Assessment of Sustainable Methods of Cyathostomin Control at The Donkey Sanctuary Devon

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Abstract

The Donkey Sanctuary Devon has, like many equine units, faced problems with anthelmintic resistance within the cyathostomin parasite population. Cyathostomins are ubiquitous, and although they are to some extent tolerated by their hosts they are also linked to potentially serious disease processes, making their management an important part of equine husbandry. Over use of the available anthelmintics has been blamed for the current situation, with cases of drug resistance reported across all the classes of anthelmintic currently licensed for use in equidae. Alternative methods of controlling cyathostomins include pasture hygiene by removal of faeces, which theoretically reduces re-infection by removing the immature larval stages from the grazing area, and targeted selective treatment (TST), which works by treating those animals shedding the highest number of eggs and leaving other animals untreated. The aim of this thesis is to evaluate these methods of parasite control at The Donkey Sanctuary Devon over an entire grazing season.

The most widely used method of monitoring parasitism is the Faecal Worm Egg Count (FWEC), which is known to be highly variable and can be difficult to analyse appropriately using traditional statistical methods. Therefore, computationally intensive Bayesian Markov chain Monte Carlo (MCMC) methods were employed, to ensure that the conclusions made are robust. A faecal egg count reduction test (FECRT) was also used to evaluate anthelmintic drug efficacy at the end of the study. There was a study population of 667 donkeys, divided into 15 groups under four different management strategies across four farms of The Donkey Sanctuary Devon. Ninety pasture larval counts and nearly 3000 FWEC were conducted from May 2010 to November 2010, followed by a FECRT on a proportion of the study population.

The principal conclusion was that twice-weekly removal of faeces from pasture, combined with a relatively high TST threshold of 2000 EPG, provides control of cyathostominosis in the donkey population studied. Manual removal of faeces (rather than using an automated sweeper) was also found to significantly reduce the requirement for anthelmintic doses when using a treatment threshold of 2000 EPG. Where faeces removal from pasture is not practical, lower TST thresholds provide greater control of cyathostomin transmission than higher thresholds, at the cost of more frequent dosing. The groups with higher dosing rates showed reduced drug efficacy at the end of the grazing season, highlighting the necessity to reduce reliance on anthelmintic use.

Table of contents

List of Tables

List of Images

List of Figures

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Declaration

This thesis, and the work contained within, was conducted from August 2010 to July 2012 by myself under the supervision of Dr Matthew Denwood and Prof. Sandy Love at the University of Glasgow.

Anna Moore

List of Abbreviations

BF – Brookfield

BZ – Benzimidazoles

CI – Confidence Interval

DIC – Deviance information criterion

DKF – Donkey Female

DKG – Gelded Male Donkey

DKS – Donkey Stallion

ELISA – Enzyme-linked immunosorbent assay

EPG – Eggs per Gram

ERP – Egg reappearance period

FECR/FECRT – Faecal Egg Count Reduction / Faecal Egg Count Reduction Test

FWEC – Faecal Worm Egg Count(s)

Hb – Haemoglobin

HOF – Horse Female

HOG – Gelded Male Horse

L1 – First Stage Larva

L2 – Second Stage Larva

L3 – Third Stage Larva

L4a – Hypobiotic Larva

L4 – Fourth Stage Larva

MUF – Mule Female

MUG – Gelded Male Mule

PCR – Polymerase Chain Reaction

PCV – Packed cell volume

PLC – Pasture Larvae Count(s)

PYF – Pony Female

PYG – Gelded Male Pony

PC - Paccombe

SHF – Slade House Farm

TR – Trow

TST – Targeted Selective Treatment

WAAVP – World Association of the Advancement of Veterinary Parasitology

Introduction

1.1 Background

The modern Donkey, *Equus asinus,* is an ancestor of the Nubian and Somalian subspecies of the African wild ass, native to arid and semi-arid climates. Domesticated around 4000BC they were gradually brought to the rest of the world. Currently 96% of the world's donkeys live in developing countries (anon 1997 from Krecek and Waller 2006), and in the developed world the numbers of donkeys kept are declining (Starkey 1997). Originally a desert species, this is reflected in much of their physiology, such as their hardiness and efficient digestive system. Another trait resulting from evolving in poor conditions is the time spent grazing. Like other equidae the donkey may spend up to 16 hours grazing a day, which was at one time necessary to obtain enough nutrition. Britain's donkeys often experience health problems related to these physiological adaptations, which are no longer appropriate to their current environment. Obesity is common (Cox, Burden *et al*. 2010), food is often available in excessive quantities and this can lead to problems such as laminitis, hyperlipidemia and gastric ulcers (Grove 2008).

