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ENDOSCOPIC AND VIROLOGICAL OBSERVATIONS ON RESPIRATORY DISEASE IN A GROUP OF YOUNG THOROUGHBRED HORSES IN TRAINING

A Thesis submitted by

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for the degree of

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in the

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DECLARATION

This work was carried out between August 1981 and October 1982. Apart from the virus isolation which was undertaken by The Equine Virology unit of The Animal Health Trust, Newmarket all the work reported in this thesis was carried out by the author. This thesis has not been previously published. A paper using data from this study is appended in support of the thesis.

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M.H. BURRELL

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ENDOSCOPIC AND VIROLOGICAL OBSERVATIONS ON RESPIRATORY DISEASE IN A GROUP OF YOUNG THOROUGHBRED HORSES IN TRAINING.

SUMMARY

A group of racehorses in a commercial training stable were examined on several occasions with a fibreoptic endoscope and monitored for evidence of clinical respiratory disease, viral infection and performance in races. Three conditions which have become easily recognisable by endoscopy were assessed at each examination: Pharyngeal lymphoid hyperplasia (PLH), exercise induced pulmonary haemorrhage (EIPH) and intratracheal mucoid exudate or mucopus.

There was only one case of possible clinical respiratory disease and in general the horses were performing to the trainers satisfaction with placings in 50 per cent of starts. Only equine herpes virus-2 (EHV-2) infection was detected by virus isolation from nasopharyngeal swabs and no serological evidence of viral infections were noted.

Pharyngeal lymphoid hyperplasia was present to some degree in all horses and its gradual decrease in severity correlated well with increasing age. There was no association between PLH severity and antibody titres to EHV-1, or with the isolation of EHV-2. Finishing position in races was not affected by PLH severity.

EIPH was evident on 23 out of 49 (47 per cent) examinations after maximal speed training exercise. The severity of PLH did not have any effect on the incidence of EIPH in this study. Eighteen out of 19 (95 per cent) horses examined on at least two occasions had EIPH but its occurence was not predictable.

Observable mucoid or mucopurulent exudate was present in the trachea in 60 out of 118 (50 per cent) examinations and the amount seen was increased following exercise. The large amounts of mucus are interpreted as resulting from lower respiratory tract inflammation, suggesting that a degree of airway inflammation is common in racing horses. Airway mucus in the dorsal phayngeal recess was associated with intratracheal exudate and it is postulated that during exercise tracheal mucus can become shifted by the airflow to eventually impinge on the tonsillar tissue of the dorsal pharyngeal recess.

CHAPTER 1 GENERAL INTRODUCTION

In recent years attention has been focused on the role of respiratory disease and its untoward effects on the racing industry of Great Britain. Such phrases as the "poor performance syndrome" have been coined by the sporting press and veterinary literature. This blanket terminology is used for any situation in which a horse fails to run to expected standards.

Sub-optimal performance in racehorses is not a new problem, but merely something which has attracted more attention in the last decade or so. It is also a worldwide problem. The British racing industry is a multimillion pound international concern of considerable status and importance. Like most large industries, there has had to be change and adaptation recently, aimed at increased efficiency and productivity. More is being expected from the horses in terms of returns on money invested in breeding and training, and decreased wastage. Cuts have also been made in the management costs, such that one lad might now be responsible for 3 or even 4 horses instead of 2 previously. The cost of racehorse owning is relatively greater in comparison to the owner's income than ever previously. All these reasons have the overall effect of increased expectations of the horses and less tolerance of poor standards.

Other factors possibly involved are the heightened awareness of performance, shifting epidemiological patterns of the infectious agents involved , and decreased resistance to disease due to breeding policies consistently aimed at speed and athletic performance. There has been an increase in travelling with mixing of populations at home and abroad. Perhaps of most significance is the increase in numbers of horses in Great Britain and the increased numbers in single stables representing an intensification of equine husbandry (Powell, D.G, personal communication).

There is no clear definition of "poor performance" and descriptions are confusing and ill-defined. Generally, it may be defined as being a detrimental change in the horse's athletic performance. It can be due to physiological or patho-physiological conditions. Poor performance in racehorses is complex and depends on the interrelationship of a variety of factors such as nutrition, infection, genetic character and environmental conditions.

Respiratory disease is thought to be one of the major causes of poor performance. Particularly when the syndrome has a high incidence within one stable or area, infectious respiratory disease is usually suspected. In lay terms, this situation is explained as being due to "the virus". Acute clinical viral respiratory disease is well recognised, and the agents are often identified. The most important agents are equine influenza and equine herpes viruses, while other groups of viruses have been known to cause respiratory disease.

Various groups of bacteria have also frequently been isolated from the upper respiratory tract although their exact role as primary or secondary pathogens is unclear (Powell 1979). Mycoplasmas have also been found in normal and diseased horses, but again their role in the aetiology of respiratory disease is yet to be defined (Poland and Lemke 1978).

Thus, while much knowledge has been obtained on the clinical signs, epidemiology, immunity and prevention of some of these infectious agents, the picture is by no means complete. Furthermore, there are many cases of sub-optimal performance, thought to be of respiratory origin, in racehorses in which no single agent of disease can be implicated. Thus, the possibility of chronic infection, sub-clinical infection, unknown or non-infectious respiratory disease must be considered.

There are other conditions of the respiratory tract whose significance or aetiology is not known. These include pharyngeal lymphoid hyperplasia (PLH), exercise induced pulmonary haemorrhage (EIPH), and lower respiratory tract (LRT) inflammatory disease. There is little published information regarding these syndromes largely due to technological difficulties in studying these conditions prior to the advent of the fibreoptic endoscope which has become available in the past decade.

Attempting to understand and define the epidemiology and pathology of these syndromes in the young racing thoroughbred is difficult due to non-availibility of post mortem samples, and experimental models. Thus, a study of a group of young throughbreds in a private commercial racing stable was undertaken. Through close surveillance and routine endoscopic examination the aim was to study PLH, EIPH and LRT inflammatory disease, with respect to:

(a) prevalence;

(b) effect on performance; and

(c) the role of viral infection in their *aetiology.*

This thesis reports those findings.

CHAPTER 2 REVIEW OF THE LITERATURE

2.1.0. Pharyngeal Lymphoid Hyperplasia (PLH)

PLH has been variously termed:- pharyngitis, chronic pharyngitis, follicular pharyngitis, and chronic lymphoid follicular hyperplasia (CLFH) (McAllister and Blakeslee, 1977)

There have been few published studies on PLH, which is surprising in view the importance ascribed to PLH as a cause of poor performance in young racing throughbreds. For example, in a survey carried out amoungst equine practitioners in the U.S.A. in 1976, half of the respondents recognised PLH as a distinct disease entity. An estimate suggested that about 50,000 cases had been seen in a 15 month period, which when translated into economic terms represented an enormous financial loss due to lost racing opportunities, maintenance and treatment. An estimate by one stable owner put the cost at $200,000 / annum (Anon 1976).

It seems strange that so many clinicians were diagnosing and treating PLH as a specific cause of decreased athletic performance before there had been any definitive publications on the subject, or more specifically, objective assessments of its effect on airway function, aetiology or pathogenesis (Anon 1976).

That this situation arose was largely due to the impact of the widespread use of the flexible fibreoptic endoscope in the early 1970's and an unprecedented ability to view the respiratory tract in standing horses. Therefore, a hitherto unrecognised lesion, was suddenly apparent, and certain effects, were rightly or wrongly attributed to its presence.

Cook (1974) reported lymphoid hyperplasia of the pharynx as being common and probably physiological in yearling and two-year-old thoroughbreds. He commented that this is prominent in certain horses and is sometimes accompanied by a coughing or "choking-up" syndrome during racing. On the aetiology he suggested that the hyperplasia may be a sequel to respiratory virus infection. A more concrete pathogenesis of the condition has yet to be suggested.

2.1.1. Incidence

Chronic PLH has been detected mainly in horses less than 4 years old (Mc Allister & Blakeslee 1977, Anon 1980, Raker & Boles 1978). The condition may be detected from a few months of age (Montgomery 1981; Baker G.J. personal communication). The frequency of detection decreases with age in horses over 4 years. However it has been diagnosed as a cause of respiratory unsoundness in horses upto 11 years old (Raker & Boles 1978). Raker and Boles in their review on PLH diagnosed and treated 207 cases out of 4,576 hospital referrals (5%).

The increased usage of fibreoptic endoscopy was responsible for the rapid increase in awareness of PLH, as the pharynx became visually accessible. It then became recognised that a degree of PLH was common to every young racehorse, but only severe cases were considered to be of clinical significance.

2.1.2 Aetiology

2.1.2.a Viruses

As mentioned in the introduction, Cook (1974) suggested that chronic inflammation of the pharynx and lymphoid proliferation was probably the result of respiratory viral infection. In all animals respiratory tract viral infections cause an acute inflammatory reaction, and horses are not exceptional. Thus, the full list of recognised respiratory viruses may have been considered as potential causes. This list includes:-

Equine Influenza A/equi/1 and A/equi/2

Equid Herpes Virus 1 (EVV-1) (subtype 1 and subtype 2)

Equid Herpes Virus 2 (EHV-2)

Equine Rhinovirus 1

Equine Rhinovirus 2

Acid Stable Picornavirus (ASPV)

Adenovirus

Evidence of a specific viral aetiology comes from Prickett (1969) who before endoscopic recognition of PLH observed that experimental infection with EHV-1 evoked a pharyngitis with lymphoid proliferation. Similarly, Blakeslee et al (1975) showed that infection of ponies with EHV-2 was followed by chronic lymphoid follicular hyperplasia. This reaction persisted for up to 232 days. The chronic nature of the reaction was associated with persistent infection by EHV-2. However, it is thought that most horses carry EHV-2 in a latent state and persistent shedding of this virus for up to 418 days has been reported (Turner 1970). McAllister (1977) considered that the nature of this viral infection could account for the persistence of PLH but no cause and effect relationship has been demonstrated.

Mansmann (unpublished) in a review of PLH listed the possible causes as:

(1) an irritant reaction to turbulent airflow;

2) the normal immunologic development of lymphoid tissue in the young horse;

3) viral disease;

4) mixed bacterial infection; and possibly

(5) an allergic reaction.

This list is echoed by most publications on PLH. However it was noted (Anon 1980) that no specific agent(s) have been identified.

Since no specific viral agent was readily identified in the aetiology, indirect evidence of viral involvement was sought by considering the effect of preventing infection using vaccination. The published evidence is conflicting.

Mansmann considered that vaccination against influenza and EHV-1 had no effect while Montgomery (1981), quoting a small number of cases considered vaccination to be beneficial. There was a variable response in a survey of clinicans to the question of whether vaccination had an effect (Anon 1976). Since there is doubt as to the efficacy of EHV-1 vaccination in preventing infection using a inactivated vacine (Mumford and Bates 1984; Burrows et al 1984) it is impossible to draw any conclusions from the literature regarding vaccine benefits.

2.1.2.b Bacteria

Various authors have quoted bacteria as being of aetiological importance. Montgomery (1981) considered bacterial invasion of the URT secondary to viral infection to be a factor in the propagation of chronic pharyngitis, and the establishment of pharyngeal oedema. However, he also suggests that these invaders may be present indefinitely and refractory to treatment. It is recognised in the human that the oropharynx constantly houses a normal mixed bacterial flora. Similarly Mansmann and Knight (1972) reported that bacteria can always be cultured from the equine *nasopharynx in particular <u>Pseudomonas spp</u>. In man non-sporing anaerobes are always present in the orophanynx in large numbers and it has been implied that the situation is no different in the horse, (Anon 1983). Recently it has been shown that potentialy pathogenic bacteria may be carried asymptomatically in the nasopharynx of horses (Burrell, unpublished data).*

Bacterial infection of one or both gutteral pouches, with mucopurulent discharge into the pharynx, has also been considered the cause of, or at least closely associated with PLH (Hackathorn T.A. 1975)

2.1.2.c. Other Agents

Other agents implicated include virtually anything inhaled, examples quoted being stable dust, fungal spores and noxious gases.

Several comments have been made pointing out the high levels of stable dust and its possible association with respiratory disease (Cook 1974; Anon 1980). Although there have been few quantitative studies of stable environment, those carried out do confirm high aerial particle levels. (Crichlow et al 1980; Sainsbury 1981). These poor stabling conditions have been implicated in the aetiology of PLH as being sources of physical and chemical injury to the pharyngeal mucosa (Anon 1976).

Specific inhaled allergens have been detected in the horse by McPherson et al (1979), the ones identified being spores of Micropolyspora faeni and Aspergillus spp. A high percentage of horses showed a cutaneous anaphylactic reponse to these antigens which has been interpreted by Schatzmann et al. (1972) as leading to latent sensitization. Since exposure is by inhalation and impingement on URT mucosa, this is presumably the basis of argument for implicating these agents as causes of PLH.

Airflow over the pharyngeal mucosa causing a physical irritation has also been suggested as a predisposing factor (Anon 1980).

Finally it has been suggested that the possibility of a "genetic weakness or nutritional deficiency may be the underlying reason for many horses, susceptibility to a diversity of pathogens, mechanical irritants, toxins and allergens" (Anon 1976).

2.1.3. Histopathology

Only Prickett (1970) has described the histological appearance of PLH, which was induced in young horses following infection by EHV-1. He described marked hyperplasia of the lymph follicles lining the pharynx due to lymphocytes with numerous mitotic figures.

An unusually large bilobed pharyngeal mass removed from a 2 year old horse was described by Meagher and Brown (1978) as comprising of an outer covering of pseudostratified columnar epithelium enclosing a vascular collection of lymphocytes sometimes organised into lymphoid follicles with some monocytes, plasma cells and eosinophils.

2.1.4. Effect on Performance

Subjective observations have resulted in PLH being implicated in causing sub-optimal performance of racehorses, a view based on subjective correlations between PLH and clinical situations. There are various hypotheses proposed for the mechanisms by which PLH could limit respiratory efficiency and, therefore performance.

Raker (1978) considered that "PLH is a common cause of partial obstruction of the upper airway in the young racehorse which may lead to a decreased tolerance of work". This suggests that in severe cases there is a reduction in airway diameter causing a significant obstruction leading to decreased airflow through the pharynx. This theory is supported by McAllister and Blakeslee (1977) who also considered the condition as an "obstructive airway disease".

In a previous paper Raker (1976) suggests the decrease in airway diameter can lead to a negative pressure in the pharynx during inspiration with resultant ventral displacement of the dorsal wall of the pharynx and elevation of the soft palate. He implies that this may predispose to palato-pharyngeal subluxation, further compounding the effect on performance. Cook (1974) previously suggested that there may be a connection between PLH and palato-pharyngeal subluxation. Thus, the basis for this argument is that hyperplastic nodules in the nasopharyngeal airway act as space occupying lesions resulting in an increased resistance to airflow due to accentuation of the normal dynamic alteration of anatomy in the region during strenuous respiration. The resultant clinical signs are considered to be a major reduction in exercise tolerance and a noise heard on inspiration and or expiration due to turbulent airflow.

All authors have considered PLH to be deleterious to performance, particularly in the more severe forms, although there has never been a reported attempt to distinguish between PLH which is "normal" and of no significance and that which is considered of clinical significance. It is conceded Anon (1980) that some 2 and 3 year old's have a degree of PLH while performing well and that therefore the pathogenicity of this condition should be re-evaluated. Montgomery (1982) says that the prolifiration of lymphoid tissue per se does not affect respiratory function unless complicated by "excessive lymphoid oedema".

2.1.5. Treatment

As might be expected in a condition where there are many unproven theories on aetiology there are at least an equal number of different methods of treatment. The methods fall into the following categories: topical applications, electrocautery, cryotherapy, rest, hyperimmunisation or systemic drug administration.

2.1.5a. Topical Applications

Spraying of the pharynx with a cocktail consisting of furacin, DMSO and prednisilone reportedly lead to a rapid remission of PLH severity within 24 to 48 hours, but this was only temporary (Raker 1976; Raker and Boles 1978). Direct spraying of organic iodide was claimed to be beneficial (McAllister and Blakeslee 1977) and there is a report of this solution being flushed into the guttural pouch diverticulum (GPD) with a disinfectant as a treatment of PLH (Hackathorn 1975).

2.1.5b. Electrocautery

Electrocautery was described first by Raker (1976) in which the entire mucosa of the dorsal and lateral walls of the pharynx were cauterised via a laryngotomy. Following cauterisation and 30-45 days rest the results showed a 100% effectiveness in reducing the PLH to at least Grade 1. It was also claimed that 90% of horses so treated had an improved performance on resumption of training.

2.1.5c. Cryotherapy

Cryotherapy of the lymphoid follicles was used particularly in cases accompanied by "inflammatory oedema" (Montgomery 1982). A good success rate in dramatically reducing the severity of pharyngitis by this method in conjunction with a monthly vaccination programme against equine influenza and EHV-1 was claimed.

2.1.5d Hyperimmunization

In a survey of American practitioners (Anon 1976) 59 per cent of respondents thought that vaccination against equine influenza was effective in preventing pharyngitis while the corresponding figure for EHV-1 was 47 per cent.

Montgomery (1981) claimed vaccination was successful in reducing PLH, as described previously.

2.1.5e Systemic Medication

Large doses of systemic corticosteroids and antibiotics led to only a temporary remission at best, while generally the response was unrewarding and unpredictable (Raker 1976). Administration of oral griseofulvin led to promising results (Churchill and Teigland, quoted by Raker 1976).

2.1.5f Rest

All reports of differing methods of treatment contain a considerable period of rest varying from 1 month to 3 months as part of the regimen. Rest alone has also been advocated. In the survey of clinicians (Anon 1976) the only fact on which all respondents agreed was that rest was an essential part of successful treatment.

2.1.6 Conclusions

A survey of the literature shows firstly that there are few publications and the majority of those are based on examination of selected populations referred for clinical problems. Theories on the aetiology are largely conjectural, or at best, based on tenuous associations between the concurrent presence of possible causes and the condition. Consequently, virtually every group of agents or condition known to influence Respiratory disease has been suggested.

Observation of the lesion in horses referred for loss of performance led the earlier workers to conclude that PLH caused a reduction in performance. Later it was only the worst cases that were thought to be important, due to obstruction of airflow through the pharynx. Recently, the first study to measure physioloical parameters at exercise and compare horses with PLH and those without, concluded that this does not affect gas exchange (Bayly, Grant and Breeze 1984).

