in its severity, ranging from remission to severe respiratory obstruction and compromise, depending on an individual horse's exposure to certain environmental factors. Therefore, the RSQ would need to extract historical as well as current information about a horse. In order to give some rationale to the questions that would best differentiate between horses with and without RAO (whether or not affected horses were in exacerbation or remission), three processes were utilised; namely a review of the scientific literature, a survey of equine practitioners and a modified Delphi consultation of experts in equine respiratory medicine.

RAO, under various names, has been the subject of many published works over the past decades. Experimental studies, including aetiological, physiological and therapeutic investigations, as well as clinical reports from case series, are available for consideration. Until recently, there has been no definitive terminology for the disease, let alone a definition of the phenotype, so it is sometimes hard to be certain that different papers are referring to the same disease. A starting point for rectifying this was the international workshop on chronic airway disease (Robinson, 2001b). It was hoped, in the current study, to perform a systematic review, including meta-analysis, of the literature with particular reference to the currently available diagnostic tests and to the clinical signs associated with RAO. The use of evidence-based medicine techniques would have helped quantify the predictive properties not only of diagnostic tools, but also the clinical signs. This would have potentially contributed to the construction and scoring of the proposed RSQ. Unfortunately, the variable, and generally poor quality (for our purpose), of published studies implied that a meta-analysis of independent studies, utilising summary ROC curves (Moses and Shapiro, 1993, Irwig *et al.*, 1995), was not feasible or appropriate. It is important when considering the extremely powerful and exciting tools of evidence-based medicine that the vital element, good quality evidence, is not obscured (Sackett *et al.*, 2000). In the review of the literature conducted in chapter 1, very few, if any studies, met the criteria for good evidence (Bossuyt *et al.*, 2003) and very few were directly comparable to each other. The use of evidence-based medicine in veterinary science is a lofty ambition but great care should be taken in its application; efforts should be concentrated on performing primary studies that produce strong evidence.

The survey of equine practitioners reported in chapter 2 confirmed that it is possible to gather useful information from busy professionals working with horses, as previously demonstrated by Christley *et al.* (2000). The veterinary surgeons were receptive to the survey, as demonstrated by the high response rate. It was reassuring that there was evidence of minimal difference between either non-responders and responders or early and late responders. Despite this, and the good response, there was still some potential for the introduction of bias, as there were differences based on a few minor characteristics. For example, the slow response by practitioners that spent greater than 90% of their time with horses is concerning if this effect was to be extrapolated to non-responders. The significantly greater response by vets working in Scotland, could introduce some bias, but also gives some weight to the suggestion that surveys could be best conducted using regional centres.

Data and information from veterinary surgeons in practice is particularly important, as this is where the majority of animals are diagnosed and treated. How information generated from research is applied in practice and conversely how vets deal with problems on which there is minimal evidence available is extremely interesting. The singular message from the study in chapter 2 that impressed this author was that over 80% of practitioners relied on history and basic clinical examination when diagnosing RAO cases, with greater emphasis placed on the former. A similar proportion became confident of their diagnosis at this point. Consequently, in most cases, the respondents did not make a definitive diagnosis, as further diagnostic tests were not used. In practice, at an individual horse level, definitive diagnoses are possibly not made as further tests are costly, enforce a delay in reaching an answer and the expectations of an owner may be that their vet should be able to make a judgment without extensive testing. There is also the impression that empirical alteration in management and treatment is often associated with resolution of signs. The minimal use of a rebreathing bag to facilitate auscultation by practitioners perhaps demonstrates that more supportive evidence is required to encourage its use, along with dissemination of its potential worth, as advocated by the experts in the Delphi consultation.

The consultation of experts described in chapter 3 is, to the author's knowledge, the first time that the modified Delphi technique has been used in veterinary research. It may well prove to be a useful tool for application throughout the veterinary world, on topics where evidence is sparse. Its use of non face-to-face consultation is particularly appealing, as veterinary experts tend to be sparingly distributed across the globe and bringing them together is a costly and logistical challenge. The consultation process generated a wealth of information. Experts, in harmony with the practitioners, placed great emphasis on historical information, especially management regimens. The output from the process supplied the basis for the RSQ and a means of scoring it, concurring, for example, with the scientific literature and vets in practice that a recurrent or prolonged period of coughing was associated with an increased likelihood of a horse having RAO.

The RSQ for RAO, using the Delphi scoring system, proved to be a relatively sensitive and specific test for the disease. In the future, it would be desirable to investigate alterations in the way questions were asked or the way scores are created in order to improve further the aforementioned measures of diagnostic performance. One avenue for further exploration is that presented by homogeneity analysis (homogeneity analysis by means of alternating least squares: HOMALS). It was satisfying that this procedure, using actual response data, identified a single dimension to the underlying 'thrust' of the questionnaire and adjustments in the questions of the RSQ may provide improvements in the resultant HOMALS scoring. Every diagnostic test will generate false negatives and false positives. In common with other diagnostic tests engaged in the diagnosis of RAO a horse, in theory, could remain unrecognised as having RAO if it has never been exposed to a challenge environment. At least the RSQ attempts to take into account historical information, although only if problems have occurred within the respondent's ownership. Thus, validation of any diagnostic test would ideally include horses being exposed to a challenge environment. This kind of validation will always be problematic if horses from the general population, and the accompanying owners, are involved.

The validation study for the RSQ was believed to follow an effective and defensible methodology, which attempted to account for the limitations of conducting such a study on a limited budget in a general horse population where owners' consent was required. The reference standard of a clinical diagnosis that included the use of respiratory cytology is a viable solution. The sensitivity and specificity of this reference procedure is unknown and both these performance criteria have previously been assumed to be 100%. No accuracy studies of the available diagnostic tests for RAO have been published that comply with the proposed criteria for diagnostic test validation (Bossuyt *et al.*, 2003). As discussed in chapter 4, an improvement to the validation study would involve a cross sectional study of randomly selected horses on which both the RSQ and a reference standard would be applied. To account for the absence of a reference standard and in order to estimate disease prevalence, as well as diagnostic test error rates, a model proposed by Hui and Walter (1980) could be utilised. In this situation, two diagnostic tests are applied to two populations with different disease prevalence. The prevalence and test performance parameters can then be obtained using maximum likelihood estimates (Hui and Walter, 1980) or Bayesian estimation (Johnson *et al.*, 2001). Alternative solutions, employing two diagnostic tests, have also been proposed for use in single populations (Joseph *et al.*, 1995).

The validation process and the subsequent horse health survey relied heavily on the co-operation of veterinary surgeons working in general practice, to a far greater degree than the requirement to complete a short 15 minute questionnaire as reported in chapter 2. The methods did achieve the intended goals but perhaps more could have been done to increase involvement. The low return in the validation study and the lack of inclination by many practices to participate in the horse health survey was frustrating. It highlights the difficulties of implementing the laudable ideals of involving practitioners in the process of research and collecting data (Camm, 2003). Efforts to engage practitioners should be used with caution. When coercing participation, there is the chance that over aggressive and intrusive interventions may disaffect contributors. Practitioners have been used with success for eliciting data on horse populations and disease via the use of sentinel practices but it can be imagined that an incredible amount of effort is required to conduct and maintain such studies (Mellor *et al.*, 2001). Surveys of equine practitioners that were members of professional associations have been performed in the past (Anon, 1965, Traub-

Dargatz *et al.*, 1991). Recently, the British Equine Veterinary Association has attempted to use the resources of its members to address specific topics (Mair, 2004). Members of an organisation may be more motivated to participate but, it must be remembered that they may not represent the whole population and that self-selected contributors provide the data. It will be interesting to observe the support that this project receives. The horse passport system does provide a potential means of surveying horse owners directly, removing the requirement to depend on veterinary practitioners (Great Britain. Parliment, 2004), providing issues regarding the Data Protection Act 1998 can be resolved (Anon, 2004b).

In this project, data for the epidemiological study of RAO were derived from observations made on a sample of the target population. If this subset of the population is representative, measurements taken from the sample can be generalised to the target population. Alternatives to the cross sectional design include use of a census or a cohort study. While a census can give an accurate measurement of variables for all members of a population, they are prohibitively expensive and often not feasible to conduct. A cohort study following a population of horses across decades would generate epidemiological data for a multitude of diseases and problems affecting horses, for now such an ambitious study will probably remain a pipe dream.

The horse health survey collated a large body of data on the types of horses that are kept throughout Great Britain and how they are kept. The main focus of the study in chapter 5 was to determine the prevalence of RAO in the horse population of Great Britain and this was estimated to be 14%. This represents a substantial concern regarding welfare, particularly as many of the owners of these horses reported that they were unaware that their horse had this condition (as previously diagnosed by a vet). This is especially worrying because information from the survey also indicated that many horses are still kept in poorly ventilated stables surrounded by fodder and bedding that can represent a challenge to the respiratory tract of any horse, let alone one with RAO. As a consequence, it could be proposed, from the management data collected, that many horses may suffer from sub-clinical inflammation of the respiratory tract, especially during winter.

The analysis presented in chapter 6 identifies risk factors that support the findings of other studies, for example the association of increasing age with the increasing likelihood of RAO. Other outcomes contribute to the generation of hypotheses that warrant further investigation, for example the association of RAO with urbanisation. The latter finding definitely warrants further investigation. In the first instance, the data from this survey could be compared to air pollution statistics that are available from the Department for Environment, Food & Rural Affairs. This governmental body, along with the regionally devolved bodies, funds the National Atmospheric Emissions Inventory (including the National Air Quality Information Archive). These resources provide geographically mapped emissions data for the UK, including the air quality index that combines information on various pollutants (including particulates) that affect human health. The second model generated in the multilevel, multivariable logistic regression addressed the possible influence of early life factors. This again brought to our attention risk factors that may contribute to the inception of RAO. The possible involvement of respiratory infection early in a horse's life contributes to an exciting hypothesis concerning the aetiology of RAO.

Some of the risk factors associated with the likelihood of horses having RAO in the first model described in chapter 6 were the converse of what logic would dictate. For example, horses fed wet hay were more likely to have a positive RSQ for RAO and vice versa for dry hay. An initial cause for concern is that, although soaking hay does dramatically reduce the quantity of respirable particles in hay, it is only effective if done for sufficient time (Blackman and Moore-Colyer, 1998, Swain, 2004). Many horses in this study were fed wet hay that had not been sufficiently soaked. A plausible conclusion of these 'contrary' risk factors is that many owners may only adopt better management practices if their horse develops an overt respiratory problem, leaving sub-clinical problems unrecognised that could potentially contribute to long term problems. It is troubling if the welfare of horses is being compromised purely because of management practices that can easily be changed. This highlights the importance that should be placed on educating horse owners as to the best practices to improve the air quality in their horse's stables.

Despite minimal dust regimens being advocated by veterinary surgeons, backed by scientific research, these regimens were not practised by a majority of owners in this study. It is important to assess the dissemination of such information. The educational booklets appeared to have had an impact with the horse owners and they hopefully increased awareness of not only respiratory health of horses but also the dangers of ragwort poisoning. These booklets have now been made more widely available to the horse owning public. Perhaps veterinary surgeons should be more proactive in encouraging owners to improve the environment of their horses, rather than just responding to problems. Written material may play a role, in combination with other more interactive forms of education, such as practice meetings. One possible use of the RSQ for RAO is that it could be linked to educational material. An owner could complete an RSQ and determine the potential risk of their horse having RAO before being either directed to information aimed at reducing the respiratory challenge or given advice to consult a health professional. This could be implemented on the World Wide Web.

It is hoped that the RSQ for RAO can be used for investigating the disease in the future. Practitioners, both anecdotally (Mair, 1995) and in the survey conducted in chapter 2, have expressed fears that the prevalence of this lifelong, debilitating disease is increasing and repetition of this survey in the future will enable this proposal to be further investigated. The RSQ can be used for assessing both specific populations of horses within the UK as well as populations throughout the world and allow comparisons to be drawn. Differences between populations may assist understanding of the aetiology of the disease. Care should be taken if the RSQ is used in populations overseas as translation into different languages may alter the meaning of questions. This is true even if the RSQ remains in English as respondents in other countries may interpret questions and management practices differently. Interest has been expressed in the RSQ as a tool for identifying individuals to allow further investigation of the aetiology of the disease. The RSQ for RAO is a novel, inexpensive and non-invasive test for identifying horses at higher risk of having RAO in large population-based studies. It has permitted the application of modern epidemiological techniques to the investigation of RAO.

APPENDICES

Appendix A1.1. Published clinical scoring systems for recurrent airway obstruction.

(Naylor *et al.*, 1992)

Severity of chronic obstructive pulmonary disease; score varied from 0 in normal horses to 8 in maximally affected horses.

Variable	Description	Score
Respiratory Rate (breaths/min)	20 21 to 30 31+	0 1 2
Respiratory effort	Normal End expiratory abdominal lift Flared nostrils/anal movement	0 1 2
Lung Auscultation (using (Roudebush, 1982))	Normal Localized wheezes or crackles Generalized wheezes or crackles	0 1 2
Decreased air movement for respiratory effort	No Yes	0 2

(Traub-Dargatz *et al.*, 1992)

Variable	Description	Score
Nasal discharge	No nasal discharge Serous nasal discharge with or without crusting at nares Purulent	0 1 2
Respiratory distress	No signs of respiratory distress Flaring of nostrils or cough but no abdominal effort to respiration Abdominal effort to respiration with no cough or flaring of nostrils Extreme abdominal effort to respiration with/without cough or flaring of nostrils, or abdominal effort to respiration with coughing or flaring of nostrils	0 1 2 3
Degree of anal movement associated with respiration	No movement Anal movement	0 1
Quality of lung sounds	Normal Crackles or wheezes heard occasionally Both crackles and wheezes heard occasionally, but not on every breath Wheezes and/or crackles easily heard on every breath Reduced lung sounds despite deep breath	0 1 2 3 4

(Erichsen *et al.*, 1994)

Entitled 'heavey score' and horses given a "heaviness rating". Based on the belief that expiratory effort correlates best with objective intrapleural pressure as a measure of the severity of bronchial obstruction. Had to show improvement of 1 score with atropine.

Heaviness	Description	Score
Not	Thoracic and abdominal excursion during respiration at rest is barely perceptible	0
Slightly	A slight increase in abdominal excursion with secondary contraction of the abdominal muscle during expiration is detected	1
Moderately	Thoracic and abdominal excursion and secondary contraction of the abdomen is moderately increased	2
Markedly	Thoracic and abdominal excursion and secondary contraction of the abdomen is markedly increased	3
Severely	Thoracic and abdominal excursion and secondary contraction of the abdomen is maximal.	4

(Tesarowski *et al.*, 1996)
Devised weighted score system based on foals that was created by (Hoffman *et al.*, 1992). Clinical signs of heaves when clinical score ≥ 6. Used by (Couetil *et al.*, 2001)

Variable	Description	Score
Respiratory rate (breaths per minute)	<15	0
	16-20	1
	21-25	2
	25-29	3
	>30	4
Nasal discharge	None	0
	Serous	1
	Mucopurulent	3
Abdominal lift	None	0
	Mild (perceptible heave line)	1
	Pronounced (abdomen, thorax and anal movement)	3
Nasal flaring	None	0
	Present	1
Tracheal sounds	Normal (tubular sound)	0
	Increase in intensity	1
	Mucus movement	3
Bronchial tones	Normal	0
	Audible ventral and dorsal sounds	2
Crackles	None	0
	Present	2
Wheezes	None	0
	Present	2
Cough	None	0
	Intermittent	1
	Paroxysmal	3
Thoracic resonance	Normal lung field	0
	Expanded dorsal and ventral lung fields	2
Total		**25**

(Pirie *et al.*, 2001)
Used in studies involving inhaled endotoxin.

Clinical Variable	Response	Score
Cough	Present?	0
	Absent?	1
Nasal discharge	Present?	0
	Absent?	1
Dyspnoea	Absent	0
	Mild	1
	Moderate	2
	Severe	3
Respiratory rate	<20 breaths/minute	0
	20-30 breaths/minute	1
	>30 breaths/minute	2
Thoracic auscultation	Normal	0
	Increased normal	1
	Adventitious lung sounds	2
	Marked adventitious noise	3
Pulse rate	<50 beats/min	0
	50-70 beats/min	1
	>70 beats/min	2
Rectal temperature	Normal	0
	Elevated (>39.5C)	1
Total score		**13**

(Robinson *et al.*, 1994b)

The magnitude of the expiratory effort, abdominal effort and the degree of nasal flaring were each scored from 1 to 4 and the scores totalled to provide a clinical score.

The following clinical scores are all derived from this study:

(Seahorn *et al.*, 1997) for Summer pasture associated obstructive pulmonary disease
Used by (Costa *et al.*, 2000, Venugopal *et al.*, 2001, Beadle *et al.*, 2002)

Variable	Description	Score
Nostril Flare	No or little movement on inspiration	0
	Slight flare during inspiration, returning to normal as inspiration ends	1
	Slight flare during inspiration, returning to near normal as inspiration ends	2
	Greater flare during inspiration and flare does not approach normal position during exhalation	3
	Nostril remains maximally flared throughout respiratory cycle	4
Abdominal lift	No or little movement in the ventral region of the flank	0
	Slight abdominal flattening with heave line just beginning to form in the cranial portion of the ventral aspect of the flank	1
	Obvious abdominal flattening and heave line extending to halfway between the tuber coxae and the cubital joint, but not to the joint	2
	Obvious abdominal flattening and heave line extending beyond halfway between the tuber coxae and the cubital joint, but not to the joint	3
	Obvious abdominal flattening and heave line extending to the cubital joint	4

Clinical score determined by the algorithm (max score was **8**):

$$CS = \frac{\text{medial flare} + \text{lateral flare}}{2} + \text{adbominal lift}$$

(Rush *et al.*, 1998b, Rush *et al.*, 2000)

Variable	Description	Score
Nostril Flaring	Normal (no signs of flaring)	1
	Slight, occasional flaring of nostrils	2
	Moderate nostril flaring	3
	Severe, continuous flaring during each respiration	4
Abdominal expiratory effort	Normal (no signs of dysfunction)	1
	Slight abdominal component	2
	Moderate abdominal component	3
	Severe, marked abdominal component	4

(Robinson *et al.*, 2000) Rating of airway obstruction (RAO).

Variable	Description	Score
Nostril Flaring	No flaring	1
	Slight, occasional flaring of nostrils	2
	Moderate nostril flaring	3
	Severe, continuous flaring during each respiration	4
Abdominal expiratory effort	No abdominal component to breathing	1
	Slight abdominal component	2
	Moderate abdominal component	3
	Severe, marked abdominal component	4

(Henrikson and Rush, 2001)

Adapted (Robinson *et al.*, 2000). Minimum RAO score = 2, max = 8. no signs = 2, mild signs = 3 or 4, moderate signs = 5 or 6, severe signs = 7 or 8. Used by (Robinson *et al.*, 1994b, Olszewski *et al.*, 1999).

Variable	Description	Score
Nostril Flaring	Normal, no signs	1
	Slight, infrequent flaring of nostrils	2
	Moderate nostril flaring	3
	Severe, continuous flaring during respiration	4
Abdominal expiratory effort	Normal	1
	Slight abdominal component	2
	Moderate abdominal component	3
	Severe abdominal component	4

(Gerber *et al.*, 2000)

Variable	Description	Score
Nostril Flare	No or little movement on inspiration	0
	Flare during inspiration, returning to normal as inspiration ends	1
	Flare during inspiration and exhalation	2
Abdominal lift	No or little movement in the ventral region of the flank	0
	Slight abdominal flattening with heave line just beginning to form in the cranial portion of the ventral aspect of the flank	1
	Obvious abdominal flattening and heave line extending no more than halfway between the cubital joint and the tuber coxae	2
	Obvious abdominal lift and heave line extending beyond halfway between the cubital joint and the tuber coxae	3

(Lavoie *et al.*, 2001)

Degree of respiratory distress determined using a respiratory score based on a visual analogue scale from 1-100 and related to the excursion of abdominal muscles and distension of the nostrils during quiet breathing.

Appendix A1.2. Published bronchoalveolar lavage techniques.
PBS = Phosphate buffered saline, SS = Sterile saline, IS = Isotonic polyionic saline

Endoscopic method

	Sedated	Bronchodilator	Twitch	Local Anaesthetic	Type of fluid	Volume of Fluid (ml)	Collection method
Multiple Authors*	-	-	-	-	-	250	-
(Derksen *et al.*, 1985c, Derksen *et al.*, 1989)	Xylazine	-	-	Lidocaine	PBS (37°C)	3x100	-
(Vrins *et al.*, 1991)	Xylazine	-	-	Lidocaine	SS (20°C)	500	Suction
(Traub-Dargatz *et al.*, 1992)	Xylazine	-	-	-	IS (37°C)	300	Suction
(Tremblay *et al.*, 1993)	-	-	-	-	-	2x 250	-
(Sweeney *et al.*, 1994)	Xylazine	Glycopyrrolate	Yes	-	SS (20°C)	3x100	Suction
(Lapointe *et al.*, 1994)	Xylazine and	-	-	Lidocaine (50-	SS (warm)	2x250	Suction
(Leguillette *et al.*, 2002)	butorphanol			100ml; 0.5%)			
(Mills *et al.*, 1996)	Detomidine	-	-	Xylocaine	SS	2x100	Syringe
(Votion *et al.*, 1998)	-	-	-	-	SS (warm)	4x50	-
(Raulo *et al.*, 2001)	-	-	-	Carbocain	SS (37°C)	300	-
(Couetil *et al.*, 2001)	-	-	-	Lidocaine	Sterile saline	250	Suction
(Sandersen *et al.*, 2001)	-	-	-	-	-	200	-

*(Mair *et al.*, 1987, McGorum and Dixon, 1994, Dixon *et al.*, 1995a, Dixon, 1997, Franchini *et al.*, 1998, Franchini *et al.*, 2000)

Blind Method

	Sedated	Twitch	Type of fluid	Volume of fluid (ml)	Collection method
(Fogarty, 1990a)	No	Yes	PBS	180	Syringe
(Watson *et al.*, 1992)	Xylazine	Yes	SS	3x100	Syringe
(Naylor *et al.*, 1992)	Xylazine	-	*	100	Syringe
(McKane *et al.*, 1993)	No	Yes	SS	65 (Cuff used)	Syringe
(McKane and Rose, 1995)	No	Yes	SS	80 (Cuff used)	Syringe
(Rush *et al.*, 1998a)	Xylazine	Yes	SS	300 (Cuff used)	Syringe

*0.145M sodium chloride, 0.0003M ethylene di-sodium tetra-acetate and 0.024M N-2-hydroxyethylpiperazine-N-ethane sulphonic acid in distilled water (plus glucose).

Appendix A2.1.1. Questionnaire used to investigate UK equine practitioners on their attitudes and opinions regarding RAO and its historical and clinical signs, diagnosis and management.
Reduced in size from A4.

2442083205

HOME of
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UNIVERSITY
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GLASGOW

Recurrent Airway Obstruction in Horses

A Confidential Survey of Equine Practitioners

Conducted by the Weipers Centre for Equine Welfare
Division of Equine Clinical Studies
University of Glasgow Veterinary School
Bearsden Road
Glasgow G61 1QH

Tel.: 0141 330 5999

Instructions

- For this survey, we are using the term **'recurrent airway obstruction'** (RAO) to identify the respiratory disease characterised by reversible lower airway obstruction in mature horses induced by environmental challenge. This reversible obstruction is widely believed to result from an airway hypersensitivity reaction following inhalation of allergens, including moulds or spores, that are present in the environment of many horses when they are stabled.
- This condition has been widely referred to in the past as chronic obstructive pulmonary disease (COPD). An international workshop on equine chronic airway disease in June 2000, attended by many leading experts in the field, recommended that it is no longer appropriate to use the term COPD in equine medicine (N.E. Robinson, *Equine Veterinary Journal* (2001) v33, pp5-19).
- When completing the survey, please remember that we are interested in your personal opinion of, and approach to, the diagnosis and treatment of recurrent airway obstruction of horses in the veterinary practice you work in.
- This survey should only take about **15 minutes** to complete.
- Please indicate the **most appropriate** response to **each and every** question using a black (or blue) pen. Where boxes are provided for written answers, please write clearly and use one box per letter like this :

H	O	R	S	E	S	

- If you feel you are unable to answer this questionnaire as you have very limited or no clinical interaction with horses, please tick here ☐ and return the form uncompleted in the pre-paid envelope.
- All responses are **completely confidential** and will be identified by code numbers only.
- Please post this survey, using the reply paid envelope, by **Monday 10th June 2002.**

Thank you for your assistance

1.0 Professional details

1.1 In the last 12 months approximately what percentage of your time during the working day have you dealt exclusively with horses?

- <25% ☐
- 26-50% ☐
- 51-89% ☐
- 90-100% ☐

1.2 Other than horses, what species makes up the major component of the remainder of your working day? (please tick one box only)

- Work exclusively with horses ☐ go to question 1.3
- Farm animal ☐
- Small animal ☐
- Other ☐

Please specify

1.3 What statement best describes the equine work you do in your practice?

- First opinion, ambulatory ☐
- First opinion, predominately ambulatory with clinic based work as required ☐
- First opinion (ambulatory or clinic based) combined with a second opinion, referred caseload ☐
- Principally referral hospital ☐
- Other ☐

Please specify

1.4 Please use percentages to give an estimate of the proportion contributed by each type of horse below to YOUR client population

- Race horses %
- Performance horses (e.g. eventing, endurance) %
- Pleasure horses (e.g. hacking) %
- Show horses %
- Stud %
- Other %

Please specify

2.0 Historical information related to recurrent airway obstruction

2.1 When obtaining a preliminary case history about a horse with suspected recurrent airway obstruction please indicate what importance YOU place on the following information?

	Definitely NOT important	Possibly important	Probably important	Definitely important	No opinion/ do not know
Presenting clinical signs					
Recent cough (within last 2 weeks)	☐	☐	☐	☐	☐
Cough when eating	☐	☐	☐	☐	☐
Cough when exercising	☐	☐	☐	☐	☐
Chronic cough for longer than 3 months	☐	☐	☐	☐	☐
Nasal discharge	☐	☐	☐	☐	☐
Decreased performance	☐	☐	☐	☐	☐
Exercise intolerance	☐	☐	☐	☐	☐
Medical history					
Recent viral respiratory infection	☐	☐	☐	☐	☐
Genetic relatives diagnosed with RAO	☐	☐	☐	☐	☐
Vaccination status	☐	☐	☐	☐	☐
Deworming history	☐	☐	☐	☐	☐
Previous diagnosis of summer pasture associated obstructive pulmonary disease	☐	☐	☐	☐	☐
Feeding management					
Type of forage and method of feeding	☐	☐	☐	☐	☐
Type of concentrate feed and method of feeding	☐	☐	☐	☐	☐
Length of time spent at pasture proportional to time spent stabled during day	☐	☐	☐	☐	☐
Stable management					
Type of bedding	☐	☐	☐	☐	☐
Location of horse when mucking out	☐	☐	☐	☐	☐
Ventilation (air quality in stable)	☐	☐	☐	☐	☐
Drainage in stable (Exposure to noxious gases e.g. ammonia)	☐	☐	☐	☐	☐
Other historical information you feel relevant	☐	☐	☐	☐	☐

Please specify ______________________

3.0 Diagnosis of recurrent airway obstruction

3.1 A wide variety of diagnostic tests are available for the diagnosis of RAO, and to assess its severity. However, often it is deemed unnecessary to perform these procedures, perhaps because of time constraints, or because the results may not affect the outcome. Using the list below, please indicate the methods YOU employ for diagnosing RAO and the frequency with which you use them.

Once you have done this, please rank what you consider to be the five most important, from 1 (most important) to 5 (least important).

	Never	Rarely	Sometimes	Frequently	Always	Rank
Obtain full history	☐	☐	☐	☐	☐	☐
Routine clinical examination (e.g. Temperature, heart and respiratory rate)	☐	☐	☐	☐	☐	☐
Thoracic auscultation **without** a rebreathing bag	☐	☐	☐	☐	☐	☐
Thoracic auscultation **with** a rebreathing bag	☐	☐	☐	☐	☐	☐
Endoscopy of lower airway	☐	☐	☐	☐	☐	☐
Cytology of tracheal aspirates (TTA)	☐	☐	☐	☐	☐	☐
Cytology of bronchoalveolar lavage (BAL)	☐	☐	☐	☐	☐	☐
Thoracic radiographs	☐	☐	☐	☐	☐	☐
Arterial blood gas	☐	☐	☐	☐	☐	☐
Pleural pressure changes (e.g. Ventigraph)	☐	☐	☐	☐	☐	☐
Other pulmonary function tests	☐	☐	☐	☐	☐	☐
Challenge/Provocation test	☐	☐	☐	☐	☐	☐
Other diagnostic test you feel relevant	☐	☐	☐	☐	☐	☐

Please specify ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

If you have not already done so please rank what you consider to be the five most important tests, from 1 (most important) to 5 (least important).

3.2 At what stage of the diagnostic process, for the majority of cases, do you tend to become confident that a horse has RAO, even if you then continue and carry out further confirmatory tests?