The Donkey Sanctuary, founded by the late Dr Elisabeth Svendsen MBE and based in Devon, has been operating to help Donkeys worldwide since 1973. Over this time more than 15,000 Donkeys have passed though their sanctuaries in the UK and Ireland and many more have been cared for through their programmes based in India, Egypt, Ethiopia, Kenya and Mexico. The Donkey Sanctuary Devon has a dedicated veterinary team dealing with the health of the donkeys under their care. These clinicians are also responsible for the prophylactic treatment, nutrition and quarantine procedures of all donkeys admitted to The Donkey Sanctuary Devon. The breeds of donkey housed at The Donkey Sanctuary Devon vary considerably in size; for

example the Miniature Mediterranean Donkey standing at less than 3ft is dwarfed in comparison to the large Poitou averaging around 5ft at the withers. The Donkey Sanctuary Devon's residents also vary in age, with much of their population being geriatric and having age related problems such as poor dentition (Sprayson 2008). Additionally, animals may come to The Donkey Sanctuary Devon due to poor treatment or on going health problems, which their owners cannot deal with. As a result the population of animals housed at The Donkey Sanctuary Devon is quite diverse, with a high number of geriatric and unhealthy animals, presenting considerable challenges for the veterinary team in developing protocols for caring for these animals.

1.2 Gastrointestinal parasites of the donkey

The common intestinal parasites of donkeys include nematodes such as small and large strongyles (*Cyathostomin spp.* and *Strongylus spp*. respectively). The symptoms of nemotodes may include weight loss and diarrhoea and in the case of large strongyles, colic. Other intestinal parasites include, but are not limited to; Pinworm (*Oxyuris equi*) presenting as anal irritation; Ascarids (*Parascaris equorum*) and threadworms (*Stongyloides westeri*), which are most commonly seen in foals. Ascarids can cause colic, ill thrift and intestinal obstruction where as threadworms normally present as diarrhoea; and finally tapeworm (*Anoplochephala spp*.) which are also often implicated in colic cases (Trawford and Getachew 2008). Other problematic endoparasites include lungworm (*Dictyocaulus arnfieldi*) and fluke (*Fasicola spp*.) but these are only rarely associated with clinical symptoms in donkeys (Trawford and Getachew 2008).

1.2.1 Cyathostomin life cycle

Cyathostomin species are found in all equidae. They have previously been known as cyathostomes, trichonemes, small strongyles and small redworm, and studies have repeatedly demonstrated a near 100% infection rate in horses and donkeys across the world (Krecek and Guthrie 1999; Matthee, Krecek *et al*. 2002; Getachew, Trawford *et al*. 2007; Nielsen, Baptiste *et al*. 2010). The adults inhabit the caecum and colon of equidae and are non migratory. Eggs are shed in faeces and develop on the pasture through the free-living L1 and L2 larval stages to become infective L3 larvae. After ingestion L3 exsheath and then penetrate the mucosal

wall, where they encyst and develop into the fourth larval stage which emerge into the intestinal lumen before progressing to become adult worms (Urquhart 1996). This process is diagrammatically represented in Figure 1.1 below. The whole process can be completed in less than 2 months, unlike large strongyles, which have a 6-month life cycle (Nielsen, Baptiste *et al*. 2010). In horses it has been shown that some encysted larvae can enter a hypobiotic state (L4a) for a period of time ranging from weeks to years (Reinemeyer 1986). It is debatable whether this same form of hypobiosis occurs in donkeys (Eysker and Pandey 1987; Getachew, Trawford *et al*. 2007); Eysker and Pandy (1987) failed to find persistent cysts on post mortem at certain times of year in donkeys in Zimbabwe and less than 1% of donkeys in The Donkey Sanctuary's care have encysted larvae at post mortem (Trawford and Getachew 2008), but as much data are derived from studies in horses, it is often assumed that hypobiosis is equally important in donkeys.