Many treatments, some less extreme than others, were claimed to be successful in reducing the degree of PLH. This should not be doubted, but perhaps it was the periods of rest subsequent to therapy which was responsible for the alleged improved performance. If it is argued that PLH has little effect on performance, then enforced rest may have allowed undiagnosed conditions to abate, for example sub-clinical lower airway disease or orthopaedic problems. In most reports describing successful treatments there is no data on follow up examinations.

2.2. Exercise Induced Pulmonary Haemorrhage (EIPH)

2.2.1 Historical Background

Epistaxis following exercise in horses has been recognised since the sixteenth century when an English gentleman quoted by Robertson (1913), observed that "many horses, especially young horses, are oft subject to this bleeding of the nose... it proceedeth from much abundance of blood, or that vein which endeth in that plane (referring to the head) is either broken, fretted or opened". Poor performance was linked with bleeding from the nostrils by Robertson (1913) when he cited the case of the famous stallion Herod who in 1776 "broke a blood vessel and was beaten off" during a race at York and concurrently noted that his "form at times was unaccountable bad and it is significant that the first and only time that he met a number of runners he showed the weakness (bleeding)".

Early recognition of post-exercise epistaxis was not followed by any further publications for some 40 years, during which time it was assumed that the bleeding originated from the upper respiratory tract, and though unpredictable in its effects and occurence was generally considered detrimental to performance and occasionally could be rapidly fatal.

2.2.2 *Prevalence*

Pfaff (1950) published the first report on the incidence of epistaxis in a population of racing thoroughbreds. Using South African racing records he found that 1-2 per cent of horses had bled from the nostrils following racing. Bleeding was assumed to originate from the nose until Cook (1974) examined 50 cases of post-exercise epistaxis from which, in the absence of other findings, he assumed the blood was of pulmonary origin. *He supported his evidence by arguing that blood from the lower airways would move cranially to appear at the nostrils and not the mouth, and that this commonly occurred when a horse was first allowed to lower its head after exercise. Furthermore, in horses with indwelling tracheostomy tubes that bled while being raced, the blood escaped from the respiratory tract via the open tracheostomy or when it was closed, from the nostrils.*

The reported prevalence of horses having blood at the nostrils following racing in Australia (Bourke 1978), South Africa (Pfaff 1950, 1973 and 1976) and Singapore (Choy 1973) varies from 0.8 to 2.5 per cent of horses racing (Table 1).

Following Cook's (1974) suggestions that the blood came from the lungs and with the introduction of fibreoptic endoscopes, endoscopic surveys of horses after racing and other exercise showed the prevalence of EIPH to be much higher than previously thought. The first such study to be published was by Pascoe (1981) who found that 43.8 per cent of horses examined after racing had EIPH of which only 0.8 per cent had blood at the nostrils. So it became obvious that cases of epistaxis represented only a small proportion of the total having pulmonary haemorrhage.

A number of endoscopic studies in different types of horse and following various levels of exercise have recently been published and are shown in Table 2.

This data shows that not only thoroughbreds, but other breeds of horse if examined after exercise may show pulmonary haemorrhage.

The incidence of EIPH increases as the examination penetrates further down the respiratory tract Mason (1982) demonstrated that by repeat endoscopic examinations 86 per cent of horses had EIPH at some time while at post-mortem, lung tissue showed evidence of previous haemorrhage in 100 per cent of horses in training. Therefore it was suggested that nearly all racing horses experience EIPH at some stage.

Table 1

Reported incidence of epistaxis in horses after racing.

REFERENCE	COUNTRY OF REPORT	INCIDENCE
Pfaff (1950)	South Africa	1.2%
Pfaff (1973)	South Africa	2.5%
Choy (1973)	Singapore	2.5%
Pfaff (1976)	South Africa	2.4%
Bourke (1978)	Australia	0.8%

Table 2

Incidence of EIPH in various breeds of horses after different types of exercise.

REFERENCE	BREED	EXERCISE	NUMBER EXAMINED	PERCENTAGE EIPH	PERCENTAGE EPISTAXIS
Pascoe et al (1981)	TB	Racing	235	43.8	0.8
Pascoe (1980)	TB	Racing	1180	42.1	3.0
"	Standard Bred	Racing	249	26.5	12.1
Raphel (1982)	TB	Racing	191	75.4	9.0
"	TB	Training	107	38.3	2.4
"	TB	Steeple-chase	31	67.7	14.3
"	TB	Timber Race	3	60.	100.
"	TB	Training	21	65.6	-
Sweeney and Soma (1983)	Mixed	Cross Country	5	40.	-
"	Mixed	Pony club Cross country	40	10.	-
"	Mixed	100 miles Endurance	10	0	-
Mason et al (1983)	TB	Racing	485	46.8	-
Pascoe and Raphel (1982)	TB/Arab	Endurance	18	0	-

2.2.3 Sex Incidence

Suspension of racehorses in South Africa occurs automatically following an episode of epistaxis on a racecourse, and data of suspensions led Pfaff (1976) to suggest that geldings were more likely to bleed than horses and mares. However, Goulden (1979) commenting on this data pointed out that geldings were likely to race more frequently and continue racing to an older age. Recent endoscopic studies have confirmed that sex has no effect on frequency of EIPH (Pascoe 1981; Pascoe 1980; Raphel 1982).

2.2.4 Age Incidence

Early reports all suggested that there was an age effect in racehorses and that the incidence was least in 2 year olds and highest in horses over 4 years old (Cook 1974, Pfaff 1976, Bourke 1978). It has remained a generally held view that older horses are more likely to bleed and this has been confirmed by endoscopic studies which showed an age related trend, with older horses showing a greater tendency to EIPH (Pascoe et al 1981). Subsequently it was reported that increasing age is directly associated with an increased incidence (Raphel and Soma 1982; Sweeny and Soma 1983; Mason et al 1983).

2.2.5 Seasonal Incidence

There are some anecdotal reports which suggest that there is an increased prevalence of bleeding at certain times of the year. In South Africa the commonest months for bleeding were during the cooler winter months (Pfaff 1976), but there was also more racing at that time of year. A simialr situation existed in Victoria State, Australia but Bourke (1977) attributed this to the greater prevalence of jumping races held at this time, while in New Zealand the difference between the higher incidence in the winter months was highly significant (Goulden 1979). No endoscopic surveys have been undertaken to show seasonal differences in prevalence, so the situation requires clarification.

2.2.6 Correlation of EIPH with Distance Exercised

There is currently disagreement on whether the incidence of EIPH correlates with distance raced. More horses bled following sprints and races upto nine furlongs in South Africa (Pfaff 1950). However, an early endoscopic survey associated a higher incidence of EIPH with longer distances raced. Horses racing eight furlongs or more were more likely to bleed than horses racing less than eight furlongs, while horses on training gallops over three, four and five furlongs showed an increasing frequency of EIPH with increasing distance (Raphel and Soma 1982). Mason et al (1982) contradict this data as they found no correlation between distance raced and incidence of EIPH. Until controlled studies under repeatable conditions, ie. on a treadmill are carried out, the discussion is unlikely to be resolved.

2.2.7 Other Epidemiological Data

Altitude was considered by Pfaff (1976) to be the main difference between racetracks on the coast at sea level (3 tracks) and at Johannesburg (5400ft above sea level) where there was less likelihood of bleeding. Mason et al (1982) found that the incidence of EIPH was not affected by location of stables, trainers, going, location of track or track type (grass or sand). There are no reported studies where the effects of ambient temperature or humidity have been considered.

2.2.8 Clinical Signs of EIPH

Apart from epistaxis and endoscopically visible free intra-tracheal haemorrhage, signs attributable to EIPH are variable or non-existant as there are few reports detailing observable signs. Horses which bleed in Australia and South Africa are banned for a period from racing because the sudden blood loss can cause a horse to fall or more commonly slow down, sometimes quite abruptly and cause interference to other runners (Bourke 1978; Pfaff 1950), but experience in North America and Europe shows this to be uncommon, and the racing authorities take no action with "bleeders".

Animals affected with post-exercise epistaxis usually have a trickle or a small amount of non-frothy blood at both nostrils. Cook (1974) explained the anatomical reasons why the blood appears at the nose and is not frothy. This is because the trachea is horizontal so the blood will flow along the ventral floor, aided by mucociliary action, through the rima glottidis of the larynx and into the nasal cavity dorsal to the soft palate. Most horses show epistaxis within a few minutes of exercise and commonly when first allowed to lower their head.

It is reported that horses with EIPH often show signs of distress such as head-shaking or pawing, "cool-out" slowly, cough and swallow frequently (Bourke 1978; Pascoe et al 1981). Some animals reportedly show hyperpnoea with a prominent diphasic expiratory effort (Bourke 1978) while dyspnoea is uncommon. If dyspnoea occurs it is usually a sign of extensive haemorrhage into the airways, exacerbation of pre-existing pulmonary disease or major structural damage to the lung (Pascoe and Raphel 1982). Thus, on auscultation there may or may not be audible abnormal respiratory sounds.

2.2.9 Pathophysiology

Only relatively recently was it realised that exercise induced epitaxis originated in the lung. The retrospective study by Cook (1974) and the first endoscopic survey (Pascoe et al 1981) established this fact, and the term exercise induced pulmonary haemorrhage was introduced.

Autopsies carried out on horses which have experienced rapidly fatal epistaxis following exercise were found to have intrathoracic haemorrhage originating from tears in the lung and visceral pleura (Sutherland 1966), or rupture of subpleural haematomas (Cook 1974). From autopsies carried out on retired racehorses, Mason et al (1983) described areas of old pulmonary haemorrhage as bilateral, near-symmetrical, with abnormal discolouration in the diaphragmatic lobes.

At the histological level, areas of recent haemorrhage are characterised by blood-filled alveoli and bronchioles, variable fibrosis, and occasional microabscesses (Cook 1974, Sutherland 1966; Johnson et al 1973). The lungs examined from retired horses with evidence of previous haemorrhage by Mason et al (1983) revealed extensive bronchiolitis involving the majority of bronchioles in sections from 96 per cent of horses examined. Little or no mucus production was evident. Most had excess peribronchiolar fibrous connective tissue with inflammatory cell infiltrates sometimes forming a cuff. In a few cases haemosiderin was present in alveolar macrophages and in some there was alveolar scarring. The authors pointed out the although they found extensive bronchiolitis, the normal situation in older thoroughbred racehorses is unknown and that it could be either a cause or effect of haemorrhage, or unrelated. These histological findings are not the same as in horses suffering from chronic pulmonary disease as described by Nicholls (1978). These findings lend support to the hypothesis that EIPH could be due in some part to subclinical lower airway disease, predisposing to bronchospasm precipitated by exercise, originally submitted by Cook (1974), and pursued by Robinson (1979 and 1980) who

explored the physiological mechanisms whereby bronchospasm could result in pulmonary haemorrhage.

For haemorrhage to occur, blood vessels must rupture, and in the lung these are either alveolar or extra-alveolar. Rupture of blood vessels occurs when there is excessive distending force which is different for alveolar and extra-alveolar vessels, respectively. For extra-alveolar vessels, the transmural pressure is the difference between the intravascular pressure and the pressure in the perivascular connective tissue. It is argued from work carried out in other species that inspiration reduces perivascular pressure resulting in dilatation of extra-alveolor vessels (Robinson and Derksen 1980).

Alveolar vessels are the pulmonary capillaries and their transmural pressure is determined by the difference between intravascular and alveolar pressures. For vascular rupture of these capillaries to occur there must be a significant increase in vascular pressure or decrease in intra-alveolar pressure. Robinson (1979) argued against increases in vascular pressure being the cause of vascular rupture since that alone should lead to fluid filtration into the interstitium resulting in lung oedema and not haemorrhage. Clinical pulmonary oedema is not a recognized feature of these horses and the blood from EIPH does not contain protein-rich oedema fluid.

Therefore, Robinson (1979) suggested that these factors point to localized decreases in perivascular pressure as a more likely cause of EIPH and that these decreases may result from (i) airway obstruction and/or (ii) scarring of the parenchyma. These proposed mechanisms which result in decreased perivascular pressure are discussed.

Airway obstruction would lead to imbalances of ventilation of adjacent pulmonary segments. The horse has an incompletely lobulated lung, and collateral ventilation is considered insufficient to equilibrate pressures between obstructed (poorly ventilated) and well ventilated segments, particularly in exercising horses (Robinson and Sorenson 1978). Bronchoconstriction would result in a drop in intra-alveolar pressure during rapid inspiration and subsequent rupture of alveolar capillaries. The airways leading to these segments need only be partially obstructed for this to occur.

The other hypothesis relating to lung parenchymal scarring or pleural adhesions is attributable to the phenomenon of interdependence, whereby all structures within the lung are interconnected, so movement of one part of lung exerts forces on adjacent lung tissue. Therefore, during inspiration expansion of lung tissue applies distending forces to adjacent immobile tissue. With exercise these distending forces are thought to be able to increase to such an extent that tearing of the tissue may result, with subsequent haemorrhage.

2.2.10 Clotting Factors

Epistaxis has been related to a low platelet count in individual horses (Franco 1979). Specific correlation between platelet count and epistaxis was made by Johnson et al (1973) who measured various coagulation parameters in "bleeders" and "non-bleeders". They found significant decreased platelet counts and increased clot retraction in bleeders but no differences between the two groups for prothrombin time, partial thromboplastin time, bleeding time and Lee-White coagulation time.

Recent studies on the effects of exercise on haemostasis (Bayly 1983) showed that there was a depression in adenosine di-phosphate (ADP)-induced platelet aggregation in all exercising horses, but a further study (Bayly 1984) failed to show any difference between EIPH susceptible and non-susceptible horses. This may be due to poor selection of normal controls, and there are platelet functions which were not investigated. Further investigations of the possible role of platelet fuctions being important in EIPH are warranted because although there were no significant differences there was a tendency from the bleeder group to be less responsive to ADP.

Recent studies on rheology (the physiology of blood flow) have suggested that the horse may have unique properties of reduced blood viscosity and erythrocyte flexibility during exercise (Colles unpublished data). Such properties may have a bearing on the pathogenesis of EIPH.

2.2.11 Upper Airway Obstuction

Rooney (1970) suggested that asphyxia from inadequate pulmonary ventilation caused by breath holding or upper airway obstruction is the most likely cause of EIPH. Endoscopic evidence does not support the contention that horses with upper airway obstruction are more likely to bleed. Pascoe (quoted in Pascoe and Raphel 1982) identified 49 (4.2 per cent) of 1180 horses with functional upper airway obstruction due to laryngeal hemiplegia and asynchrony, arytenoid cartilage chondroma and epiglottic abnormalities. Twenty (40.8 per cent) of these 49 horses also had EIPH on examination which is a similar level to that found by the same authors in an other study (Pascoe 1981). It has been argued that on physiological principles breath holding and upper airway obstruction are unlikely causes of pulmonary haemorrhage (Robinson 1979). Furthermore, Attenburrow (1982) showed by telemetric recordings that the breathing cycle is irrevocably linked to the cycle of limb movements at the canter and gallop. From this evidence it is most unlikely that horses could hold their breath whilst galloping.

2.2.12 Treatment of EIPH

Since the haemorrhages are generally short-lived and inconsequential in themselves, treatments have been aimed at prevention rather than haemostasis or supportive therapy. There are a large number of treatments that have been used at one time or another and serve to emphasise the lack of understanding of the pathophysiology of EIPH. Reports of the efficacy of various preparations have been mostly anecdotal and unsupported by clinical trials. The fact that no one therapy has consistently been adopted probably reflects a failure of response in most instances, or possibly difficulties in assessing therapy results.

Bourke (1978) reviewed the treatments being used at that time in Australia. Various clotting agents, oxalic and malonic acids, hormones and tranexamic acid are recommended; Vitamins A and C to strengthen capillaries; Vitamin K to assist in clotting; corticosteroids to elevate platelet counts; intranasal insufflation with thromboplastin to enhance fibrinogen formation, adrenalin to produce local vasoconstriction and local or systemic antibiotics to resolve foci of infection. Lastly he mentions the use of furosemide an injectable diuretic, which was being used widely in America at that time (Gabel et al 1977).

Furosemide is generally administered four to six hours prior to racing and is considered to limit or prevent epistaxis, however, a rational basis for its use is lacking. It has been hypothesized that the cardiovascular effects of furosemide, reduced right atrial pressure and pulmonary arterial pressure may be the reason for its supposed effects (Milne et al 1977). This has not been demonstrated. Doubt has also been cast on the idea that exercise-related pulmonary hypertension is related to the occurrence of EIPH (Robinson and Derksen, 1980).

It seems most unlikely that furosemide entirely prevents EIPH, and recent studies have failed to resolve whether furosemide is beneficial in reducing the amount or incidence of haemorrhage.

It has been reported that of a group of 56 horses examined endoscopically after furosemide administration and then raced, 53.6 per cent had blood in the tracheal lumen, which was no different to the untreated horses (Pascoe et al 1981).

On the other hand a recent study (Bayly et al 1983) suggested that there may be a basis for the belief that furosemide is of use in treating EIPH. Although it was not demonstrated statistically, there was a tendency for the post-exercise inhibition of ADP-induced platelet aggregation to be less marked after furosemide administration. This was particularly evident in horses known to have recently had epistatic episodes. Whether this effect was real or coincidental requires further study on a larger group of animals. The mechanism by which furosemide could exert such an effect is unclear.

Other medications aimed at alleviating possible bronchoconstriction during exercise are the β2 agonist clenbuterol, the mast cell stabilizing agent, sodium cromoglycate, and atropine. However, there have been published trials and anecdotal evidence suggesting that their effect on EIPH is variable. The one trial published (Raphel Sweeney et al 1984) used only 3 horses in a small number of exercise trials (3-8) for each drug given prior to an exercise test. The drugs tested were atropine, sodiume cromoglycate, furosemide and ipratropium (an anticholinergic bronchodilator). Only the latter appeared to have any beneficial effect, but the data is insufficient to reach conclusions.