- History only ☐
- History and clinical examination ☐
- History, clinical examination and endoscopy ☐
- History, clinical examination, endoscopy and cytology of respiratory samples ☐

3.3 **The following clinical signs have sometimes been reported as being associated with RAO. Please indicate below which signs you associate with the three different degrees of severity: mild, moderate and severe. Some of the clinical signs may occur in relation to more than one degree of severity. For example, if you think a clinical sign is associated with all three degrees of severity, then you should tick all three boxes; if only two apply, please tick only two boxes etc.**

	Mild RAO	Moderate RAO	Severe RAO	Never observe this clinical sign
Respiratory rate 20 - 30 breaths per minute	☐	☐	☐	☐
Respiratory rate >30 breaths per minute	☐	☐	☐	☐
Cough during the examination period	☐	☐	☐	☐
Serous or mucopurulent nasal discharge	☐	☐	☐	☐
Nostril flare at rest	☐	☐	☐	☐
Double expiratory effort at rest (Abdominal breathing)	☐	☐	☐	☐
Presence of a heave line	☐	☐	☐	☐
Cough when squeeze trachea	☐	☐	☐	☐
Coughing or distress when use a rebreathing bag	☐	☐	☐	☐
Wheeze or crackles on auscultation — **Without** a rebreathing	☐	☐	☐	☐
Wheeze or crackles on auscultation — **With** a rebreathing bag	☐	☐	☐	☐
Regions of silence (reduced lung sounds) on auscultation	☐	☐	☐	☐
Other clinical sign you feel relevant	☐	☐	☐	☐

Please specify ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

3.4 **The following endoscopic findings have sometimes been reported as being associated with RAO. Please indicate below which signs you associate with the three different degrees of severity: mild, moderate and severe. You may wish to indicate a particular finding occurs in relation to more than one degree of severity by ticking all the boxes that apply.** If you do not perform endoscopy please go to the next question on page 6.

	Mild RAO	Moderate RAO	Severe RAO	Never observe this finding
Excessive coughing in response to presence of endoscope within trachea	☐	☐	☐	☐
Few isolated mucopus globules in trachea on endoscopy	☐	☐	☐	☐
A pool of mucopus at thoracic inlet on tracheal endoscopy	☐	☐	☐	☐
Oedema (blunting) of the carina at the tracheal bifurcation on endoscopy	☐	☐	☐	☐

4.0 Management and therapy of recurrent airway obstruction

4.1 Once you have made a diagnosis of RAO, do you find any of the following management practices useful when treating the disease?

	Never	Sometimes	Frequently	Almost always
Soaking hay	☐	☐	☐	☐

If **YES,** how long do you recommend soaking for ?

☐☐ : ☐☐ hours : minutes

	Never	Sometimes	Frequently	Almost always
Alternative forage (e.g. haylage, silage)	☐	☐	☐	☐
Complete feed cubes to provide total nutrition	☐	☐	☐	☐
Change of bedding	☐	☐	☐	☐

If **YES,** what do you generally recommend a change to ? (if combination please tick all applicable boxes)

Shavings ☐ Peat ☐ Good quality straw ☐

Rubber matting ☐ Paper/Cardboard ☐ Commercial ☐

Other ☐ **Please specify** ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

	Never	Sometimes	Frequently	Almost always
Changing the environment of other horse's stables in the local vicinity	☐	☐	☐	☐
Improvement of stable location (e.g. away from muck heap, hay store)	☐	☐	☐	☐
Improvement of stable ventilation or design	☐	☐	☐	☐
Increase turnout to pasture	☐	☐	☐	☐
Reduction in level of exercise	☐	☐	☐	☐
Other change in management	☐	☐	☐	☐

Please specify ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

4.2 How often do you prescribe each of the following medications when treating recurrent airway obstruction?

	Never	Sometimes	Frequently	Almost always
Clenbuterol (e.g. Ventipulmin)	☐	☐	☐	☐
Clenbuterol/antibiotic combination (e.g. Ventipulmin TMP/S)	☐	☐	☐	☐
Antibiotics	☐	☐	☐	☐
Mucolytics (e.g. Sputolosin, Bisolvon)	☐	☐	☐	☐
Oral corticosteroids (e.g. prednisolone tablets)	☐	☐	☐	☐
Inhaled corticosteroids (e.g. beclomethasone, fluticasone)	☐	☐	☐	☐
Injectable corticosteroids (e.g. dexamethasone)	☐	☐	☐	☐
Inhaled ß2-agonist bronchodilators (e.g. salbutamol, salmeterol)	☐	☐	☐	☐
Inhaled anticholinergic (e.g. ipratropium)	☐	☐	☐	☐
Systemic anticholinergic (e.g. atropine)	☐	☐	☐	☐
Sodium cromoglycate	☐	☐	☐	☐
Other therapy	☐	☐	☐	☐

Please specify ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

4.3 If YOU prescribe clenbuterol, have you ever administered the drug in progressively higher doses (in a stepwise manner over a number of days) until clinical improvement has occurred?

Yes ☐

No ☐

Do not prescribe clenbuterol ☐

4.4 From your own clinical experience, do you consider the prevalence of RAO to have increased in the last 10 years?

Yes ☐

No ☐

Do not know ☐

Unable to answer ☐

If you have additional information or comments regarding this questionnaire please use the space provided below

Would you be willing to take part in a similar survey in the future? Yes ☐ No ☐

Please return the completed questionnaire using the pre-paid envelope provided.

Thank you for your participation in this survey.

Appendix A2.1.2. Pre-paid reply envelope provided with each questionnaire.

NO
STAMP
REQUIRED

WEIPERS CENTRE FOR EQUINE WELFARE
FREEPOST SCO7397
GLASGOW
G61 1BR

UNIVERSITY
of
GLASGOW

Appendix A2.1.3. Cover letter sent with the initial mailing of the practitioner survey.

Printed on University of Glasgow Veterinary School notepaper (not reproduced).

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«Title» «FirstName» «MiddleName» «LastName» «Suffix»
«BusinessStreet»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

28th May 2002

Dear «Title» «FirstName» «LastName»,

We are writing to invite you to contribute your opinions on the disease recurrent airway obstruction (RAO) in horses. This disease is also known as heaves and formerly as chronic obstructive pulmonary disease (COPD). Please find enclosed a questionnaire booklet and a pre-paid return envelope.

This survey, conducted by the University of Glasgow and funded by the Home of Rest for Horses, forms part of a 3-year study into the epidemiology of RAO in the United Kingdom. This is the first time such a study has been undertaken. By completing the questionnaire you will contribute an important insight into the historical and clinical information used by veterinary surgeons in the diagnosis of RAO, and the thought processes involved. The results of the survey will be used, in combination with other information, to construct a screening questionnaire that will be applied as a tool to study the prevalence of RAO in the UK horse population.

Hopefully you will be able to find time during your busy day to complete this short questionnaire. You have been contacted, in addition to approximately 300 other veterinary surgeons, as it is believed you work with horses in a clinical situation. If this is an incorrect assumption as you have little or no contact with equines please tick the box on the cover of the booklet and return it uncompleted in the envelope provided. All information provided will be kept in the strictest confidence and code numbers will be used for administrative purposes only.

A pre-paid envelope is included for your convenience and it would be helpful if the completed questionnaire could reach us by 10th June 2002. We would like to thank you in anticipation of the valuable information you will provide in your returned questionnaire. If you have any questions regarding this survey please do not hesitate to contact Joel Hotchkiss.

Yours sincerely,

Joel Hotchkiss	Professor Sandy Love
BSc BVSc CertEM(Int.Med) MRCVS	BVMS PhD MRCVS
Home of Rest for Horses' Postgraduate Student	Head of Division of Equine Clinical Studies

Appendix A2.1.4. Cover letter sent with the second mailing to non-responders to the practitioner survey.
Printed on University of Glasgow Veterinary School notepaper (not reproduced).

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«Title» «FirstName» «MiddleName» «LastName» «Suffix»
«BusinessStreet»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

25th June 2002

Dear «Title» «FirstName» «LastName»,

Recently Professor Love and I wrote to you inviting you to contribute your opinions on the disease recurrent airway obstruction (RAO) in horses (also known as heaves and formerly as COPD). I would like to take this opportunity to encourage you to participate in the survey, as I believe the information you can provide is extremely important to my study of the epidemiology of RAO. Please find enclosed a second copy of the questionnaire booklet and a pre-paid return envelope to facilitate your participation.

This survey, conducted by the University of Glasgow and funded by the Home of Rest for Horses, forms part of a 3-year study into the epidemiology of RAO in the United Kingdom. By completing the questionnaire you will contribute an important insight into the historical and clinical information used by veterinary surgeons in the diagnosis of RAO, and the thought processes involved. The results of the survey will be used, in combination with other information, to construct a screening questionnaire that will be applied as a tool to study the prevalence of RAO in the UK horse population.

To date we have had an excellent response to the survey. However, we wish to encompass the opinions of as many people as possible and hope that you can find the time to contribute. If you have been contacted in error as you have little or no contact with equines please tick the box on the cover of the booklet and return it uncompleted in the envelope provided. All information provided will be kept in the strictest confidence and code numbers will be used for administrative purposes only. If you have recently returned the questionnaire, please ignore this letter.

I hope you will not find this follow up communication too intrusive and I would like to thank you for your tolerance. If you have any questions regarding this survey please do not hesitate to contact myself.

Yours sincerely,

Joel Hotchkiss
BSc BVSc CertEM(Int.Med) MRCVS
Home of Rest for Horses' Postgraduate Student

Appendix A2.1.5. Reminder postcards sent in third mailing to all non-responders to the practitioner survey.

HOME of
REST for HORSES

UNIVERSITY of GLASGOW

8th July 2002

Dear «Title» «FirstName» «LastName»

Recently a questionnaire seeking your opinion on recurrent airway obstruction was mailed to you. If you have already completed and returned it to us please accept our thanks. If not, please do so today. It would be greatly appreciated if you could find the time to complete the questionnaire and return it in the prepaid envelope. We believe the information you can provide is extremely important to our study. If you require a copy of the questionnaire please do not hesitate to contact me.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS
DIVISION OF EQUINE CLINICAL STUDIES, WEIPERS CENTRE FOR EQUINE WELFARE
University of Glasgow Veterinary School
Bearsden Road, Glasgow G61 1QH
Telephone: 0141-330 5999 *Fax:* 0141-330 6025

Appendix A2.1.6. Description of response rate to the practitioner survey.

	Number of responses	Proportion (n = 227) (%) [95%CI]	Number declining participation	Proportion (n = 15) (%) [95%CI]	Total response	Proportion (n = 242) (%) [95%CI]
By first deadline	111	48.9 [42.4-55.4]	6	40.0 [16.3-67.7]	117	48.3 [42.1-54.6]
By second mailing	31	13.7 [9.2-18.2]	0	0 [0-21.8]	31	12.8 [8.6-17.0]
By postcard mailing	49	21.6 [16.2-26.9]	4	26.7 [7.8-55.1]	53	21.9 [16.7-27.1]
By final deadline	36	15.9 [11.1-20.6]	5	33.3 [11.8-61.6]	41	16.9 [12.2-21.7]

Appendix A2.1.7. Cox regression or proportional hazards model of time to return of the practitioner questionnaire on receipt of a signed postcard.
Antilog of the coefficient: the hazard ratio, (n = 101).

	Coefficient	Standard Error	exp(coefficient) (hazard ratio)	95% Confidence Interval	P
Signature	0.61	0.32	1.85	0.98 to 3.49	0.059

Appendix A2.1.8. Number of responders, non responders and declines by university of graduation to the practitioner survey.
* International veterinary schools

	Response	No response	Decline
Bristol	29	8	1
Cambridge	35	10	3
Edinburgh	37	10	5
Glasgow	21	6	0
Liverpool	40	8	2
London	40	11	2
Other*	25	7	2

Appendix A2.1.9. Response by university of graduation for each stage of the practitioner questionnaire.
* International veterinary schools

	Bristol	Cambridge	Edinburgh	Glasgow	Liverpool	London	Other*
By deadline	15	16	16	12	20	23	9
By second mailing	5	5	5	2	3	8	3
By reminder postcard	7	6	9	5	11	4	7
By final deadline	2	8	7	2	6	5	6

Appendix A2.1.10. Median number of days to respond to the practitioner survey by university of graduation.
* International veterinary schools

	Median	Inter-quartile range	Range
Bristol	14	9 to 36	3 to 91
Cambridge	16	11 to 42	4 to 88
Edinburgh	21	4 to 39	3 to 60
Glasgow	11	4 to 35	3 to 96
Liverpool	15.5	5 to 36	2 to 81
London	12	9 to 26	3 to 63
Other*	31	11 to 42	2 to 94

Appendix A2.1.11. Time to respond (mean and median numbers of days) to the practitioner survey for different number of years qualified.

	Mean	Median
Up to 5 years	31	35
6 to 10 years	20	14
11 to 15 years	20	14
16 to 20 years	25	14
21 to 25 years	26	35
26 to 30 years	18	12
31 to 35 years	34	29
36 to 45 years	34	39

Appendix A2.1.12. Response to the practitioner survey by number of years qualified.

	Response	No Response
Up to 5 years	23	7
6 to 10 years	36	11
11 to 15 years	55	8
16 to 20 years	32	8
21 to 25 years	32	13
26 to 30 years	27	5
31 to 35 years	15	6
36 to 40 years	6	1
41 to 45 years	2	0
46 to 50 years	0	1

Appendix A2.1.13. The number of responses, by years qualified, for each stage of the practitioner survey process.

	By deadline	By second mailing	By reminder postcard	By final deadline	Total response
Up to 5 years	9	2	7	5	23
6 to 10 years	18	7	7	4	36
11 to 15 years	28	10	11	6	55
16 to 20 years	16	4	4	8	32
21 to 25 years	13	3	11	4	31
26 to 30 years	18	3	3	3	27
31 to 35 years	6	2	2	5	15
36 to 45 years	3	0	4	1	8
Total response	**111**	**31**	**49**	**36**	**227**

Appendix A2.1.14. Cox regression or proportional hazards model of time to return of questionnaire on ownership of a further professional qualification. Antilog of the coefficient: the hazard ratio, z: test of significance (n = 287).

	Coefficient	Standard error	exp(coefficient) (hazard ratio)	95% Confidence interval	P
Further professional qualification	0.22	0.073	1.24	1.07 to 1.43	0.003

Appendix A2.1.15. The number of responses from veterinary surgeons, by region, to the questionnaire, including the proportion declining to participate.

	Response	No response	Declined	Totals (excluding declined)	Prevalence
Scotland	28	1	3	29	0.97
Eastern England	36	4	2	40	0.90
Midlands	21	4	2	25	0.84
Wales	7	2	1	9	0.78
North West	19	6	2	25	0.76
South & South East	63	21	2	84	0.75
South West	34	13	1	47	0.72
North East	17	7	2	24	0.71
Northern Ireland	2	2	0	4	0.50
Totals	227	**60**	**15**	**287**	

Appendix A2.1.16. Response to the practitioner survey from each region of the UK by the stage of mailing.

	By deadline	By second mailing	By postcard mailing	By final deadline	Totals
Scotland	18	4	3	3	28
Eastern England	21	4	7	4	36
Midlands	12	3	3	3	21
Wales	0	2	3	2	7
North West	7	1	9	2	19
South & South East	26	8	17	12	63
South West	14	7	5	8	34
North East	11	2	2	2	17
Northern Ireland	1	0	1	0	2

Appendix A2.1.17. Breakdown of costs for the practitioner survey.

	Cost (£)
First Mailing (n = 302)	
Questionnaire Booklets	139.10
Return Envelopes	28.39
Cover Letters	30.20
A4 envelopes	8.72
Postage first mailing (first-class = 27p)	81.54
Second Mailing (n = 154)	
Questionnaire Booklets	70.93
Return Envelopes	14.48
Cover Letters	15.40
A4 envelopes	4.44
Postage second mailing (first-class = 27p)	41.58
Reminder Postcard Mailing (n = 102)	
Reminder postcard	15.58
Postage postcard	19.38
Return Mailing (n = 242)	
Response Service Licence	30.00
Cost of return postage (second-class = 19.5p)	44.27
Total Cost First Mailing	**287.95**
Total Cost Second Mailing	**146.83**
Total Cost Reminder Postcard	**34.96**
Total Cost of Return Postage	**74.27**
Total Cost of Survey	**544.01**
Total Cost per Useable Return (n = 227)	**2.40**

Appendix A2.2.1. Other types of horse population tended to by respondents to the practitioner survey.

	Number of Respondents
Donkeys	9
Miniature	3
Retired	2
Riding Schools	2
Ponies	2
Heavy Horses	1
Polo	1
Pets	2
Blood Donors	1
Driving	4
Hunters	2
Police	2

Appendix A2.2.2. Comparison of the responses by veterinary surgeons in Scotland to the rest of the UK, on the importance of various historical information factors.

	Scotland	Rest of the UK
A previous diagnosis of SPAOPD		
Definitely not or possibly important	8	21
Probably or definitely important	20	176
Location of a horse when mucking out		
Definitely not or possibly important	9	27
Probably or definitely important	17	172
Drainage in stable (exposure to noxious gases)		
Definitely not or possibly important	8	23
Probably or definitely important	19	175

Appendix A2.2.3. Table of additional diagnostic tests recommended by respondents to the practitioner survey.

Diagnostic test	Frequency
Response to treatment	17, 5 of which cited atropine
Post exercise auscultation/endoscopy	10
Results of haematology	6
Nasal occlusion with auscultation	4
Response to environment change (turn out)	3
Listen at nostrils	3
Elicit cough	2
Allergen testing	2
Culture of BAL or TTA	2
Rule out lungworm	2
Type of cough	1
Percussion	1

Appendix A2.2.4. Comparison of the frequency of use of different diagnostic tests by veterinary surgeons depending on exposure to a referral caseload.

	No referral work	Exposure to referral work
Routine clinical Exam		
Never, Rarely or Sometimes	2	11
Frequently, Always	146	66
Thoracic auscultation with rebreathing		
Never, Rarely or Sometimes	113	48
Frequently, Always	35	28
Endoscopy		
Never, Rarely and Sometimes	111	28
Frequently, Always	38	48
TTA		
Never, Rarely and Sometimes	115	32
Frequently, Always	34	45
BAL		
Never, Rarely and Sometimes	143	65
Frequently, Always	7	12
Thoracic radiographs		
Never, Rarely and Sometimes	149	74
Frequently, Always	0	3

Appendix A2.2.5. Stage of diagnosis that clinicians become confident that a horse has RAO depending on percentage of equine work and exposure to referral work.

	< 90% Equine	> 90% Equine
History	2	7
History & clinical examination	67	115
History, clinical examination, endoscopy & cytology	4	29
	No referral work	**Exposure to referral work**
History	4	5
History & clinical examination	128	54
History, clinical examination, endoscopy & cytology	16	16

Appendix A2.2.6. Degree of RAO severity that respondents associated with different clinical signs.

	Response proportion (%)(n = 227)				
	Mild RAO	Moderate RAO	Severe RAO	Never observe this clinical sign	Not complete
Squeeze cough	**49.3**	26.0	8.4	12.3	4.0
Nasal discharge	**49.8**	37.4	7.9	2.6	2.2
Wheeze (rebrth)	**34.8**	30.0	4.4	3.1	27.8
Cough (exam)	39.2	**50.2**	8.8	0.4	1.3
20 to 30 brths/min	37.9	**51.1**	7.0	0.9	3.1
Cough (rebrth)	13.2	**38.8**	12.8	8.4	26.9
Abdominal	17.6	**53.3**	**29.1**	0.0	0.0
Wheeze (no rebrth)	13.7	**48.0**	**35.7**	0.9	1.8
Heave line	6.6	**48.5**	**44.1**	0.9	0.0
Nostril flare	4.8	36.6	**57.7**	0.4	0.4
> 30 brths/min	2.6	27.8	**67.0**	1.3	1.3
Auscultate silence	4.4	9.3	**40.5**	37.9	7.9

Appendix A2.2.7. Additional clinical signs that respondents believed important for assessing the severity of RAO.

Clinical sign	Frequency
Cough and/or nasal discharge during and/or post exercise	4
Poor exercise tolerance	4
Weight loss	3
Body movement/anal movement with respiration	3
Auscultate at base of trachea	3
Increased heart rate	2
Type of cough	2
Nasal occlusion resentment/cough	2
Audible crackles at nostril	1
Mucus near water bucket	1
Pass wind when cough	1

Appendix A2.2.8. Additional clinical signs that respondents believed important for assessing the severity of RAO as separated by the proportion of equine work they performed.

	< 90% Equine	> 90% Equine
Cough during examination		
Mild RAO	21	68
Moderate RAO	46	68
Severe RAO	6	14
Never	0	0
Presence of heave line		
Mild RAO	8	7
Moderate RAO	29	81
Severe RAO	38	62
Never	0	0
Cough when squeeze trachea		
Mild RAO	21	68
Moderate RAO	46	68
Severe RAO	6	14
Never	14	14

Appendix A2.2.9. Additional management changes found useful in treating cases of RAO.

Management change	Frequency
Feeding from the floor	9
Remove horse from stable when muck out	5
Groom outside	3
Permanent turn out to pasture	4
Increase exercise	4
Regular exercise	1
Herbal feed additives	1
Remove deep litter	1
Ensure adequate drainage	2
Stable design	1
Clean stable (vacuum)	1
Encourage weight loss	2
Move geographically	2
No straw in transport	1

Appendix A3.1. Letter inviting the selected experts to participate in the Delphi consultation process.
Printed on University of Glasgow Veterinary School notepaper (not reproduced).

«Title»«FirstName»«LastName»
«BusinessStreet»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»
«BusinessCountry»

HOME OF REST FOR HORSES

UNIVERSITY of GLASGOW

16th October 2002

Dear «Title»«FirstName»«LastName»

I am an equine veterinary surgeon undertaking a PhD in the Department of Veterinary Clinical Studies at the University of Glasgow, Scotland. The main purpose of my PhD is to conduct quantitative epidemiological studies on recurrent airway obstruction (RAO, 'heaves') in United Kingdom horses using a risk-screening questionnaire (RSQ). The study is being funded by the Home of Rest for Horses. The aim is to construct the RSQ using three main components: 1, the available literature, 2, a survey of the opinions of UK equine practitioners and 3, the opinions of experts in the field of equine respiratory diseases; in particular RAO. Following validation the RSQ would be used in epidemiological surveys in the UK and, if effective, it would be hoped to apply it to other populations overseas.

We have written to you, as we believe you are an acknowledged expert in the respiratory field with publications on the subject of RAO and participation in the International Workshop on Equine Chronic Airway Disease (June 2000). We would like to invite you to participate in this consultation of experts on the relative importance of different clinical signs in the diagnosis of RAO. For this project we will be using the Delphi method, a technique that has been widely used for consulting experts in a range of fields, including that of human medicine. The Delphi method utilises anonymous written responses and permits feedback to participants in the form of statistical summaries of the group's responses. The technique aims to establish some degree of consensus amongst participants.

If you kindly agree to participate in this process it should entail a maximum of three questionnaires over a time period of 2 to 3 months, each taking only 15 minutes to complete. In the rounds subsequent to the first questionnaire a summary of the results of the previous round is included and evaluated by the participants. A formal summary of results will be provided to the participants on completion of the process. All information obtained will be kept in the strictest confidence and code numbers used to identify responses. If desired, future Delphi rounds may be completed utilising e-mail.

If you are willing to participate in this Delphi process please complete the enclosed questionnaire and return it using the prepaid envelope by 31st October 2002. If you do not wish to participate please return the questionnaire uncompleted. I would like to emphasise that only 25 people have been contacted in this survey so your opinion is extremely important to me. I appreciate how valuable your time is, but we hope you will find the process rewarding. Thank you for your assistance and time

Yours sincerely

Joel Hotchkiss
BSc BVSc CertEM(Int.Med) MRCVS
Home of Rest for Horses' Postgraduate Student

Professor Sandy Love
BVMS PhD MRCVS
Head of Division of Equine Clinical Studies

Appendix A3.2. Questionnaire booklet for first round of the Delphi consultation.
Reduced in size from A4.

2158208556

HOME of
REST for HORSES

UNIVERSITY
of
GLASGOW

Recurrent Airway Obstruction in Horses

An Expert Panel Delphi Consultation Process on the Relative Importance of History and Clinical Signs

Round One

Conducted by the Weipers Centre for Equine Welfare
Division of Equine Clinical Studies
University of Glasgow Veterinary School
Bearsden Road
Glasgow G61 1QH
UK
Tel.: (0044) (0)141 330 5999

Instructions

- For this process, we are using the term **'recurrent airway obstruction' (RAO)** to identify the respiratory disease characterised by reversible lower airway obstruction in mature horses induced by environmental challenge that can be associated with housing.
- When completing the questionnaire, please utilise your personal experience including your knowledge of the available literature, research you may have performed and clinical judgement in the field of RAO. In answering this questionnaire there should be no need to consult the literature.
- This questionnaire should only take about **15 minutes** to complete.
- Please indicate the **most appropriate** response to **each and every** question, use a black (or blue) pen to place a tick or cross in the corresponding box, thus : ☒
- Each section has space provided for additional criteria you believe that should have been included. Please justify, or explain the reasoning behind, these added criteria in the comments area at the bottom of each page.
- All responses are **completely confidential** and will be identified by code numbers only.
- Please post this survey, using the reply paid envelope, by
- Once all the participants have returned their questionnaires the responses will be analyzed. The second questionnaire, with participant suggested modifications, and a feedback report of the first round will then be mailed. This will provide an opportunity for you to reconsider or refine your opinions in the light of the group response.
- If you have any queries please do not hesitate to contact me using the above details or by e-mail at j.hotchkiss@vet.gla.ac.uk

Thank you for your assistance

1.0 Questions Related to Historical Information

Of all the diagnosed cases of RAO that you have observed in the past, how important did you consider the following historical information in reaching that diagnosis?

On a scale from 0 to 100, where 0 = Definitely NOT important, to 100 = Definitely important.

Historical Information	0 Definitely NOT important	1-20	21-40	41-60	61-80	81-99	100 Definitely important
Age of horse	☐	☐	☐	☐	☐	☐	☐
Breed of horse	☐	☐	☐	☐	☐	☐	☐
Activity or use of horse	☐	☐	☐	☐	☐	☐	☐
Genetic relative already diagnosed with RAO	☐	☐	☐	☐	☐	☐	☐
Vaccination history	☐	☐	☐	☐	☐	☐	☐
History of poor performance	☐	☐	☐	☐	☐	☐	☐
History of exercise intolerance	☐	☐	☐	☐	☐	☐	☐
History of viral infection (febrile respiratory disease)	☐	☐	☐	☐	☐	☐	☐
History of recent viral infection (febrile respiratory disease)	☐	☐	☐	☐	☐	☐	☐
Geographical location of horse (prevalent weather conditions)	☐	☐	☐	☐	☐	☐	☐
Season in which disease developed (owner reported)	☐	☐	☐	☐	☐	☐	☐
Stable design - ventilation	☐	☐	☐	☐	☐	☐	☐
Stable design - drainage	☐	☐	☐	☐	☐	☐	☐
Type of bedding provided in stable	☐	☐	☐	☐	☐	☐	☐
Type of forage provided	☐	☐	☐	☐	☐	☐	☐
Length of time spent each day at pasture	☐	☐	☐	☐	☐	☐	☐
Other, please specify below: ______________________	☐	☐	☐	☐	☐	☐	☐
Other, please specify below: ______________________	☐	☐	☐	☐	☐	☐	☐

Any comments on this section/justification for additional criteria

2.0 Questions Related to Clinical Signs (Part 1)

Of all the diagnosed cases of RAO that you have observed in the past, approximately what percentage have exhibited each of the following signs, either during clinical examination or in the month previous to the examination, as a result of the disease?

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
A cough	☐	☐	☐	☐	☐	☐	☐
Coughing more than four times in 24 hrs	☐	☐	☐	☐	☐	☐	☐
Coughing at the start of exercise	☐	☐	☐	☐	☐	☐	☐
Coughing during exercise	☐	☐	☐	☐	☐	☐	☐
Cough more than four times during exercise	☐	☐	☐	☐	☐	☐	☐
Coughing when eating	☐	☐	☐	☐	☐	☐	☐
Coughing during an examination period	☐	☐	☐	☐	☐	☐	☐
Coughing when squeeze larynx/trachea	☐	☐	☐	☐	☐	☐	☐
A nasal discharge	☐	☐	☐	☐	☐	☐	☐
A nasal discharge following exercise	☐	☐	☐	☐	☐	☐	☐
Other, please specify below: ______________________	☐	☐	☐	☐	☐	☐	☐
Other, please specify below: ______________________	☐	☐	☐	☐	☐	☐	☐

Of all the coughing horses that you have observed in the past, what would the likelihood be of them being diagnosed with RAO if they have been coughing for:

On a scale from 0 to 100, where 0 = Could not be less likely, to 100 = Could not be more likely.

	0 (Not likely)	1-20	21-40	41-60	61-80	81-99	100 (Very likely)
Two weeks	☐	☐	☐	☐	☐	☐	☐
Four weeks	☐	☐	☐	☐	☐	☐	☐
Three months	☐	☐	☐	☐	☐	☐	☐

Any comments on this section/justification for additional criteria

3.0 Questions Related to Clinical Signs (Part 2)

Of all the diagnosed cases of RAO that you have observed in the past, approximately what percentage have exhibited each of the following signs, either during clinical examination or in the month previous to the examination, as a result of the disease?