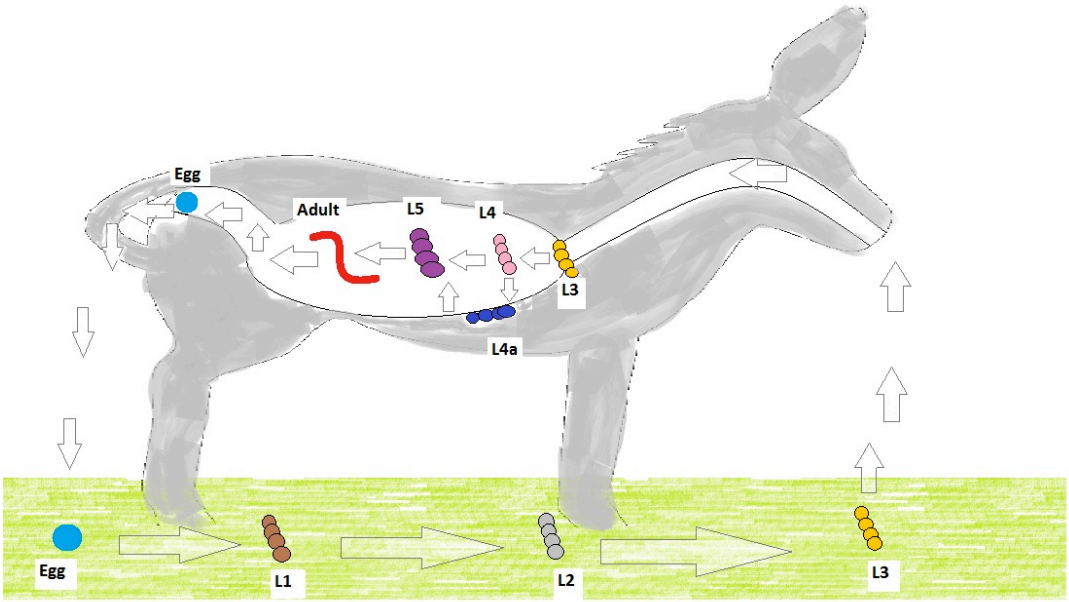

Figure 1.1 Diagrammatic representation of the cyathostomin life cycle in the donkey. Eggs passed in faeces develop through the larval stages (L1, L2, L3) on pasture before being ingested. Once within the digestive system they exsheath and develop through to more larval stages before becoming adults and producing eggs, which are passed in the faeces. Between the 4th and 5th larval stages some larva may encyst in the mucosal wall of the gut (L4a). The whole life process can take as little as 2 months.

1.2.2 Cyathostomin - Free living stages

Only part of the cyathostomin life cycle occurs within the host. The remainder of the life cycle takes place on pasture, and plays an important role in the perpetuation of infections.

These 'free living stages' are affected by climatic influences such as temperature and humidity, which vary dramatically with geographical location. Devon UK, where The Donkey Sanctuary Devon is based, is considered to be in the 'Northern Temperate Climate' (Reinemeyer 1986; Nielsen, Kaplan *et al*. 2007), which has considerably different climatic conditions to 'Southern Tropical Climates'. This may limit the relevance of many field studies to the Donkey Sanctuary Devon.

The free-living stages of cyathostomins are as follows. Eggs passed in faeces first become embryonated eggs containing visible larva. These become L1 then L2 stages, (the pre-infective stages,) which are free feeding and develop into the L3, which has a double, layered cuticle and cannot ingest food (Nielsen, Kaplan *et al*. 2007). These differences in structure and biology leave each stage vulnerable to different climatic influences. Variations in temperature and humidity can affect the speed of development between stages and indeed the survival of each stage. Laboratory studies show egg development ceases at less than 4°C (Lucker 1941), with an optimum temperature for development of around 25°C (Ogbourne 1973), and destruction of eggs occurring at temperatures of greater than 40°C. Freezing can damage free-living stages, and unembryonated eggs seem to withstand frost more readily than L1 and L2 stages (Lucker 1941). It has also been shown that L3 can survive freezing and thawing to an extent (Lucker 1941).

Moisture is also important in larval development, with no development occurring in low humidity (<15-20%) (Mfitilodze and Hutchinson 1987). Intact faecal balls can provide protection for eggs and larvae (Enigk 1934 as cited by Nielsen, Kaplan *et al.* 2007) slowing desiccation, which can improve survival compared to rapid drying (Ogbourne 1973). Table 1.1, adapted from Nielsen, Kaplan *et al*. (2007), summarises the survival of free-living stages of equine strongyles exposed to different climatic conditions.

Table 1.1 The Survival of free-living stages of equine strongyles exposed to different climatic conditions

Free-Living Stage	Frost	Alteration between frost & thaw	Dessication	Heat (*a*)
Unembyronated egg	++	++	*b*	++
Embryonated egg	+	-	*b*	++
First stage larva	-	-	-	++
Second stage larva	-	-	-	++
Third stage larva	+++	+	+++	-

- \- Indicates very susceptible, + weakly resistant, ++ moderately resistant, +++very resistant.
- *a* Temperatures in the range 30-38°C
- *b* No data available

(After Nielsen, Kaplan *et al*. 2007)

The capability of L3 to survive is thought to be related to the presence of fat granules in their intestinal cells (Giovannola 1936). Over time these energy stores become depleted, and it is thought that L3 with exhausted fat granules are less able to penetrate host tissue (Medica and Sukhdeo 1997). It may therefore be the case that although L3 larvae survive on pasture over winter, their subsequent infectivity is reduced.