2.2.13 Conclusions

It was not until endoscopy became widespread that the true incidence of EIPH became apparent; epistaxis is obviously only the tip of the iceberg. Endoscopic examinations to the tracheal bifurcation considerably increases the chance of observation of EIPH, but in fact is still a long way from the site of haemorrhage in the diaphragmatic lobe. Probably every horse in training experiences EIPH, the evidence coming from the post mortem studies by Mason et al (1983) and more recently surveys using tracheal washing have shown that thoroughbreds in training all have macrophages containing haemosiderin, which is indicative of vascular leakage into the alveoli (Burrell, M.H., unpublished data).

Reproducable exercise tests, on a treadmill, should resolve the arguments as to whether speed and distance significantly effect the amount and degree of EIPH. Measurement of air flow, gas exchange, ventilation: perfusion ratios and blood pressure at exercise should also help in future to determine the mechanisms which lead to vascular rupture.

Recent unpublished post mortem studies by Pascoe et al may determine the exact site of haemorrhage and which circulation is involved. Further descriptions of lung pathology of racing horses would also be helpful in determining the prevalence of sub-clinical pathology in racehorses.

Although aetiologies are sometimes worked out by happening upon a prevention or cure, it is more likely that successful treatment or prevention will become apparent after the aetiology is known. The multitude of therapies that have been used underlines the lack of an understood pathogenesis.

2.3 Lower Respiratory Tract (LRT) Inflammatory Disease

The earliest recognised form of LRT inflammatory disease is that known as chronic obstructive pulmonary disease (COPD) or colloquially as "heaves" or "broken-wind". The condition is thought to have been recognised by Aristotle in 333 BC who described the "heave" or characteristic expiratory effort associated with this condition.

COPD is now considered to be primarily a bronchiolitis, characterised by epithelial hyperplasia, goblet cell metaplasia, peribronchiolar cellular accumulations and an exudate of mucus and cells in the airway lumen (Nicholls 1978). The last pathological characteristic, the mucoid exudate is of importance in the context of this study, since this material may be readily observed in the trachea using a fibreoptic endoscope (Cook 1974). It may be assumed that such exudate is of lower airway origin and that it is rapidly carried cranially primarily by mucociliary transport and coughing. Recently the mean tracheal mucous transport rate of the horse was determined to be 1.66 +/- 0.24.cm/min (Nelson and Hampe 1983)

COPD is considered to be a chronic pathological state probably of multifactorial origin, predisposed to by such factors as age, climatic changes, acute infection and exposure to environmental aeroallergens (Gerber 1973). It is likely that excessive production of mucus and egression of cells into the airway lumen can occur in response to other stimuli, because it is a non-specific component of airway inflammation. For example, infection with equine herpes virus (Prickett 1969) and equine influenza (Gerber 1970) may cause acute bronchopneumonia, which features serofibrinous exudation and cell necrosis.

The aims of the present study were to record the prevalence of visible mucopus in the trachea of thoroughbreds in training and to make an assessment of the amount and whether exercise had any effect on the amount seen.

2.4. Viral Respiratory Infection

Respiratory viral infection can, as already described, lead to lower respiratory tract inflammatory disease which may be manifested by the presence of visible mucoid or mucopurulent discharge in the trachea.

Since PLH and LRT inflammation may both be a result of viral infection, it was pertinent to sample horses for evidence of viral infection in this study. Therefore a brief review of the commonly recognised equine respiratory viruses is given.

2.4.1. Epidemiology of Respiratory Viral Infection in UK

The only comprehensive epidemiological study of respiratory viral infection in racehorses in training in the UK was by Powell et al (1978). Up to 600 two to four year old thoroughbreds in training were surveyed between 1972 and 1976, from different parts of Britain. The following viruses were isolated from nasopharyngeal swabs: equine influenza A (Subtype 1 and subtype 2), EHV-1, EHV-2, Adenovirus, Rhinovirus2 (ERV-2) and an acid stable picornavirus (ASPV). Evidence of infection, either serological or by isolation, associated with outbreaks of upper respiratory disease was found for both subtypes of influenza, EHV-1, equine adenovirus and equine rhinovirus-1 (ERV-1). Apart from these viruses, a third rhinovirus and a reovirus have been isolated from clinically affected horses in other countries (Erasmus, Pieterse and Boshoff 1978; Steck el al 1978).

2.4.2. Influenza

Influenza A virus was first isolated in 1958 from horses in an outbreak of disease. The type of virus became designated Influenza A/equi 1/Prague 56 (H7,N7) and is sometimes referred to as equine influenza, subtype 1. Subsequently the same virus was isolated from other parts of the world, first being recognised in Britain in 1963 (Beveridge, Mahaffey and Rose 1965).

A second equine serotype was isolated in 1963 in Miami, Florida (Waddell, Teigland and Sigel, 1963) and was found to have surface antigens unrelated to subtype 1, and therefore was designated A/equi 2/Miami/'63 (H3,N8), and is sometimes referred to as influenza, subtype 2.

Subsequently epizootics due to influenza equi 2 occurred in Britain in 1964/65 and 1969 (Rose 1965; Rose, Round and Beveridge 1970).

The first commercially available vaccine against influenza was an adjuvenated killed vaccine which became available in 1965, but after limited use was withdrawn due to injection site reactions. Two more inactivated vaccines were licensed for use in Britain between 1965 and 1969, but it was not until 1970 that widespread vaccination of thoroughbreds occurred (Mumford and Rossdale 1980).

The increase in vaccination was probably a result of the severe disruption of racing and financial losses to the industry caused by the 1969 epizootic. In the early seventies four commercial inactivated vaccines were available ("Prevac", Hoechst, Germany; "Fluvac-equine", Fort Dodge, U.S.A.; Duvaxyn, Phillips Duphar, Holland, and T.V.L.influenza vaccine, France).

An epizootic caused by localized infections due to A/equi-2 occurred during 1971 and 1972 in unvaccinated horses but in 1976 both unvaccinated and recently vaccinated thoroughbreds in Newmarket were susceptible to a strain of A/equi-2 introduced by a horse imported from America (Thomson et al 1977). The last recorded outbreak of influenza occured in Britain in 1979 when A/equi-2 caused a widespread epidemic which appeared in Britain at the Olympia International Horseshow in December 1978. Initially it was primarily unvaccinated animals which were infected but later on vaccinated horses were also succumbing to infection, many infections being mild or subclinical (Burrows et al 1982). No significant antigenic difference was found between the virus isolated in this epizootic (A/England-1/79) and the prototype A/equi-2 virus A/Miami-1/63 (H3.N8) (Burrows et al 1981).

Laboratory diagnosis of influenza virus is possible by either isolating the virus from nasopharyngeal swabs, in embryonated hens eggs or by detecting a four-fold rise in haemagglutinating antibody between acute and convalescent serum samples.

2.4.3. Equid Herpesvirus-1

Three antigenically different types of herpesviruses are known to infect horses, equid herpes virus type-1 (EHV-1), equid herpes virus type-2 (EHV-2) and equid herpes virus type-3 (EHV-3).

2.4.3.1 Epidemiology

Equid herpesvirus-1 is a major cause of equine respiratory disease, abortion and occasionally may cause a paralytic syndrome due to myeloencephalitis. Two serotypes are recognised on the basis of differences in cross-neutralization between strains, subtype 1 isolates are regarded as being able to cause abortion, respiratory disease and paresis, while subtype 2 isolates have only been associated with respiratory disease (Bagust 1971; Burrows and Goodridge 1972).

More recently analysis of virus DNA by restriction endonucleases which cleave the viral genome at specific nucleotide sequences have shown highly characteristic patterns for each group suggesting that most, if not all, EHV-1 isolates fall into either subtype-1 (foetal) or subtype-2 (respiratory) isolate categories (O'Callaghan, Allen and Randall 1978; Sabine, Robertson and Whalley 1981; Studdert, Simpon and Roizmann 1981; Turtinen, Allen and Darlington 1981). This review will be confined to the respiratory effects of both subtypes of EHV-1.

EHV-1 is regarded as being endemic in most equine populations, with the majority of individuals experiencing repeated infections in early life (Bryans 1969; Studdert 1974; Powell et al 1978). Repeated reinfection is due to a short lived immunity (3-5 months), and generally re-exposure results in a mild or inapparent infection, with the exception of pregnant mares which may abort (Doll and Bryans 1963 b).

2.2.3.b Clinical Signs

Clinical respiratory disease associated with EHV-1 infection occurs primarily in young horses and is characterized by fever, anorexia and profuse serous nasal discharge which tends later to become mucopurulent. Mortality is rare, except in young foals. Hartley and Dixon (1979) reported 29 deaths in 150 full term foals, in which some were stillborn, some were born alive but weak and others, initially healthy, died within 3 days of birth.

Other early cases of mortality are attributed to lethal secondary bacterial invasion aided by depletion of lymphocytes due to EHV-1 (Bryans et al 1977).

2.4.3.c Pathology

Prickett (1970) described the pathological changes seen following experimental infection by aerosolised EHV-1, in weanling foals. The only clinical signs were a mild pyrexia two and three days after exposure. Autopsy at this stage showed an acute bronchopneumonia with an early massive infiltration of the smaller airways and necrosis of the respiratory epithelial lining where typical herpes inclusion bodies were noted. In another series of experimental infections by nasal spray the most notable difference was the induction of marked hyperplasia of the lymphoid follicles present in the pharynx, particularly in those horses with some pre-existing immunity to the disease.

The pathology consisted primarily of lung lesions characterised by pulmonary oedema, alveolitis and necrotising bronchitis and bronchiolotis with intranuclear inclusions. Microscopic lesions were also seen in the liver, adrenal, thymus and spleen of some foals (Hartley and Dixon 1979). Patel et al (1982) compared the pathogenesis of infection for two isolates of EHV-1 subtype 1 (a paresis isolate and an abortion isolate) and a subtype 2 virus isolated from an outbreak of respiratory disease. None of the foals given the respiratory isolate had pyrexia in contrast with foals given either the foetal or paresis isolates in which the majority had temperatures up to 103 deg. F for at least 24 hours. All the foals developed a rhinitis and conjunctivitis irrespective of virus strain.

All three isolates infected nasal mucosa, bronchial epithelium, alveolar cells and conjunctival epithelium. The fetal and paresis subtype 1 isolates spread systemically outwith the respiratory tract by infecting lymphocytes and the latter showed a particular tropism for endothelial cells of arteries and capillaries.

2.4.3.d. Immunity

Immunity resulting from natural infection of the respiratory tract is reported to be of short duration. Despite persistence of virus neutralizing (VN) antibody, the respiratory mucosa may be asymptomatically reinfected within 3-4 months (Doll 1961). In general, in the first year of life the antibody responses to EHV-1 are poor but increase by the second year of life (Gerber et al 1977). Bryans (1969) and Frymus (1980) noted that even high antibody levels in horses one year old were not always protective, and therefore cell-mediated immunity (CMI) is obviously involved in protection against infection. In general, CMI activity was found in antibody negative ponies (Wilks & Coggins 1976). There is little published work on the nature of the CMI response to EHV-1 although lymphocyte-associated cytotoxic activity of virus infected cells has been demonstrated (Wilks and Coggins 1977).

2.4.3.e. Vaccination

Many vaccines to EHV-1 have been developed, the earliest attempts dating from 1959. Four live and modified-live virus vaccines have also been marketed, however, two at least have been withdrawn due to complicating side effects and inefficacy (Campbell and Studdert 1983).

All vacccines developed have used the subtype 1 (abortion) strain. To date, none incorporating the subtype 2 (respiratory) strain have been marketed. Licences for the vaccines have been granted primarily for protection against abortion, however, many doses are used as prophylaxis for the respiratory disease.

At the time of writing there are two vaccines marketed in the UK. "Pneumabort K" (Fort Dodge, Iowa) is an adjuvanted, formalin inactivated tissue culture grown subtype 1 vaccine. Recommended immunisation programmes are three intamuscular doses at five month intervals.

"Rhinomune" (Smith Kline) is the other vaccine marketed. This is a modified live virus vaccine incorporating the subtype 1 (strain RAC-H) virus. The virus is grown firstly in pig kidney cells and subsequently on equine cell culture. Recommended usage is for two intramuscular doses 4-8 weeks apart with revaccination every 6 months. Foals are recommended to be vaccinated after 3 months old. This vaccine was initially licensed for protection against abortion and respiratory disease. However, due to failure to protect against abortion claims for potency in pregnant mares were withdrawn in 1977.

2.4.4. *Equid Herpesvirus - 2 (EHV-2)*

In 1963 an equine herpes virus was isolated in England by Plummer and Waterson (1963) which was serologically distinct from the equine rhinopneumonitis virus (EHV-1). Since then, EHV-2 has been isolated frequently from the tissues of both clinically normal and diseased horses (Bagust 1971; Hadden et al 1974; Studdert 1974; Kemeny and Pearson 1970).

The main distinguishing feature of EHV-2 from EHV-1 is the length of time required to produce cytopathic effect (CPE) in tissue culture. Although isolates have produced CPE within 24 hours (Erasmus 1970), most isolates take two to three passages in permissive cell culture before CPE is seen.

Equine Herpesvirus type 2 is an extremely successful parasite as the incidence of infection in horses based on virus isolation (Kemeny and Pearson 1970) and on serology (Burrows 1968; Bagust et al 1972), is very high, frequently exceeding 80 per cent. Foals become infected within 30 days post-natally, often in the face of high VN antibody titres. Once infected, virus may persist for prolonged periods (Wilks and Studdert 1974).

Because of its ubiquitous nature it has been difficult to attribute clinical or pathological significance to EHV-2. Blakeslee et al (1975) suggested that following experimental infection foals develop chronic pharyngitis due to lymphoid proliferation in response to persistent EHV-2 infection. Thein (1978) isolated EHV-2 from foals with keratoconjunctivitis and suggested that this virus had been the causative agent.

Palfi et al (1979) isolated EHV-2 from an outbreak of clinical respiratory disease in a large group of foals. The lung tissue contained histopathological lesions of viral origin and they suggested EHV-2 was the initiating agent of the disease, followed by secondary bacterial complications. In a second paper (Belak et al 1980) they reported an apparent protection from respiratory disease in the same group of foals by the administration fo hyperimmune serum to EHV-2. With repeated use of the serum the disease could be prevented while non-immunized foals succumbed once colestral protection waned. They suggested that these findings served as indirect proof that certain EHV-2 strains may initiate respiratory disease in young foals. Despite these reports EHV-2 is generally considered to be non-pathogenic, at least to the adult horse.

2.4.5. Picornaviruses

The picornaviruses comprise three genera of RNA viruses which are characterised by their small size (30-40 nm), and distinguished from each other by their physico-chemical properties. They comprise the enteroviruses, rhinoviruses and caliciviruses. There are three strains of equine rhinoviruses and one acid-stable picornavirus which have been isolated from the respiratory tract of horses.

The first isolate described by Plummer (1962) was recovered from faeces but was subsequently found to multiply in the respiratory tract. This was designated equine rhinovirus-1 (ERV-1). A second rhinovirus, later classified as ERV-2, was isolated from Swiss Army horses in 1965 (Hofer et al 1973). The same authors isolated a third strain, ERV-3 in 1975 (Steck et al 1978). In the same year Mumford and Thomson (1978) isolated an acid-stable picornavirus designated 4442/75, which because of its acid stability could not be classified as a rhinovirus.

2.4.5.a. Equine Rhinovirus 1 (ERV-1)

ERV-1 has been associated with both clinical and subclinical disease, and serological surveys have indicated that infection is common in most horse populations (Burrows 1979). High neutralizing antibody titres result from infections and are maintained for long, if not indefinite, periods and it is likely that immunity extends for life. (Burrows and Goodridge 1978).

An early serological survey in Britain (Burrows 1970) showed that 60% of adult thoroughbred horses had significant levels of neutralising antibody to ERV-1. Further studies in British training stables (Powell et al 1978) indicated that up to 25 per cent of horses may have experienced infection before entering training stables and that approximately one third of susceptible horses aquire infection during their first winter in training. The numbers of susceptible horses which became infected varied considerably between individual stables and between training areas of different locations.

The majority of infections were of an inapparent nature and a direct association between infection, whether alone or with other viruses, and upper respiratory tract disease was found in only 11 of 84 outbreaks.

Clinically apparent infection with ERV-1 is characterised by pyrexia, nasal discharge and swelling of the pharyngeal lymph nodes (Plummer and Kerry 1962). Studdert and Gleeson (1978) gave a detailed description of signs seen in a four year old mare which they associated with the isolation of ERV-1. The mare developed an acute febrile illness characterised by anorexia, lethargy, limb oedema, increased heart and respiratory rates, abnormal lung sounds and pyrexia (up to 41.4 deg.C). The mare remained ill for 7 days. ERV-1 was isolated from nasal swabs taken 4 days after the onset of clinical signs. These signs appear to be extreme and the clinical signs are generally considered milder but variable (Hofer et al 1973).

2.4.5.b. *Equine Rhinovirus-2. (ERV-2)*

Fifty-six per cent of horses in training in Great Britain were found to be seropositive to ERV-2 (Powell et al 1978). Serological observations during that survey indicated that inapparent infection was common. Many infections occured before the horses entered training stables. Sixteen of 42 thoroughbred foals and yearlings sampled in 1971 and 1972, had already experienced infection (Burrows and Goodridge 1973) and it was noted that 49 per cent of seven to twelve month-old Swiss bred foals had antibody to the virus (Steck et al, 1978). Most isolations of ERV-2 have been obtained from clinically normal horses and often in the presence of pre-existing antibody (Steck et al 1978; Mumford and Thomson 1978). However, Steck and colleagues (1978) concluded that infection in young horses could cause pyrexia and mild symptoms which could lead to secondary bacterial infection. Two out of three isolates obtained by Powell (1979) were associated with respiratory disease.

Burrows (1979) gives conclusive evidence of the existence of a carrier state in ponies. Recrudescence was stimulated by corticosteroid treatment and grass sickness. Thus recovered animals may carry the virus for long periods, providing a source of infection.

2.4.5.c. Acid Stable Picornavirus

There is little information regarding this virus other than the original report of Mumford and Thomson (1978). The majority of isolates were obtained from a single animal, which showed no signs of respiratory disease, over an 18 month period, suggesting the existence of a long-term inapparent carrier state. One isolate was recovered during an outbreak of respiratory disease among a group of horses in training. The majority of these horses showed a concurrent significant rise in antibody titre to the same virus.