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
Increased expiratory abdominal effort:							
at rest	☐	☐	☐	☐	☐	☐	☐
when stabled in high dust conditions	☐	☐	☐	☐	☐	☐	☐
when fed dry hay	☐	☐	☐	☐	☐	☐	☐
following/during mucking out	☐	☐	☐	☐	☐	☐	☐
Increased/prolonged expiratory abdominal effort post-exercise	☐	☐	☐	☐	☐	☐	☐
A visible heave line	☐	☐	☐	☐	☐	☐	☐
Movement of anus with respiration	☐	☐	☐	☐	☐	☐	☐
Other, please specify below: ______________________	☐	☐	☐	☐	☐	☐	☐

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
Flaring of nostrils:							
at rest	☐	☐	☐	☐	☐	☐	☐
when stabled in high dust conditions	☐	☐	☐	☐	☐	☐	☐
when fed dry hay	☐	☐	☐	☐	☐	☐	☐
following/during mucking out	☐	☐	☐	☐	☐	☐	☐
Increased/prolonged flaring of nostrils post-exercise	☐	☐	☐	☐	☐	☐	☐
Other, please specify below: ______________________	☐	☐	☐	☐	☐	☐	☐

Any comments on this section/justification for additional criteria

4.0 Questions Related to Clinical Signs (Part 3)

Of all the diagnosed cases of RAO that you have observed in the past, approximately what percentage have exhibited each of the following signs, either during clinical examination or in the month previous to the examination, as a result of the disease?

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
Elevated respiratory rate	☐	☐	☐	☐	☐	☐	☐
Elevated heart rate	☐	☐	☐	☐	☐	☐	☐
Wheezing audible at nostrils	☐	☐	☐	☐	☐	☐	☐
Abnormal tracheal auscultation	☐	☐	☐	☐	☐	☐	☐
Thoracic auscultation:							
Audible wheezes	☐	☐	☐	☐	☐	☐	☐
Audible crackles	☐	☐	☐	☐	☐	☐	☐
Silence	☐	☐	☐	☐	☐	☐	☐
Thoracic auscultation, with rebreathing bag:							
Audible wheezes	☐	☐	☐	☐	☐	☐	☐
Audible crackles	☐	☐	☐	☐	☐	☐	☐
Silence	☐	☐	☐	☐	☐	☐	☐
Other, please specify below: ______________________	☐	☐	☐	☐	☐	☐	☐

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
Weight loss	☐	☐	☐	☐	☐	☐	☐
Exercise intolerance	☐	☐	☐	☐	☐	☐	☐
Prolonged recovery from exercise	☐	☐	☐	☐	☐	☐	☐
Exacerbation of clinical signs when challenged by hay/straw	☐	☐	☐	☐	☐	☐	☐
Other, please specify below: ______________________	☐	☐	☐	☐	☐	☐	☐
Other, please specify below: ______________________	☐	☐	☐	☐	☐	☐	☐

Any comments on this section/justification for additional criteria

Thank You for Your Participation.

Please use the space below for any comments or further information you would like to contribute

If you would prefer to participate in the next Delphi round using e-mail combined with a Word for Windows document, please tick the box below and write your e-mail address in the space provided

I would prefer to use e-mail ☐

E-mail address:

Appendix A3.3. Cover letter and questionnaire booklet for second round of the Delphi consultation – sent by e-mail.

HOME of REST for HORSES

UNIVERSITY *of* GLASGOW

«Title»«FirstName»«LastName»
«BusinessStreet»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»
«BusinessCountry»

14th November 2002

Dear «Title»«FirstName»«LastName»

Thank you for returning the questionnaire so promptly and in doing so agreeing to participate in this Delphi consultation. A total of 21 out of the 26 experts contacted have agreed to participate. Please find enclosed the questionnaire for the second round of this process. The questionnaire is very similar to the previous one. As far as possible, additional criteria that were suggested have been incorporated. Other existing criteria have required further explanation. In addition as many respondents' comments as possible have been addressed and **all** have been noted.

All the participants' responses from the last round have been summarised for each criteria. These responses have been summarised in a graphical form as laid out in the example below. A red star indicates the median and the red line indicates the interquartile range (first to third quartiles) that represents the central 50% of the responses. The red shading around the tick box indicates your response to the criteria in the first round.

	0 Definitely **NOT** important	1-20	21-40	41-60	61-80	81-99	100 Definitely important
				Interquartile range		Median *	
Age of horse	☐	☐	☐	☐	☐	☐	☐
					Your response		

We would request that you reconsider the responses you gave last time in the light of the group response. This is to encourage a degree of consensus but in no way should it force you to alter you views, unless desired.

This is the second of three rounds and should only take 15 minutes to complete. Depending on the responses to this round the third round may be a similar process or it may be a final summary. All information obtained will be kept in the strictest confidence and code numbers used to identify responses. Please complete the attached questionnaire following the instructions below and return it as an email attachment by 29th November 2002. Thank you again for your assistance and time

Yours sincerely

Joel Hotchkiss
BSc BVSc CertEM(Int.Med) MRCVS
Home of Rest for Horses' Postgraduate Student

Doctor Rob Christley
BVSc MACVSc PhD MRCVS

Professor Sandy Love
BVMS PhD MRCVS

HOME of REST for HORSES

UNIVERSITY of GLASGOW

Recurrent Airway Obstruction in Horses

An Expert Panel Delphi Consultation Process on the Relative Importance of History and Clinical Signs

Round Two

Conducted by the Weipers Centre for Equine Welfare
Division of Equine Clinical Studies
University of Glasgow Veterinary School
Bearsden Road
Glasgow G61 1QH
United Kingdom

Tel.: +44 (0)141 330 5999

Instructions

- This is the second round of our consultation of experts on the relative importance of different clinical signs in the diagnosis of **recurrent airway obstruction (RAO).**
- When completing the questionnaire, please utilise your personal experience including your knowledge of the available literature, research you may have performed and clinical judgement in the field of RAO. In answering this questionnaire there should be no need to consult the literature.
- This questionnaire should only take about **15 minutes** to complete.
- Please indicate the **most appropriate** response to **each and every** question using your mouse for tick boxes. For written answers, mouse click over the **top left corner** of the appropriate yellow box and type your response (see right). Once you have completed the survey please save it and e-mail the file, as an attachment, to me at j.hotchkiss@vet.gla.ac.uk by November 2002.

Click here

- Each section has space provided for additional comments at the bottom of each page.
- All responses are **completely confidential** and will be identified by code numbers only.
- If you have any queries please do not hesitate to contact me using the above details or by e-mail at j.hotchkiss@vet.gla.ac.uk

Thank you for your assistance

Further Explanation in Response to Comments by Participants

In the last round the term recurrent airway obstruction (RAO) was used to identify the respiratory disease characterised by reversible lower airway obstruction in mature horses induced by environmental challenge that can be associated with housing. This statement was made taking into account the recommendations of the International Workshop on Equine Chronic Airway Disease as published in the Equine Veterinary Journal (2001), volume 33, pages 5-19. It was an omission on my part not to draw attention to this reference.

This definition of reversible obstruction in relation to RAO is crucial as it is this that sets it apart from other respiratory diseases such as Inflammatory Airway Disease (IAD). Recently there has been a workshop concerning this syndrome. However, the results of this meeting are not generally available to all the participants in this Delphi consultation process at the present time.

It is accepted that the clinical signs present at any particular time will vary depending on the individual animal and the stage of disease at the time of examination e.g. remission or crisis. In an attempt to address this problem, the questions relating to clinical signs contain the phrase ***"or in the month previous to the examination".*** One month was chosen as a realistic time period over which the presence or absence of a clinical sign could be estimated.

All the information you have supplied has been based on **"all diagnosed cases of RAO that you have observed"**. This wording was selected to encourage participants to consider all cases of RAO that they had diagnosed using their particular "gold standard" ancillary diagnostic tool e.g. tracheal wash, bronchoalveolar lavage, lung function tests etc. Participants could then indicate what proportion of all these cases actually presented with a particular clinical sign. Thus, theoretically, the multitude of different types of presentation (e.g. those in crisis, remission, long or short duration etc) could be considered in each question.

The intensity of examination and number of times the horse is examined (repetition) will affect what clinical signs are detected. Ancillary diagnostic tools have not been discussed to attempt to shorten this consultation process and allow concentration on history and clinical signs only.

1.0 Questions Related to Historical Information

Of all the diagnosed cases of RAO that you have observed in the past, how important did you consider the following historical information in reaching that diagnosis?

On a scale from 0 to 100, where 0 = Definitely Not important, to 100 = Definitely important

Historical Information	**0** Definitely **NOT** important	**1-20**	**21-40**	**41-60**	**61-80**	**81-99**	**100** Definitely important
Age of horse	☐	☐	☐	☐	☐	* ☐	☐
Breed of horse	* ☐	☐	☐	☐	☐	☐	☐
Activity or use of horse	☐	☐	* ☐	☐	☐	☐	☐
Genetic relative already diagnosed with RAO	☐	* ☐	☐	☐	☐	☐	☐
Vaccination history	☐	* ☐	☐	☐	☐	☐	☐
History of poor performance (subtle effect on athletic potential)	☐	☐	☐	☐	* ☐	☐	☐
History of exercise intolerance (intolerance to low level of exercise)	☐	☐	☐	☐	* ☐	☐	☐
History of viral infection (febrile respiratory disease)	☐	☐	* ☐	☐	☐	☐	☐
History of <u>recent</u> viral infection (febrile respiratory disease)	☐	☐	* ☐	☐	☐	☐	☐
Geographical location of horse (prevalent weather conditions)	☐	☐	☐	☐	* ☐	☐	☐
Season in which disease first developed (owner reported)	☐	☐	☐	☐	* ☐	☐	☐
Stable design – ventilation	☐	☐	☐	☐	☐	* ☐	☐
Stable design – drainage	☐	☐	* ☐	☐	☐	☐	☐
Type and quality of bedding provided in stable	☐	☐	☐	☐	☐	☐	* ☐

Historical Information (continued)	**0** Definitely **NOT** important	**1-20**	**21-40**	**41-60**	**61-80**	**81-99**	**100** Definitely important
Type and quality of forage provided	☐	☐	☐	☐	☐	☐	* ☐
Length of time spent each day at pasture	☐	☐	☐	☐	☐	* ☐	☐

Recommendations for additional criteria made by participants

Location of horse for feeding/mucking out/grooming	☐	☐	☐	☐	☐	☐	☐
Storage of forage/bedding and location of muck heap in relation to stable/stall	☐	☐	☐	☐	☐	☐	☐
History of exposure to donkeys	☐	☐	☐	☐	☐	☐	☐
Duration of problem (Recurrent?)	☐	☐	☐	☐	☐	☐	☐
Alterations to normal routine exposing horse to high dust conditions e.g. transportation, stabling at competitions etc	☐	☐	☐	☐	☐	☐	☐
Improvement when at pasture	☐	☐	☐	☐	☐	☐	☐
Health of contemporaries, including others diagnosed with RAO	☐	☐	☐	☐	☐	☐	☐

Status of contemporaries may give an indication of exposure to antigens in their management system. Otherwise health of contemporaries might indicate the presence of an infectious disease.

Please type any comments on this section

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2.0 Questions Related to Clinical Signs (Part 1)

Of all the diagnosed cases of RAO that you have observed in the past, approximately what percentage have exhibited each of the following signs, either during clinical examination *or in the month previous to the examination*, as a result of the disease?

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
A cough	☐	☐	☐	☐	☐	* ☐	☐
Coughing more than four times in 24 hrs	☐	☐	☐	☐	* ☐	☐	☐
Coughing at the start of exercise	☐	☐	☐	☐	☐	* ☐	☐
Coughing during exercise	☐	☐	☐	☐	* ☐	☐	☐
Cough more than four times during exercise	☐	☐	☐	☐	* ☐	☐	☐
Coughing when eating	☐	☐	☐	* ☐	☐	☐	☐
Coughing during an examination period	☐	☐	* ☐	☐	☐	☐	☐
Coughing when squeeze larynx/trachea	☐	☐	☐	* ☐	☐	☐	☐
A nasal discharge	☐	☐	☐	* ☐	☐	☐	☐
A nasal discharge following exercise	☐	☐	☐	* ☐	☐	☐	☐

Recommendations for additional criteria made by participants

Evidence of previous nasal discharge i.e. dried debris	☐	☐	☐	☐	☐	☐	☐
Nasal discharge after sedation or when head lowered after travelling	☐	☐	☐	☐	☐	☐	☐

Of all the coughing horses that you have observed in the past, what would be the likelihood of them being diagnosed with RAO if they have been coughing for:

On a scale from 0 to 100, where 0 = Could not be less likely, to 100 = Could not be more likely.

Time period of cough	**0** (Not likely)	**1-20**	**21-40**	**41-60**	**61-80**	**81-99**	**100** (Very likely)
Two weeks	☐	☐	☐	☐	☐	☐	☐
Four weeks	☐	☐	☐	☐	☐	☐	☐
Three months	☐	☐	☐	☐	☐	☐	☐

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3.0 Questions Related to Clinical Signs (Part 2)

Of all the diagnosed cases of RAO that you have observed in the past, approximately what percentage have exhibited each of the following signs, either during clinical examination *or in the month previous to the examination*, as a result of the disease?

Sign	**0%** (None)	**1-20%**	**21-40%**	**41-60%**	**61-80%**	**81-99%**	**100%** (All)
Increased expiratory abdominal effort:							
• at rest	☐	☐	☐	☐	☐	☐	☐
• when stabled in high dust conditions	☐	☐	☐	☐	☐	☐	☐
• when fed dry hay (poor quality)	☐	☐	☐	☐	☐	☐	☐
• following/during mucking out	☐	☐	☐	☐	☐	☐	☐
Increased/prolonged expiratory abdominal effort post-exercise	☐	☐	☐	☐	☐	☐	☐
A visible heave line	☐	☐	☐	☐	☐	☐	☐
Movement of anus with respiration	☐	☐	☐	☐	☐	☐	☐

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
Flaring of nostrils:							
• at rest	☐	☐	* ☐	☐	☐	☐	☐
• when stabled in high dust conditions	☐	☐	☐	☐	☐	* ☐	☐
• when fed dry hay (poor quality)	☐	☐	☐	☐	* ☐	☐	☐
• following/during mucking out	☐	☐	☐	* ☐	☐	☐	☐
Increased/prolonged flaring of nostrils post-exercise	☐	☐	☐	☐	* ☐	☐	☐

Please type any comments on this section

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4.0 Questions Related to Clinical Signs (Part 3)

Of all the diagnosed cases of RAO that you have observed in the past, approximately what percentage have exhibited each of the following signs, either during clinical examination *or in the month previous to the examination*, as a result of the disease?

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
Increased respiratory rate	☐	☐	☐	* ☐	☐	☐	☐
Increased heart rate	☐	* ☐	☐	☐	☐	☐	☐
Wheezing audible at nostrils	☐	*	☐	☐	☐	☐	☐
Abnormal tracheal auscultation	☐	☐	☐	☐	* ☐	☐	☐
Thoracic auscultation:							
• Audible wheezes	☐	☐	☐	* ☐	☐	☐	☐
• Audible crackles	☐	☐	* ☐	☐	☐	☐	☐
• Silence (regions of minimal air flow as a result of severe obstruction)	☐	* ☐	☐	☐	☐	☐	☐

Of all the diagnosed cases of RAO that you have observed in the past, approximately what percentage have exhibited each of the following signs, either during clinical examination *or in the month previous to the examination*, as a result of the disease?

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
Thoracic auscultation, with rebreathing bag:							
• **Audible wheezes**	☐	☐	☐	☐	* ☐	☐	☐
• **Audible crackles**	☐	☐	☐	* ☐	☐	☐	☐
• **Regions of silence** (regions of minimal air flow as a result of severe obstruction)	☐	* ☐	☐	☐	☐	☐	☐

Recommendations for additional criteria made by participants

Coughing following use of rebreathing bag or nasal occlusion	☐	☐	☐	☐	☐	☐	☐
Increased area of auscultation	☐	☐	☐	☐	☐	☐	☐

Sign	0% (None)	1-20%	21-40%	41-60%	61-80%	81-99%	100% (All)
Weight loss	☐	* ☐	☐	☐	☐	☐	☐
Exercise intolerance	☐	☐	☐	☐	☐	* ☐	☐
Prolonged recovery from exercise	☐	☐	☐	☐	☐	* ☐	☐
Exacerbations of clinical signs when challenged by hay/straw	☐	☐	☐	☐	☐	* ☐	☐

Please use the space below for any comments or further information you would like to contribute

Click here

Thank You for Your Participation

Appendix A3.4. Final letter concluding the Delphi consultation process.
Printed on University of Glasgow Veterinary School notepaper (not reproduced).

«Title»«FirstName»«LastName»
«BusinessStreet»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»
«BusinessCountry»

HOME OF REST FOR HORSES

UNIVERSITY of GLASGOW

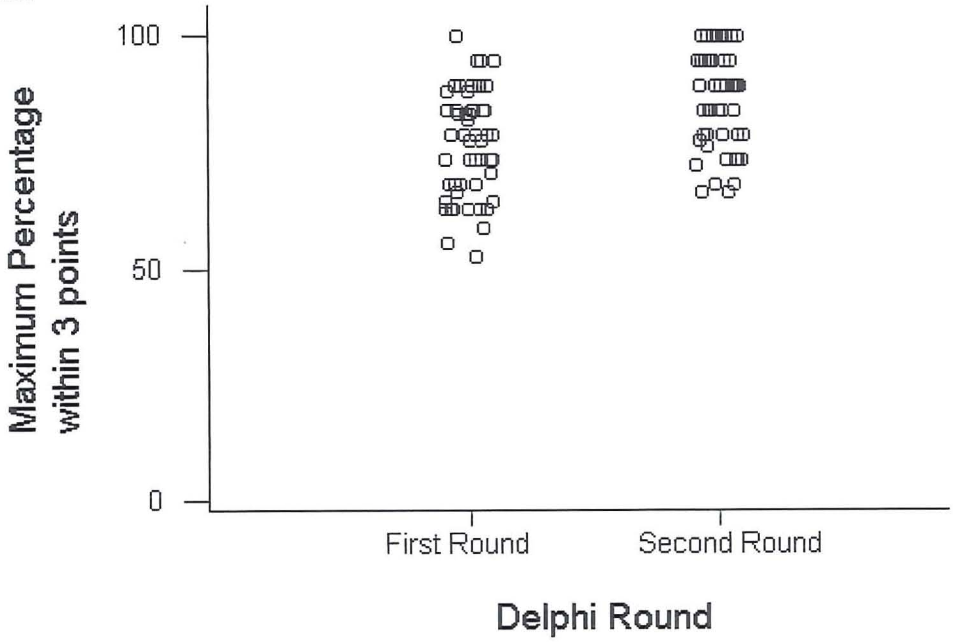
17th February 2003

Dear «Title»«FirstName»«LastName»

Thank you for returning your second round questionnaire to me; you will be relieved to know that that was the final round. This is just a short letter to summarise the results of the process. The initial analysis has already been used to guide the construction of a risk-screening questionnaire (RSQ) for recurrent airway obstruction (RAO). This RSQ is now in the early stages of testing and validation.

A total of 26 experts were invited to participate in this Delphi consultation process on the relative importance of history and clinical signs in RAO. Twenty-one agreed to contribute to the first round and 19 completed both rounds. Item omission was minimal and was virtually eliminated in the second round by clarification of some criteria. Responses were on a 7-point scale with a total of 55 criteria in the first round. Thirteen additional criteria were recommended and these were included in the second round of the Delphi process.

Consensus was pre-defined as 75% or more of the participants placing their choice within 3 points of each other. In the first round the mean maximum percentage of participants within 3 points of each other was 77.17% (sd= 11.06; n=55). This increased to a mean of 86.73% (sd= 9.88, n=55); the mean difference being –9.56% (confidence intervals =-11.41% to -7.71%). This implies that the second round encouraged, on average per question, between 1 and 2 people to alter their position to within the most popular 3 points.



For each criterion the group position (median) did not change greatly between rounds but responses became more closely grouped about the median. The number of outliers was greatly reduced in the second round.

Examples of Boxplots to Demonstrate Changes in Response from First to Second Round

Figure 1. Boxplots of the responses to the question:

"Of all the diagnosed cases of RAO that you have observed in the past, approximately what percentage have exhibited each of the following signs, either during clinical examination *or in the month previous to the examination,* as a result of the disease?"

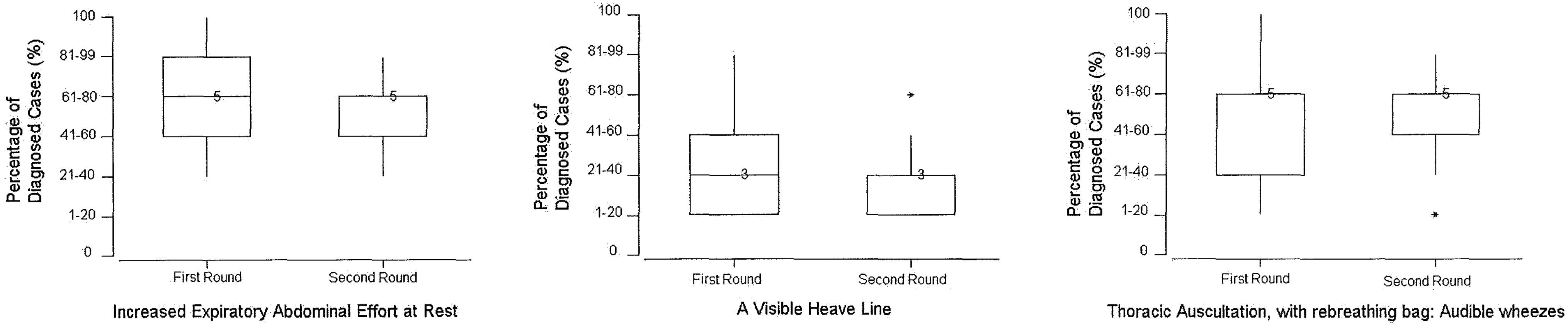
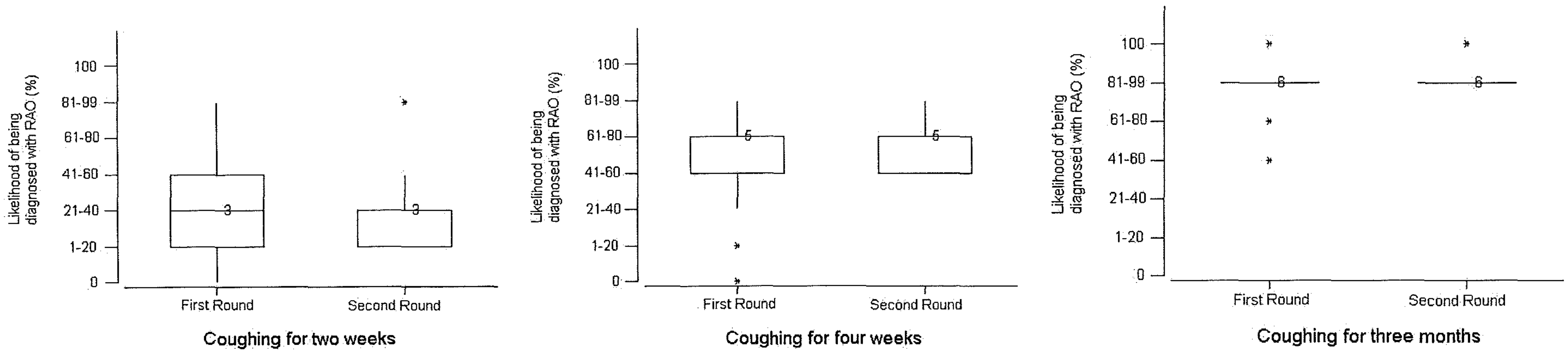


Figure 2. Boxplots of the responses to the question:

"Of all the coughing horses that you have observed in the past, what would be the likelihood of them being diagnosed with RAO if they have been coughing for: 2 weeks, 4 weeks or 3 months. On a scale from 0 to 100, where 0= Could not be less likely, to 100= Could not be more likely"



All the criteria were considered for inclusion in the RSQ. Reasons for exclusion of possible criteria included:

1. Criteria that were ambiguous and/or achieved no measure of consensus
2. Criteria that related to the results of an actual clinical examination by a veterinary surgeon.
3. Criteria that were related to the environment, i.e. those that could be considered to be risk factors.

The remaining criteria had achieved a degree of consensus, with greater than 75% of participants placing their opinions within 3 points. The tables below contain the major remaining criteria (shaded region includes criteria requiring clinical examination):

Importance of Historical Information

Criteria	n	Median	Interquartile range	Consensus	
				%	Range
Age	19	81-99	61-100	78.9	61-100
Poor Performance/Exercise Intolerance	19	61-80	50-90	78.9	21-80
Season in which disease first developed	19	61-80	50-99	78.9	41-99
Duration of problem	18	81-99	61-99	88.9	61-100
Improvement at pasture	18	89-99	81-99	88.9	81-100

Percentage of Diagnosed Cases Exhibiting Clinical Signs

Criteria	n	Median	Interquartile range	Consensus	
				%	Range
A cough	19	81-99	61-99	89.5	61-99
Cough at start of exercise	19	81-99	61-99	78.9	61-99
Cough during exercise	19	61-80	41-90	94.7	41-99
Nasal discharge	19	41-60	10-60	84.2	1-60
Coughing for two weeks	19	21-40	1-40	78.9	1-40
Coughing for four weeks	19	61-80	41-80	89.5	41-80
Coughing for three months	19	81-99	81-99	89.5	81-99
Increased expiratory abdominal effort:					
At rest	19	61-80	41-80	94.7	21-80
When stabled in high dust	19	81-99	61-99	89.5	61-100
When fed dry hay	19	61-80	61-99	78.9	61-99
Prolonged post-exercise	19	61-80	61-99	78.9	61-100
A heave line	19	21-40	1-40	78.9	1-40
Movement of anus with respiration	19	1-20	1-30	100	1-40
Flaring of nostrils:					
At rest	19	21-40	1-50	84.2	1-60
When stabled in high dust	18	61-80	50-99	77.8	41-99
Prolonged post-exercise	17	61-80	41-99	82.4	41-99
Increased respiratory rate	19	41-60	21-80	84.2	21-80
Prolonged recovery from exercise	19	81-99	61-99	84.2	81-99
Exacerbations when hay challenge	19	81-99	70-100	89.5	61-100
Increased heart rate	19	1-20	1-20	94.7	1-40
Wheeze audible at nostrils	19	1-20	1-20	84.2	1-20
Abnormal tracheal auscultation	19	61-80	41-80	84.2	21-80
Auscultation – wheeze	19	21-40	21-60	94.7	1-40
Auscultation – crackles	19	21-40	21-60	78.9	21-60
Auscultation – silence	19	1-20	0-20	94.7	0-20
Auscultation-rebreathing – wheeze	19	61-80	41-80	89.5	21-80
Auscultation-rebreathing – crackles	19	41-60	41-80	84.2	21-80
Auscultation-rebreathing – silence	19	1-20	0-20	89.5	0-20
Weight loss	19	1-20	1-20	84.2	0-20

This information was combined with that generated from a survey of equine practitioners and a literature review to help construct the RSQ. Obviously the information generated from this process is only qualitative and any conclusions made will reflect this. Complicating factors are probably innumerable, not least those of disease definition. It is intended to use the data from the Delphi process to provide decision rules as one way of interpreting the RSQ.

In the future it is intended to publish details of how this Delphi process was conducted and copies of the manuscript will be forwarded to the participants at this time. All information will remain anonymous unless requested. Thank you again for your assistance in this project and I hope it has proved interesting.

Yours sincerely

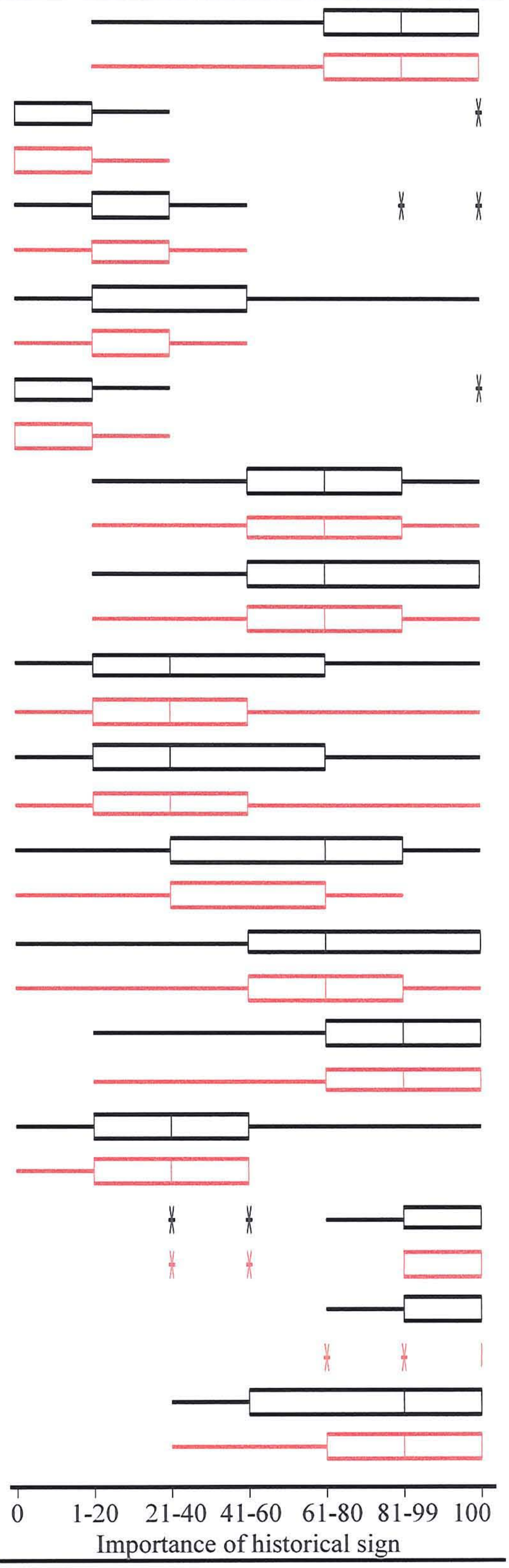
Joel Hotchkiss
BSc BVSc CertEM(Int.Med) MRCVS
Home of Rest for Horses' Postgraduate Student

Doctor Rob Christley
BVSc MACVSc PhD MRCVS

Professor Sandy Love
BVMS PhD MRCVS

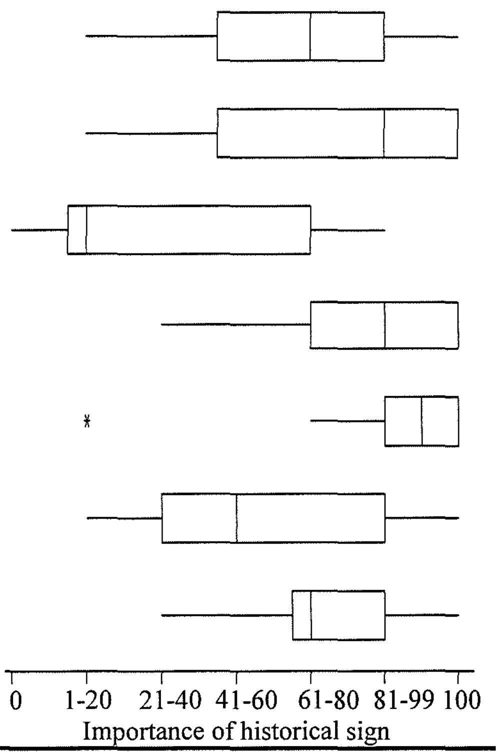
Appendix A3.5a. Summary table for Delphi group responses after round one and round two for items included in the historical information section.
Box plots: Black = first round. Red = second round.