2.4.6. Adenovirus

There are many reports from throughout the world showing that adenovirus infection is common (Studdert et al 1974; Burrows 1979), and that many of these infections occur during the first year of life. Investigations at five breeding studs disclosed that 17 of 41 sero-negative foals and yearlings developed antibody within one year (Powell, Burrows and Goodridge 1974). Following experimental infection, adenovirus was recovered from nasopharyngeal swabs for up to 68 days, suggesting virus persistence (Burrows and Goodridge 1978). Burrows (1979) also reports that foals can acquire infection from their dams during the suckling period. Serological studies have shown a varying prevalence of antibody in different parts of the world.

Burrows (1979) notes that the serological response to infection can be detected earlier and for a longer period using antigens prepared from the homologous virus and this may indicate minor antigenic differences between strains. In the survey of horses in training in Britain, Powell et al (1978) found some serologic evidence of adenovirus activity, but there was little evidence to suggest that the virus was involved in any of the outbreaks of disease investigated.

2.4.7. Summary on Picornavirus and Adenovirus

The natural history of picornaviruses and adenovirus in horse populations is succinctly summarized by Burrows (1979), using data obtained from long term studies of an isolated pony herd, studs and training stables in Britain. With the exception of rhinovirus-1, these viruses can persist naturally in a small population kept in relative isolation from others horses. Most of the infections in all populations studied were sub clinical, and it appeared unlikely that single infections by any of these viruses were of great consequence in the training stable. However, multiple infections involving various combinations of EHV-1, rhinovirus-1, rhinovirus-2, acid-stable picornavirus and adenovirus were recognised in 15 of 69 outbreaks of disease which affected training programmes between 1972 and 1975 (Powell et al 1978) and such mixed infections were thought to be of greater clinical significance.

Chapter 3 Materials and Methods

3.1. Introduction

The fibreoptic endoscope has been available for the last decade, enabling direct visual assessment of the internal features of the gastro-intestinal and respiratory tracts. It has provided a practical and simple means of examining the upper respiratory tract of horses. In the first instance it has been used in diagnosis of long recognised functional obstructive problems, such as laryngeal hemiplegia. Subsequently it has been the prime reason for the description and frequent diagnosis of PLH and widespread EIPH. It has therefore become necessary to carry out epidemiological studies which will define the incidence of such entities in a sample population, representative of the equine population as a whole. Close monitoring of such a population for a period of a year allows changes in these entities to be observed, and some of the factors which affect changes may be recognised.

Data obtained from one selected population does not of course automatically apply to the equine population as a whole. However, assumptions may be made for the general population, provided there is statistical support for the conclusions reached and that any bias is recognised.

3.2. Study Design

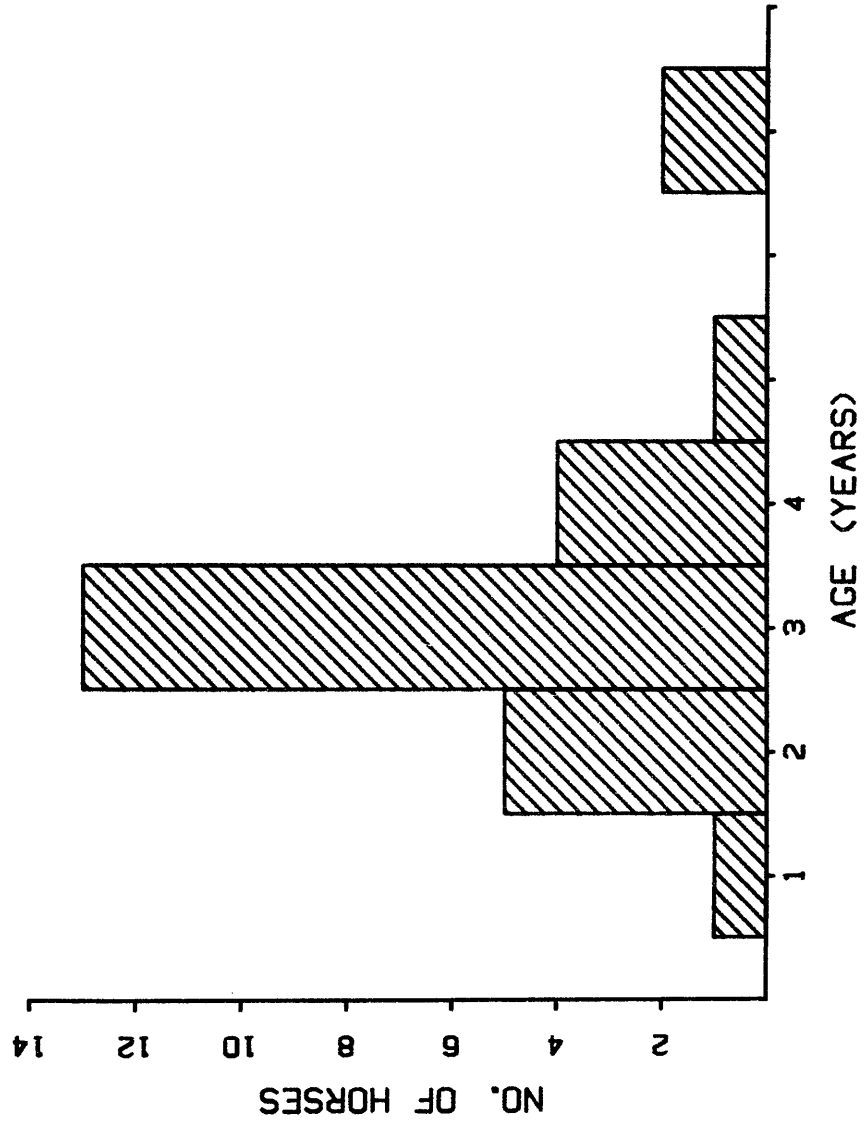
The group of horses studied were in a flat-racing training stable situated in the West of Scotland. These horses were examined from June 1981 to August 1982 (14 months). There was unfortunately a period of 4 months during which time it was not possible to make any examinations, because the stable temporarily had to close for financial reasons.

The stable was situated adjacent to a racecourse, and the gallops available there were used in training. There was a grass gallop and an all-weather shavings surface.

The stable population fluctuated and horses were present in the stable for varying periods of time. A total of thirty-one different horses were examined endoscopically. Of these sixteen were examined on three or more occasions and eleven on five or more occasions. Ages ranged from one year to seven years at the time the first examinations were made (fig 1.) with a median of 3 years (birthdates assumed to be January 1st).

Endoscopic examinations were carried out either at rest or within one hour of various levels of training exercise. The speed of training exercise was recorded and classified as walk/trot only (less than 240 m/min), canter (500-700 m/min) or maximal speed (700-1000 m/min). The distances covered at the canter varied from 0.8 to 2.4 km (0.5 to 1.5 miles) and at maximal speed from 0.8 to 1.6 km (0.5 to 1.0 miles).

FIG1. AGE DISTRIBUTION AT FIRST EXAMINATION



3.3. Endoscopic Examination Technique

A flexible fibreoptic endoscope of 100 cm length and 9mm diameter (Olympus GIF Type P2) was used for all examinations. Examinations were carried out in a loose box with the horse restrained using a bridle and a twitch. The endoscope was passed through the right nares, along the ventral meatus to the nasopharynx. After observation of the nasopharynx the endoscope was then guided through the aditus laryngus and into the tracheal lumen. Using a 100 cm length endoscope it was possible to visualise about 60 cms of the tracheal lumen which is approximately equivalent to the cranial two-thirds.

3.4. Endoscopic Observations

3.4.a Pharyngeal Lymphoid Hyperplasia

Pharyngeal lymphoid hyperplasia consisted of nodules or follicles of varying size and colour which covered varying amounts of the roof and lateral walls of the pharynx, generally extending caudally from the lymphoid tissue of the dorsal pharyngeal recess (DPR). Raker and Boles (1978) described a system for grading the severity of PLH and is described as: Grade 1 - a few small white follicles over the dorsal pharyngeal wall; Grade 2 - mainly small white follicles with occasional larger pink follicles over the dorsal pharyngeal wall and extending laterally to the level of the gutteral pouch ostia; Grade 3 - pink and white follicles covering the entire dorsal and lateral pharyngeal walls and often also involving the nasal surface of the soft palate; Grade 4 - large pink, oedematous follicles covering all visible mucosa of the pharynx and sometimes including polyps. (Figs 2,3,4 and 5).

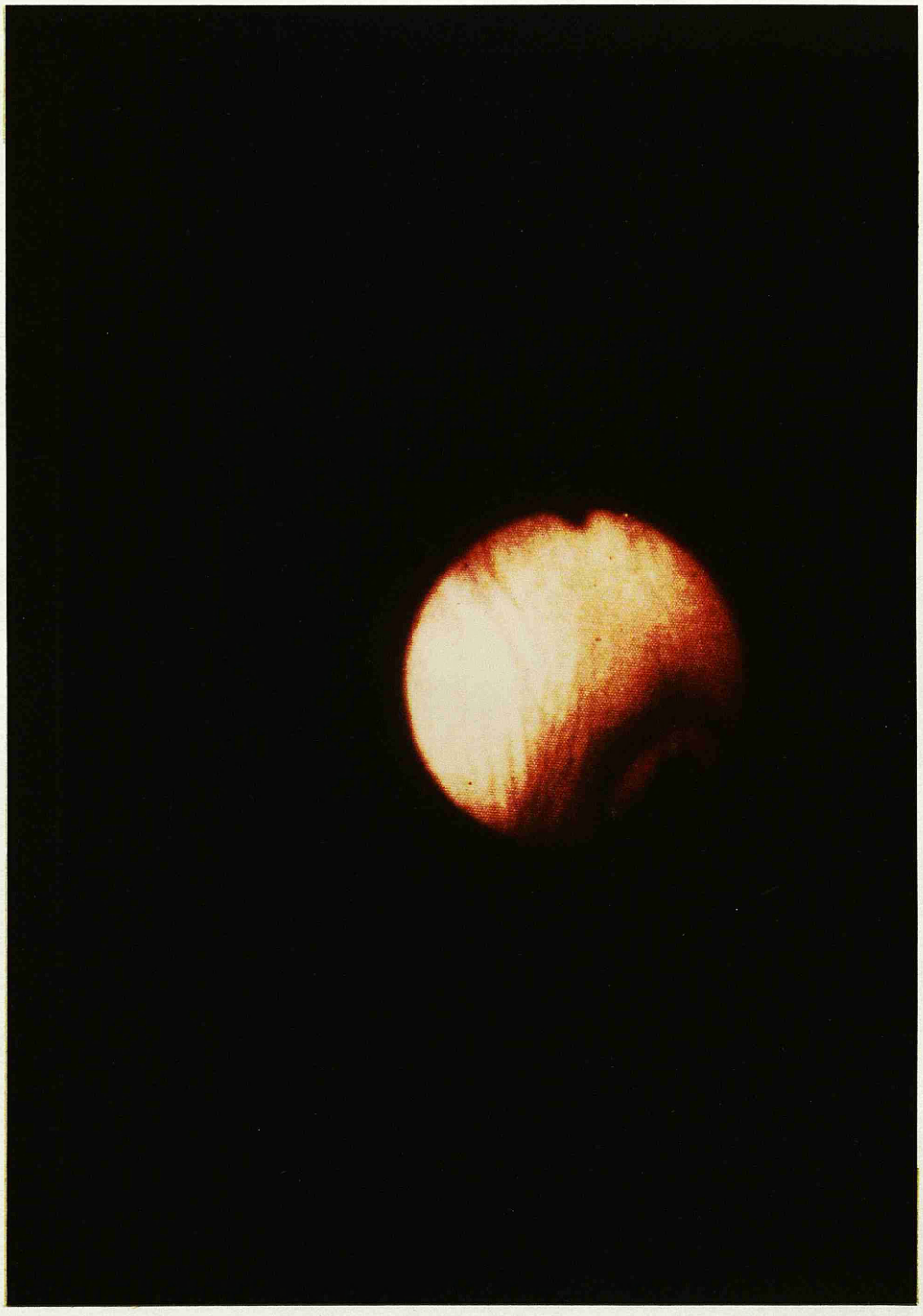


Fig.2. Endoscopic view of the pharynx showing smooth dorsal and lateral walls with no lymphoid hyperplasia (PLH Grade 0).

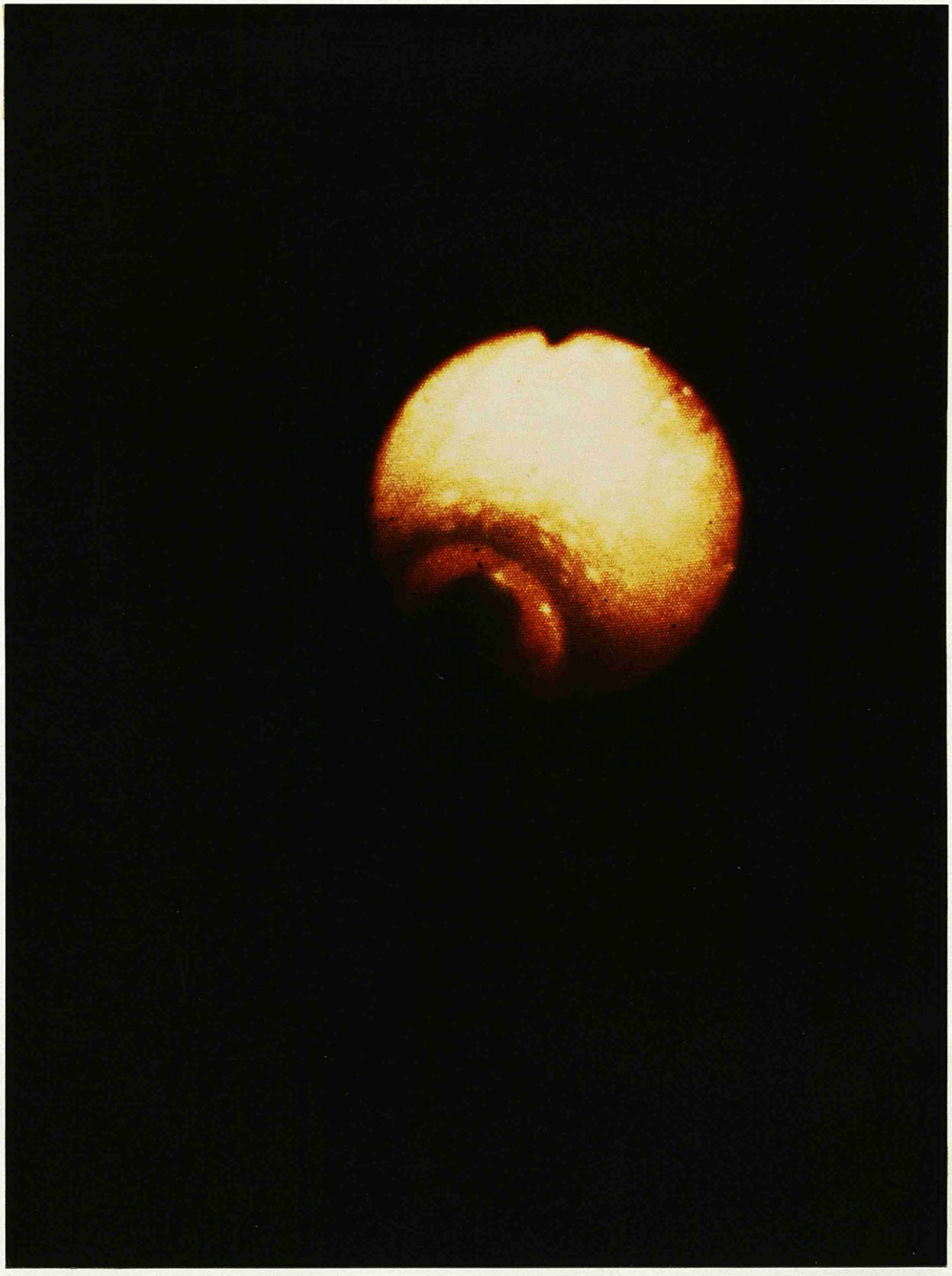


Fig.3. Endoscopic view of the pharynx with PLH grade I. Small white raised nodules are visible.

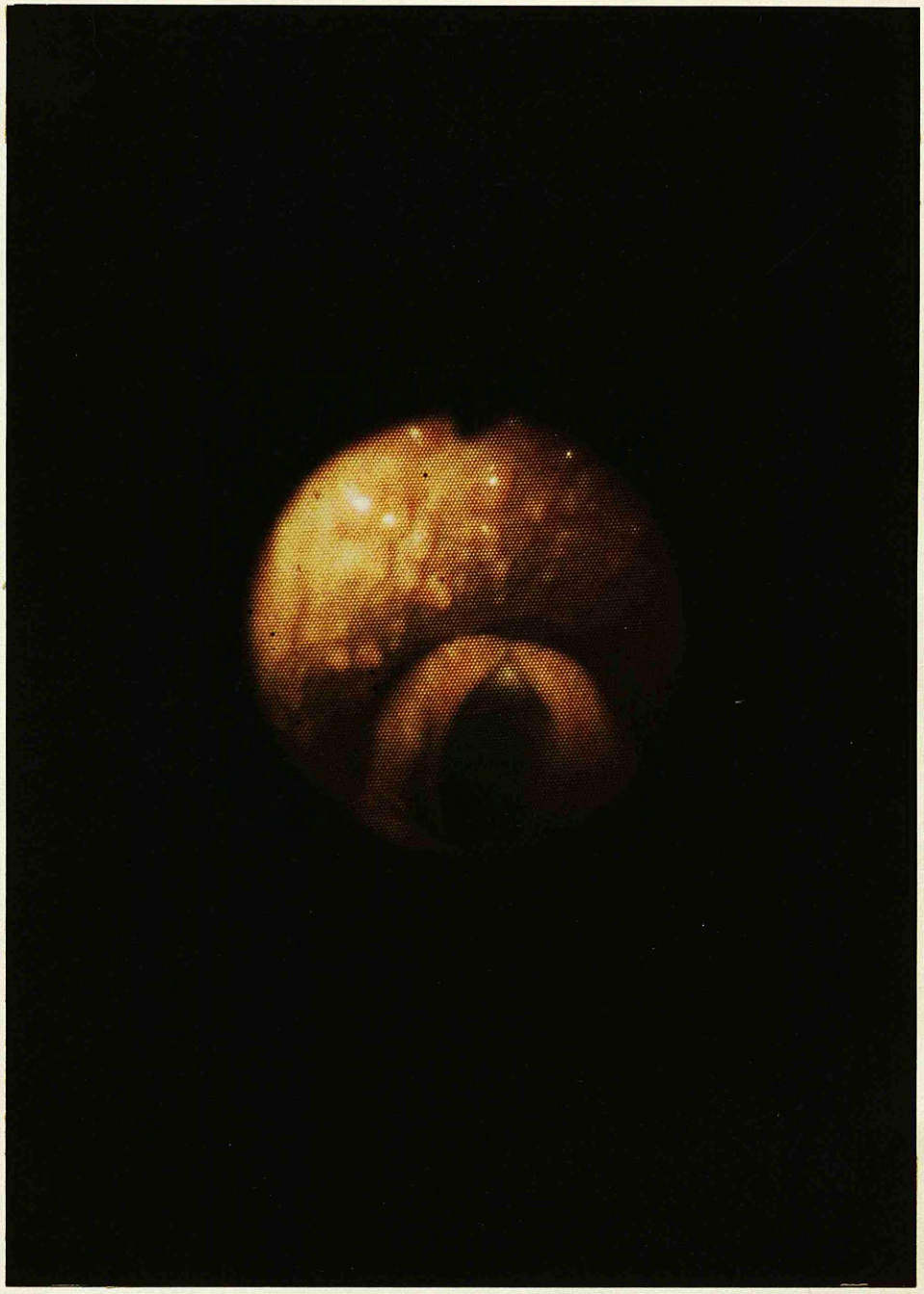


Fig.4. Endoscopic view of pharynx and larynx showing PLH grade 3. Large, oedematous lymphoid follicles cover the dorsal and lateral pharyngeal walls.

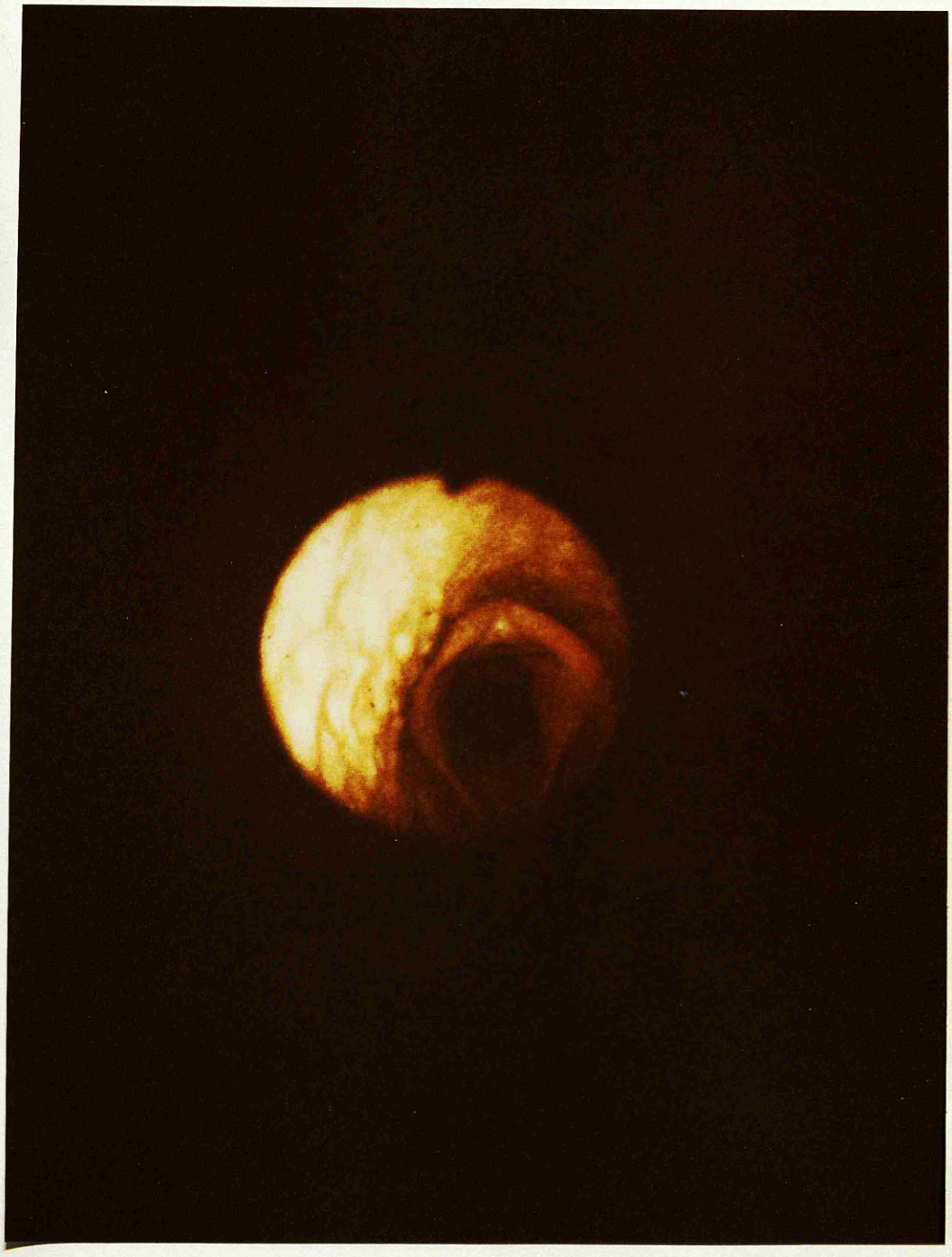


Fig.5. *Endoscopic view of larynx and lateral wall of the pharynx wall with PLH grade 3 or 4. All visible mucosa is covered in large lymphoid follicles.*

3.4.b Exercise Induced Pulmonary Haemorrhage

Exercise induced pulmonary haemorrhage was usually visible as free blood on the floor of the tracheal lumen. A grading system describing four grades was given by Pascoe et al (1981), dependant on the continuity and width of the stream of free blood. However in the present study the grading was simplified to two grades. If there was blood-stained mucous, flecks or thin streaks of blood, it was graded as "mild"; a continuous stream of blood covering at least half the floor of the trachea was graded severe. With the severe grades there was sometime blood-spattering over the whole tracheal lumen and occasionally blood clots could be seen adhering to the dorsal of the epiglottis or even in the nasopharynx. (Figs 6 and 7).

3.4.c. Tracheal Mucopus

Respiratory secretions, assumed to be of lower respiratory tract origin, were seen as accumulations of mucoid or muco-purulent exudate within the tracheal lumen. No distinction was made as to whether the material was predominantly mucoid or purulent in appearence, so the term mucopus was used. The mucopus was seen as globules or as a continuous stream lying on the floor of the trachea. The amount of mucopus was graded on a four-point stream: Grade 1-isolated globules; Grade 2-a thin continuous stream (15mm wide, and Grade 3- a thick continuous stream)15mm wide. (Figs 8,9,10 and 11).

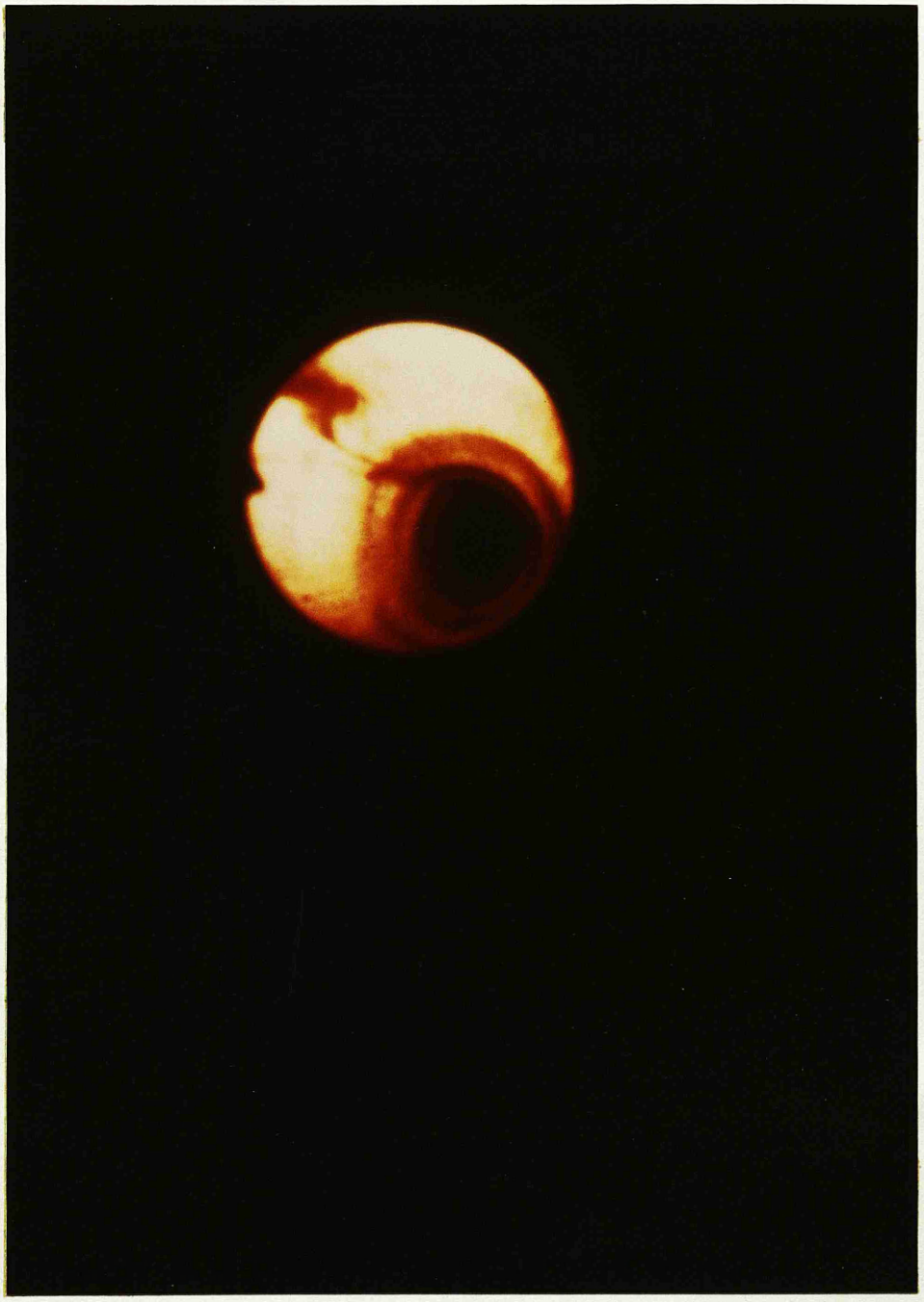


Fig.6. Endoscopic view of the trachea showing mild EIPH.

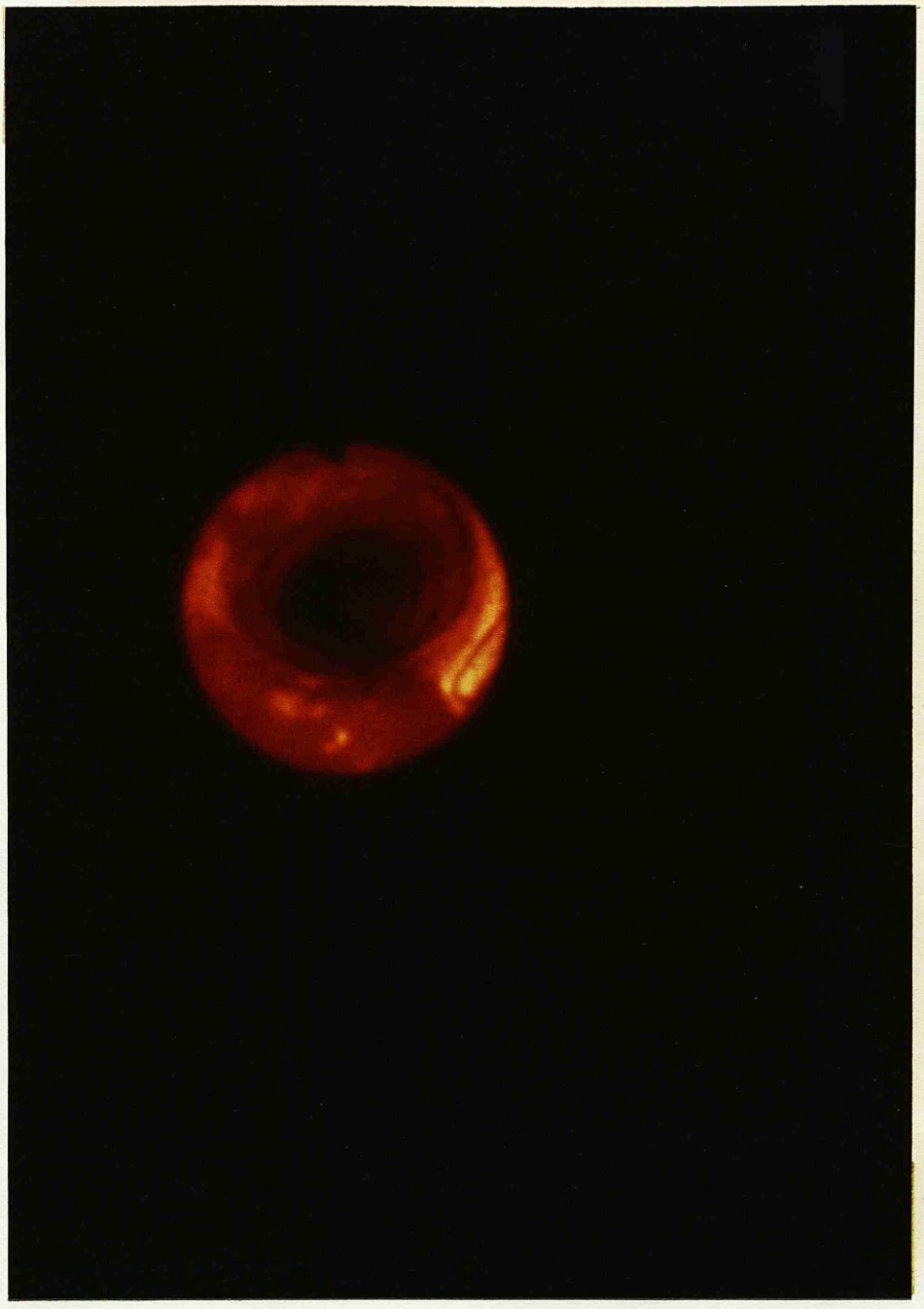


Fig.7. Endoscopic view of the trachea showing severe EIPH.