	Round	Median (range)	Inter-quartile range
Age of horse	1	81 to 99	61 to 80
	2	81 to 99	61 to 80
Breed of horse	1	0	0 to 20
	2	0	0 to 20
Activity or use of horse	1	21 to 40	1 to 40
	2	21 to 40	1 to 40
Genetic relative already diagnosed with RAO	1	1 to 20	1 to 60
	2	1 to 20	1 to 40
Vaccination history	1	1 to 20	0 to 20
	2	1 to 20	0 to 20
History of poor performance (subtle effect on athletic potential)	1	61 to 80	41 to 99
	2	61 to 80	41 to 99
History of exercise intolerance (intolerance to low level of exercise)	1	61 to 80	41 to 100
	2	61 to 80	41 to 99
History of viral infection (febrile respiratory disease)	1	21 to 40	1 to 80
	2	21 to 40	1 to 60
History of <u>recent</u> viral infection (febrile respiratory disease)	1	21 to 40	1 to 80
	2	21 to 40	1 to 60
Geographical location of horse (prevalent weather conditions)	1	61 to 80	21 to 99
	2	61 to 80	21 to 80
Season in which disease first developed (owner reported)	1	61 to 80	41 to 100
	2	61 to 80	41 to 99
Stable design – ventilation	1	81 to 99	61 to 100
	2	81 to 99	61 to 100
Stable design – drainage	1	21 to 40	1 to 60
	2	21 to 40	1 to 60
Type and quality of bedding provided in stable	1	100	81 to 100
	2	100	81 to 100
Type and quality of forage provided	1	100	81 to 100
	2	100	100
Length of time spent each day at pasture	1	81 to 99	41 to 100
	2	81 to 99	61 to 100



Appendix A3.5b. Summary table for Delphi group responses after round two for items included in the historical information section that were introduced by participants after the first round.

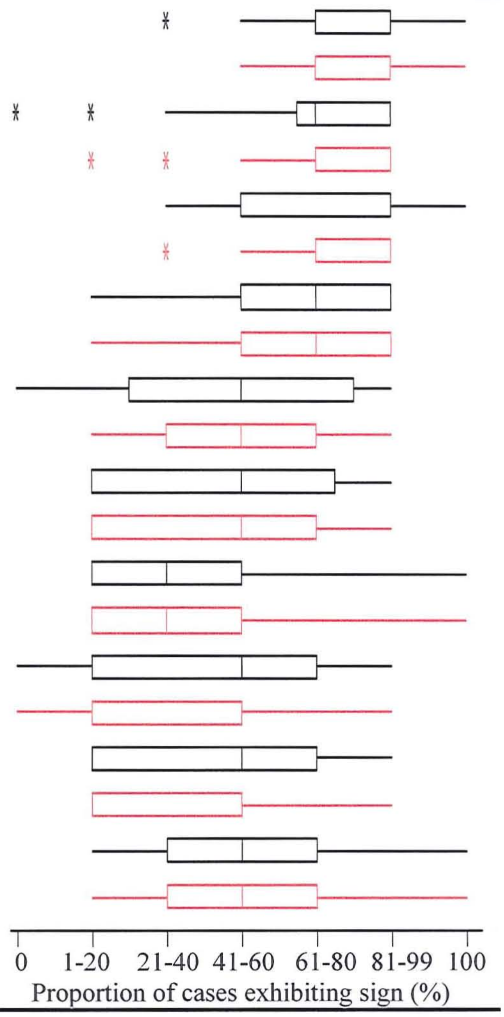
	Median (range)	Inter-quartile range
Location of horse for feeding/mucking out/grooming	61 to 80	41to 99
Storage of forage/bedding and location of muck heap in relation to stable/stall	8 to 99	41 to 100
History of exposure to donkeys	1 to 20	1 to 80
Duration of problem (Recurrent?)	81 to 99	61 to 100
Improvement when at pasture	90	81 to 100
Health of contemporaries, including others diagnosed with RAO	41 to 60	21 to 99
Alterations to normal routine exposing horse to high dust conditions e.g. transportation, stabling at competitions etc	61 to 80	61 to 99



Appendix A3.6a. Summary table for Delphi group responses after round one and round two for items included in the clinical sign section (part 1; respiratory inflammation).

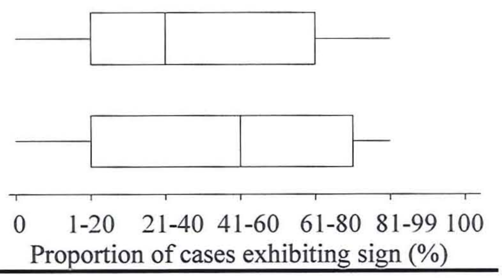
Box plots: Black = first round. Red = second round.

	Round	Median (range)	Inter-quartile range
A cough	1	81 to 99	61 to 80
	2	81 to 99	61 to 80
Coughing more than four times in 24 hrs	1	61 to 80	41 to 99
	2	61 to 80	61 to 99
Coughing at the start of exercise	1	81 to 99	41 to 99
	2	81 to 99	61 to 99
Coughing during exercise	1	61 to 80	41 to 99
	2	61 to 80	41 to 99
Cough more than four times during exercise	1	41 to 60	1 to 99
	2	41 to 60	21 to 80
Coughing when eating	1	41 to 60	1 to 99
	2	41 to 60	1 to 80
Coughing during an examination period	1	21 to 40	1 to 60
	2	21 to 40	1 to 60
Coughing when squeeze larynx/trachea	1	41 to 60	1 to 80
	2	41 to 60	1 to 60
A nasal discharge	1	41 to 60	1 to 80
	2	41 to 60	1 to 60
A nasal discharge following exercise	1	41 to 60	21 to 80
	2	41 to 60	21 to 80



Appendix A3.6b. Summary table for Delphi group responses after round two for items included in the clinical sign section (part 1; respiratory inflammation) that were introduced by participants after the first round.

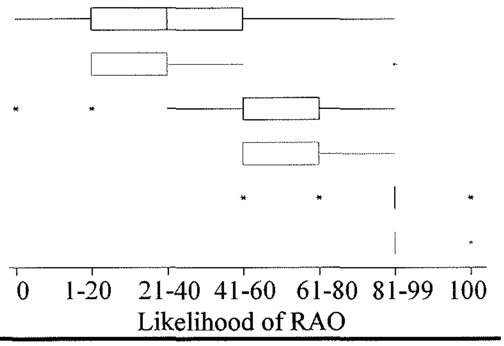
	Median (range)	Inter-quartile range
Evidence of previous nasal discharge i.e. dried debris	21 to 40	1 to 80
Nasal discharge after sedation or when head lowered after travelling	41 to 60	1 to 80



Appendix A3.6c. Summary table for Delphi group responses after round one and round two for items included in the clinical sign section (part 1; chronicity of cough).

Box plots: Black = first round. Red = second round.

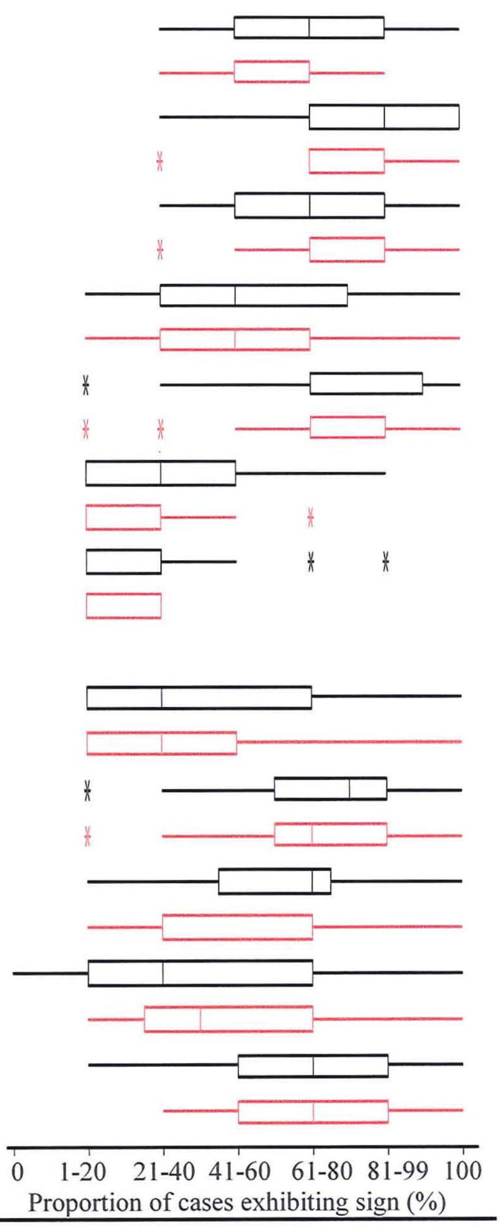
Time period of cough	Round	Median (range)	Inter-quartile range
Two weeks	1	81 to 99	61 to 80
	2	81 to 99	61 to 80
Four weeks	1	61 to 80	41 to 99
	2	61 to 80	61 to 99
Three months	1	81 to 99	41 to 99
	2	81 to 99	61 to 99



Appendix A3.7. Summary table for Delphi group responses after round one and round two for items included in the clinical sign section (part 2; respiratory obstruction).

Box plots: Black = first round. Red = second round.

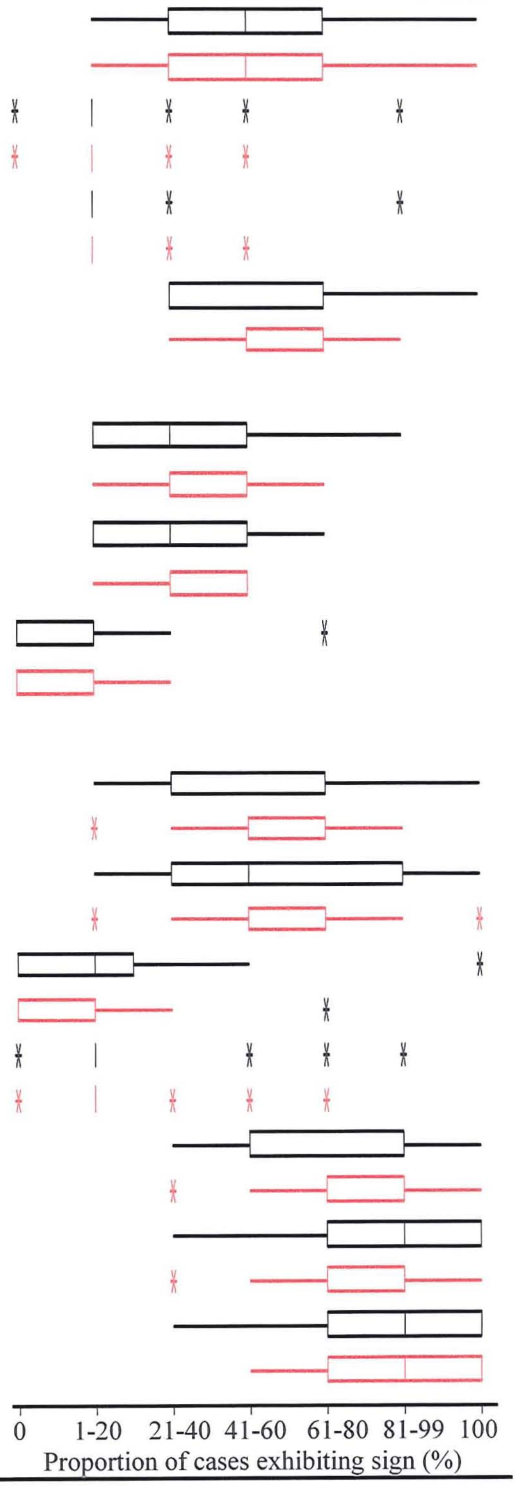
	Round	Median (range)	Inter-quartile range
Increased expiratory abdominal effort:			
• **at rest**	1	61 to 80	41 to 99
	2	61 to 80	41 to 80
• **when stabled in high dust conditions**	1	81 to 99	61 to 100
	2	81 to 99	61 to 99
• **when fed dry hay** (poor quality)	1	61 to 80	41 to 99
	2	61 to 80	61 to 99
• **following/during mucking out**	1	41 to 60	21 to 99
	2	41 to 60	21 to 80
Increased/prolonged expiratory abdominal effort post-exercise	1	61 to 80	61 to 100
	2	61 to 80	61 to 99
A visible heave line	1	21 to 40	1 to 60
	2	21 to 40	1 to 40
Movement of anus with respiration	1	1 to 20	1 to 40
	2	1 to 20	1 to 40
Flaring of nostrils:			
• **at rest**	1	21 to 40	1 to 80
	2	21 to 40	1 to 60
• **when stabled in high dust conditions**	1	61 to 80	41 to 99
	2	61 to 80	41 to 99
• **when fed dry hay** (poor quality)	1	61 to 80	21 to 80
	2	61 to 80	21 to 80
• **following/during mucking out**	1	21 to 40	1 to 80
	2	21 to 40	21 to 80
Increased/prolonged flaring of nostrils post-exercise	1	61 to 80	41 to 99
	2	61 to 80	41 to 99



Appendix A3.8a. Summary table for Delphi group responses after round one and round two for items included in the clinical sign section (part 3; detailed clinical examination).

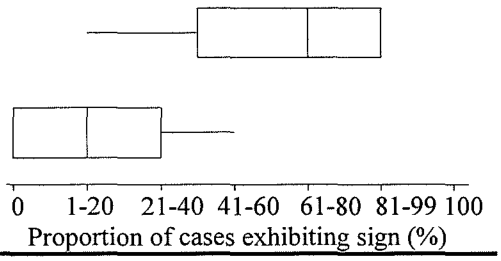
Box plots: Black = first round. Red = second round.

	Round	Median (range)	Inter-quartile range
Increased respiratory rate	1	41 to 60	21 to 80
	2	41 to 60	21 to 80
Increased heart rate	1	1 to 20	1 to 20
	2	1 to 20	1 to 20
Wheezing audible at nostrils	1	1 to 20	1 to 20
	2	1 to 20	1 to 20
Abnormal tracheal auscultation	1	61 to 80	21 to 80
	2	61 to 80	41 to 80
Thoracic auscultation:			
• **Audible wheezes**	1	21 to 40	1 to 60
	2	21 to 40	21 to 60
• **Audible crackles**	1	21 to 40	1 to 60
	2	21 to 40	21 to 60
• **Silence** (regions of minimal air flow as a result of obstruction)	1	1 to 20	0 to 20
	2	1 to 20	0 to 20
Thoracic auscultation, with rebreathing bag:			
• **Audible wheezes**	1	61 to 80	21 to 80
	2	61 to 80	41 to 80
• **Audible crackles**	1	41 to 60	21 to 99
	2	41 to 60	41 to 80
• **Silence** (regions of minimal air flow as a result of obstruction)	1	1 to 20	0 to 40
	2	1 to 20	0 to 20
Weight loss	1	1 to 20	1 to 20
	2	1 to 20	1 to 20
Exercise intolerance	1	81 to 99	41 to 99
	2	81 to 99	61 to 99
Prolonged recovery from exercise	1	81 to 99	61 to 100
	2	81 to 99	61 to 99
Exacerbations of clinical signs when challenged by hay/straw	1	81 to 99	61 to 100
	2	81 to 99	61 to 100



Appendix A3.8b. Summary table for Delphi group responses after round two for items included in the clinical sign section (part 3; detailed clinical examination) that were introduced by participants after the first round.

	Median (range)	Inter-quartile range
Coughing following use of rebreathing bag or nasal occlusion	61 to 80	21 to 99
Increased area of auscultation	1 to 20	0 to 40



Appendix A4.1. Invitation cover letter for first round of the validation study.
Printed on University of Glasgow Veterinary School notepaper (not reproduced).

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«Title» «FirstName» «MiddleName» «LastName» «Suffix»
«BusinessStreet»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

19th February 2003

Dear «Title» «FirstName» «LastName»,

Last year you kindly completed and returned a questionnaire that investigated your opinions on recurrent airway obstruction (RAO), also known as heaves and formerly as chronic obstructive pulmonary disease (COPD). We would like to take this opportunity to thank you for your valuable contribution. The overall response was exceedingly high (over 80%) and the information generated has been invaluable for constructing a risk-screening questionnaire that is to be used to study the prevalence of RAO in the UK horse population.

We have now started the second phase of the 3-year study into the epidemiology of RAO in the United Kingdom that is being conducted by the University of Glasgow and funded by the Home of Rest for Horses. This involves the validation of our risk-screening questionnaire against other diagnostic tests used in the diagnosis of RAO. In your returned questionnaire you indicated that you were prepared to participate in a future survey and that in appropriate circumstances you do use cytological analyses of tracheal washes (TW) or bronchoalveolar lavage (BAL) in clinical investigations.

We would request that the next time you or a practice colleague propose to use cytological analyses (either TW or BAL) in the investigation of any case (not necessarily RAO) that you and the animal's owner complete the attached questionnaire package. Each packet contains a **blue** form requesting limited details of the case from yourself and a **yellow** risk-screening questionnaire for completion by the horse's owner. A second **green** questionnaire for owner completion is also provided. Please ask the owner of the next healthy horse you encounter (after collection of a TW or BAL) to complete this green form. I have included 2 questionnaire packages.

It is important to emphasise that all information provided will be kept in the strictest confidence and will be used for validating the risk-questionnaire only. Code numbers will be used for administrative purposes only. If you do not wish to participate in this process but believe a practice colleague will, please pass on this communication. Pre-paid envelopes are included for returning completed questionnaires. We would like to thank you in anticipation of the important information you will provide in your returned questionnaire. If you have any questions regarding this survey or would like further questionnaire packages please do not hesitate to contact Joel Hotchkiss.

Yours sincerely,

Joel Hotchkiss
BSc BVSc CertEM(Int.Med) MRCVS
Home of Rest for Horses' Postgraduate Student

Professor Sandy Love
BVMS PhD MRCVS
Head of Division of Equine Clinical Studies

Appendix A4.2. Case record sheet for completion by veterinary surgeon for the validation study.
Printed on blue paper.

9825043506

HOME of REST for HORSES

Case Details

UNIVERSITY of GLASGOW

Form for Completion by Veterinary Surgeon

Conducted by the University of Glasgow Veterinary School
Funded by the Home of Rest for Horses

Instructions for administration of the questionnaires

1. Please complete the reverse of this page for a horse on which you are performing either a tracheal wash or a bronchoalveolar lavage. Please indicate the appropriate answers using a cross. This form deliberately only requests a limited amount of information and does not attempt to replicate the process by which you will have reached your final diagnosis.

2. Please ask the horse's owner to complete a **yellow** questionnaire with the corresponding **ID number** ________

3. Where possible please request the next client you encounter, whom you consider to own a horse with a healthy respiratory tract, to complete the second **green** questionnaire. This might be a horse that you are vaccinating or that you are examining for a lameness problem.

4. When completed please return all three forms in the pre-paid envelope provided.

Thank You for Your Assistance

COLOUR CODES FOR ADMINISTRATION OF QUESTIONNAIRE

BLUE FORM	=	For completion by Veterinary Surgeon
YELLOW FORM	=	For completion by the owner of the horse that is having endoscopy/tracheal wash/BAL performed
GREEN FORM	=	For completion by an owner of a horse seen by you for a non-respiratory problem

7060373433

Case Details

Today's date: Day __ / Month __ / Year ____ ID: ______

Clinical Signs

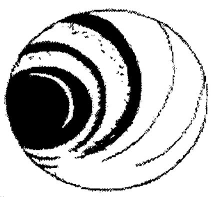
Please indicate all the clinical signs the horse has demonstrated or you have detected on clinical examination.

Coughing	No ☐	Yes ☐
A mucopurulent nasal discharge	No ☐	Yes ☐
Elevated respiratory rate	No ☐	Yes ☐
Exercise intolerance	No ☐	Yes ☐
Exagerrated abdominal effort at rest	No ☐	Yes ☐
Nostril flare at rest	No ☐	Yes ☐
A heave line	No ☐	Yes ☐
Elevated rectal temperature	No ☐	Yes ☐
Crackles or Wheezes on Thoracic Auscultation (with or without a rebreathing bag)	No ☐	Yes ☐

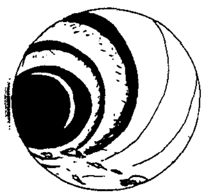
Diagnosis

What was the appearance of the trachea on endoscopy? Please tick the description that best applies

☐ Normal - No mucus



☐ Mild mucus



☐ Moderate mucus

☐ Marked mucus

Tracheal Wash Results

Cell Count ($x10^9/l$)	OR	Proportion of Cells (%)
	Neutrophils	
	Macrophages	
	Lymphocytes	
	Eosinophils	
	Mast Cells	
	Epithelial Cells	
.	**Total Cell Count** ($x10^9/l$)	.

Bronchoalveolar Lavage Results

Cell Count ($x10^9/l$)	OR	Proportion of Cells (%)
	Neutrophils	
	Macrophages	
	Lymphocytes	
	Eosinophils	
	Mast Cells	
	Epithelial Cells	
.	**Total Cell Count** ($x10^9/l$)	.

Culture of pathogenic organisms? (bacteria or mycoplasma) No ☐ Yes ☐ Not done ☐

Final Diagnosis

What was the final diagnosis in this case? Normal Lower Respiratory Tract ☐

RAO (COPD, Heaves) ☐ SPAOPD ☐ IAD ☐

Infectious Respiratory Problem ☐ Other ☐

Please specify ______

Appendix A4.3. Cover letter for RSQ for owners of cases in the validation study.

Printed on yellow paper. Cover letter for owners of controls had the title deleted (printed on green paper).

HOME of REST for HORSES

UNIVERSITY of GLASGOW

FOR COMPLETION BY THE OWNER OF A HORSE THAT HAS HAD ENDOSCOPY AND/OR A TRACHEAL WASH AND/OR A BRONCHOALVEOLAR LAVAGE

Dear Owner,

Your veterinary surgeon has requested you to complete the attached questionnaire as part of a study being funded by the charity, The Home of Rest for Horses, and conducted by the University of Glasgow Veterinary School. This is the initial validation of a questionnaire that will be used to investigate respiratory problems in the whole of the horse population in the United Kingdom. The request to complete this anonymous questionnaire does not imply that your horse has a problem; in fact the majority of people who take part will own perfectly healthy horses.

The whole questionnaire should only take 10 minutes to complete. Some background information is provided on the reverse of this page, please read this before answering the questions.

All questionnaires returned are completely anonymous and all information provided will be kept in the strictest confidence. Code numbers are used for administrative purposes only.

When you have completed your questionnaire please return it to your veterinary surgeon. We would like to thank you in anticipation of the valuable information you will provide in your completed questionnaire that will contribute to our knowledge of the UK horse population.

Yours faithfully,

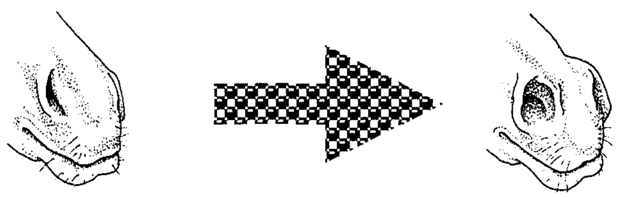
Joel Hotchkiss
BSc BVSc CertEM(Int.Med) MRCVS
Home of Rest for Horses' Postgraduate Student

Background Information

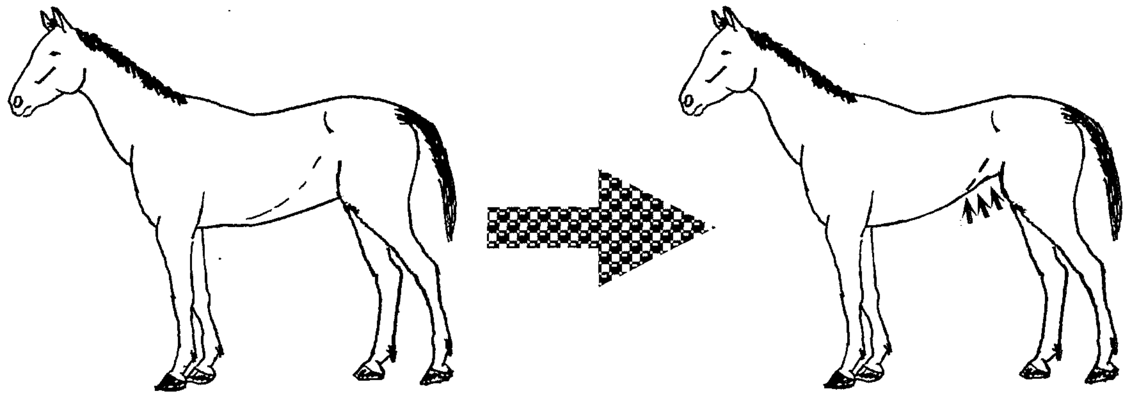
Horses are performance animals and as such need a highly efficient respiratory system that extends from the nostrils to the lung. Horses use the muscles of the chest, diaphragm and abdomen to help them breathe air in and out of their lungs.

When a horse has a respiratory problem they can have a wide range of symptoms. These include obvious symptoms such as a cough or a nasal discharge, as well as other symptoms related to an increased effort associated with breathing.

Normally when your horse is at rest it is very difficult to see them breathing, the chest hardly moves and the nostrils remain still. When they have been exercising hard it is possible to see that they are breathing faster and with increased effort. This increased effort of breathing is shown by increased movement of the chest, **flaring and movement of the nostrils** and **exaggerated abdominal effort.**



Flaring and Movement of the Nostrils



Exaggerated Abdominal Effort

Sometimes when at rest horses will show subtle signs of increased effort of breathing by having **exaggerated abdominal effort** and **flaring and movement of the nostrils**. If the horse has not recently been exercising and is not excited or anxious, this may be a sign of increased breathing effort.

In the questionnaire that follows, we ask whether your horse has had these signs, and others, **whilst at rest . . . . .**

Appendix A4.4. Risk-screening questionnaire for use in the validation study. Printed on either yellow (for case) or green (for control) paper.

9208102990

ID number: ______

HOME of REST for HORSES

Confidential Horse Health Questionnaire

UNIVERSITY of GLASGOW

How to Complete the Questionnaire

On this sheet are questions about **your** horse. Some of the questions require you to write your answers in the spaces provided. All the other questions require you to **tick** your answer in a box. If you make a mistake fill in the box in which you made the mistake and tick the correct answer.

Examples of how to mark the questionnaire: **How old is your horse?:** 0 8 years 0 6 months

To answer **"no"** Yes ☐ No ☑ To answer **"yes"** Yes ☑ No ☐

Why was a vet seeing your horse today? ☐

The Following Questions are About Your Horse's Respiratory Health and Breathing

How old is your horse? ☐☐ years ☐☐ months **Today's date:** ☐☐ / ☐☐ / ☐☐☐☐ (Day / Month / Year)

How long have you owned or been in possession of your horse? ☐☐ years ☐☐ months

What breed is your horse? ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

What sex is your horse? Mare/Filly ☐ Stallion/Colt ☐ Gelding ☐

Whilst under your care, at ANY time in the past, has your horse ever had any of the following symptoms?

Coughing	No ☐	Yes ☐	Don't know ☐
A discharge from the nose	No ☐	Yes ☐	Don't know ☐
Exaggerated abdominal effort **at rest**	No ☐	Yes ☐	Don't know ☐
Nostril flare **at rest**	No ☐	Yes ☐	Don't know ☐

Has your horse had any of the following symptoms in the LAST 12 months?