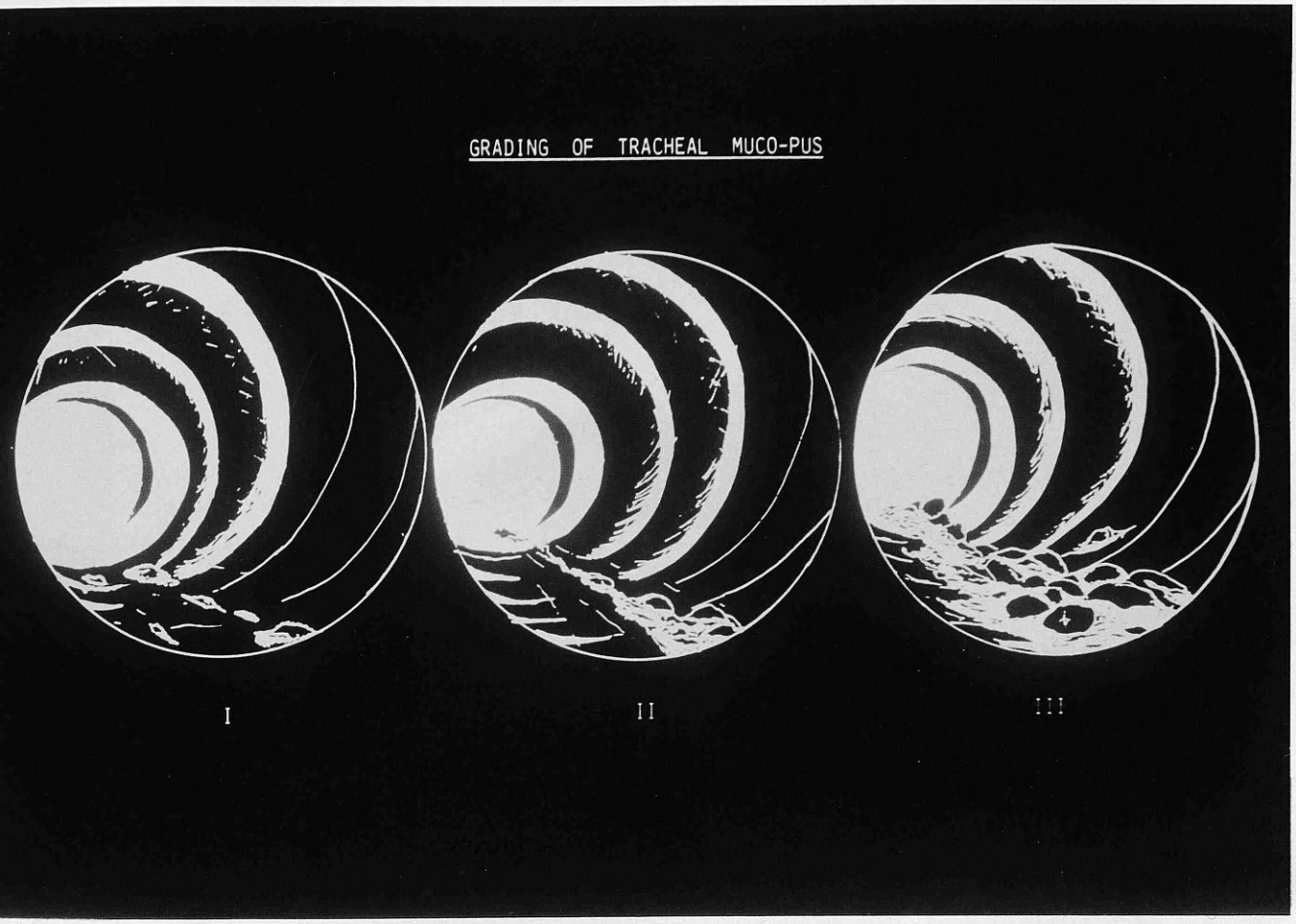


Fig.8. Diagram depicting the grading on intra-tracheal muco-pus

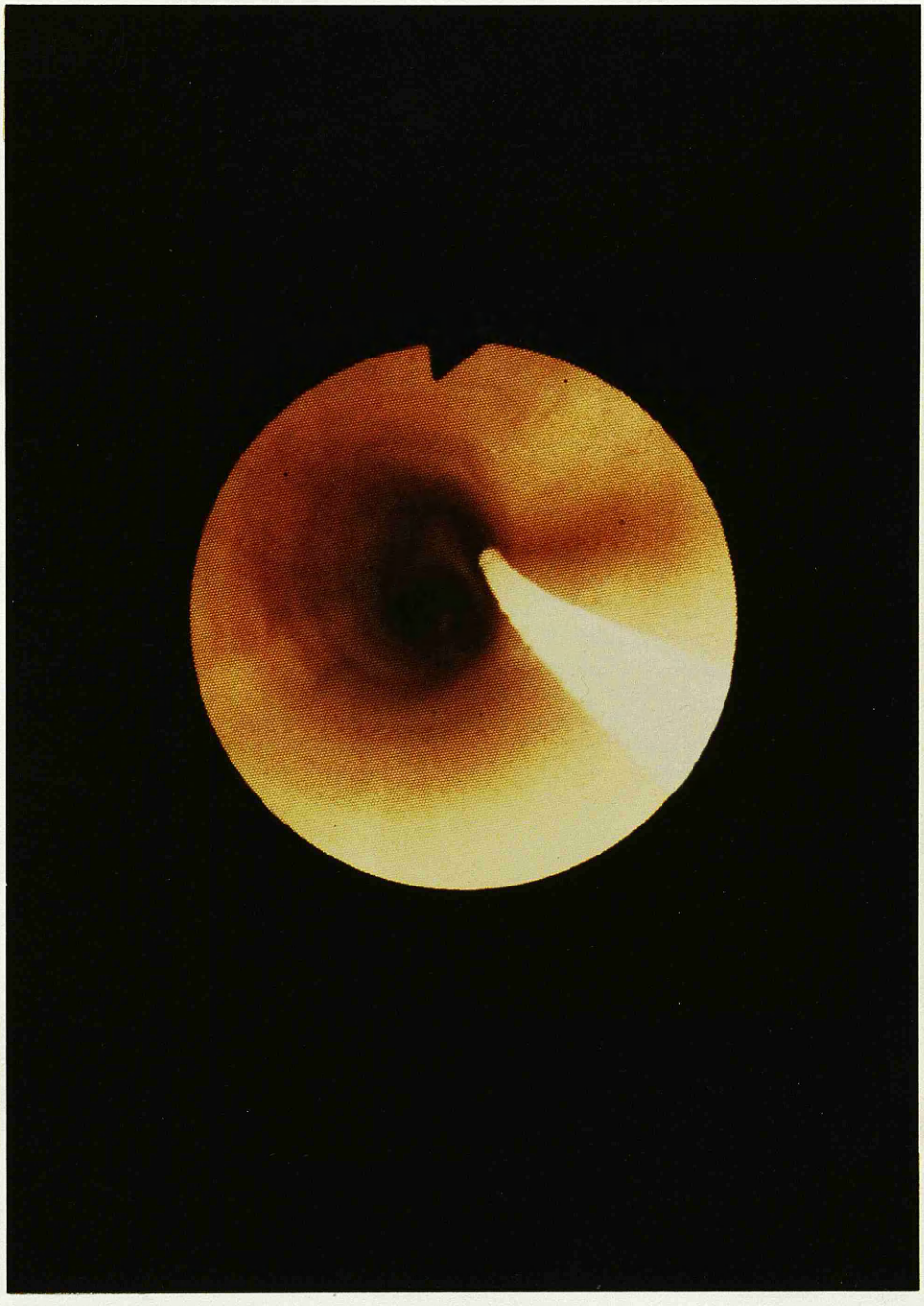


Fig.9. Endoscopic view of the caudal trachea showing the carina. No mucopus is visible. (A catheter emerging from the endoscope is present in the photograph).

Fig.10. Endoscopic view of the trachea showing a moderate amount of mucopus (Grade 2).

Fig.11. Endoscopic view of the trachea showing a large amount of mucopus (Grade 3).

3.5. Clinical Observations

Clinical observations relied upon signs of illness being recognised by stable staff and being brought to the attention of the trainer. To aid this procedure a form for each horse was filled in daily by the head lad recording rectal temperatures, nasal discharges and coughs. Any cases with pyrexia or signs of respiratory disease were given a full clinical examination, by the author within twelve hours of being noted.

3.6. Vaccination Histories

All horses in the study had been vaccinated against equine influenza in accordance with Jockey Club rules. The vaccine used was a killed adjuvanted (aluminium hydroxide) vaccine incorporating the prototype strains of equine influenza 1 and 2 (Prevac, Hoechst, Germany). All horses received an annual booster during the period of study. All horses were also vaccinated against EHV-1 with a killed adjuvanted vaccine incorporating the subtype 1 (abortion) strain prototype virus Army - 183 (Pneumabort-K, Fort Dodge Laboratories, USA). During the 10 month period in which sera were collected each horse received three booster doses of vaccine, at intervals of 3 months. (See appendix 2).

3.7. Laboratory Procedures

All laboratory specimens were examined at the Animal Health Trust Equine Virology Unit, Newmarket.

3.7.1 Virus Isolation

A large gauze swab mounted on a 40-50 cm soft metal wire was inserted via the external nares and passed along the ventral meatus into the pharynx. After a few seconds the swab was withdrawn and placed in a sterile screw-top container holding about 2 ml of transport medium, the excess wire having been removed. The medium contained phosphate buffered saline including 0.1 per cent albumin plus an antifungal (50 units per ml of nystatin) and an antibacterial 70 units per ml of neomycin). The transport medium tubes were kept in a cool container at 0-4 deg.C. for transport to the laboratory by post. Once in the laboratory swabs were processed in an isolation hood to exclude possible contamination. Samples were added to confluent monolayers of rabbit kidney (RK 13) continuous cell line and primary equine embryonic semi-continuous cell lines (Kidney or Lung) obtained from foetuses. Samples were considered negative if there was no visible cytopathic effect (CPE) after 2 passages of 7 days each. CPE was identified by appearance with differentiation between EHV-1 and EHV-2 by indirect immunofluorescence, using rabbit hyperimmune serum detected by goat anti-rabbit gammaglobulin labelled with fluoroscein isothiocyanate. Thus, infected cells fluoresce with the homologous antibody when viewed under ultra-violet light.

Thirty-eight nasopharyngeal swabs were taken for virus isolation randomly on five seperate occasions from 13 horses, over a period of 10 months. The gap between samples was between 2 and 4 months.

3.7.2. Serological Tests

Ten ml of whole blood was collected on 7 occasions from 21 horses over a 10 month period, serum was removed after centrifugation and stored at -20 deg.C. Collection was at approximately monthly intervals, but there was an unavoidable gap in sampling of 3 months.

All sera from each horse was processed in parallel. Antibody titres to EHV-1 subtype 1 (strain RACH) and subtype 2 (strain MD) and equine rhinovirus -1 (ERV-1) were measured by the complement fixation (CF) test as described by Thomson and Mumford (1976). Serum samples were diluted to 1:5 with CF diluent (Oxoid) and heat inactivated at 60 deg.C. for 30 minutes. All tests were carried out in round-bottomed microtitre plates using 0.025 ml volumes. Two-fold dilutions of serum were used (1:5 to 1:640). All antigens were grown in tissue culture, stored at -70 deg.C., and titrated using a chequer-board system against specific homologous antiserum raised in gnotobiotic foals. Young guinea pig complement was used at 4 complement fixing units /0.025 mls. Rabbit haemolytic serum was used as the haemolysin and 2% sheep red blood cells were used. Control wells were incorporated for the complement titration, antigen control, sheep cell control and a titration against the gnotobiotic foal homologous antiserum.

Antibody levels to influenza virus were measured against the prototype strains of equine influenza subtype 1, (A/Equine 1/Prague/56)(H7 N7) and subtype 2 (A/Equine 2/Miami/63)(H3 N8) and a recent strain of influenza 2 (A/Equine 2/Newmarket/79) (H3 N8). The serum haemagglutination inhibition (HI) test was used as described by Powell, Thomson, Spooner, Plowright, Burrows and Schild (1974). All tests were carried out in "v" bottomed microtitre plates using 0.05 ml volumes and phosphate buffered saline as diluent. The antigens used were grown in chicken eggs and ether treated. Sera were diluted 1:8 and heat treated for 30 minutes at 56 deg.C. Two-fold dilutions of sera were tested. Eight haemagglutinating units of antigen were used and tested with 4% adult chicken erythrocytes.

Adenovirus antibodies were also measured by the same HI test, using a strain recently isolated at the Equine Virology Unit.

For an antibody rise between two samples to be considered diagnostic of infection, at least a four fold rise in antibody titre, known as a seroconversion, was required.

Sixteen horses present in the stable for the duration of the study, were tested to investigate the possible association between prevailing EHV-1 CF antibody titres and PLH grade on 56 occasions. The horses tested comprised 10 three year olds, 4 two year olds, 1 yearling, and 1 five year old. The prevailing antibody titre at the time of serum collection was linked with the PLH grade observed in that month. The observations of PLH were grouped according to grade and the mean of the corresponding antibody titres calculated. The means of the groups were then tested for association.

3.8. Assessment of Performance

The training exercise was carried out on a racecourse adjacent to the stable. There was either an all-weather bark surface or a grass track over which the horses were trained. However, the actual amount of work each horse was given, and the speed and distance covered by each would have been difficult to measure and impossible to standardise without undue disruption of the trainer's programme. Therefore, the only practical means of assessing satisfactory performance was by recording finishing position in races.

3.9. Statistical Analysis

The data were analysed statistically using the chi-squared test for association with continuity correction (X2 c(1)), the student's unpaired T test or by Linear Regression Analysis (Yates 1960). The 5% (P=0.05) level of significance was selected.

Chapter 4 Results

4.1. Clinical Disease

No confirmed cases of acute viral respiratory disease occurred during the time of study. One horse was pyretic and anorexic for two days (horse no. 16), but showed no other signs of upper or lower respiratory tract disease. Coughing more than two or three times at exercise was never noted.

4.2. Virus Isolation

From the 38 nasopharyngeal swabs taken randomly on five seperate occasions from 13 healthy horses, EHV-2 was isolated nine times from 5 of the 13 horses (table 3). In addition, EHV-2 was isolated from one pyretic horse. No other viruses were isolated.

4.3. Serology

4.3.1. Evidence of Infection

There were no four-fold rises in antibody titre to the viruses tested. All horses had HI titres to influenza, due to vaccination. Four horses had low levels (1:8 to 1:16) of HI antibody to equine adenovirus, while the rest were negative. All horses had low levels of antibody to ERV-1 (1:5 to 1:40) indicating previous exposure, but no four-fold rises were seen.

Table 3

Virus Isolates from 38 Nasopharyngeal Swabs

From 13 Horses

Horse	Date of Sample 2.12.81	16.12.81	30.4.82	9.6.82	2.9.82
4	*	-	-	-	-
5	*	-	*	*	*
6	*	*	EHV-2	EHV-2	-
7	*	*	*	-	-
9	*	*	-	-	-
10	*	EHV-2	EHV-2	-	-
11	*	-	-	-	-
12	*	-	EHV-2	EHV-2	-
13	*	-	-	-	-
16	EHV-2	-	-	-	EHV-2
18	-	-	*	*	*
21	*	*	*	*	-
34	*	EHV-2	*	*	*

Legend :

* No sample taken

\- No Virus isolated

EHV-2 Equine Herpes Virus-2 isolated

4.4. Endoscopy

4.4.1. Pharyngeal Lymphoid Hyperplasia

One hundred and thirty-six endoscopic examinations were made on thirty-one different horses, after various levels of work, over a period of 14 months. The results of each examination for each horse are shown in appendix 3. All horses had a degree of PLH, which in those present on the stable for the duration of the study showed a gradual decrease in severity. Similarly, there was an inverse relationship between the PLH grade and the age (months since date of birth) of the horse at the time of examination (Fig.12). Analysis of the data by Linear Regression Analysis showed that the mean grading value regressed with age and showed a high degree of correlation ($r = -0.9819$). Similarly, horses in the age group 19-42 months ($n = 35$ examinations) had a significantly lower degree of PLH than those $>$ 43 months of age ($n = 72$ examinations) when analysed using an unpaired student's t-test ($P < 0.001$).

Sixteen horses, present in the stable for the duration of the study, were tested to investigate the possible association between prevailing EHV-1 CF antibody titre and PLH grade on 56 different occasions. The horses tested comprised 10 three year olds, 4 two year olds, 1 yearling and 1 five year old. The mean titre corresponding to each grade of PLH was very similar showing no correlation between CF antibody level to EHV-1 and PLH grade ($p > 0.05$) (Fig.13).

The mean PLH grade for the group of 5 horses from which EHV-2 was isolated was not significantly different from the group of horses from which the virus was not isolated.

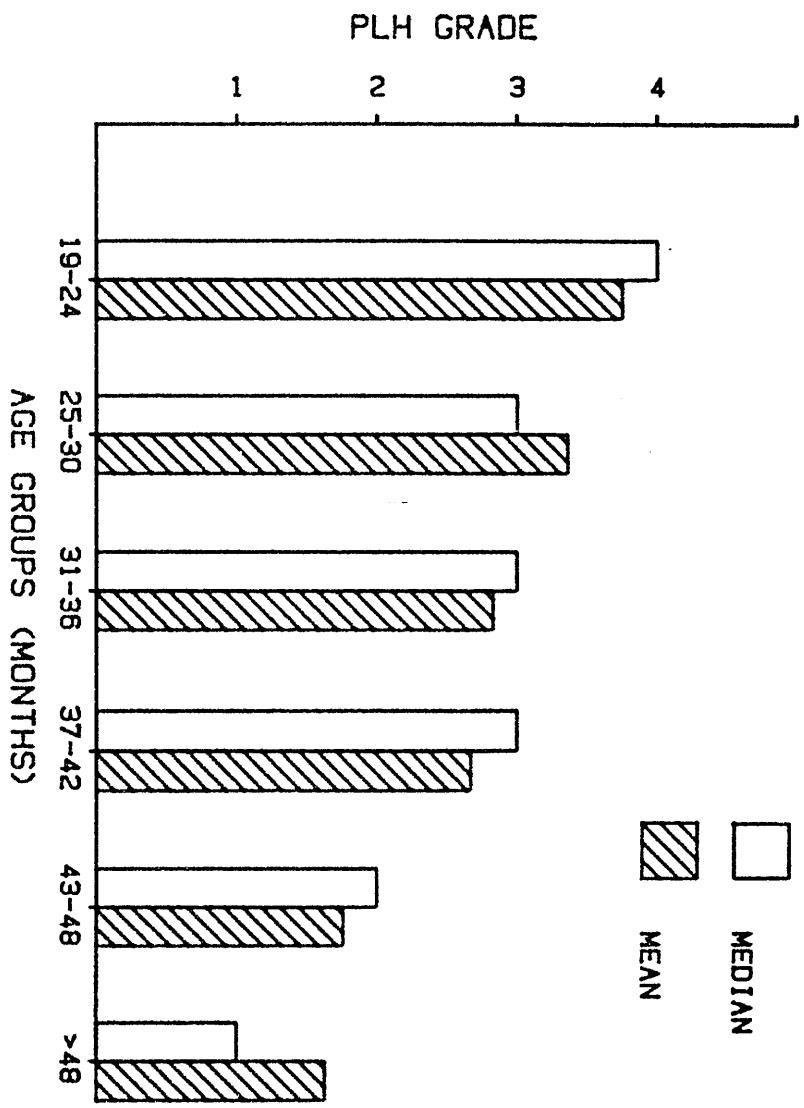


FIG. 12 THE EFFECT OF AGE AT EXAMINATION ON PLH GRADE

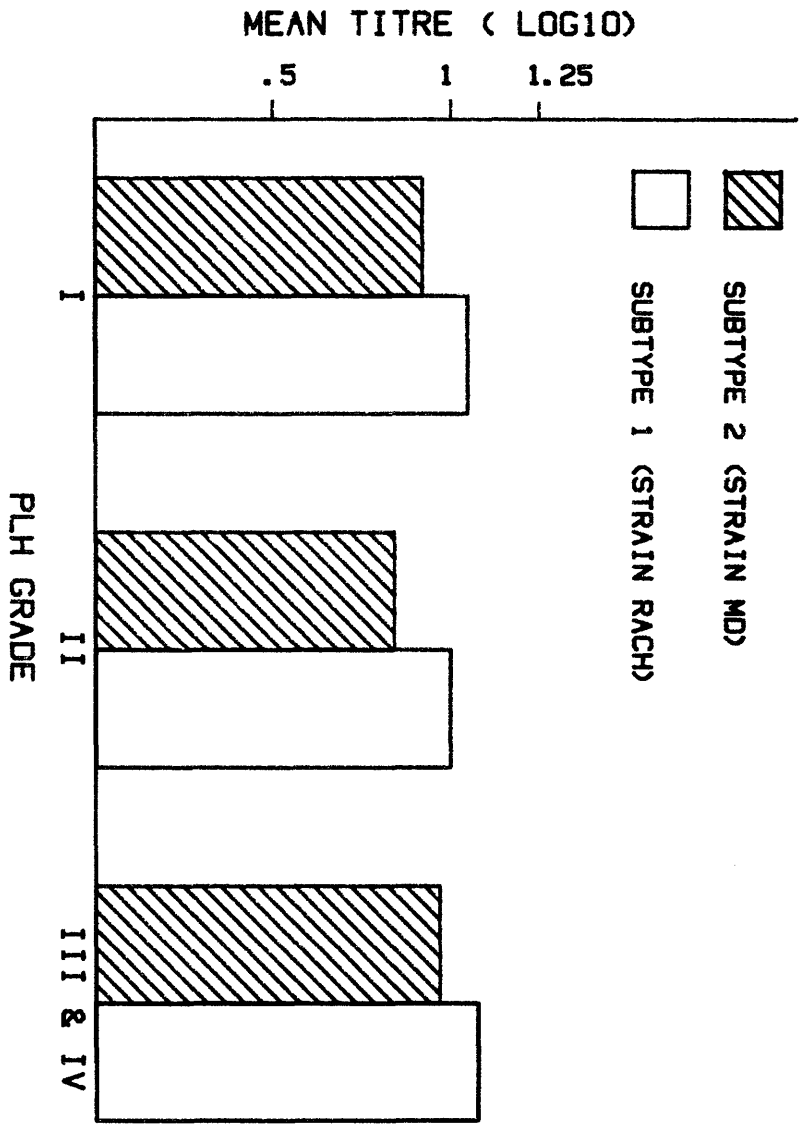


FIG 13 EHV-1 TITRES &PLH GRADE AFTER VACCINATION WITH KILLED EHV-1

4.4.2. Exercise Induced Pulmonary Haemorrhage

EIPH was seen predominantly after cantering or maximal speed training exercise, but was seen on one occasion after a horse had only walked and trotted. Cantering or maximal speed exercise resulted in free blood in the trachea on 32 of 86 (37 per cent) occasions. The incidence was greater after maximal speeds (23 of 49, 47 per cent of examinations), than after cantering (9 of 37, 24 per cent of examinations) (table 4). However this increase in incidence with increased speed was not statistically significant ($x = 3.70$, $p > 0.05$).

The mild grade of haemorrhage was more commonly seen than the severe grade. Twenty-four out of 33 (73%) positive observations were graded mild, whereas 9 of 33 (27%) were graded severe (table 4). The severe grade of haemorrhage occurred at cantering and maximal speed, and was not significantly more common following maximal speed ($p > 0.05$).

Most horses examined more than once after cantering or maximal speed exercise showed EIPH. Eighteen out of 19 (95 per cent) showed evidence of EIPH on at least one occasion.

Table 4

Incidence of Exercise Induced Pulmonary Haemorrhage after different levels of exercise

	Exercise Level		
EIPH Grade	*Maximal*	*Cantering*	*Walk/Trot*
Mild	*17*	*6*	*1*
Severe	*6*	*3*	*0*
Total number of observations	*49*	*37*	*17=103*
(No. Positive Observations)	*23*	*9*	*1*
(Percentages of total observations)	*(47%)*	*(24%)*	*(5%)*

The occurrence of observable EIPH was not repetitive when horses were examined after the same level of work and under similar conditions. The proportion of examinations in which EIPH was seen varied considerably between horses. For example, Horse no.4 had EIPH on 2 out of 8 examinations, Horse no. 10 on 6 out of 8 examinations and Horse no. 6 on 1 out of 10 examinations (table 5).

The effect of the presence *of PLH of differing severity on the incidence EIPH was examined. The 86 examinations after cantering or maximal speed exercise were grouped according to the prevailing PLH grade and the incidence of EIPH in each group was calculated (table 6). There was no correlation between PLH and incidence of EIPH.*

4.4.3. Tracheal Mucopus

Observable amounts of mucoid or mucopurulent exudate were present in varying quantities in 57 out of 118 (48 per cent) examinations. The occurence of tracheal mucopus was widespread amongst all the horses. Twenty-three of the thirty one horses examined had tracheal mucopus at one or more examinations (Appendix 3). Only one horse examined 4 or more times (Horse no. 1) did not have mucopus present on at least one occasion.

The amount, or grade of mucopus varied, but the smallest amount (Grade 1) was most commonly seen. Of the 57 positive observations, 35 (56 per cent) were grade 1, 16 (28 per cent) were grade 2 and 8 (16 per cent) were grade 3 (table 7).

Table 5

Positive Observations of EIPH on Horses examined on at least 3 seperate occasions

	Horse Identification No.								
	4	5	6	9	10	11	16	21	23
No. of times EIPH Positive	2	1	1	2	6	2	2	1	2
No. of examinations after cantering or maximal speed exercise	8	6	10	3	8	3	5	3	3

Table 6

The Effect of PLH on the Incidence of EIPH after cantering or maximal speed exercise

PLH Grade	EIPH Positive	Number of Observations (Percentage Positive)
I	13	27 (48%)
II	10	17 (59%)
III	6	22 (27%)
IV	3	17 (17%)

Table 7

Incidence of different grades of tracheal mucopus

Mucopus Grade	No. of Observations	(percentage of total observations of mucopus)
I	35	(60%)
II	16	(27%)
III	8	(13%)

Exercise, faster than walking or trotting, appeared to increase the incidence of tracheal mucopus. The incidence was similar at examinations made at rest (2 of 15, 13 per cent of examinations) and after only walking and trotting (5 of 17, 20 per cent of examinations). After cantering the incidence was similar (29 of 49, 40 per cent of examinations) (table 8). The increase in incidence after cantering or maximal speed when compared to walking/trotting or rest was highly significant ($x = 13.2, p < 0.001$). There was no significant difference when the incidence after cantering and maximal speed was compared.

4.4.4. Mucus in the Dorsal Pharyngeal Recess

Collections of mucus were frequently seen on the mucosal surface of the pharynx in the region of the dorsal pharyngeal recess (DPR) as shown in the photograph (fig 14).

Mucus in the DPR was seen on 48 of 118 (40 per cent) observations. Mucus was never seen in the DPR at rest. The incidence was increased by exercise up to cantering, but the incidence was the same after maximal speed exercise (table 9.)

Table 8

Incidence of tracheal mucopus after different levels of exercise

Mucopus Grade	Exercise Level: Maximal	Canter	Walk/Trot	Rest
0	20	12	12	13
I	15	13	5	2
II	9	8	0	0
III	5	3	0	0
Total no. Observations	49	37	17	15= 118
Total no. positive observations	29	24	5	2= 57
(Percentage of total)	(60%)	(65%)	(20%)	(13%)

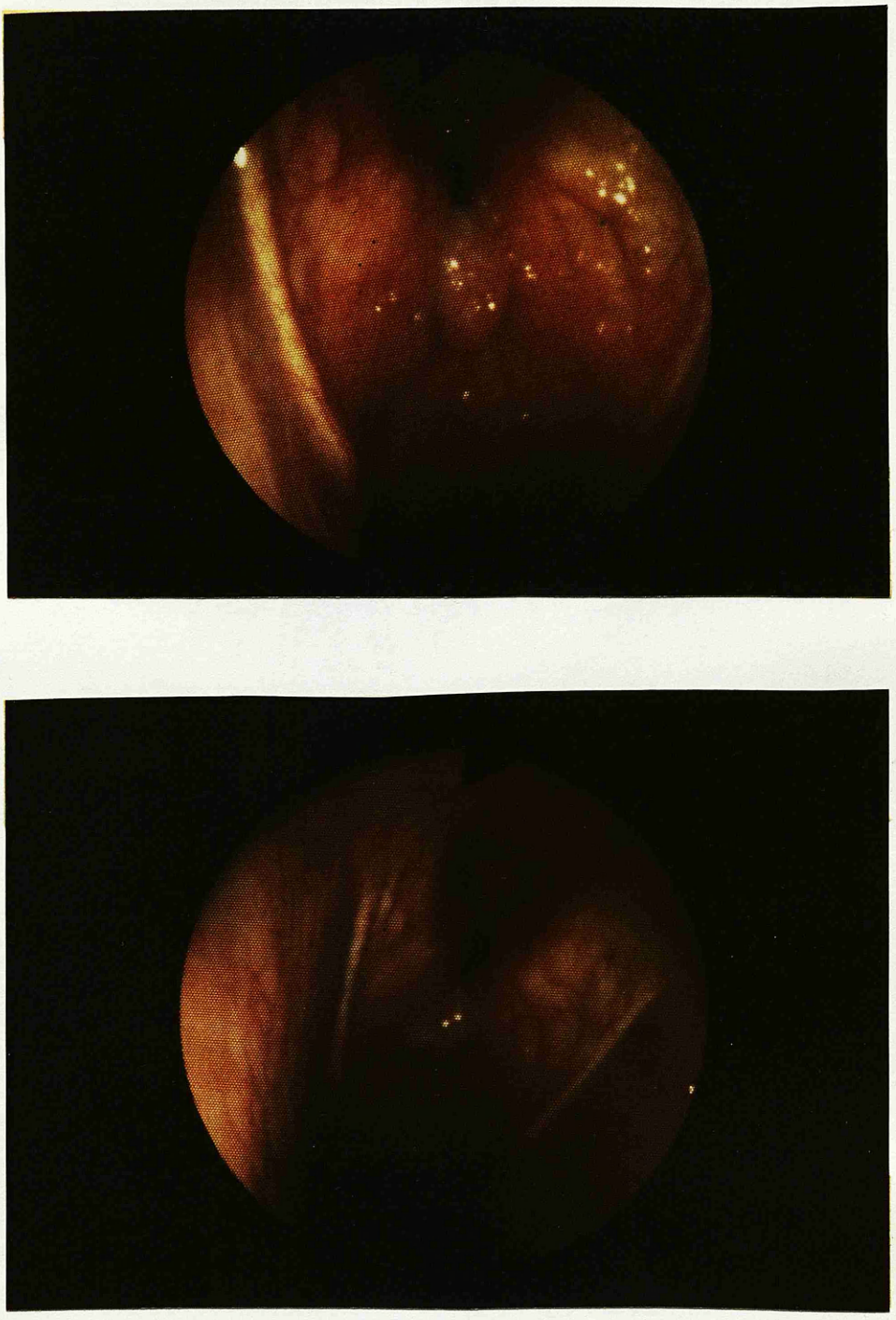


Fig.14. Two endoscopic views of the dorsal pharyngeal recess showing accumulations of mucus.

Table 9

The Incidence of Mucopus in the DPR after different levels of exercise

Mucopus in DPR	Exercise Level			
	Rest	Walk/Trot	Canter	Maximal
Present	0	4	19	25
(Percentage of Observations)	(0%)	(23%)	(51%)	(51%)
No. of Observations	15	17	37	49

Because of the similarity in appearance between the material accumulating in the DPR and that found in the trachea, a possible association between the two was examined. The occurence of mucopus in the DPR and the trachea at the same time or seperately is shown in Table 10. It was over three times more common to find mucopus in the trachea and the DPR concurrenctly than in the DPR alone, suggesting a common origin.

4.4.5. Performance

Generally the horses in the stable were performing satisfactorily with placings in 50% of starts. The association between PLH and finishing position in races was examined (Table 11). Although the sample was small (48 races), there was no difference in the proportion of times placed for those horses with Grade 1 or 2 at the time of the race, when compared to Grade 3 or 4.

Table 10

Occurence of Mucopus in the Trachea and DPR

Mucopus in DPR only	*Mucopus in Trachea Only*	*Mucopus in DPR and Trachea*
11	*23*	*37*

TABLE 11

The Effect of PLH on Race Results

	RACE RESULTS		
PLH	*UNPLACED*	*PLACED*	*SAMPLE*
I & II	*20*	*18*	*38*
III & IV	*5*	*5*	*10*

Chapter 5. Discussion

Fibreoptic endoscopy has increased the ability to detect conditions of the equine respiratory system. Two of the three conditions considered in this thesis, PLH and EIPH have become common diagnoses, but with little background knowledge of their incidence, aetiology, pathogenesis and effects. The third condition, LRT inflammation has not reportedly been diagnosed by endoscopy apart from its use as an aid in assessment of cases of chronic obstructive pulmonary disease.

5.1. Validity of Sample as being Representative of the Population

A popyilongit study of this sort should preferrably involve many more horses and more than one stable. However, at the time these investigations were undertaken, repeated endoscopic examination was not accepted as being non-detrimental to the horse by all trainers and owners. Financial limitations also prevented a more extensive survey.

Therefore in this study only a small group of race horses in training were examined although the data may not reflect the situation in the general population. There was only one case of possible clinical respiratory disease noted and on no occassion did the trainer complain that any horse was performing markedly below expectations, and the success rate of placings in 50 per cent of starts supported this. Thus outwardly, the horses in this stable did not have any clinical respiratory disease problems which would normally warrant veterinary attention.

5.2. Validity of Endoscopic Technique and Evaluation.

The technique of endoscopy used was comparable to that employed by other workers. The use of a 100 cm fibreoptic endoscope permits visualisation of only the cranial two-thirds of the trachea only and therefore, it is possible that the incidence of observable material in the trachea might be greater if a longer endoscope were used to provide visualisation to the level of the carina.

The assessment of the amount and speed of exercise can only be made subjectively when observing horses training on open gallops, hence the broad categories of classification. However, within these broad categories, the evaluation of the speed of exercise is likely to be accurate.

Visual assessment of the severity of the three conditions being examined in the study is entirely subjective. However, guidelines for the various grades as laid down are fairly exact for PLH and evaluation is therefore likely to be accurate in the 4 categories. In the case of EIPH grading, a simpler two-tier system was adopted in contrast to that described by Pascoe et al (1981). This was adopted because the amount of blood visible in the tracheal lumen could vary considerably throughout the length of trachea examined. Use of a longer endoscope may have increased the number of mild EIPH cases seen, but is is unlikely that the incidence of severe cases would have been increased.

5.3. Pharyngeal Lymphoid Hyperplasia

5.3.1. Virus Isolates from Nasopharyngeal Swabs

Pharyngitis with lymphoid proliferation and oedema has been reported following experimental infection with EHV-1 (Prickett 1969), EHV-2 (Blakeslee et al 1975) and in the field following influenza (Montgomery 1981). Blakeslee et al (1975) considered the chronic nature of infection by EHV-2 with replication in the pharynx to be a primary factor in the concurrent development of PLH, however, there were no uninfected control horses examined in this study.

The isolation of EHV-2 from 5 of 13 clinically normal horses in the present study confirms that this virus commonly circulates asymptomatically (Studdert 1974). In the present study there was no difference in the mean PLH grades in those horses from which EHV-2 was isolated, when compared to those from which it was not isolated. This does not preclude EHV-2 from being involved in the pathogenesis of PLH, because it is known that isolation rates can be increased by further passage of samples in tissue culture, than the two seven-day passages used in this present study. Indeed the almost ubiquitous prescence of EHV-2 in equine tissues (Kemeny and Pearson 1972) makes it difficult to attribute any disease significance to this virus. On the one hand, it may be postulated that early and chronic infection of foals (Wilks and Studdert 1974) with antigenic expression on the surface of infected cells in the pharynx may stimulate a low grade cellular immune response;

but on the other hand, the viral genome appears to be able to become incorporated into the cellular DNA with minimal expression, so it is questionable whether it would provoke a visible immune response recognisable as lymphoid hyperplasia. The present study does not provide any data to enable speculation on the involvement of other viruses in PLH, because there was no evidence of infection by other respiratory viruses or overt clinical respiratory disease during the time of study.

5.3.2. *Incidence and Correlation with Age*

There have been no published studies on the incidence of PLH in young racing thoroughbreds although it has frequently been reported to be of major clinical importance in racehorses (Raker and Boles 1978). Raphel (1982) looking at a population of mixed ages and breeds reported an overall incidence of 29.4 per cent of horses examined at a referral clinic, and showed that prevalence of PLH decreased with age. In the present study, PLH was evident in all horses, of which the oldest horse was 7 years old. However, in support of Raphel's findings there was a highly significant age associated decrease in severity of PLH.

The increased usage of fibreoptic endoscopy was responsible for the rapid increase in awareness of PLH, as the pharynx became visually accessible. It should now be recognised that a degree of PLH is common to every young racehorse, and even severe cases should not be considered to be of clinical significance.

5.3.3. Influence on Racing Results

The statement that PLH in it's severe forms contributes to loss of performance in racing horses (Raker and Boles 1978; Montgomery 1981; Anon 1980) is not confirmed by the results of this study (Table 2); furthermore, no correlation has been found between racing results and PLH grade in a much larger sample, from a number of different training establishments (Greet and Powell, unpublished data).

A recent study found acid-base balance and blood gas tensions during and after an exercise test were not significantly different before and after the chemical induction of pharyngeal lesions similar to those found in naturally occurring cases of PLH (Bayly, Grant and Breeze 1984). They concluded that PLH probably does not affect gas exchange during exercise, unless the lesions are extemely severe and question whether or not PLH is a genuine cause of decreased performance in the athletic horse. This report lends support to the findings in the present study.

5.3.4. Association with Antibody Titres

It has been suggested (Montgomery 1981) that hyperimmunisation of young thoroughbreds using vaccines against EHV-1 and equine influenza specifically reduce the severity of PLH, and that the grade of PLH was consistently reduced 30 days after EHV-1 vaccination. Because CF antibody titres are boosted by vaccination and therefore reflect the recent stimulation of humoral immunity to that antigen, according to Montgomery's (1981) hypothesis, it might be expected that high CF titres, due to vaccination, would be associated with lower grades of PLH. This hypothesis was tested in the present study.

In the present study all horses were vaccinated with a killed EHV-1 vaccine that stimulated CF antibody levels (Appendix 1). The data showed no correlation between EHV-1 CF antibody titre and PLH grade, indicating that hyperimmunisation does not reduce PLH grade. Vaccinated horses did show a gradual decline in PLH severity but this was closely correlateed with increasing age of the horse. Again, this data does not necessarily infer that EHV-1 is not involved in the pathogenesis of PLH, simply that vaccination with a killed vaccine should not be expected to reduce the severity of PLH.

The equivalent of PLH in humans is chronically enlarged adenoids, which is infrequently observed in adults (Stewart and Birrell 1968; Birrell 1978). Birrell (1978) considered there to be a physiological enlargement of adenoids in children in response to unfamiliar viruses or strains of bacteria. It seems likely that the situation in the young horse may be similar. PLH will only abate with exposure to and immune recognition of, the whole range of antigens encountered in a training yard as the horse ages, allowing a moderation of mononuclear cell activity in tonsillar tissue.

5.4. Exercise Induced Pulmonary Haemorrhage

5.4.1. Incidence

The incidence of EIPH in the present study was lower than that reported after racing by Pascoe et al (1981) 43.8 per cent, Mason, Collins and Walkins (1983) 46.8 per cent, or other competetive exercise by Raphel and Soma (1982) 75.4%. However, the results reported here followed training exercise and a similar figure, 38.3 per cent was reported by Raphel and Soma (1982) in horses following training exercise. Mason, Collins and Watkins (1983) showed by repeat examinations and autopsy that almost all horses have EIPH at some stage. This study supports the finding that most horses if examined frequently after fast exercise will be seen to be EIPH positive at some time.