Coughing	No ☐	Yes ☐	Don't know ☐
A discharge from the nose	No ☐	Yes ☐	Don't know ☐
Exaggerated abdominal effort **at rest**	No ☐	Yes ☐	Don't know ☐
Nostril flare **at rest**	No ☐	Yes ☐	Don't know ☐

If you have answered YES to ANY of these questions please complete both parts A and B.

Otherwise please complete Part B on the next page.

Part A

The following questions are about the symptoms you have indicated as occurring above

Did two or more of these symptoms occur at the same time as each other?

No ☐ Yes ☐ Don't know ☐

Did these symptoms improve when the horse spent more time at pasture?

No ☐ Yes ☐ Got worse ☐ Don't know ☐

Were these symptoms associated with any of the following situations? (please tick all that apply)

Feeding dry hay ☐ Being at pasture ☐ Stabling ☐

Use of straw bedding ☐ Mucking out ☐ Don't know ☐

continued over

Part A (continued)

During which times of the year did these symptoms occur? (please tick all the months that apply)

Winter		Spring			Summer			Autumn			Winter
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Did these symptoms occur more than once, i.e. recurrent ? (please tick all that apply)

No ☐ More than once in one year ☐

Repeatedly over a period of several years ☐ Don't know ☐

Were any of these symptoms associated with your horse being "off colour" or having a raised temperature? (please consider all the options before selecting the most appropriate)

No ☐ Yes ☐

Yes, but not on every occasion ☐ Don't know ☐

Were these symptoms associated with a respiratory infection, as diagnosed by your vet?

No ☐ Yes ☐ Yes, but not on every occasion ☐

<u>If you indicated earlier that your horse has had a COUGH, please answer the following questions:</u>

How long has/did your horse cough for? Less than 2 weeks ☐ About a month ☐

Between 1 and 3 months ☐ 3 months or more ☐

During this period how often, on average, did your horse cough per day?

Rarely ☐ Infrequently ☐ Frequently ☐ Constantly ☐

Was this cough associated with any particular activity? (please tick all that apply)

No particular pattern to cough ☐ Coughed at start of exercise ☐

Coughed during exercise ☐ Don't know ☐

Please now continue to part B below

Part B

Has your horse ever taken longer to recover from exercise than you would normally have expected, given its level of fitness? No ☐ Yes ☐ Don't know ☐

If **YES**, did this coincide with any of the symptoms mentioned on the **first** page?

No ☐ Yes ☐

Has your horse ever had a period of time during which they did not perform as well as you would expect, or were unable to exercise at their normal level? No ☐ Yes ☐ Don't know ☐

If **YES**, did this coincide with any of the symptoms mentioned on the **first** page?

No ☐ Yes ☐

Has a vet ever told you that your horse has COPD (chronic obstructive pulmonary disease), also known as heaves or recurrent airway obstruction (RAO), a dust or hay allergy? No ☐ Yes ☐ Don't know ☐

If **YES**, how old was your horse when this problem was diagnosed? ☐☐ years ☐☐ months

Has a vet ever told you that your horse has SPAOPD (Summer Pasture Associated Obstructive Pulmonary Disease), an allergic respiratory disease associated with being at pasture in the summer?

No ☐ Yes ☐ Don't know ☐

If **YES**, how old was your horse when this problem was diagnosed? ☐☐ years ☐☐ months

Appendix A4.5. Reminder postcard sent to all invitees in the validation study.

HOME *of* REST *for* HORSES

UNIVERSITY *of* GLASGOW

24th March 2003

Dear «Title» «FirstName» «LastName»

Recently you were sent a letter inviting you to assist in the validation of a risk-screening questionnaire (RSQ) for recurrent airway obstruction (RAO). I would like to take this opportunity to remind you that if you do propose to use respiratory cytological analyses for further investigation of any case, we would be grateful if you could, with owners' assistance, complete one of the questionnaire packages provided with the letter. Recently, BEVA reiterated their support for clinically based research such as this. We would be most appreciative if you were able to contribute to the development of this RSQ, which, once validated, will be used to investigate RAO in the UK horse population. Thank you for your time - and tolerance.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS
Division of Equine Clinical Studies, Weipers Centre for Equine Welfare, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, *Tel.:* 0141-330 5999 *Fax:* 0141-330 6025

Appendix A4.6. Invitation cover letter for second round of the validation study.

Printed on University of Glasgow Veterinary School notepaper (not reproduced).

HOME of REST for HORSES

UNIVERSITY of GLASGOW

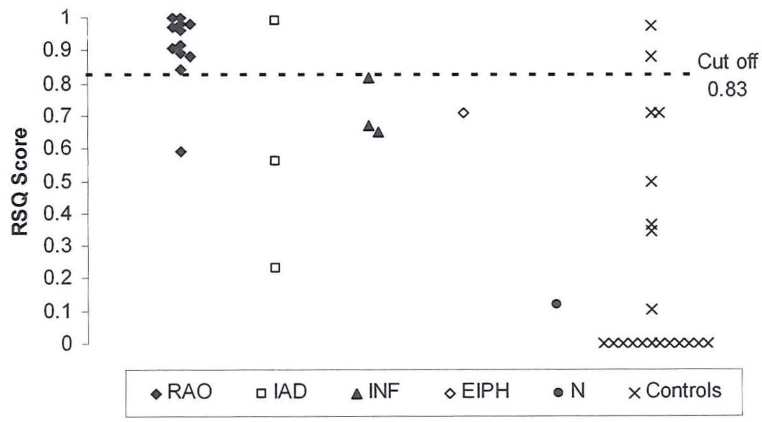
«Title» «FirstName» «MiddleName» «LastName» «Suffix»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessStreet3»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

25th November 2003

Dear «Title» «FirstName» «MiddleName» «LastName»

We would like to request the help of you and your practice colleagues in the further validation of an epidemiological tool we have developed for investigating the prevalence of recurrent airway obstruction (RAO; COPD). We have developed a risk-screening questionnaire (RSQ) for the diagnosis of apparent RAO that can be completed by horse owners (for use only in surveys). In return for help with validation of our RSQ we are offering **Oddbins vouchers** as a small recompense.

A horse owner completed RSQ determines a score for the likelihood of their horse having RAO, the cut off for a positive diagnosis being determined by ROC analysis. The RSQ has been validated against a clinical diagnosis of RAO that includes the use of respiratory cytology. The validation so far has obtained results for 40 horses (20 cases and 20 controls). This has suggested that the RSQ has a sensitivity = 0.92 (confidence interval=0.64-0.99) and specificity = 0.89 (0.73-0.96) for the diagnosis of apparent RAO (compared to all other diagnoses).



RAO=Recurrent airway obstruction, IAD=Inflammatory airway disease, INF=Infection, EIPH=Exercise induced pulmonary haemorrhage, N=Normal. Each symbol represents one horse and there are 40 horses in total

Over the coming months, with your help, we would like to increase the number of horses involved in our study to provide a better estimate of the RSQ's diagnostic ability. This would entail obtaining an owner completed RSQ (yellow form) for horses on which you have performed a tracheal wash (TW) and/or a bronchoalveolar lavage (BAL), **for whatever reason** - not necessarily because of a suspicion of RAO. There is a short one-sided form (blue form) for the recording of clinical findings, the results of the respiratory cytology and the final diagnosis. An owner completed RSQ (green form) would also be obtained for the next horse, without obvious or known respiratory problems, that you encountered in your working day. The RSQ takes owners less than 5 minutes to complete. For this validation we are using the term recurrent airway obstruction (also known as heaves and formerly as COPD) to identify the inflammatory respiratory disease characterised by reversible lower airway obstruction in mature horses induced by environmental challenge.

The senders of the first **sixty** completed RSQ validations will receive **Oddbins vouchers**, one five-pound voucher for every completed RSQ that is returned. It is important to emphasise that all information provided will be kept in the strictest confidence and will be used for validating the risk-screening questionnaire only. Code numbers will be used for administrative purposes only. If you do not wish to participate in this process but believe a practice colleague would, please pass on this communication. Pre-paid envelopes are included for returning completed questionnaires. We would like to thank you in anticipation of the important information you will provide in your returned questionnaire. If you have any questions regarding this survey or would like further questionnaire packages please do not hesitate to contact me.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS

Appendix A4.7. Probabilities associated with the items/responses utilised for the Delphi scored RSQ for RAO.

	No	Yes
Whilst at ANY time in the past has your horse ever had any of the following symptoms?		
Coughing	-	0.61
Nasal discharge	-	0.3
Exaggerated abdominal effort **at rest**	-	0.41
Has your horse had any of the following symptoms in the LAST 12 months?		
Coughing	-	0.81
Nasal discharge	-	0.41
Exaggerated abdominal effort **at rest**	-	0.61
Nostril flare **at rest**	-	0.21
Did these symptoms improve when the horse spent more time at pasture?		
Improve at pasture	-	0.81
Were these symptoms associated with any of the following situations?		
None of the situations		0.23
Feeding dry hay	-	0.61
Stabling	-	0.61
Use of straw bedding	-	0.61
Mucking out	-	0.61
Did these symptoms occur more than once, i.e. recurrent?		
More than once in one year	-	0.61
Repeatedly over a period of several years	-	0.81
Were any of these symptoms associated with your horse being "off colour" or having a raised temperature?		
Temperature	-	0.17
Were these symptoms associated with a respiratory infection as diagnosed by your vet?		
Respiratory infection	-	0.17
How long has/did your horse cough for?		
$<$ 2 weeks	-	0.21
$\approx$ 1 month	-	0.61
1-3 months	-	0.71
$>$ 3 months	-	0.81
During this period how often, on average, did your horse cough per day?		
Frequently or constantly	-	0.61
Was this cough associated with any particular activity?		
Cough at start of exercise	-	0.81
Cough during exercise	-	0.61
Has your horse ever taken longer to recover from exercise than you would normally have expected, give its level of fitness?		
Not coinciding with symptoms	-	0.61
Coincide with symptoms	-	0.81
Has your horse ever had a period of time during which they did not perform as well as you would expect, or were unable to exercise at their normal level?		
Not coinciding with symptoms	-	0.61
Coincide with symptoms	-	0.81

Appendix A4.8. Descriptive data for the 40 cases in the validation study.
Blue shading indicates cases from first round (winter/spring 2003). PP=private practice, U=University clinic.

ID	Vet	Reason for being seen	Age	Length of ownership	Breed	Gender
1	PP	Cough/nasal discharge	10y	1y	Clydesdale x Highland	G
2	PP	Coughing	22y	2y 6m	Connemarax	M
3	PP	For BAL	10y 11m	2y 6m	Connemara	M
4	PP	Coughing	10y 11m	8y	TB	G
5	PP	Epistaxis	12y	2y 9m	TB	G
6	PP	Persistent coughing	9y 9m	2m	TB	G
7	PP	Coughing	6y 10m	2y 11m	Cob	M
8	PP	Cough/nasal discharge	6y 10m	4y	TBx Cleveland Bay	G
9	PP		11y	8y	Welsh Cob x Hackney	G
10	PP	Coughing	18y	6y 6m	TBx Cleveland Bay	G
11	PP		15y	8y	TBx Irish Draught	G
12	PP	Cough	5y 6m	1m	TBx	G
13	PP	Cough/nasal discharge	18y 8m	8m	TBx	M
14	U	Bad breathing	15y	9y	Connemara x Arab	M
15	PP	Coughing	12y	5y	Highland x	G
16	U	Cough/nasal discharge	16y 10m	6y	TB	M
17	U	Coughing	11y 2m	4y	TB	M
18	PP	Persistent coughing	5y 4m	2y	TB	G
19	PP	Respiratory distress	22y	2y	TBx	G
20	U	Nasal discharge	8y	2y	Riding Pony	G
21	PP	Post choke	10y	3m	Welsh Section A	M
22	PP	Coughing	9y 7m	2y	TBx Warmblood	G
23	PP	Chronic cough	10y 2m	5y 6m	TBx	G
24	U	Poor performance	9y	2y 6m	TB	G
25	U	Possible RAO	8y	1m	Irish Cob	G
26	U	Coughing	6y 9m	1y 4m	Connemara	G
27	U	Coughing	16y	6m	Crossbred	M
28	U	Poor performance	8y 11m	1y	TB	G
29	U	Poor performance	9y	1y 6m	TB	G
30	U	Respiratory problem	13y	3y	Connemara x	G
31	U	Cardiac problem	9y	5m	Warmblood	S
32	U	Possible RAO	10y	5y 2m	TBx Irish Draught	G
33	U		14y 9m	3y	TB	M
34	U	Cough/nasal discharge	10y	3y	Hunter	M
35	U	Coughing	9y 3m	9y 3m	TBx Warmblood	G
36	PP		8y	10m	Coloured Cob	G
37	PP	Coughing	13y	8y	Anglo Arab	M
38	U	Colic	9y 1m	9y 1m	Arab	S
39	U	Poor performance	3y	1y	TB	G
40	PP	Respiratory problem	8y 6m	6y 6m	Cob	G

Appendix A4.9. Veterinary surgeons' clinical findings for the 40 cases included in the validation study.
Blue shading indicates cases from first round (winter/spring 2003).

ID	Cough	Nasal discharge	Respiratory rate	Exercise intolerance	Exaggerated abdominal effort	Nostril flare	Heave line	Elevated rectal temp	Crackles or wheeze	Endoscopic mucus score	Comments
1	1	0	1	1	0	0	0	0	0	3	Viral
2	1	1	1	1	1	0	0	0	0	-	-
3	0	1	0	0	0	0	0	0	0	-	-
4	1	0	0	0	0	0	0	0	0	1	-
5	0	0	0	1	0	0	0	0	0	1	EIPH
6	1	1	1	-	0	0	0	1	1	4	-
7	1	0	1	0	1	1	0	0	0	3	-
8	1	1	1	1	1	0	0	0	1	4	-
9	1	1	1	1	1	1	1	0	1	4	-
10	1	0	0	0	0	0	0	0	0	2	-
11	1	0	0	1	0	0	0	0	0	2	Post tie-back
12	1	1	1	1	1	0	0	0	0	2	Secondary bacterial infection
13	1	1	1	1	1	0	0	0	1	4	Viral with secondary bacterial infection
14	1	0	1	1	1	1	0	0	0	2	-
15	1	1	0	1	1	0	0	0	1	3	-
16	1	1	0	0	1	0	0	0	1	3	-
17	1	1	1	0	1	0	0	0	1	4	-
18	1	0	0	1	0	0	0	0	0	2	History of EIPH
19	0	0	1	1	1	1	1	0	1	3	Secondary bacterial infection
20	0	1	0	0	0	0	0	0	0	4	-
21	0	0	0	0	0	1	0	0	0	1	-
22	0	1	1	0	1	0	0	0	0	-	-
23	1	0	0	1	0	0	0	0	0	3	Lower airway infection secondary to choke
24	0	0	0	1	0	0	0	0	0	1	Upper airway inflammation/ Tracheitis
25	0	0	1	1	0	0	0	0	0	3	Post-viral
26	1	1	0	0	0	0	0	0	1	4	Allergic airway disease (upper and lower)
27	1	1	0	0	0	0	0	0	1	2	-
28	0	0	0	1	0	0	0	0	0	2	-
29	0	0	0	1	0	0	0	0	0	2	-
30	0	0	1	0	0	0	0	0	1	3	-
31	0	0	0	0	0	0	0	0	0	1	-
32	1	1	0	0	0	0	0	0	0	3	-
33	0	0	0	1	0	0	0	0	0	3	-
34	1	0	1	1	1	1	0	0	0	4	-
35	1	0	0	0	0	0	0	0	0	2	-
36	0	0	0	1	0	0	0	0	0	1	Persistent eosinophilia of unknown origin
37	1	0	0	1	0	0	0	0	0	2	Pulmonary granular cell tumour
38	0	0	0	0	0	0	0	0	0	2	-
39	0	0	0	0	0	0	0	0	0	2	Upper airway inflammation
40	0	0	0	1	0	0	0	0	0	1	Secondary bacterial infection

Appendix A4.10. Two by two tables for veterinary reported clinical signs comparing cases according to a diagnosis of RAO or non RAO in the validation study.

	Yes	**No**
	Cough ($\chi^2_1 = 1.1$, p = 0.29)	
RAO group (n=18)	12	6
Non RAO group (n=22)	11	11
	Nasal discharge ($\chi^2_1 = 4.4$, p = 0.04)	
RAO group (n=18)	10	8
Non RAO group (n=22)	5	17
	Elevated respiratory rate ($\chi^2_1 = 7.6$, p = 0.006)	
RAO group (n=18)	11	7
Non RAO group (n=22)	4	18
	Exercise intolerance ($\chi^2_1 = 1.4$, p = 0.23)	
RAO group (n=18)	8	10
Non RAO group (n=22)	14	8
	Abdominal effort ($\chi^2_1 = 11.9$, p = 0.0006)	
RAO group (n=18)	11	7
Non RAO group (n=22)	2	20
	Nostril flare (Fisher's exact p = 0.005)	
RAO group (n=18)	6	12
Non RAO group (n=22)	0	22
	Heave line (Fisher's exact p = 0.2)	
RAO group (n=18)	2	16
Non RAO group (n=22)	0	22
	Elevated rectal temperature (Fisher's exact p = 0.5)	
RAO group (n=18)	1	17
Non RAO group (n=22)	0	22
	Crackles or wheezes on auscultation (Fisher's exact p = 0.2)	
RAO group (n=18)	7	11
Non RAO group (n=22)	4	18

Appendix A4.11. Tracheal wash cytology results for cases in the validation study.

Blue shading indicates cases from first round (winter/spring 2003).

ID	Proportion of cells (%) or (-,+/-,1+,2+, or 3+ scale)						Total cell count ($x10^9/l$)	Pathogenic bacteria cultured
	Neutrophils	Macrophages	Lymphocytes	Eosinophils	Mast cells	Epithelial cells		
1	81	14	4	1			3.25	Not done
2	70	22	2		2	4	67	Not done
4	+/-		+/-				0.7	Yes
5	+/-		+/-					No
6	3+					1+	58.2	Yes
7	3+	1+				1+		No
8	94	5	1					No
9	70	5					23.4	Not done
10	3+			+/-		2+	4.8	Not done
11	7	57	2		1	33		No
12	1+	1+	1+				1	Not done
13	97	1	4	1			26.2	No
15	85	8	6.5		0.5		2	No
18	2+	2+	+/-			1+	0.5	Yes
19	3+	1+	+/-				54.1	Yes
21	+/-	3+	+/-				1.3	Yes
23	3+							Yes
24	64	19	17					No
25	8	82	8		2			Not done
27	31	64	3		2			Not done
29	26	61	13					Not done
30	93	1	6					No
32	77	16	7					Not done
33	6	94			1			Not done
34	54	35	10	1			1.072	Not done
35	6	82	10	1	2			Not done
37	No sample taken as tumours identified in airways during endoscopy							Not done
38	24	67	9				2.11	No
39	71	22	7					Not done
40	1+						1.6	Yes

Appendix A4.12. Bronchoalveolar lavage fluid cytology results for cases included in the validation study.
Blue shading indicates cases from first round (winter/spring 2003).

ID	Proportion of cells (%)						Total Cell Count ($x10^9/l$)
	Neutrophils	Macrophages	Lymphocytes	Eosinophils	Mast cells	Epithelial cells	
3	9	36	44	1	10		0.35
9	28	18	54				0.3
11	7	55	31			7	
14	49	42	3		6		0.067
16	22	60	16		2		0.259
17	29	57	13		1		0.138
20	26	37	28	5	4		0.84
22	20	60	20				
24	1	78	18		3		
25	2	40	50				
26	3	54		25	18		
27	7	57	35		1		
28	5	79	13		3		
29	3	60	34		3		
30	19	58	23		3		
31	2	94	3		1		
32	24	50	13		3		
33	2	71	21		6		
36	6	2	4			71	
37	No sample taken as tumours identified in airways during endoscopy						
39	1.2	74.2	23		1.6		

Appendix A4.13. Descriptive data for the 40 controls in the validation study.
Blue shading indicates controls from first round (winter/spring 2003). PP=private practice, U=University clinic.

ID	Vet	Reason for being seen	Age	Length of ownership	Breed	Gender
41	PP	Vaccination	7y 10m	8m	Fell pony	M
42	PP	-	9y 10m	3y 6m	TB	M
43	PP	Vaccination	3y 10m	3y 10m	TBx Connemara	M
44	PP	-	19y 3m	15y 1m	TB	M
45	PP	Pastern injury	11y	1y 10m	TB	M
46	PP	Lameness	10y 7m	-	TB	G
47	PP	Recheck injury	3y 10m	3y 10m	TB	M
48	PP	Lameness	3y 10m	5m	TB	M
49	PP	Recheck fracture	10y 2m	2y	Irish sports horse	G
50	PP	-	17y 1m	6y 4m	TBx	G
51	PP	Post operative check	8y	6y	Irish	G
52	PP	-	12y	8y 2m	TBx Irish draught	G
53	PP	Vaccination	10y	6y	TBx	M
54	U	-	2y	2y	Welsh section D x Connemara x Arab	M
55	PP	-	13y	5y	Cob	G
56	U	-	9y 6m	7y 2m	Welsh	M
57	U	-	2y	2y 3m	TB	G
58	PP	Vaccination	10y 5m	6y	TB	G
59	PP	Lameness	16y	8y	TBx	G
60	U	-	6y	2y	Unknown	M
61	PP	General	7y 6m	6y	Apaloosa	M
62	PP	Lameness	11y	6y 6m	Cob	G
63	PP	Wound on withers	8y 11m	5m	TB	M
64	U	Dental exam	10y	3y	Irish sports horse	G
65	U	Lameness	8y 2m	1y	Welsh Cob	M
66	U	Lameness	8y	2y 2m	TB	M
67	U	Dental exam	38y	26y	Connemara x	G
68	U	Lameness	8y 7m	8m	Irish draught x Welsh Cob	M
69	U	Cardiac problem	14y	4y	TB	G
70	U	Lameness	13y 10m	2y 4m	TBx Warmblood	G
71	U	-	2y	2y	TB	M
72	U	Headshaking	5y 10m	2m	Connemara	G
73	U	Lameness	12y	1y	Welsh x Shetland	G
74	U	Lameness	9y 11m	1y 10m	Highland pony	M
75	U	Recheck lameness	9y	1y	TBx Welsh section D	G
76	PP	-	6y 2m	7m	Connemara	G
77	PP	Vaccination	8y	1y	Irish draught x	M
78	U	Lameness	8y	5y	TBx	M
79	U	Sarcoids	17y	15y	Arab	G
80	PP	Vaccination	6y 2m	1y	Connemara	G

Appendix A4.14. RSQ scores for the 40 cases included in the validation study.
Blue shading indicates cases from first round (winter/spring 2003).

ID	Final diagnosis made by veterinary surgeon	Delphi Score	HOMALS Score
1	Infectious. Viral in origin.	**0.3356**	2.26
2	**RAO.**	**0.9999**	8.14
3	**RAO.**	**0.5136**	5.05
4	Normal respiratory tract.	**0.0472**	4.69
5	Other. EIPH.	**0.4233**	0.36
6	**RAO.**	**0.9594**	6.85
7	**RAO.**	**0.9986**	5.66
8	IAD.	**0.6090**	2.18
9	**RAO.**	**0.7933**	6.75
10	**RAO.**	**0.9102**	4.52
11	IAD. Post tieback.	**0.9968**	5.97
12	**RAO.** Secondary bacterial infection.	**0.9380**	6.08
13	Infectious. Viral in origin with secondary bacterial infection.	**0.9380**	6.14
14	**RAO.**	**0.9448**	4.74
15	**RAO.**	**0.9890**	9.21
16	**RAO.**	**0.9993**	7.05
17	**RAO.**	**0.9993**	7.88
18	Infectious. History of EIPH.	**0.8055**	4.78
19	**RAO**. Secondary bacterial infection.	**0.9816**	9.44
20	IAD.	**0.4256**	3.71
21	**RAO.**	**0.8696**	4.06
22	**RAO.**	**0.8293**	3.22
23	Infectious. Lower airway infection secondary to choke.	**0.7989**	4.76
24	Other. Upper airway inflammation/tracheitis.	**0.3194**	0.36
25	Infectious. Post viral.	**0.7476**	1.35
26	Other. Allergic airway disease (upper and lower).	**0.6603**	6.03
27	Infectious.	**0.2705**	5.32
28	Normal respiratory tract.	**0.4233**	0.36
29	Normal respiratory tract.	**0.3194**	0.36
30	**RAO.**	**0.9840**	6.73
31	Normal respiratory tract.	**0.3194**	0.36
32	**RAO.**	**0.8837**	5.38
33	Normal respiratory tract.	**0.9796**	5.87
34	**RAO.**	**0.9728**	8.61
35	Normal respiratory tract.	**0.3031**	3.71
36	Other. Persistent eosinophilia of unknown origin.	**0.2308**	0
37	Other. Pulmonary granular cell tumour.	**0.9780**	3.77
38	Normal respiratory tract.	**0.6651**	2.66
39	Normal respiratory tract. Upper airway inflammation.	**0.3194**	0.36
40	**RAO.** Secondary bacterial infection.	**0.9701**	8.62

Appendix A4.15. RSQ scores for the 40 controls included in the validation study.

Blue shading indicates controls from first round (winter/spring 2003).

ID		Delphi Score	HOMALS Score
41	Control	0.2308	0
42	Control	0.9821	5.51
43	Control	0.2308	0
44	Control	0.2308	0
45	Control	0.2308	0
46	Control	0.0772	2.73
47	Control	0.2308	0
48	Control	0.2308	0
49	Control	0.4233	0.36
50	Control	0.2308	0
51	Control	0.6574	2.82
52	Control	0.3751	3.08
53	Control	0.2308	0
54	Control	0.2308	0
55	Control	0.9125	2.35
56	Control	0.2308	0
57	Control	0.2308	0
58	Control	0.2308	0
59	Control	0.8463	5.08
60	Control	0.9699	3.6
61	Control	0.8463	5.45
62	Control	0.2308	0
63	Control	0.8960	5.43
64	Control	0.2308	0
65	Control	0.2308	0
66	Control	0.2308	0
67	Control	0.2308	0
68	Control	0.2308	0
69	Control	0.3194	0.36
70	Control	0.3194	0.36
71	Control	0.0077	4.57
72	Control	0.0988	1.29
73	Control	0.2308	0
74	Control	0.2308	0
75	Control	0.2308	0
76	Control	0.2308	0
77	Control	0.2308	0
78	Control	0.2308	0
79	Control	0.9735	6.9
80	Control	0.2321	1.53

Appendix A4.16. ROC curve coordinates for the Delphi scoring of the RSQ.

		RAO	Not RAO	Sensitivity	Specificity	1-Specificity
0	Yes	18	62	1.00	0.00	1.00
0	No	0	0			
>=0.0077	Yes	18	62	1.00	0.00	1.00
<0.0077	No	0	0			
>=0.0472	Yes	18	61	1.00	0.02	0.98
<0.0472	No	0	1			
>=0.0772	Yes	18	60	1.00	0.03	0.97
<0.0772	No	0	2			
>=0.0988	Yes	18	59	1.00	0.05	0.95
<0.0988	No	0	3			
>=0.2308	Yes	18	58	1.00	0.06	0.94
<0.2308	No	0	4			
>=0.2321	Yes	18	32	1.00	0.48	0.52
<0.2321	No	0	30			
>=0.2705	Yes	18	31	1.00	0.50	0.50
<0.2705	No	0	31			
>=0.3031	Yes	18	30	1.00	0.52	0.48
<0.3031	No	0	32			
>=0.3178	Yes	18	29	1.00	0.53	0.47
<0.3178	No	0	33			
>=0.3194	Yes	18	28	1.00	0.55	0.45
<0.3194	No	0	34			
>=0.3356	Yes	18	23	1.00	0.63	0.37
<0.3356	No	0	39			
>=0.3751	Yes	18	22	1.00	0.65	0.35
<0.3751	No	0	40			
>=0.4233	Yes	18	21	1.00	0.66	0.34
<0.4233	No	0	41			
>=0.4256	Yes	18	18	1.00	0.71	0.29
<0.4256	No	0	44			
>=0.5136	Yes	18	17	1.00	0.73	0.27
<0.5136	No	0	45			
>=0.6090	Yes	17	17	0.94	0.73	0.27
<0.6090	No	1	45			
>=0.6574	Yes	17	16	0.94	0.74	0.26
<0.6574	No	1	46			
>=0.6603	Yes	17	15	0.94	0.76	0.24
<0.6603	No	1	47			
>=0.7476	Yes	17	14	0.94	0.77	0.23
<0.7476	No	1	48			
>=0.7933	Yes	17	13	0.94	0.79	0.21
<0.79333	No	1	49			
>=0.7989	Yes	16	13	0.89	0.79	0.21
<0.7989	No	2	49			
>=0.8055	Yes	16	12	0.89	0.81	0.19
<0.8055	No	2	50			
>=0.8293	Yes	16	11	0.89	0.82	0.18
<0.8293	No	2	51			
>=0.8463	Yes	15	11	0.83	0.82	0.18
<0.8463	No	3	51			
>=0.8696	**Yes**	**15**	**9**	**0.83**	**0.85**	**0.15**
<0.8696	**No**	**3**	**53**			
>=0.8837	Yes	14	9	0.72	0.85	0.15
<0.8837	No	4	53			

		RAO	Not RAO	Sensitivity	Specificity	1-Specificity
>=0.8960	Yes	13	8	0.72	0.87	0.13
<0.8960	No	5	54			
>=0.9102	Yes	13	7	0.72	0.89	0.11
<0.9102	No	5	55			
>=0.9125	Yes	12	7	0.67	0.89	0.11
<0.9125	No	6	55			
>=0.9380	Yes	12	6	0.67	0.90	0.10
<0.9380	No	6	56			
>=0.9448	Yes	11	5	0.61	0.92	0.08
<0.9448	No	7	57			
>=0.9594	Yes	10	5	0.56	0.92	0.08
<0.9594	No	8	57			
>=0.9699	Yes	9	5	0.50	0.92	0.08
<0.9699	No	9	57			
>=0.9701	Yes	9	4	0.50	0.94	0.06
<0.9701	No	9	58			
>=0.9728	Yes	8	4	0.44	0.94	0.06
<0.9728	No	10	58			
>=0.9780	Yes	7	4	0.39	0.94	0.06
<0.9780	No	11	58			
>=0.9796	Yes	7	3	0.39	0.95	0.05
<0.9796	No	11	59			
>=0.9816	Yes	7	2	0.39	0.97	0.03
<0.9816	No	11	60			
>=0.9821	Yes	6	2	0.33	0.97	0.03
<0.9821	No	12	60			
>=0.9840	Yes	6	1	0.33	0.98	0.02
<0.9840	No	12	61			
>=0.9899	Yes	5	1	0.28	0.98	0.02
<0.9899	No	13	61			
>=0.9968	Yes	4	1	0.22	0.98	0.02
<0.9968	No	14	61			
>=0.9986	Yes	4	0	0.22	1.00	0.00
<0.9986	No	14	62			
>=0.9993	Yes	3	0	0.17	1.00	0.00
<0.9993	No	15	62			
>=0.9999	Yes	1	0	0.06	1.00	0.00
<0.9999	No	17	62			
>=1	Yes	0	0	0.00	1.00	0.00
<1	No	18	62			

Appendix A4.17. Collapsed responses from the RSQ to form 12 items for the HOMALS analysis.