5.4.2. Relationship with PLH

It has been suggested that severe PLH might predispose to EIPH by compromising upper airway airflow, thereby increasing the negative pressure in the lower airways and alveoli during inspiration. This suggestion has been refuted on physiological principles by Robinson (1979). The severity of PLH did not have any effect on the incidence of EIPH in this study, supporting Robinson's premise.

5.4.3. Unpredictability

Raphel and Soma (1982) considered that EIPH was a repeatable event on the basis of similar results on two examinations. However, Mason, Collins and Watkins (1983) found little repeatability of results on several examinations. The results presented here support the latter view and show that it is not consistently possible to predict presence of haemorrhage on the basis of a previous examination since horses were not found to have EIPH consistently after similar levels of exercise.

In a more recent publication (Raphel, Sweeney and Soma 1984) a group of 24 horses which formed a control group in an EIPH treatment trial, all of which had a history of previous EIPH, were examined after exercise and 17 (70.8 per cent) were found to have bled. The authors suggest in this paper that there was approximately a 20 per cent remission of EIPH cases, a supposition presumably based on two examinations. In another EIPH medication trial (Raphel, Sweeney et al 1984) 3 thoroughbred horses were used which had consistantly shown EIPH on 5 examinations, suggesting that these horses always had EIPH after exertion.

All this data would appear to suggest that some thoroughbred horses do bleed repeatedly, all bleed at some time or another, but in between these extremes there is great variability in the probability of bleeding for each individual after similar exercise tests, and the occurence of EIPH for most individuals is unpredictable.

5.4.4. Relationship with Level of Exertion.

There are conflicting views on whether the speed and distance of exercise affect the incidence of EIPH (Raphel and Soma 1982; Mason, Collins and Watkins 1983; Sweeney and Soma 1983). The data in the present study suggests that neither incidence nor severity of EIPH were greatly affected by the speed of exercise over and above a medium paced training canter. On one occasion a horse had EIPH after only walking and trotting, but this perhaps ought to be regarded as an extreme case. The implication is, therefore, that whereas a certain threshold exertion is required for free blood to reach the trachea, this varies between individuals and also varies for each individual at different times.

5.4.5. A Consideration of the Aetiology of EIPH

The data presented in this study suggests that rupture of blood vessels in the lung is not a purely routine physiological consequence of increased vascular and/ or pulmonary function, but is influenced by other factors. One such factor may be concurrent bronchiolitis as proposed by Robinson and Derksen (1980), and found to be common in EIPH positive horses by Mason, Collins and Watkins (1983). Robinson and Derksen suggest that bronchiolitis may predispose to bronchoconstriction causing poor ventilation of some parts of the lung. The poor collateral ventilation of the horse lung would then lead to extreme differences in the degree of inflation between well ventilated and poorly ventilated (obstructed) neighbouring areas of lung due to the property of inter-dependence of lung lobules with one another. This situation could lead to haemorrhage in two possible ways:-

a) The poorly ventilated segment unable to inflate synchronously with the surrounding areas of lung, would have a large decrease in intra-alveolar pressure during inspiration, resulting in capillary rupture.

or

b) the tearing forces between the inflated and non-inflated segments could result in parenchymal tearing.

In both instances the key step for capillary rupture to occur is the presence of bronchoconstriction at exercise. In humans, inflamed airway mucosa is considered hyperreactive to bronchoconstricting stimuli, and in exercise-induced bronchoconstriction, heat loss from the mucosa caused by increased airflow is a triggering stimulus. The temperature and humidity of inhaled air is therefore important in the development of exercise-induced bronchoconstriction (McFadden and Ingram 1982). Thus, ambient air temperature and humidity may be other factors important in the pathogenesis of EIPH.

5.5.0. Tracheal Mucus

At present it is not possible to ascertain the significance of the visible exudate in the trachea. The small amounts (Grade 1) commonly seen may only reflect an agitation of normal lining of mucus during exercise. However, the larger amounts may be assumed to result from lower airway inflammation, because increased mucus production due to the proliferation of goblet cells is a feature of the airway inflammation seen in chronic obstructive pulmonary disease (Nicholls 1978).

The present study suggests, on the basis of excessive intra-tracheal mucoid exudate, that some degree of airway inflammation is common in racing horses, as found by Mason, Collins and Watkins (1983). However, in their population, increased mucus production was not a feature of the bronchiolitis observed, but the population examined cannot be compared with the present study, since the animals examined were retired, and therefore older, racing thoroughbreds.

Fast exercise significantly increases the chances of observing exudate in the trachea, although obviously a profuse exudate will be present at rest. The presence of such exudate does not in any way presuppose the presence of concurrent EIPH. There was no association between the grade of PLH and the presence of tracheal mucus.

Further studies on the nature and constituents of the exudate and histological studies of the airways are necessary before any conclusions can be drawn regarding its characterisation, causes and significance. Long term observation of affected horses are necessary to determine any link with EIPH.

5.6.0. Mucus in the Dorsal Pharyngeal Recess (DPR).

It has been suggested (Baker G.J., personal communication) that the collection of mucus commonly seen in the DPR is a transudate originating from the lymphoid tissue of that region. If that were the case, one would expect such an exudate to be visible at rest, but in the present study it was never seen at rest, only after exercise (Fig.9). It was most commonly present after cantering or maximal speed exercise. From the original premise, it might be expected that an exudate would be more commonly seen in horses with the greater degree of inflammation in that region. This, would appear to be supported by the present data since the incidence of mucopus in the DPR was increased with the severity of PLH (Table 5).

However, the observation of mucus in the trachea and the DPR was 3 times more common than in the DPR alone, suggesting an association between the two occurrences.

It is postulated that when mucus is present in excessive quantities in the tracheal lumen, at exercise small flecks of this material will be shifted back and forth by rapid airflow into the pharynx or nasal passages. By considering the anatomy, it is apparent that airflow from the nasal passages impinges against the DPR prior to being directed into the rima glottidis. This is an obvious adaptation to enhance the primary function of the tonsillar tissue in the DPR, namely the appraisal of inhaled particles. Thus, the presence of mucoid exudate which seems only to occur after exercise is thought to be related to its presence in the trachea, rather than as a transudate from the lymphoid tissue. The association with the severity of PLH, might be explained on the grounds that lymphoid tissue in the severe grades of PLH will protrude further into the airflow from the DPR enhancing the likelihood of catchment of mucus flecks. Only by collection and detailed comparison by analysis of exudates present in the DPR and trachea can this theory be fully proven.

Appendix 1

Antibody levels to EHV-1 (subtype 1 and subtype 2), ERV-1

Equine influenza (three strains) and equine adenovirus.

(titres given as the reciprocal of the dilution)

Horse Number	Collection Date	EHV-1 I	EHV-1 II	ERV-1	Influenza Pr.	Influenza Mi.	Influenza '79	Adeno
1	7.10.81	5	5	10	64	64	64	0
	12.11.81	0	10	10				
2	7.10.81	0	5	20	256	128	128	0
	12.11.81	0	5	20				
	14.12.81	5	5	20				
3	7.10.81	5	10	20	128	64	128	0
4	7.10.81	5	5	5	64	32	32	0
	12.11.81	5	5	10				
	25. 3.82	5	10	10				
	20. 4.82	10	10	20	128	128	128	0
	1. 7.82	10	20	20	128	128	128	0
	29. 7.82	10	40	20	128	128	128	0
5	7.10.81	5	10	20	256	128	128	0
	14.12.81	5	10	20	128	64	64	0
6	7.10.81	5	5	20	128	128	128	0
	12.11.81	5	5	20				
	14.12.81	5	10	20				
	25. 3.82	20	20	20				
	20. 4.82	20	20	20	64	64	64	0
	1. 7.82	40	20	40	128	64	64	0
	29. 7.82	20	10	10	128	128	64	0

Appendix 1 Contd.

Antibody levels to EHV-1 (subtype 1 and subtype 2), ERV-1

Equine influenza (three strains) and equine adenovirus.

(titres given as the reciprocal of the dilution)

Horse	Collection	EHV-1		ERV-1	Influenza			Adeno
Number	Date	I	II		Pr.	Mi.	'79	
7	7.10.81	5	0	10	64	32	32	0
	12.11.81	0	0	10				
	14.12.81	10	20	10	64	32	64	0
	1. 7.82	10	20	20	64	32	32	0
	29. 7.82	10	10	10	64	16	32	0
8	7.10.81	5	0	10	32	16	64	0
9	7.10.81	0	5	20	16	32	64	0
	2. 4.82	10	5	40	256	256	256	0
	20. 4.82	10	5	20	64	128	128	0
	1. 7.82	10	10	40	64	64	256	0
	29. 7.82	10	10	40	64	128	256	0
10	18.12.81	5	20	10				
	25. 3.82	20	20	20				
	20. 4.82	10	40	10	128	64	128	0
	1. 7.82	20	40	10	128	64	128	0
	29. 7.82	20	20	10	128	64	128	0
11	12.11.81	20	20	10				
	14.12.81	10	80	10	32	16	32	0
	25. 3.82	20	20	10				
	20. 4.82	10	20	20	64	32	128	0
	1. 7.82	10	10	10	64	64	128	0
	29. 7.82	10	10	20	64	64	128	0

Appendix 1 Contd.

Antibody levels to EHV-1 (subtype 1 and subtype 2), ERV-1, Equine influenza (three strains) and equine adenovirus.

(titres given as the reciprocal of the dilution)

Horse Number	Collection Date	EHV-1 I	EHV-1 II	ERV-1	Influenza Pr.	Influenza Mi.	Influenza '79	Adeno
12	12.11.81	10	10	5	128	128	128	16
	14.12.81	10	80	10	32	64	16	16
	25. 3.82	20	10	10				
	20. 4.82	10	10	5	64	64	16	16
	1. 7.82	10	40	10	32	64	16	16
	27. 7.82	10	20	20	32	64	16	16
13	12.11.81	20	5	10				
	14.12.81	10	40	10	0	0	0	0
	25. 3.82	20	5	10				
	20. 4.82	10	10	20	0	0	0	0
	1. 7.82	10	20	20	0	0	0	0
	29. 7.82	10	20	10	0	0	0	0
14	12.11.81	20	20	10				
15	14.12.81	0	0	0	128	128	64	0
16	12.11.81	20	20	10				
	14.12.81	10	10	10	16	32	8	16
	25. 3.82	20	40	10				
	20. 4.82	10	20	10	32	32	16	16
	1. 7.82	10	20	10	16	32	16	16

Appendix 1 Contd.

Antibody levels to EHV-1 (subtype 1 and subtype 2), ERV-1

Equine influenza (three strains) and equine adenovirus.

(titres given as the reciprocal of the dilution)

Horse Number	Collection Date	EHV-1 I	EHV-1 II	ERV-1	Influenza Pr.	Influenza Mi.	Influenza '79	Adeno
17	14.12.81	0	20	0	64	128	32	16
	1. 7.82	10	10	40	0	0	0	16
18	14.12.82	10	10	10	64	128	16	0
	1. 7.82	10	10	10	64	64	16	0
	29. 7.82	20	10	20	64	64	8	0
19	14.12.81	10	10	10	16	16	0	0
	1. 7.82	10	10	10	64	32	0	0
	29. 7.82	20	40	20	32	16	0	0
20	14.12.81	5	5	10	0	128	16	0
	1. 7.82	20	20	10	16	64	32	0
	29. 7.82	10	10	10	64	128	64	0
21	29. 7.82	10	10	10	0	128	16	0

Abreviations

EHV-1 (1): equine herpesvirus 1, subtype 1 (abortion strain)

" (11): " " " " 2 (respiratory strain)

ERV-1 equine rhinovirus 1

Influenza Pr: equine influenza subtype 1, (A/Equine 1/Prague/56) (H7N7)

" Mi: " " " 2, (A/Equine 2/Miami/63) (H3N8)

" '79: " " " 2, (A/Equine 2/Newmarket/79) (H3N8)

Adeno Equine adenovirus

Appendix 2

Vaccination Dates for Horses tested Serologically

Horse Number	Influenza		EHV-1			
	10.12.81	9.6.81	14.11.81	20.2.82	25.5.82	1.9.82
1	*	*	*			
2	*		*			
3	*					
4	*	*	*	*	*	*
5	*		*			
6	*		*	*	*	*
7	*	*	*		*	
8	*	*				
9	*	*		*	*	
10	*		*	*	*	*
11	*		*	*	*	
12	*		*	*	*	
13	*,21.6.82		*	*	*	
14	*		*			
15	*	*	*			
16	*		*	*	*	
17	*		*	*	*	
18	*		*	*	*	
19	*		*	*	*	
20	*		*	*	*	
21					*	

Appendix 3

Results of Observations of PLH, EIPH and Tracheal Mucopus for individual horses, in chronological order

Horse Number	Age at Examination (Months)	PLH Grade	Amount of Exercise	EIPH Grade	Tracheal Mucopus Grade
1	38	4	Max	M	0
	40	3	Max	0	0
	43	2	Rest	0	0
	43	2	W/T	0	0
	44	1	W/T	0	0
2	63	2	W/T	0	0
	66	1	Cant	0	2
	66	1	Max	0	1
	67	1	W/T	0	0
3	37	3	Cant	0	2
	39	2	W/T	0	1
	41	1	W/T	0	0
	42	1	W/T	0	0
4	26	4	Cant	0	2
	29	3	Rest	0	1
	30	3	W/T	0	1
	35	3	Max	0	0
	35	3	Cant	0	1
	35	3			
	40	3	Max	0	1
	43	2	Cant	M	1
	43	3	Max	M	0
	43	3	Max	0	0

Appendix 3

Results of Observations of PLH, EIPH and Tracheal Mucopus for individual horses, in chronological order continued

Horse Number	Age at Examination (Months)	PLH Grade	Amount of Exercise	EIPH Grade	Tracheal Mucopus Grade
5	37	4	Max	0	3
	38	4	Max	M	3
	40	4	Cant	0	3
	43	2	Cant	0	0
	43	2	Max	0	1
	44	2	W/T	0	1
	44	2	Cant	0	1
6	36	2	W/T	0	0
	37	3	Cant		
	39	3	Max	M	2
	42	1	Cant	0	1
	42	2	Rest	0	0
	43	2	Rest	0	0
	48	2	Max		
	48	1	Rest	0	0
	48	2	Max	0	0
	49	2	Max	0	0
	51	1	Max	0	0
	52	1	Max	0	2
	52	1	Max	0	2
	52	1	Max	0	1

Appendix 3

Results of Observations of PLH, EIPH and Tracheal Mucopus for individual horses, in chronological order continued

Horse Number	Age at Examination (Months)	PLH Grade	Amount of Exercise	EIPH Grade	Tracheal Mucopus Grade
7	37	4	Cant	0	3
	40	4	Cant	0	1
	43	2	Cant	0	0
	44	1	Cant	0	0
	52	1	Max	M	2
	53	1	Cant	0	0
	53	2	Max	2	1
	53	1	Cant	0	0
8	26	4	Cant	0	3
	29	3	Cant	M	2
	29	3	Rest	0	0
9	37	4	Cant	0	0
	39	2	W/T	M	1
	41	1	Rest	0	0
	41	2	W/T	0	0
	47	1	Max	M	0
	47	1	Rest	0	0
	47	1	Rest	0	0
	48	1	Max	S	0

Appendix 3

Results of Observations of PLH, EIPH and Tracheal Mucopus for individual horses, in chronological order continued

Horse Number	Age at Examination (Months)	PLH Grade	Amount of Exercise	EIPH Grade	Tracheal Mucopus Grade
10	38	3	Max	0	0
	40	3	Max	0	2
	43	3	Cant	M	1
	44	1	W/T	0	0
	44	2	W/T	0	1
	49	1	Rest	0	0
	49	1	Rest	0	0
	49	2	Rest		
	50	2	Max	S	1
	52	1	Max	M	0
	53	1	Cant	S	2
	53	2	Max	S	1
	53	2	Cant	S	2
11	43	1	W/T	0	0
	48	1	Rest		
	48	1	Cant	0	1
	48	1	Max	M	1
	49	1	Max	S	1
12	34	3	Rest	0	0
	39	3	Rest		
	39	3	Rest	0	0
	40	2	Max	M	1
	43	1	Max	M	0

Appendix 3

Results of Observations of PLH, EIPH and Tracheal Mucopus

for individual horses, in chronological order continued

Horse Number	Age at Examination (Months)	PLH Grade	Amount of Exercise	EIPH Grade	Tracheal Mucopus Grade
13	20	3	Rest	0	1
	24	4	Rest		
	24	4	Rest	0	0
	25	3	Cant	0	1
	27	3	Max	0	2
	28	3	Max	0	1
14	27	4	Max	0	3
	31	3	W/T	0	0
15	37	4	Max	M	2
	39	3	Max	0	2
16	43	3	Max	0	0
	48	1	Max	M	0
	48	2	Cant	0	0
	48	2	Max		
	49	2	Cant	M	2
19	41	2	Max	S	1
	42	2	Cant	M	1

Appendix 3

Results of Observations of PLH, EIPH and Tracheal Mucopus for individual horses, in chronological order continued

Horse Number	Age at Examination (Months)	PLH Grade	Amount of Exercise	EIPH Grade	Tracheal Mucopus Grade
22	48	3	Cant	0	0
	50	4	Cant	0	0
23	85	2	Max	M	3
	90	1	Cant	M	1
	87	1	Cant	0	2
25	54	1	Cant	S	1
26	40	3	Max	S	1
27	24	4	Cant	0	0
	26	4	Max	0	0
28	49	3	Max	0	0
	51	2	Cant	0	1
29	52	4	Max	0	0
	55	1	Cant	0	0
21	88	1	Cant	0	1
	88	1	Max	M	0
	88	1	Max	0	0

Appendix 3

Results of Observations of PLH, EIPH and Tracheal Mucopus

for individual horses, in chronological order continued

Horse Number	Age at Examination (Months)	PLH Grade	Amount of Exercise	EIPH Grade	Tracheal Mucopus Grade
31	50	4	Cant	0	0
	52	4	Max	0	0
17	37	3	Cant	0	0
18	39	1	Max	M	0
20	27	3	W/T	0	0
24	53	1	Max	0	1
30	45	3	Max	M	3

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