RSQ responses	Collapsed responses for HOMALS scoring	
Whilst at ANY time in the past has your horse ever had any of the following symptoms? Coughing Nasal discharge Exaggerated abdominal effort **at rest** Nostril flare **at rest** Has your horse had any of the following symptoms in the LAST 12 months? Coughing Nasal discharge Exaggerated abdominal effort **at rest** Nostril flare **at rest**	**Cough** **Nasal discharge** **Exaggerated abdominal effort** **Nostril flare**	**No Yes** **No Yes** **No Yes** **No Yes**
Did two or more of these symptoms occur at the same time as each other?	**Symptoms coincide**	**No Yes**
Did these symptoms improve when the horse spent more time at pasture?	**Improve at pasture**	**No Yes**
Were these symptoms associated with any of the following situations? None of the situations Feeding dry hay Stabling Use of straw bedding Mucking out	**Worse with risk factor**	**No Yes**
Did these symptoms occur more than once, i.e. recurrent? More than once in one year Repeatedly over a period of several years	**Recurrent**	**No Yes**
How long has/did your horse cough for? 1-3 months > 3 months	**Cough for > 1 month**	**No Yes**
During this period how often, on average, did your horse cough per day? Frequently Constantly	**Frequent cough**	**No Yes**
Was this cough associated with any particular activity? Cough at start of exercise Cough during exercise	**Cough when exercise**	**No Yes**
Has your horse ever taken longer to recover from exercise than you would normally have expected, give its level of fitness? Has your horse ever had a period of time during which they did not perform as well as you would expect, or were unable to exercise at their normal level?	**Poor performance**	**No Yes**

Appendix A4.18. Correlation matrix, means and standard deviations of HOMALS RSQ items (p =12, n = 80).

	Cough	Nasal discharge	Exaggerated abdominal effort	Nostril flare	Symptoms coincide	Improve at pasture	Worse with risk factor	Recurrent	Coughed for >1 month	Frequent cough	Cough when exercise	Poor performance
Cough	1.000											
Nasal discharge	0.750	1.000										
Exaggerated abdominal effort	0.236	0.236	1.000									
Nostril flare	0.102	0.197	0.466	1.000								
Symptoms coincide	0.573	0.573	0.290	0.399	1.000							
Improve at pasture	0.410	0.227	-0.012	-0.032	0.275	1.000						
Worse with risk factor	0.529	0.529	0.316	0.218	0.431	0.441	1.000					
Recurrent	0.453	0.562	0.247	0.435	0.513	0.193	0.441	1.000				
Cough for >1 month	0.651	0.541	0.344	0.239	0.481	0.278	0.346	0.428	1.000			
Frequent cough	0.711	0.605	0.303	0.208	0.521	0.227	0.396	0.536	0.680	1.000		
Cough when exercise	0.472	0.406	0.124	0.244	0.158	0.163	0.257	0.345	0.434	0.453	1.000	
Poor performance	0.148	0.200	0.242	0.358	0.246	-0.037	0.073	0.263	0.232	0.105	0.039	1.000
Mean	0.488	0.488	0.125	0.075	0.338	0.213	0.313	0.300	0.288	0.325	0.175	0.388
SD	0.503	0.503	0.333	0.265	0.476	0.412	0.466	0.462	0.455	0.471	0.382	0.490

Appendix A4.19. HOMALS category quantifications of the RSQ item responses.

	Yes	No
Cough	0.861	-0.819
Nasal discharge	0.83	-0.79
Exaggerated abdominal effort	1.206	-0.172
Nostril flare	1.555	-0.126
Symptoms coincide	1.029	-0.524
Improve at pasture	0.768	-0.207
Worse with risk factor	0.98	-0.445
Recurrent	1.101	-0.472
Cough for >1 month	1.208	-0.487
Frequent cough	1.155	-0.556
Cough when exercise	1.181	-0.251
Poor performance	0.387	-0.245

Scaled HOMALS category quantifications were calculated for each criterion by calculating the difference between each Yes and No, dividing this by the sum of the differences between all of the Yes/No and multiplying by 10. This gave the Yes score and the No score was set as 0. For example, for Cough the scaled quantification = (0.861- ⁻0.819/17.355) x 10 = 0.91.

Appendix A4.20. ROC curve coordinates for the HOMALS scoring of the RSQ

		RAO	Not RAO	Sensitivity	Specificity	1-Specificity
>=0	Yes	18	62	1.00	0.00	1.00
<0	No	0	0			
>=0.36	Yes	18	37	1.00	0.40	0.60
<0.36	No	0	25			
>=1.29	Yes	18	28	1.00	0.55	0.45
<1.29	No	0	34			
>=1.35	Yes	18	27	1.00	0.56	0.44
<1.35	No	0	35			
>=1.53	Yes	18	26	1.00	0.58	0.42
<1.53	No	0	36			
>=2.18	Yes	18	25	1.00	0.60	0.40
<2.18	No	0	37			
>=2.26	Yes	18	24	1.00	0.61	0.39
<2.26	No	0	38			
>=2.35	Yes	18	23	1.00	0.63	0.37
<2.35	No	0	39			
>=2.66	Yes	18	22	1.00	0.65	0.35
<2.66	No	0	40			
>=2.73	Yes	18	21	1.00	0.66	0.34
<2.73	No	0	41			
>=2.82	Yes	18	20	1.00	0.68	0.32
<2.82	No	0	42			
>=3.08	Yes	18	19	1.00	0.69	0.31
<3.08	No	0	43			
>=3.22	Yes	18	18	1.00	0.71	0.29
<3.22	No	0	44			
>=3.6	Yes	17	18	0.94	0.71	0.29
<3.6	No	1	44			
>=3.71	Yes	17	17	0.94	0.73	0.27
<3.71	No	1	45			
>=3.77	Yes	17	15	0.94	0.76	0.24
<3.77	No	1	47			
>=4.06	Yes	17	14	0.94	0.77	0.23
<4.06	No	1	48			
>=4.52	Yes	16	14	0.89	0.77	0.23
<4.52	No	2	48			
>=4.57	Yes	15	14	0.83	0.77	0.23
<4.57	No	3	48			
>=4.69	Yes	15	13	0.83	0.79	0.21
<4.69	No	3	49			
>=4.74	**Yes**	15	12	**0.83**	**0.81**	**0.19**
<4.74	**No**	3	50			
>=4.76	Yes	14	12	0.78	0.81	0.19
<4.76	No	4	50			
>=4.78	Yes	14	11	0.78	0.82	0.18
<4.78	No	4	51			
>=5.05	Yes	14	10	0.78	0.84	0.16
<5.05	No	4	52			
>=5.08	Yes	13	10	0.72	0.84	0.16
<5.08	No	5	52			
>=5.32	Yes	13	9	0.72	0.85	0.15
<5.32	No	5	53			
>=5.38	Yes	13	8	0.72	0.87	0.13
<5.38	No	5	54			

		RAO	Not RAO	Sensitivity	Specificity	1-Specificity
>=5.43	Yes	12	8	0.67	0.87	0.13
<5.43	No	6	54			
>=5.45	Yes	12	7	0.67	0.89	0.11
<5.45	No	6	55			
>=5.51	Yes	12	6	0.67	0.9	0.01
<5.51	No	6	56			
>=5.66	Yes	12	5	0.67	0.92	0.08
<5.66	No	6	57			
>=5.87	Yes	11	5	0.61	0.92	0.08
<5.87	No	7	57			
>=5.97	Yes	11	4	0.61	0.94	0.06
<5.97	No	7	58			
>=6.03	Yes	11	3	0.61	0.96	0.04
<6.03	No	7	59			
>=6.08	Yes	11	2	0.61	0.97	0.03
<6.08	No	7	60			
>=6.14	Yes	10	2	0.56	0.97	0.03
<6.14	No	8	60			
>=6.73	Yes	10	1	0.56	0.98	0.02
<6.73	No	8	61			
>=6.75	Yes	9	1	0.5	0.98	0.02
<6.75	No	9	61			
>=6.85	Yes	8	1	0.44	0.98	0.02
<6.85	No	10	61			
>=6.9	Yes	7	1	0.39	0.98	0.02
<6.9	No	11	61			
>=7.05	Yes	7	0	0.39	1.00	0.00
<7.05	No	11	62			
>=7.88	Yes	6	0	0.33	1.00	0.00
<7.88	No	12	62			
>=8.14	Yes	5	0	0.28	1.00	0.00
<8.14	No	13	62			
>=8.61	Yes	4	0	0.22	1.00	0.00
<8.61	No	14	62			
>=8.62	Yes	3	0	0.17	1.00	0.00
<8.62	No	15	62			
>=9.21	Yes	2	0	0.11	1.00	0.00
<9.21	No	16	62			
>=9.44	Yes	1	0	0.06	1.00	0.00
<9.44	No	17	62			
>=10	Yes	0	0	0.00	1.00	0.00
<10	No	18	62			

Appendix A5.1. Letter of invitation to practices to participate in the horse health survey.
Printed on University of Glasgow Veterinary School notepaper (not reproduced).

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«Name»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

6th August 2003

Dear «Name»,

We would like to invite you to collaborate in a client survey of the horse population of the United Kingdom. This study is being funded by the Home of Rest for Horses and is being conducted by the University of Glasgow Veterinary School. We are investigating the prevalence of recurrent airway obstruction in the horse population of the United Kingdom. Recurrent airway obstruction is also known as chronic obstructive pulmonary disease (COPD) or 'heaves'. The prevalence of this disease in the UK is unknown. We are also interested in the related disease of summer pasture associated obstructive disease (SPAOPD); anecdotal evidence suggests the incidence of this disease is increasing.

We have developed a risk-screening questionnaire (RSQ) that can be used to investigate the probability of a horse having RAO (or SPAOPD) based on signs and history reported by its owner. This approach has been used to investigate the prevalence of asthma in children and adults throughout the world, human asthma being very similar in many aspects to RAO in horses. Asthma diagnosis is not straightforward and tests considered to be gold standards, such as bronchial responsiveness, may have lower validity than risk-screening questionnaires. A study using an RSQ has never been attempted in the veterinary field before. Initial validation results are very encouraging, indicating that the RSQ has reasonable sensitivity and specificity for diagnosing RAO when compared to a veterinary diagnosis that includes the use of respiratory cytology.

Our intention is to survey a representative proportion of horse owners from throughout the UK using the RSQ to establish the prevalence of the disease RAO. The RSQ has been embedded within a more general questionnaire that focuses on feeding, stable management, geographic location, early life etc; this may help identify risk factors for the disease.

From preliminary investigations we calculate that we need to target approximately 2000 horse owners via 19 veterinary practices from throughout the UK. Your practice was randomly selected from the RCVS database of veterinary practices that stated they conducted equine work and we invite you to participate in the survey. This would involve you allowing access to your list or database of equine clients in order that we could randomly select 110 clients. These clients would be asked to participate in the mailed questionnaire. We would administer this process and the costs involved would be covered by our study. All the information provided would remain completely confidential and would only be used for the purpose of this study. The survey would be anonymous with identifying code numbers used for the administration process involved in mailing the questionnaire. You would be kept fully informed as to the progress of the survey and its final results.

This is a valuable opportunity to be involved in practice-based research. If you would like your practice to participate in this study or you would like further information before making a decision please complete the attached form and return it in the pre-paid envelope (or fax to 0141 330 6025). We will then contact you and discuss the study and your participation in more detail. If you have any queries please do not hesitate to contact Joel Hotchkiss. We hope you will agree to collaborate with us in this survey process and look forward to talking with you.

Yours sincerely

Joel Hotchkiss
BSc BVSc CertEM(Int.Med) MRCVS
Home of Rest for Horses' Postgraduate Student

Professor Sandy Love
BVMS PhD MRCVS
Head of Division of Equine Clinical Studies

Appendix A5.2. Form for veterinary practices' response to an invitation to participate in a horse health survey.
Reduced in size from A4.

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HOME of REST for HORSES

UNIVERSITY of GLASGOW

Confidential Horse Health Survey

Conducted by the Weipers Centre for Equine Welfare
Division of Equine Clinical Studies
University of Glasgow Veterinary School
Bearsden Road
Glasgow G61 1QH
Tel.: 0141 330 5999

Please return this form, indicating whether or not you are interested in partcipating in the survey. Please note that all information will remain confidential.

We are interested in our practice participating in a questionnaire survey of a sample of our equine clients

Yes, I/We are interested in participating ☐

If you would like to participate approximately how many equine clients do you have?
Please write the number of clients in the boxes below

Number of equine clients: ☐☐☐☐

Name of person to contact: ______________________

Best time of day to make phone contact: ______________

We do not wish to participate in this survey

No, I/We do not want our practice to participate ☐

Thank You for Your Time

Identification number for administrative purposes only: ☐☐

Appendix A5.3. Letter to practices that had indicated an interest in participating in the horse health survey.
Printed on University of Glasgow Veterinary School notepaper (not reproduced).

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«Name»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

2003

Dear «Name»,

Thank you for agreeing to consider allowing us to survey a sample of your clients. A sample of the questionnaire is enclosed and the final professionally printed booklet will differ little in content from this. The client would be sent one copy of the questionnaire enclosed with a cover letter and a pre-paid return envelope in early October.

The cover letter will:

1. Invite the horse owner to participate in the survey
2. Highlight the collaboration of your practice with the study and that this is how they were contacted.
3. Explain that the study is a survey of horse general health; the exact purpose of the study (investigation of respiratory disease) would not be mentioned.
4. Ask them to answer the questionnaire for only one horse (the first name in the alphabet).
5. Emphasise the confidential and anonymous nature of the survey. The horse owner would be reassured that their contact details would only be used for the purposes of conducting this survey.
6. Invite them to participate in a short further study where only a small selected sample (400 across the whole of the UK) would receive some information regarding the care of horses that would be followed by a short questionnaire. This is a small educational project where information would be provided on either environmental respiratory management or ragwort awareness. The short questionnaire would assess if the material changed peoples attitudes.
7. Encourage people who no longer own horses to return a blank questionnaire
8. Direct all queries to us at the University of Glasgow.

Horse owners who do not respond to the initial mailing would receive a polite postcard reminder two weeks later. If they had still not responded by 4 weeks a second copy of the questionnaire would be mailed with an invitation emphasising the importance of responding. These would be the only interventions and non-responders would not be contacted after this final mailing.

There are a number of methods available to randomly select the 110 horse clients from your practice list. We would recommend whatever method was most convenient for your practice. You would have veto on any clients you did not wish to be surveyed. We hope you will be able to take part in this survey and I shall be in touch to discuss the specific details of participation in the near future. Thank you for your time

Yours sincerely

Joel Hotchkiss
BSc BVSc CertEM(Int.Med) MRCVS
Home of Rest for Horses' Postgraduate Student

Appendix A5.4. Horse owner questionnaire for horse health survey.
Reduced in size from A4.

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HOME of REST for HORSES

Confidential Horse Health Questionnaire

Conducted by the Welpers Centre for Equine Welfare
University of Glasgow Veterinary School
Bearsden Road
Glasgow G61 1QH
Tel.: 0141 330 5999

UNIVERSITY of GLASGOW

How to Complete the Questionnaire

- This booklet contains questions about **your** horse. This horse might be one you own, part own or even have on long term loan. If you have more than one horse please complete the questionnaire for the horse who's **name comes first in the alphabet**
- Some of the questions require you to write your answers in the spaces provided. All the other questions require you to **tick** your answer in a box. If you make a mistake fill in the box in which you made the mistake and tick the correct answer

  Examples of how to mark the questionnaire: How old is your horse?: 0 8 years 0 6 months

  To answer "**no**" Yes ☐ No ☑

  To answer "**yes**" Yes ☑ No ☐
- If it is possible please use a black or blue pen to complete the questionnaire
- Please consider all options before indicating the **most appropriate** response to **each and every** question
- This survey should only take about **15 minutes** to complete
- All responses are **completely confidential** and will be identified by code numbers only
- Once completed please post this survey, using the **pre-paid envelope** provided

Thank you for your assistance

ID number for administrative purposes only:

About Your Horse

- **How old is your horse?** ☐☐ years ☐☐ months
- **How long have you owned or looked after your horse?** ☐☐ years ☐☐ months
- **What breed is your horse?**
- **What sex is your horse?** Mare/Filly ☐ Stallion/Colt ☐ Gelding ☐
- **Is your horse?** Less than 15 hands ☐ Greater than 15 hands ☐
- **What activity do you mainly use your horse for?**

  Performance ☐ e.g. racehorse, eventing, endurance

  Pleasure ☐ e.g. hacking, small competitions, riding club, riding school

  Showing ☐ Breeding/Stud ☐ Retired ☐
- **Approximately how many hours is your horse exercised for per week?** ☐☐ hours per week
- **Is your horse vaccinated for?**

  Influenza (flu) ☐ Tetanus ☐ Equine Herpes Virus ☐
- **Approximately how frequently do you worm your horse?**

  Never ☐ Annually ☐ 6 monthly ☐

  3 monthly ☐ 2 monthly ☐ More often than every 2 months ☐

  Worm irregularly depending on result of worm egg count in faeces ☐
- **How much time, on average, do you think you spend with your horse each day (or week), including time spent feeding, mucking out, grooming, riding etc?** (please complete either of the boxes below)

  ☐☐ hours per day **OR** ☐☐ hours per week
- **Who usually looks after your horse (feeding, mucking out etc), on a day to day basis?** (please tick the most appropriate description)

  Livery staff (full time) ☐

  Livery staff (part time) ☐ (you the rest of the time)

  Own private staff (full time) ☐

  Own private staff (part time) ☐ (you the rest of the time)

  You personally look after your own horse ☐
- **Does your horse suffer from the following?**

  Sweet itch ☐ Food allergy ☐ Neither of these problems ☐
- **How many horses do you own, or look after?** Number of horses: ☐☐☐

If you have owned or looked after your horse since birth or since before it was FIVE years old please answer the questions BELOW

If you acquired your horse, or started taking care of it, AFTER it was FIVE years of age please go to the NEXT page (page 4)

Your Horse's Early Life

- **Have you owned or looked after your horse since birth?**

  Yes ☐ No ☐ **If NO, at what age did you acquire your horse or start taking care of it?** ☐☐ years ☐☐ months

- **At what age was your horse first vaccinated against the following diseases?** (please tick the appropriate box for each vaccine)

	Never	Less than one year old	One to two years of age	Three to five years of age	Greater than five years of age
Influenza (flu)	☐	☐	☐	☐	☐
Tetanus	☐	☐	☐	☐	☐

- **Whilst under your care, between the ages of TWO and FIVE years, how often was your horse kept stabled for all, or part, of the day on the following kinds of bedding?** (please tick the appropriate box for each type of management)

	Never	Occasionally	Often	Always
Straw bedding	☐	☐	☐	☐
NOT straw bedding e.g. shavings, paper	☐	☐	☐	☐

- **Whilst under your care, what was the most common type of roughage or forage your horse was fed between the ages of TWO and FIVE years?** (if more than one type please tick all the appropriate boxes)

  Dry Hay ☐ Wet or soaked hay ☐ Haylage ☐ Silage ☐

- **Whilst under your care, as a youngster (below the age of 5 years), did your horse ever have a respiratory infection, perhaps a snotty nose and a cough, for more than a few days?**

  No ☐ Yes ☐ Don't know ☐

  IF YES,

  At what age(s) did respiratory infection occur? (your horse may have had more than one infection so please tick all the boxes that apply)

  As a foal, before weaning from their mother ☐ From weaning to one year old ☐

  As a yearling ☐ Between the ages of 2 and 5 years ☐

- **Did this respiratory infection require treatment by your vet?**

  No ☐ Yes ☐ Don't know ☐

  IF YES,

  What diagnosis did your veterinary surgeon make? (at different times your horse may have had more than one infection so please tick all the boxes that apply)

  Viral respiratory infection ☐ Bacterial respiratory infection ☐ Strangles ☐

  Flu (influenza) ☐ Other ☐ **If so please specify**

Please continue to the NEXT page (page 4)

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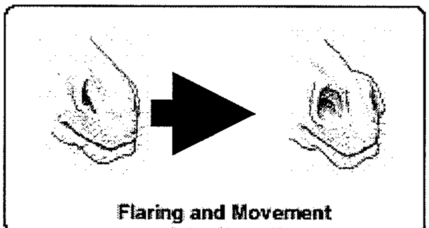
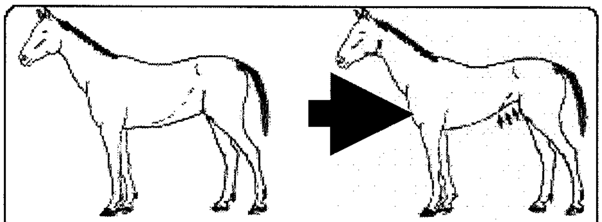
The Following Questions are About Your Horse's Respiratory Health and Breathing

When a horse has a respiratory problem they can have a wide range of symptoms. These include obvious symptoms such as a cough or a nasal discharge, as well as other symptoms related to an increased effort associated with breathing.

Sometimes, when at rest, horses will show subtle signs of increased effort of breathing by having **exaggerated abdominal effort** and **flaring and movement of the nostrils.** If the horse has not recently been exercising and is not excited or anxious, this may be a sign of increased breathing effort. In the set of questions that follow, we ask whether your horse has had these signs, and others, **whilst at rest.**



Flaring and Movement of the Nostrils



Exaggerated Abdominal Effort

- **Whilst under your care, at ANY time in the past, has your horse ever had any of the following symptoms?**

Coughing	No ☐	Yes ☐	Don't know ☐
A discharge from the nose	No ☐	Yes ☐	Don't know ☐
Exaggerated abdominal effort at rest	No ☐	Yes ☐	Don't know ☐
Nostril flare at rest	No ☐	Yes ☐	Don't know ☐

- **Has your horse had any of the following symptoms in the LAST 12 months?**

Coughing	No ☐	Yes ☐	Don't know ☐
A discharge from the nose	No ☐	Yes ☐	Don't know ☐
Exaggerated abdominal effort at rest	No ☐	Yes ☐	Don't know ☐
Nostril flare at rest	No ☐	Yes ☐	Don't know ☐

If you have answered YES to ANY of these questions please complete both parts A and B.
Otherwise please complete Part B on the next page (page 5).

Part A

The following questions are about the symptoms you have indicated as occurring above

- **Did two or more of these symptoms occur at the same time as each other?**
  No ☐ Yes ☐ Don't know ☐
- **Did these symptoms improve when the horse spent more time at pasture?**
  No ☐ Yes ☐ Got worse ☐ Don't know ☐
- **Were these symptoms associated with any of the following situations?** (please tick all that apply)
  Feeding dry hay ☐ Being at pasture ☐ Stabling ☐
  Use of straw bedding ☐ Mucking out ☐ Don't know ☐
- **During which times of the year did these symptoms occur?** (please tick all the months that apply)

Winter		Spring			Summer			Autumn			Winter
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

- **Did these symptoms occur more than once, i.e. recurrent?** (please tick all that apply)
  No ☐ More than once in one year ☐
  Repeatedly over a period of several years ☐ Don't know ☐

continues on next page

Part A (continued)

- **Were any of these symptoms associated with your horse being "off colour" or having a raised temperature?** (please consider all the options before selecting the most appropriate)

  No ☐ Yes ☐

  Yes, but not on every occasion ☐ Don't know ☐

- **Were these symptoms associated with a respiratory infection, as diagnosed by your vet?**

  No ☐ Yes ☐ Yes, but not on every occasion ☐

<u>If you indicated earlier that your horse has had a COUGH, please answer the following questions:</u>

- **How long has/did your horse cough for?**

  Less than 2 weeks ☐ About a month ☐

  Between 1 and 3 months ☐ 3 months or more ☐

- **During this period how often, on average, did your horse cough per day?**

  Rarely ☐ Infrequently ☐ Frequently ☐ Constantly ☐

- **Was this cough associated with any particular activity?** (please tick all that apply)

  No particular pattern to cough ☐ Coughed at start of exercise ☐

  Coughed during exercise ☐ Don't know ☐

Please now continue to part B below

Part B

- **Has your horse ever taken longer to recover from exercise than you would normally have expected, given its level of fitness?**

  No ☐ Yes ☐ Don't know ☐

  If YES, did this coincide with any of the symptoms mentioned on page 4?

  No ☐ Yes ☐

- **Has your horse ever had a period of time during which they did not perform as well as you would expect, or were unable to exercise at their normal level?**

  No ☐ Yes ☐ Don't know ☐

  If YES, did this coincide with any of the symptoms mentioned on page 4?

  No ☐ Yes ☐

- **Has a vet ever told you that your horse has COPD (chronic obstructive pulmonary disease), also known as heaves or recurrent airway obstruction (RAO), a dust or hay allergy?**

  No ☐ Yes ☐ Don't know ☐

  If YES, how old was your horse when this problem was diagnosed? ☐☐ years ☐☐ months

- **Has a vet ever told you that your horse has SPAOPD (Summer pasture associated obstructive pulmonary disease), an allergic respiratory disease associated with being at pasture in the summer?**

  No ☐ Yes ☐ Don't know ☐

  If YES, how old was your horse when this problem was diagnosed? ☐☐ years ☐☐ months

- **Do any relatives of your horse suffer from COPD (chronic obstructive pulmonary disease), also known as heaves or recurrent airway obstruction (RAO), a dust or hay allergy?**

  No ☐ Yes ☐ Don't know ☐

  If YES, how was/were the horse or horses related to yours? (please tick all the boxes that apply)

  Mother (Dam) ☐ Father (Sire) ☐ Brother/Sister ☐ Foals (offspring) ☐

About Your Horse's Management

- **How is your horse currently kept during each season of the year?** (please tick the appropriate box for each season of the year)

	Spring	Summer	Autumn	Winter
Entirely outdoors	☐	☐	☐	☐
Partly outdoors/partly stabled	☐	☐	☐	☐
Entirely stabled	☐	☐	☐	☐

- **On average, how long does your horse spend each day at pasture?** Please put 0 (zero) if stabled all day

In summer ☐☐ hours per day **In winter** ☐☐ hours per day

- **If you keep your horse stabled at any point during the year, what bedding do you use in the stable?** (if you use more than one product please tick all the boxes that apply)

Never stabled ☐ Straw ☐ Paper ☐

Wood Shavings ☐ Rubber mats ☐ Hemp based product ☐

Other ☐ If so please specify ______

- **Do you operate a deep bed system, also known as a deep litter system, when your horse is stabled ?**

No ☐ Yes ☐ Don't know ☐

- **Where is your horse during mucking out?** Kept in the stable ☐ Taken outside the stable ☐

- **Where is your horse usually groomed?** In the stable ☐ Outside of the stable ☐

- **What feed do you currently give during each season of the year, including grass?** (please tick the appropriate boxes for each season)

		Spring	Summer	Autumn	Winter
	Grass (grazing/at pasture)	☐	☐	☐	☐
	Dry hay	☐	☐	☐	☐
	Soaked (wet) hay	☐	☐	☐	☐
	Haylage or silage	☐	☐	☐	☐
Bucket Feed	**Concentrate** - straights e.g. oats/barley etc	☐	☐	☐	☐
Bucket Feed	**Concentrate** - compound e.g. cubes/nuts etc	☐	☐	☐	☐
Bucket Feed	**Chop or chaff**	☐	☐	☐	☐
	Other, If so please specify	☐	☐	☐	☐

______

- **If you wet hay before feeding by full immersion in water, how long do you soak it for?**

☐☐:☐☐ hours : minutes Don't fully immerse in water ☐

- **Do you wet or dampen concentrate (bucket) feeds?** No ☐ Yes ☐

- **Approximately how long have you been keeping you horse under the management detailed above?**

☐☐ years ☐☐ months

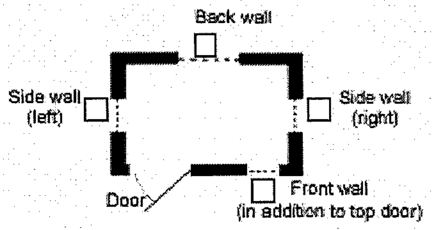
About Your Horse's Stable

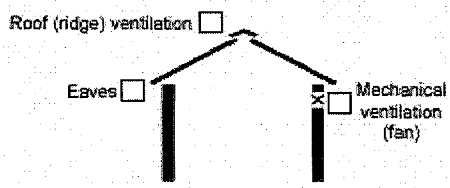
- **What best describes the type of building your horse is stabled in?**

Not stabled ☐ Field shelter ☐ Stall in an american type barn (enclosed) ☐

Loose box/stable, open fronted ☐ Other ☐ If so please specify

If you keep your horse in a loose box/stable, where does it have ventilation points or open windows?
(please tick all the appropriate boxes on the diagrams below that correspond to an open window or open ventilation point)





- **How close is the muck heap to your horses stable?**

Less than 6 metres (20 ft) from the stable ☐ 6 metres (20 ft) or over from the stable ☐

- **Is there a hay or straw store, sharing air space, in the same building as your horse?**

No ☐ Yes ☐ Don't know ☐

- **What bedding are horses in neighbouring stables kept on?** (please tick all the boxes that apply)

Straw ☐ Hemp based product ☐ Paper ☐ Wood Shavings ☐ Rubber mats ☐

Other ☐ If so please specify

- **Which of the following feeds are horses in neighbouring stables given?**

Dry hay ☐ Soaked (wet) hay ☐ Haylage/silage ☐

Other ☐ If so please specify

About The Premises Where You Keep Your Horse

- **Which of the types of premises listed below best describes where you keep your horse?**
(please tick one box only)

Livery stables ☐ Livestock Farm ☐ (cattle, sheep etc) Arable Farm ☐ (crops etc) Riding school ☐

Racing yard ☐ Stables on own premises ☐ Rented pasture only ☐ Private yard ☐

Other ☐ If so please specify

- **What is the total size (acreage) of the premises, including available grazing?**

Size of premises to nearest acre: ☐☐☐☐☐ acres

- **Apart from this horse, how many other horses are usually kept at the premises?**

Number of other horses: ☐☐☐

Please continue over . . .

About The Premises Where You Keep Your Horse (cont.)

- **What type of land immediately surrounds the pasture where your horse grazes?** (please tick all that apply)

  Grazing pasture ☐ Hay/haylage/silage fields ☐ Agricultural crops ☐ Woodland ☐

  Urbanized ☐ e.g. Housing, roads, industry etc Other ☐ If so please specify ☐

- **How would you describe the area local to where your horse is kept?**

  Urban ☐ Semi-rural ☐ Rural ☐ Industrial ☐

- **How long have you kept your horse at the current premises?** ☐☐ years ☐☐ months

- **What is the address of the premises where you keep your horse?** (only limited details have been requested, please do **NOT** provide the name of the premises e.g. name of farm, stable etc)

> PLEASE NOTE:
> **This information will be used only for geographically identifying the type of area where you keep your horse, for example elevation above sea level, local weather conditions. The address will not be used for any other purposes.**

Street/lane/road: ☐

Village: ☐

Town: ☐

County: ☐

Postcode: ☐☐☐☐ ☐☐☐

- **Would you like to participate in a further study in which we would provide information regarding the care of horses? This information would be followed by a short questionnaire.**

  Yes ☐ No ☐

That is the end of the questionnaire
Please return the completed questionnaire in the pre-paid envelope provided

Thank You For Your Participation In This Survey

Appendix A5.5. Cover letter sent with first owner mailing of the horse health questionnaire.

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«Title»«FirstName»«MiddleName»«LastName»
«BusinessStreet»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

**th October 2003

Dear «Title» «FirstName» «LastName»,

Together with your veterinary practice, "noahs ark", we would like to invite you to participate in a questionnaire survey investigating the health of horses throughout the United Kingdom. You were randomly selected to participate in this survey, which is intended to reflect the horse population of the UK. The study is being funded by the charity The Home of Rest for Horses and is being conducted by the University of Glasgow Veterinary School.

This survey will generate important information on the horse population including aspects of its health, care and environment. By completing the questionnaire you will contribute an important insight into how horses are cared for and how this potentially affects their general health. This is the first time that a survey of this kind has been attempted and the results will hopefully benefit all horses in the future.

The short questionnaire is self-explanatory and should only take about 15 minutes to complete. If you own or look after more than one horse we would request that you complete the questionnaire for the horse with the name that comes first in the alphabet. All information provided will remain completely confidential and will only be used for the purpose of this study. The survey will be anonymous with identifying code numbers used for the administration process involved in mailing the questionnaire. This is a one off survey; you will not be approached to take part in any further surveys unless you volunteer to participate in a small follow up study detailed in the questionnaire booklet. A pre-paid envelope is provided for the return of the questionnaire. If you no longer own a horse it would be greatly appreciated if you could return the questionnaire uncompleted.

We look forward to receiving your completed questionnaire. If possible please return it by **Friday, **th October 2003**. If you have any questions please contact me at the address below. We would like to thank you in anticipation of the valuable information you will provide in your returned questionnaire.

Yours sincerely
Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS

WEIPERS CENTRE FOR EQUINE WELFARE
University of Glasgow Veterinary School
Bearsden Road, Glasgow G61 1QH
Telephone: 0141 330 5999

Appendix A5.6. Reminder postcards mailed to non-responders to the horse health survey by the time of the deadline.

«FirstName»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

HOME of REST for HORSES

UNIVERSITY of GLASGOW

21st October 2003

Dear «FirstName»

Recently a questionnaire was mailed to you as part of a national survey investigating the horse population. If you have already completed and returned it to us please accept our thanks. If not, please do so today. It would be greatly appreciated if you could find the time to complete the questionnaire and return it in the prepaid envelope. We believe the information you can provide is extremely important to our survey. If you require a copy of the questionnaire please do not hesitate to contact me.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS
Weipers Centre for Equine Welfare, University of Glasgow Veterinary School,
Bearsden Road, Glasgow G61 1QH, *Tel.:* 0141-330 5999

Appendix A5.7. Cover letter sent as part of second mailing to non-responders in the horse health survey.

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«FirstName»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

2003

Dear «FirstName»,

Recently I wrote to you inviting you to participate in a questionnaire survey investigating the health of horses throughout the United Kingdom. I would like to take this opportunity to encourage you to participate in the survey, as I believe the information you can provide is extremely important to our study. Please find enclosed a second copy of the questionnaire booklet and a pre-paid return envelope to facilitate your participation. This study is being funded by the charity The Home of Rest for Horses and is being conducted by the University of Glasgow Veterinary School.

The short questionnaire is self-explanatory and should only take about 15 minutes to complete. If you own or look after more than one horse we would request that you complete the questionnaire for the horse with the <u>name that comes first in the alphabet</u>. All information provided will remain completely confidential and will only be used for the purpose of this study. The survey is anonymous with identifying code numbers used for the administration process involved in mailing the questionnaire. This is a one off survey; you will not be approached to take part in any further surveys unless you volunteer to participate in the small follow up study detailed in the questionnaire booklet.

To date we have had an excellent response to the survey. However, we wish to encompass information from as many people as possible and hope that you can find the time to contribute. If you have been contacted in error as you do not own or look after a horse it would be greatly appreciated if you could return the questionnaire uncompleted. If you have recently returned the questionnaire, please ignore this letter.

I hope you will not find this follow up communication too intrusive and I would like to thank you for your tolerance. If you have any questions regarding this survey please do not hesitate to contact myself.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS

WEIPERS CENTRE FOR EQUINE WELFARE
University of Glasgow Veterinary School
Bearsden Road, Glasgow G61 1QH
Telephone: 0141 330 5999

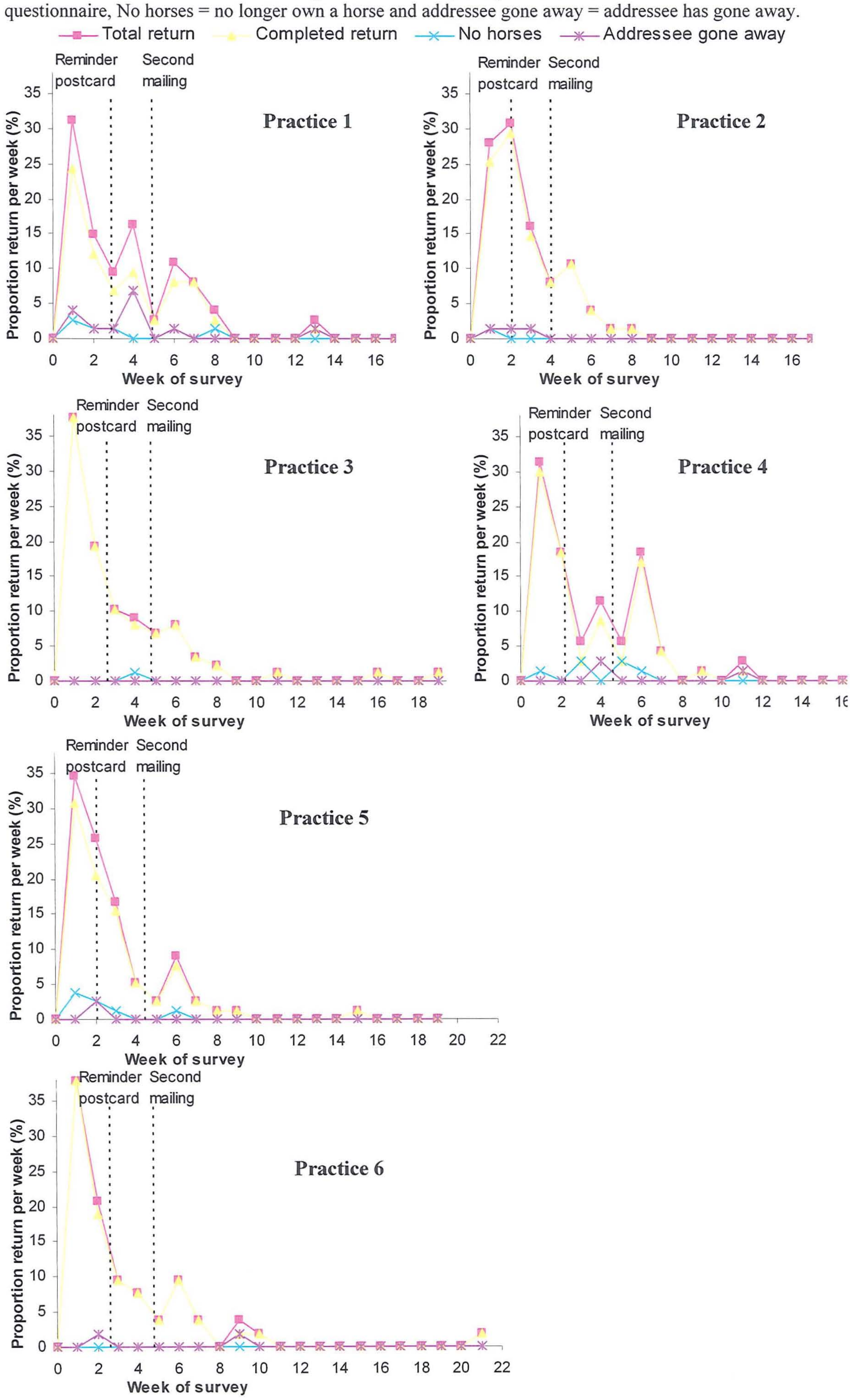
Appendix A5.8. Mailing numbers, return categories and observed return, response and completion proportions of the mailed horse health questionnaire for the fourteen practices.

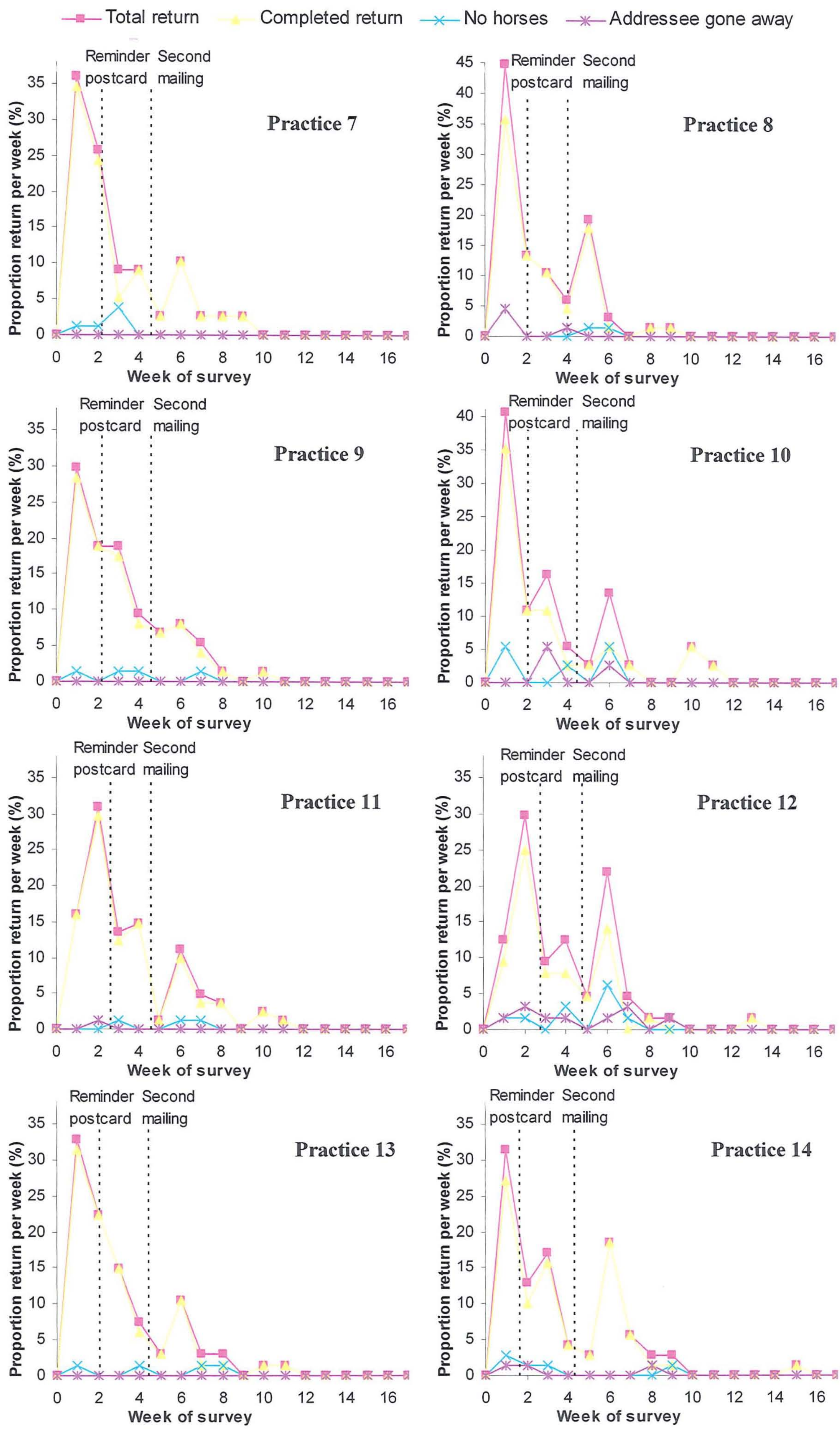
*Corrected total mailed = Total mailed – (no longer own horses + addressee has gone away)

Practice	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Total mailed (N)	110	110	110	110	105	69	103	110	114	54	111	110	110	106
First mailing	55	62	73	51	66	42	64	50	62	23	62	44	54	48
First mailing/N (%)	(50.0)	(56.4)	(66.4)	(46.4)	(62.9)	(60.9)	(62.1)	(45.5)	(54.4)	(42.6)	(55.9)	(40.0)	(49.1)	(45.3)
Second mailing	19	13	15	19	12	11	14	17	12	6	19	20	13	22
Second mailing/N (%)	(17.3)	(11.8)	(13.6)	(17.3)	(11.4)	(15.9)	(13.6)	(15.5)	(10.5)	(11.1)	(17.1)	(18.2)	(11.8)	(20.8)
No longer own a horse	6	1	1	6	7	0	5	5	4	5	3	9	4	5
No horse/N (%)	(5.5)	(0.9)	(0.9)	(5.5)	(6.7)	(0.0)	(4.9)	(4.5)	(3.5)	(9.3)	(2.7)	(8.2)	(3.6)	(4.7)
Addressee has gone away	12	3	0	3	2	2	0	4	0	3	1	9	0	3
Addressee gone away/N (%)	(10.9)	(2.7)	(0.0)	(2.7)	(1.9)	(2.9)	(0.0)	(3.6)	(0.0)	(5.6)	(0.9)	(8.2)	(0.0)	(2.8)
Returned proportion (also response proportion)	74	71	88	70	78	53	78	67	74	29	81	64	67	70
Return proportion/N (%)	(67.3)	(64.5)	(80.0)	(63.6)	(74.3)	(76.8)	(75.7)	(60.9)	(64.9)	(53.7)	(73.0)	(58.2)	(60.9)	(66.0)
No return by end of study period	36	39	22	40	27	16	25	43	40	25	30	46	43	36
No return/N (%)	(32.7)	(35.5)	(20.0)	(36.4)	(25.7)	(23.2)	(24.3)	(39.1)	(35.1)	(46.3)	(27.0)	(41.8)	(39.1)	(34.0)
Completion proportion (useable surveys)	56	71	87	61	69	51	73	58	70	29	77	46	63	62
Completion proportion/N (%)	(50.9)	(64.5)	(79.1)	(55.5)	(65.7)	(73.9)	(70.9)	(52.7)	(61.4)	(53.7)	(69.4)	(41.8)	(57.3)	(58.5)
Corrected total mailed* (n)	**92**	**106**	**109**	**101**	96	**67**	**98**	**101**	**110**	**46**	**107**	**92**	**106**	**98**
Corrected completion proportion Completion proportion/n (%)	**60.9**	**67.0**	**79.8**	**60.4**	**71.9**	**76.1**	**74.5**	**57.4**	**63.6**	**63.0**	**72.0**	**50.0**	**59.4**	**63.3**

Appendix A5.9. Return frequencies over time for practices one to fourteen.

Total return = total number of returns regardless of reason; Completed return = useable returned questionnaire, No horses = no longer own a horse and addressee gone away = addressee has gone away.





Total return
Completed return
No horses
Addressee gone away
Reminder postcard
Second mailing
Practice 7
Practice 8
Practice 9
Practice 10
Practice 11
Practice 12
Practice 13
Practice 14
Proportion return per week (%)
Week of survey

Appendix A5.10. Breakdown of the cost of the horse health survey.

	Cost (£)
First Mailing (n = 1431)	
Questionnaire Booklets	299.86
Return Envelopes	134.52
Cover Letters	143.10
A4 envelopes	41.32
Postage first mailing (first-class = 28p)	400.68
Reminder Postcard Mailing (n = 883)	
Reminder postcard	134.87
Postage postcard (second class = 21p)	185.43
Second Mailing (n = 680)	
Questionnaire Booklets	142.39
Return Envelopes	63.94
Cover Letters	68.00
A4 envelopes	19.61
Postage second mailing (first-class = 28p)	190.40
Return Mailing (n = 976)	
Response Service Licence	60.00
Cost of return postage (second-class = 21.5p)	209.84
Total Cost First Mailing	**1019.48**
Total Cost Reminder Postcard	**320.30**
Total Cost Second Mailing	**484.34**
Total Cost of Return Postage	**269.84**
Total Cost of Survey	**2093.96**
Total Cost per Useable Return (n = 873)	**2.40**

Appendix A5.11. Table of the reported frequencies of different breeds and cross breeds of horses for which completed questionnaires were received.
Continued on page 283.

	Arab	Anglo Arab	Andalucian	Appaloosa	American Quarter Horse	Cleveland Bay	Clydesdale	Cob	Connemara	Dales pony	Dole	Exmoor	Fell pony	Gelderlander	Hackney	Haflinger	Hanovrian	Highland	Holsteiner	Hunter	Irish Cob	Irish Draught	Irish Sports Horse	Lipizzaner
Arab	21																							
Anglo Arab		13																						
Andalucian			1																					
Appaloosa	1			7																				
American Quarter Horse					5																			
Cleveland Bay						1																		
Clydesdale							1																	
Cob							1	42																
Connemara	6	1							13															
Dales pony								1		2														
Dole											1													
Exmoor									1			1												
Fell pony								2					5											
Gelderlander														1										
Hackney															1									
Haflinger																1								
Hanovrian						1	1	1		1							4							
Highland					1													14						
Holsteiner																			1					
Hunter																				6				
Irish Cob																					9			
Irish Draught	1	1		1			1		1						1		1		1			8		
Irish Sports Horse																							11	
Lipizzaner																								2
Minature Shetlands																								
Morgan																								
Native	1																							
New Forest																								
Nonius																								
Norwegian Fjord																								
Palamino																								
Percheron																								
Polo																								
Riding pony																								
Standard Bred																								
Selle Francais																								
Shetland Pony																								
Shire								1		2							1					1		
Thoroughbred	5			2		7	6	5	8				1				16	1		5		77		
Trakehner																								
Trotter																								
Welsh	18														2			1						
Welsh Section A								1																
Welsh Section B																								
Welsh Section C								1																
Welsh Section D (Cob)						1				1												5		
Warmblood						1	1										1			1		2		
Unknown	10					1	1	7	8	4		1	2				3	3		1		10		

	Miniature Shetlands	Morgan	Native	New Forest	Nonius	Norwegian Fjord	Palamino	Percheron	Polo	Riding pony	Standard Bred	Selle Francais	Shetland Pony	Shire	Thoroughbred	Trakehner	Trotter	Welsh	Welsh Section A	Welsh Section B	Welsh Section C	Welsh Section D	Warmblood	Unknown
Miniature Shetlands	4																							
Morgan		2																						
Native			2																					
New Forest				8																				
Nonius					0																			
Norwegian Fjord						2																		
Palamino							1																	
Percheron								1																
Polo									6															
Riding pony										10														
Standard Bred											3													
Selle Francais												1												
Shetland Pony													7											
Shire														2										
Thoroughbred				2						2		1		8	99									
Trakehner															3	5								
Trotter																	1							
Welsh															5			5						
Welsh Section A																			28					
Welsh Section B																				12				
Welsh Section C															1						5			
Welsh Section D (Cob)															10						1	60		
Warmblood												1		1	9			1	1				39	
Unknown			1		1							2		4	42			8	3			7	2	31

Appendix A6.1. Descriptive statistics and multilevel, univariable logistic regression analyses of host and environmental risk factors associated with a positive RSQ for RAO in a randomly selected population of horses in Great Britain.
Results of the multilevel, multivariable logistic regression model (table 6.3) are provided for comparison.

	Control % (n)	Case % (n)	Univariable odds ratio	Univariable p	Multivariable odds ratio	Multivariable p
Age						
Less than five years	97 (70)	3 (2)	Ref.		Ref.	
Five to seven years	88 (98)	13 (14)	5.02	0.04	5.08	0.04
Seven to nine years	80 (117)	20 (30)	8.87	0.003	8.13	0.005
Nine to eleven years	75 (99)	25 (33)	11.63	0.001	11.38	0.001
Eleven to fifteen years	77 (128)	23 (39)	10.54	<0.001	9.45	0.003
Greater than fifteen years	62 (150)	38 (93)	21.13	0.001	18.30	<0.001
Breeding						
Not used for breeding	75 (613)	25 (206)	Ref.		Ref.	
Used for breeding	91 (49)	9 (5)	0.33	0.02	0.37	0.045
Urbanisation						
Rural	77 (589)	23 (178)	Ref.		Ref.	
Semi-urban, urban	69 (73)	31 (33)	1.54	0.06	2.20	0.002
Access to grass						
Grass for 2 seasons or more	75 (574)	25 (195)	Ref.		Ref.	
None or only one season	85 (88)	15 (16)	0.55	0.04	0.50	0.04
Forage						
2 seasons or less exposure to dry hay	73 (465)	27 (172)	Ref.		Ref.	
3 seasons or more exposure to dry hay	83 (197)	17 (39)	0.52	0.001	0.60	0.02
1 season or less exposure to wet hay	79 (582)	21 (159)	Ref.		Ref.	
2 seasons or more exposure to wet hay	61 (80)	39 (52)	2.34	<0.001	1.88	0.004
Time at pasture in winter						
Centred (hours/day)				0.02		0.03
Centred squared				<0.001		0.001

Appendix A6.2. Descriptive statistics and multilevel, univariable logistic regression analyses of early life factors associated with a positive RSQ for RAO in a randomly selected population of horses in Great Britain.
Results of the multilevel, multivariable logistic regression model (table 6.6) are provided for comparison.

	Control % (n)	Case % (n)	Univariable odds ratio	Univariable p	Multivariable odds ratio	Multivariable p
Age acquired						
Bred as foal or acquired before 2 years	84 (122)	16 (24)	Ref.		Ref.	
Acquired after 2 years	74 (125)	26 (45)	1.78	0.043	1.81	0.045
Early exposure to hay						
No	82 (222)	18 (49)	Ref.		Ref.	
Yes	56 (25)	44 (20)	3.17	0.02	3.92	0.009
Early respiratory infection						
No	91 (51)	9 (5)	Ref.		Ref.	
Yes	75 (196)	25 (64)	4.00	<0.001	4.62	<0.001

Appendix A7.1. Educational booklet on ragwort.

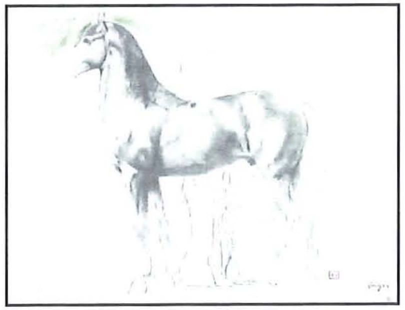
Front cover

RAGWORT:

A DANGER TO HORSE HEALTH

Helping you maintain your horses health

Back cover



Funded by	The Home of Rest for Horses
Produced by	Weipers Centre for Equine Welfare University of Glasgow Veterinary School

Ragwort Poisoning

Ragwort is a common weed that grows throughout the British Isles. Ragwort has always been a problem but recently it has become apparent that the weed may be getting out of control and potentially posing a real threat to the horse population.

Ragwort, also known as *Senecio jacobea*, contains the toxic compounds **pyrrolizidine alkaloids.** Horses are particularly susceptible to ragwort poisoning although other grazing animals are also at risk. Pyrrolizdine alkaloids principally damage the liver, resulting in severe disease and in many cases death.

In this short article we describe:

How to recognise ragwort and where it grows

How ragwort can damage your horses' health

How ragwort can be controlled

Facing pages

Ragwort

The Life of Ragwort

Ragwort is normally a biennial – taking two years to fully grow and flower. Seedlings have a spade shaped leaf that is notched at the top. In the first year of growth ragwort has a dense rosette of leaves low to the ground.

Plants in their second year grow to between 30 and 100cm high and have woody stems and dark green leaves with ragged, irregular edges. They produce bright yellow, densely packed flowers from May to October.

Ragwort can behave like a perennial (flowering every year) if the long stems are cut or mown. Each plant produces thousands of seeds that are dispersed widely by the wind resulting in the rapid spread of the weed. Seeds can also lie dormant for years before germinating.

Ragwort and Horses

Ragwort thrives on wasteland, road verges and railway land and from these locations it can spread to pasture. Poor quality and poorly managed horse pastures are particularly susceptible to ragwort infestations. Closely growing grass sward prevents ragwort growth but when the grass becomes thinned out, due to poaching or over grazing, the seeds are able to germinate in the exposed soil.

Most animals will avoid eating ragwort as long as they have an alternative source of good food. This can therefore be a problem on sparse, overgrazed pastures which ragwort can thrive on.

There are anecdotal reports that some horses can develop an acquired taste for the plant, especially if there is little else to eat.

When cut or wilted (during hay or haylage making) ragwort loses its bitter taste and becomes more palatable to horses. Drying does not destroy the toxins and dried grass, hay and haylage are common sources of ragwort poisoning.

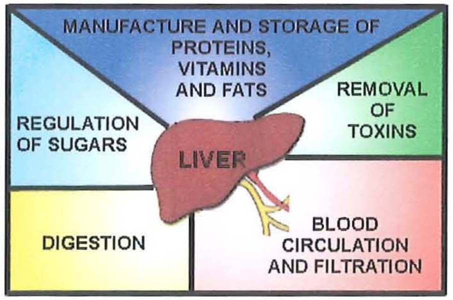
Ingestion of the pyrrolizidine alkaloid toxin contained in ragwort typically results in the delayed onset of chronic, progressive liver failure.

Facing pages

The Normal Liver of a Horse

The liver plays a vital role in the body and is one of the most active organs. The majority of nutrients absorbed from the intestines pass straight to the liver for "processing". The liver is responsible for regulation of these nutrients to ensure the body has enough protein, carbohydrate and fats.

The liver's other functions include manufacture of many of the proteins essential for life, removal of toxins from the body, aiding the intestinal digestion of food, as an important part of the immune system and storage for several vitamins and trace minerals.



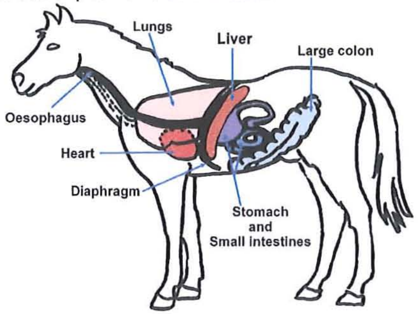
FUNCTIONS OF THE LIVER

Large cells called **hepatocytes** perform most of the liver's work. The liver is constantly regenerating itself – hepatocytes die and are replaced by new cells on a regular basis.

Liver failure (or hepatic failure) occurs when the liver can no longer perform its essential functions. Liver failure only tends to happen when at least 80% of the liver has been damaged. The liver is able to regenerate under certain conditions.

Liver Damage by Ragwort

The effects of ragwort toxins are cumulative, thus it is common for ragwort poisoning to occur following **consumption of small quantities of the plant over a long period of time.** Development of disease can be delayed from four weeks to six months after eating the plant. Different individuals appear to have different susceptibilities to the toxin.



When a horse eats ragwort the pyrrolizidine alkaloids are absorbed into the body from the intestines. The alkaloids then pass straight to the liver where they are further metabolised to produce toxic agents that damage liver cells (hepatocytes). These damaged liver cells can no longer manufacture protein and cannot multiply in order to replace themselves.

When these cells die they are replaced by fibrous tissue. Slowly, as more and more cells are damaged, the liver shrinks and becomes more fibrous in structure. Eventually there are not enough functional liver cells left to conduct the essential functions of the liver and liver failure is inevitable.

The clinical signs (or symptoms) of ragwort poisoning tend to only become apparent when liver failure has occurred. There is often no warning of impending failure. The signs of liver failure are a direct reflection of the loss of liver function.

Ragwort Poisoning – Clinical Signs

A principle sign is bizarre or depressed behaviour due to altered mental status (**hepatic encephalopathy**). This is thought to occur because the liver is no longer able to remove chemicals or toxins from the blood that have harmful effects on the brain.

A second common clinical sign is inflammation of white, unpigmented areas of the skin when they have been exposed to sunlight, known as **photosensitization.** The degradation of grass in the intestines produces a photodynamic agent (that reacts to sunlight). This agent is normally removed and eliminated by the liver. In hepatic failure this does not occur and concentrations of the agent increase in the body. In areas of unpigmented skin the activation of the photodynamic agent by sunlight causes damage to and inflammation of the skin. This is not the same as the common problem of sunburn, as suffered by some horses.

Other clinical signs of liver failure may also occur, these include jaundice, weight loss and diarrhoea.

Diagnosis

Diagnosis of liver failure can be aided by laboratory analyses of blood samples that often show evidence of liver damage and reduced liver function. Performing a liver biopsy can make a definitive diagnosis.

Treatment

Treatment is difficult once liver failure has occurred. It relies on supportive therapies in the hope that the liver can regenerate. Unfortunately in many cases the liver is too damaged for this to occur, although some horses can survive.

Field Companions

Attention should also be turned to companion horses that are showing no clinical signs of liver failure. It is essential that the possible source of ragwort be eliminated from their diet.

Prevention

Prevention of ragwort poisoning is the best option. Control methods for ragwort such as pulling, spraying and cutting should all be viewed as short-term methods. Maintaining or improving the quality of pasture should be the long-term priority to ensure the prevention of ragwort growth.

If ragwort begins to grow in a paddock it must be immediately removed before it can spread new seed.

Pulling Ragwort Plants Up

This is the most basic control method and is particularly appropriate when the ragwort is not an extensive problem. Rubber gloves should be worn, as the plants are potentially harmful to humans. Fragments of root will remain in the ground so new growth will have to be monitored and removed, year after year.

All pulled plant material should be **removed and burnt** to prevent all animals (including cows and sheep) from eating it.

Facing pages

Mowing/Cutting

Cutting will not kill the ragwort plant; in fact it may actually encourage growth. However, in an emergency situation mowing may prevent seed production.

Chemical Spraying

This is effective against the less mature rosette form of ragwort; however, older stemmed plants are more resistant. Spring is the ideal time for spraying pasture for grazing, but is too late if hay production is intended. Spraying for hay production should be carried out in the previous autumn.

All affected paddocks should be sprayed at the same time to avoid ragwort spreading to the bare areas left by dead weeds. As the ragwort dies it should be removed and burnt before pasture can be grazed.

Improving Pasture Quality

Ploughing is very effective at removing ragwort as long as it is ensured that the grass reseeding produces a healthy, thick sward Fertiliser encourages thick, vigorous sward growth. Good grazing management to prevent overstocking, overuse and poaching is essential.

What to do if you are concerned about Ragwort spreading to your land

The control of Ragwort comes under two government acts the Weeds Act 1959 (for the whole of the UK) and the Ragwort Act 2003 (England and Wales only). Under these laws governmental authorities can serve clearance notices to prevent the weed from spreading.

If appropriate, in the first instance, approach the owner/occupier of the land on which ragwort is growing and request them to take steps to clear the weed.

Ragwort on road verges
If ragwort is growing on the verges of motorways or trunk roads the Highways Agency should be contacted.

Telephone: 08457 50 40 30
Website: http://www.highways.gov.uk

If the ragwort is on the verges of minor roads the complaint should be directed to the local highway authority.

Ragwort on railway land
Where ragwort is growing on land associated with the railways Network Rail should be contacted.

Telephone: 0845711 41 41
Website: http://www.networkrail.co.uk

Facing pages

Governmental agencies

In England and Wales the Department of Environment, Farming and Rural Affairs (Defra) is responsible for enforcing the Weeds Act 1959 and Ragwort Act 2003. If the problem of ragwort growing on adjacent land cannot be resolved then a Weed Act complaint form can be completed. These forms, and further information, can be obtained from either:

Website: http://www.defra.gov.uk/rural/horses/topics/ragwort.htm.

Or from the following rural development services (depending on the region)

Bristol - South West Rural Development Service
Block 3
Government Buildings
Burghill Road
Westbury-on-Trym
Bristol BS10 6NJ
Tel: 0117 959 8622

Crewe - North West Rural Development Service Office
Electra Way
Crewe
Cheshire CW1 6GJ
Tel: 01270 754262

In Scotland the responsibility for the Weed Act 1959 is that of the Scottish Executive Environment and Rural Affairs Department (SEERAD) and pressure is underway to update legislation inline with that in England and Wales.

Conservation Branch
Scottish Executive Rural Affairs Department
Pentland House
47 Robb's Loan
Edinburgh EH14 1TY

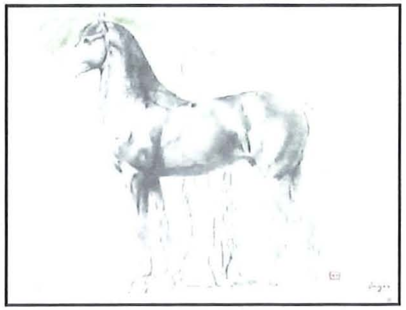
Appendix A7.2. Educational booklet on respiratory health.

Front cover

HORSE RESPIRATORY HEALTH

Helping you maintain your horses health

Back cover



Funded by	The Home of Rest for Horses
Produced by	Weipers Centre for Equine Welfare
	University of Glasgow Veterinary School

The Respiratory System of the Horse

The horses' respiratory system is very efficient and has developed to make the horse a unique athlete.
The respiratory system is adapted for the horses' natural environment, which is open pasture where there is limitless fresh air.
By removing horses from their natural environment we risk upsetting the natural balance of their health.

In this short article we:

Describe how the respiratory system works

Describe how the way we keep horses can affect their health

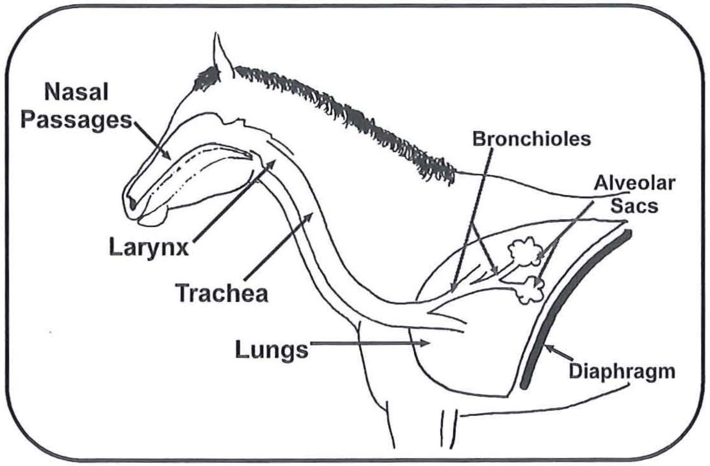
Suggest ways to minimise the challenge to your horse's respiratory health

Facing pages
Facing pages

The Structure of the Respiratory System

Horses use the muscles of the chest, diaphragm and abdomen to move air in and out of their lungs. During breathing, air is drawn in through the nostrils, passes along the nasal passages through the larynx and into the trachea (or windpipe).

The trachea then branches into two bronchi, one passing into the left lung and the other into the right lung. Within each lung these bronchi repeatedly branch forming smaller and smaller airways known as bronchioles. Eventually the bronchioles end as alveolar sacs where oxygen is drawn into the blood and waste gases are expelled from the body.



Defence Mechanisms of the Lungs

The respiratory system has a defence mechanism for preventing infection and also removing any airborne particles or dust that a horse inhales. The majority of harmful material is filtered out by the nasal passages. Smaller particles including dust, bacteria and viruses may pass further into the lungs, even as far as the alveolar sacs, and be deposited there. The lining of the airways provides protection against the potential harmful effects of these small particles in a number of ways:

- It secretes mucus and other substances that form a liquid barrier.
- Much of the airway lining has millions of microscopic finger like projections that sweep dust and other substances upwards away from the lower airways and into the back of the throat.
- The respiratory system also has a very active immune system that is able to react to, fight and remove material ranging from bacteria to tiny particles of dust.

Breathing Zone

It is the quality of the air in the zone directly around a horses' nostrils that is critical to its respiratory health - this is termed the **breathing zone,** as this is the air that a horse will draw into its lungs

Even if a horse is in a well-ventilated stable if it has its nose buried deep in mouldy and dusty hay, or is rooting in its bed, it will be inhaling thousands of airborne particles from the immediate surrounding breathing zone

Facing pages

Stabling

A stable is an unnatural environment for the horse, potentially exposing its respiratory system to a multitude of airborne challenges including:

- Dust
- Fungi
- Toxins
- Ammonia

Research has shown that some stables exceed the dust levels that are deemed safe for people working in factories.

When stabled, a horse's defence mechanism is constantly challenged and has to work overtime to remove harmful substances from the lungs. One component of the defence mechanism is the immune system, which may be particularly active and result in inflammation of the respiratory tract.

When stabled, even healthy horses have been shown to have inflamed respiratory tracts. For most horses, the respiratory tract is at its healthiest when the horse is at pasture 24 hours a day.

Even though apparently healthy horses may not show obvious clinical signs such as coughing or nasal discharge, these horses may not be able to perform at their full potential and may have respiratory problems in later life.

Recurrent Airway Obstruction (RAO)

Recurrent airway obstruction (RAO) or 'heaves' is a respiratory disease associated with horses generally over 7 years of age. Signs of RAO include coughing, nasal discharge and, in more severe cases, increased respiratory effort with involvement of the abdominal muscles, hence the name 'heaves'.

The disease is linked to stabling (and is therefore often seen in winter). Horses with RAO are believed to have developed an **allergy** to one or more of the airborne particles (often termed **allergens**) associated with housing. Most evidence points to **fungal spores**, present in both hay and straw, as being the principle agents that cause RAO.

Some Confusing Terminology
Respiratory disease associated with stabling has been recorded since the time of the ancient Greeks – as documented by Aristotle.

Since then the condition has gone under many names including:

- Hay or Dust Allergy
- Chronic Obstructive Pulmonary Disease (COPD)
- Small Airway Disease (SAD)
- Broken Wind

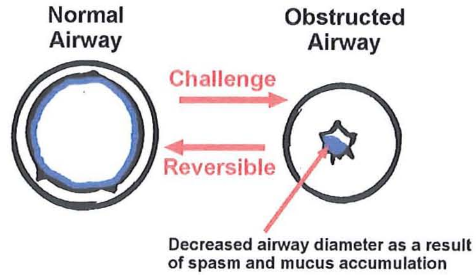
Nowadays veterinary surgeons throughout the world refer to this disease as either:

Recurrent Airway Obstruction (abbreviated to **RAO**)
Or **'Heaves'**

Facing pages

What happens to the lungs of horses with RAO?

When a susceptible horse breathes in an allergen the immune system of the lungs "overreacts" and the bronchioles go into "spasm" reducing their diameter. The airways also become inflamed and, as a result, clog with increased quantities of mucus.



All of these changes lead to obstruction of the airways, making it more difficult for the horse to breathe air in and out of its lungs. To compensate, horses have to increase the effort associated with breathing (heaves). The respiratory inflammation and excess mucus that occur in RAO result in the clinical signs of coughing and nasal discharge. A horse with RAO has a less efficient respiratory system and often cannot perform athletically to their full potential.

Once this process has been set in motion, an affected horse's lungs react not only to allergens but also to other irritants, such as dust and ammonia from urine.

Horses with RAO have become hypersensitive and hyperreactive to allergens associated with stabling. Medication is sometimes used to control the disease, however, in the long term control of the environment is essential.

Improving Air Quality

Maximise Turnout
The natural environment for a horse is out of doors – away from all sources of dust present in a stable. For many reasons this is often not practical and there has to be a compromise between stabling and turnout.

Reducing the Challenge
All horses would benefit from being exposed to reduced dust and this is especially true for horses with RAO. Ideally when stabled a horse should be provided with:

1. **A well-ventilated stable**
2. **Low dust bedding**
3. **Low dust feeds**

Ventilation

Stables should be well ventilated. This means that there should be proper circulation of air with no stagnant regions. Fresh air should be able to enter the stable and stale air able to leave it. Ventilation relies on two main processes:

1. Fresh air should enter a stable via the open top door, as well as through windows or vents at the back, sides or front. Horses body heat warms the air, which then rises and leaves the stable at the highest point of the stable. This circulation of air draws fresh air from the sides and stale air leaves via the roof.
2. The prevailing wind also plays a part forcing fresh air into the stable and drawing stale air out of it. However, it is important to avoid draughts by using baffled windows or vents.

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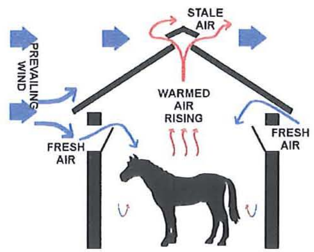
Facing pages

Ventilation

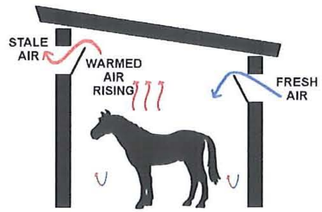
Many modern stable designs provide warm and comfortable accommodation but do not provide sufficient ventilation, as they do not contain enough inlets or outlets in addition to the open top door, which should always be open. Ventilation must be maintained even in cold weather – it is better to put an extra rug on your horse than close vents, windows or doors.

Stables with pitched roofs

Windows or vents in the walls allow fresh air to enter the stable.
An alternative arrangement is to have vents in the eaves allowing fresh air in.



Stables with mono-pitched roofs and lean to stables



Windows or vents in the front and back walls allow fresh air to enter the stable (through the lower opening) and stale air to leave (though the upper opening)

Barns
American type barns, although apparently spacious and airy, may not allow proper circulation of air, which result in regions of stagnant air within the barn. Barns require multiple outlets and inlets to counteract these limitations – some barns even incorporate mechanical ventilation.

Creating a Minimum-Dust Environment

Good ventilation is essential for the health of your horse, but is undermined if the breathing zone of a horse is surrounded by bedding and forage containing dust and fungal spores.

Bedding
Wood shavings (dust extracted), paper, cardboard or hemp consistently contain minimal dust and fungal spore content. Good quality straw that has been well stored can have low dust content however it is hard to guarantee that this is consistent for every bale.

Deep litter systems may encourage the growth of fungi and can be associated with other agents that inflame the respiratory system, for example ammonia and endotoxins.

Drainage
Ensuring proper drainage prevents urine and other liquids from collecting in the stable. Urine contains ammonia, a noxious gas, which can be irritating to the respiratory tract.

Feeding
Fungal spores develop in hay because our climate results in it usually being cured in damp, humid conditions. Again it is pointless having a perfect environment if a horse has its muzzle, and therefore nostrils, buried in hay containing fungal spores. Soaking hay does prevent the majority of fungal spores becoming airborne, as long as it does not dry out. Prolonged soaking will leech nutrients and vitamins from hay. A balance is probably complete immersion in water for between 30 minutes and two hours.

Alternatives such as haylage can be considered. The baling and wrapping process involved in making haylage creates an environment that prevents the formation of fungal spores.

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Facing pages

Minimise the challenge to your horse's respiratory health

Bucket Feeds

Straights and concentrates in bucket feeds can produce dust, which can be inhaled when the horse has its nose buried in the bucket. Therefore it is beneficial to damp down hard feed with a small amount of water.

Groom outside

Grooming can generate a lot of dust and should be done outside where the dust cannot accumulate and be breathed in by the horse.

Hay and Bedding stores
These can also be a source of dust and fungal spores. Try to avoid storing in a common air space with a horse's stable, for example an overhead hayloft.

Muck out with horse absent
Ideally a horse should not be present in its stable during mucking out and bedding down, as this generates lots of airborne particles. If possible allow dust to settle after putting down a new bed before returning your horse to its stable.

Muck heaps should be remote from a horse's stable as they are a source of dust and generate fungal spores.

Appendix A7.3. Questionnaire for educational survey.
Reduced in size form A4.

0431471718

HOME of REST FOR HORSES

Confidential Horse Questionnaire

UNIVERSITY of GLASGOW

Conducted by the Weipers Centre for Equine Welfare
University of Glasgow Veterinary School
Bearsden Road
Glasgow G61 1QH
Tel.: 0141 330 5899

- This questionnaire is about the horse health information booklet that was recently mailed to you.
- Some of the questions require you to write your answers in the spaces provided. All the other questions require you to **tick** your answer in a box. If you make a mistake please fill in the box in which you made the mistake and tick the correct answer.
- All responses are **completely confidential** and will be identified by code numbers only.
- Once completed please post this survey, using the **pre-paid envelope** provided.

Thank you for your assistance

About The Horse Health Booklet

- **How would you rate the overall presentation of the booklet?**

  Poor ☐ Fair ☐ Good ☐ Very good ☐ Excellent ☐

- **Were you interested in the information provided in the booklet?**

  Not interested ☐ Mildly interested ☐ Moderately interested ☐ Extremely interested ☐

- **Would you have liked more or less detailed information in the booklet, or was it about right?**

  Less detail ☐ About right ☐ More detail ☐

- **Did the booklet contain any information you were not already aware of?**

  No ☐ Yes ☐

  If **YES** please briefly describe just one item that made an impression on you

- **Where have you obtained information on these issues from in the past?** Please tick all the boxes that apply.

  The media (TV and newspapers) ☐
  Horse press, books and magazines ☐
  Horse and breed societies ☐
  World wide web ☐
  Fellow horse owners, relatives and friends ☐
  Equine courses (BHS examinations, evening classes, college, university etc) ☐

- **Were there any issues in the booklet that you did not agree with or believe not to be true in your experience?** Nothing is ever black and white, there is never only one correct answer. Your honest criticism would be greatly appreciated.

  No ☐ Yes, one or two items ☐ Yes, a large proportion of the items ☐

  If **YES** please describe just one item you particularly objected too

- **Would you consider adopting any of the management practices suggested in the booklet ?**

  Definitely not ☐ Possibly ☐ Definitely ☐ Already use many or most of the management practices ☐

ID number for administrative purposes only: ☐☐☐

1287471718

The next six questions are designed to find out how much you know about two horse health problems associated with the way we keep horses, for example:

1. Stabling places horses in a unique set of conditions that may affect their health.
2. Ragwort is a weed that grows throughout the United Kingdom and it contains toxins that are harmful to horses that eat it.

Please read the questions below carefully and consider all the options before deciding which single answer is the most appropriate. Try to answer the questions without looking anything up.

1) Is it possible for healthy horses to have an inflamed respiratory system just because they are being kept in a stable?

False ☐ True ☐

2) If the weather turns very cold and your horse needs extra warmth whilst it is stabled, it is best to do which of the following?

Close windows or vents ☐
Put an extra rug on your horse ☐
Both of the above ☐
Close the top door ☐

3) Recurrent airway obstruction (also known as COPD or heaves) is a respiratory disease caused by which of the following?

A viral infection ☐
An allergy to fungal spores and dust contained in hay and bedding ☐
Excessive exercise ☐
Poor worming ☐

4) When the weed, ragwort, is fully grown and flowering what does it look like?

A weed that is low to the ground with yellow flowers ☐
A tall weed with many small white flowers ☐
A weed that is low to the ground with white flowers ☐
A tall weed with many small yellow flowers ☐

5) Is it true that the toxins contained in ragwort damage the liver of horses that eat it?

False ☐ True ☐

6) One way to get rid of ragwort is to pull it up. What should be done with ragwort after pulling it up?

Allow it to dry out as it is then not harmful to horses ☐
Place it on the muck heap ☐
It can be left in the field to rot ☐
It should be taken away from the pasture and burnt ☐

That is the end of the questionnaire
Please return the completed questionnaire in the pre-paid envelope provided

Thank You For Your Participation In This Survey

Appendix A7.4. Cover letter for mailing of educational booklet.

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«FirstName»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

7th April 2004

Dear «FirstName»,

We would like to thank you for returning a completed Horse Health Questionnaire as part of a survey that is being funded by The Home of Rest for Horses and carried out by the University of Glasgow Veterinary School. Your participation is greatly appreciated.

At the end of the questionnaire you indicated that you would be willing to receive some further information regarding the care of horses. We have prepared two different information booklets, one of which is enclosed. We hope you will find the booklet interesting. It contains information on current understanding of how the way we keep horses can affect their health. The booklets are intended as a guide, if you have any concerns regarding your horses health you should contact your veterinary surgeon. In two weeks time we will send you a very short follow-up questionnaire. If you return the questionnaire completed, we will mail to you the second booklet as a small gesture of thanks.

This process is completely confidential. You have been randomly selected to be sent this booklet – it is not based on information provided in the questionnaire that you returned as this was anonymous. We would like to thank you again for your time and effort in taking part in this study; it would not be possible without you.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS

WEIPERS CENTRE FOR EQUINE WELFARE
University of Glasgow Veterinary School
Bearsden Road, Glasgow G61 1QH
Telephone: 0141 330 5999

Appendix A7.5. Cover letter for the first mailing of the educational survey.

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«FirstName»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

21st April 2004

Dear «FirstName»,

Recently we sent you a horse health information booklet that is being funded by The Home of Rest for Horses and carried out by the University of Glasgow Veterinary School.

We hope you have found horse health booklet of some interest. Please find enclosed a short questionnaire regarding the booklet – it should take less than 5 minutes to answer. The survey is anonymous with identifying code numbers used for the administration process involved in mailing the questionnaire. It would be greatly appreciated if you could find the time to complete and return the questionnaire using the prepaid envelope provided. When we receive your completed questionnaire we will mail you a second information booklet. That will be the end of the study and there will be no further contact from this project.

We look forward to receiving your completed questionnaire. If possible please return it by **Friday, 7th May 2004**. If you have any questions please contact me at the address below. We would like to thank you in anticipation of the valuable information you will provide in your returned questionnaire.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS

WEIPERS CENTRE FOR EQUINE WELFARE
University of Glasgow Veterinary School
Bearsden Road, Glasgow G61 1QH
Telephone: 0141 330 5999

Appendix A7.6. Postcard sent to non-responders in the educational survey.

«FirstName»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

HOME of
REST for HORSES

UNIVERSITY
of
GLASGOW

5th May 2004

Dear «FirstName»

Recently a questionnaire was mailed to you regarding a horse health information booklet that we sent you. If you have already completed and returned it to us please accept our thanks. If not, please do so today. It would be greatly appreciated if you could find the time to complete the questionnaire and return it in the prepaid envelope. We believe the information you can provide is extremely important to our survey. If you require a copy of the questionnaire please do not hesitate to contact me.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS
Weipers Centre for Equine Welfare, University of Glasgow Veterinary School,
Bearsden Road, Glasgow G61 1QH, *Tel.:* 0141-330 5999

Appendix A7.7. Cover letter for the second mailing in the educational survey.

HOME of
REST for HORSES

UNIVERSITY
of
GLASGOW

«FirstName»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

27th May 2004

Dear «FirstName»,

Recently I wrote to you inviting you to complete a short questionnaire regarding a horse health information booklet that we sent you. This is part of a study being funded by the charity The Home of Rest for Horses and being conducted by the University of Glasgow Veterinary School. Please find enclosed a second copy of the questionnaire and a pre-paid return envelope to facilitate your participation. I would like to take this opportunity to encourage you to participate in the survey, as I believe the information you can provide is extremely important to our study.

The short questionnaire is self-explanatory and should take less than 5 minutes to complete. The survey is anonymous with identifying code numbers used for the administration process involved in mailing the questionnaire. To date we have had an excellent response to the survey. However, we wish to encompass information from as many people as possible and hope that you can find the time to contribute. If you have recently returned the questionnaire, please ignore this letter. When we receive your completed questionnaire we will mail you a second information booklet. That will be the end of the study and there will be no further contact from this project.

I hope you will not find this follow up communication too intrusive and I would like to thank you for your tolerance. If you have any questions regarding this survey please do not hesitate to contact myself.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS

WEIPERS CENTRE FOR EQUINE WELFARE
University of Glasgow Veterinary School
Bearsden Road, Glasgow G61 1QH
Telephone: 0141 330 5999

Appendix A7.8. Cover letter sent with the second educational booklet.

HOME of REST for HORSES

UNIVERSITY of GLASGOW

«FirstName»
«Company»
«BusinessStreet»
«BusinessStreet2»
«BusinessCity»
«BusinessState»
«BusinessPostalCode»

April 2004

Dear «FirstName»,

Thank you for returning a completed questionnaire. Please find enclosed the second horse health information booklet. Your help has been vital in conducting this study, which has now concluded.

Yours sincerely

Joel Hotchkiss BSc BVSc CertEM(Int.Med) MRCVS

WEIPERS CENTRE FOR EQUINE WELFARE
University of Glasgow Veterinary School
Bearsden Road, Glasgow G61 1QH
Telephone: 0141 330 5999

Appendix A7.9. Breakdown of cost for the educational study.

	Cost (£)
First booklet (n = 400)	
Educational booklets	344.00
Envelopes	7.55
Cover letters	40.00
Postage (first-class = 28p)	112.00
First Mailing (n = 396)	
Questionnaires	39.60
Return Envelopes	37.23
Cover Letters	39.60
A4 envelopes	11.43
Postage first mailing (first-class = 28p)	110.88
Reminder Postcard Mailing (n = 251)	
Reminder postcard	38.34
Postage postcard (second class = 21p)	52.71
Second Mailing (n = 168)	
Questionnaires	16.80
Return Envelopes	15.80
Cover Letters	16.80
A4 envelopes	4.84
Postage second mailing (first-class = 28p)	47.04
Return Mailing (n = 292)	
Response Service Licence	20.00
Cost of return postage (second-class = 21.5p)	62.78
Second booklet (n = 292)	
Educational booklets	251.12
Envelopes	5.51
Cover letters	29.20
Postage (first-class = 28p)	81.76
Total cost of survey	**513.85**
Survey cost per useable return (n = 292)	1.76
Total cost of booklets including mailing	**871.13**
Total cost of study	**1384.99**
Overall cost per useable return (n = 292)	**4.74**

Appendix A7.10. Table of item omissions for the educational survey.

Question subject	Number of omissions	Percentage omission rate (n = 292)
Booklet		
Presentation	0	0
Interest	4	1.4
Degree of detail	6	2.1
Information not aware of	22	7.5
Sources of information in the past	0	0
Did not agree with content	11	3.8
Adoption of content	5	1.7
Knowledge		
Question 1	0	1.0
Question 2	1	0
Question 3	1	0.3
Question 4	1	0.3
Question 5	0	0.3
Question 6	0	0
Total	54	1.4

LIST OF SUPPLIERS

Epi Info v6.04d	Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA.
fi-4110cu scanner	Fujitsu Limited, Shiodome City Center, 1-5-2 Higashi-Shimbashi Minato-ku, Tokyo 105-7123, Japan.
Microsoft Excel 2000	Microsoft Corporation, One Microsoft Way, Redmond, WA 98052-6399, USA.
Microsoft Word 2000	Microsoft Corporation, One Microsoft Way, Redmond, WA 98052-6399, USA.
Minitab v12.21	Minitab Inc., 3081 Enterprise Drive, State College, PA 16801-3008, USA.
QuickAddress Batch v3	QAS Ltd., George West House, 2-3 Clapham Common North Side, London, SW4 0QL.
R v1.9.1	R Foundation for Statistical Computing, Vienna, Austria.
S-Plus 2000 v3	1700 Westlake Avenue North, Suite 500, Seattle, WA 98109-3044, USA.
SPSS v11	SPSS Inc., 233 S. Wacker Drive, 11th floor, Chicago, Illinois 60606, USA.
TELE*form* Elite v8	Verity Inc., 894 Ross Drive, Sunnyvale, CA 94089, USA (formerly produced by Cardiff Software, USA).

REFERENCES

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