1.2.3 Pathogenesis of cyathostomins

In contrast to the large strongyle *S. Vulgaris*, which exerts its pathogenic effects while migrating through blood vessels in the gut, causing intestinal ischaemia and often leading to colic (Duncan 1974), the adult stage of the cyathostomin is non migratory. This combined with the fact many horses and donkeys harbour large burdens of cyathsotomins with no deleterious effects being apparent, often leads to the perception that cyathostomins are non-pathogenic. However, the reduction in prevalence of *S. vulgaris* has exposed the pathogenic effects of cyathostomin species (Love, Murphy *et al*. 1999; Kaplan and Nielsen 2010). The decreased prevalence of colic seen on premises where cyathostomin control is effective

compared to those with poorer control suggests that cyathostomins may also play a direct role in the pathogenesis of colic (Uhlinger 1990).

The main pathogenic effects of cyathostomins occur in the prepatent stages, when mucosal damage is caused by the penetration and emergence of the parasites from the intestinal wall. After ingestion L3 larvae exsheath in the small intestine and enter the glands of Lieberkuhn in the caecum and colon, and then penetrate mucosal cells at the base of the glands (Urquhart 1996). Different species have preferences for either the mucosa or the submucosa (Reinemeyer and Herd 1986) as well as the caecal wall versus ventral colon; very few are found in the dorsal colon (Reinemeyer and Herd 1986; Kuzmina, Kharchenko *et al*. 2007). Damage is caused on entering the glands and as the larvae develop fibroblasts surround them, this leads to disruption of the gland architecture and goblet cell hyperplasia and hypertrophy (Rupasinghe and Ogbourne 1978). There may also be infiltration by lymphocytes, plasma cells and eosinophils (Love, Escala *et al*. 1992).

Field, experimental and clinical studies on cyathostomin infections have identified pathological symptoms such as: diarrhoea, pyrexia, weight loss or growth checks in growing animals (Murphy and Love 1997; Love, Murphy *et al*. 1999), disrupted enterocolic motility (Bueno, Ruckebusch *et al*. 1979), as well as haematological changes such as anaemia, lowered haemoglobin concentrations (Hb) and packed cell volume (PCV) (Matthee, Krecek *et al*. 2002), eosinophilia and leucocytosis (Reinemeyer 1986). Increases in beta-globulins and hypoalbuminemia have also been observed (Reinemeyer, Smith *et al*. 1986). None of these symptoms are pathognomonic for cyathostomin infection, but studies have demonstrated a link to the introduction of infection (Murphy and Love 1997), or observed a reversal of symptoms after treatment to remove the cyathostomins (Matthee, Krecek *et al*. 2002).

An extreme form of cyathostomin parasitism is larval cyathostominosis, also known as acute verminous enteritis. In the donkey, it is characterized by anorexia, abdominal pain and depression followed by hyperlipaemia, submucosal oedema and in some cases diarrhoea (Trawford and Getachew 2008). It is often a fatal condition, caused by the mass emergence of encysted larvae (Love and McKeand 1997) and most commonly occurs in young horses

and donkeys. Affected animals often have low or negative faecal egg counts (Reinemeyer 1986), as the infection is caused by the pre-patent stages of infection; therefore diagnosis is made on history and clinical signs. Furthermore, as encysted larvae are refractory to most anthelmintic drugs, this syndrome can potentially occur in animals that are regularly dosed with anthelmintic (Reid, Mair *et al*. 1995). In addition to severe pathogenic effects of mass larval emergence, high numbers of adult parasites can cause a loss of condition, drop in Hb and PCV as described above (Matthee, Krecek *et al*. 2002). This can be more severe in young animals and working animals, which tend to carry higher burdens (Getachew, Trawford *et al*. 2010).

1.2.4 Species of cyathostomins

Although the eggs of equid strongyle species are morphologically indistinguishable from each other (Rupashinge and Ogbourne 1978), laboratory culture to further life stages can be performed to distinguish large strongyles from cyathostomins. Individual cyathostomin species are usually differentiated in their adult form. Over 50 species of cyathostomins have been identified (Lichtenfels, Kharchenko *et al*. 1998). Some are present in all equidae, others appear to be species specific to zebras or donkeys, or both zebras and donkeys (Lichtenfels, Kharchenko *et al*. 1998). Studies have found a predominance of certain species in horses across continents, with *Cylicocyclus nassatus, Cylicostephantus longibursatus, Cyathostomum catinatum, Cylicocyclus goldi,* and *Cyathostomum pateratum* being identified as common species in Europe, the US and Australia (Traversa, Milillo *et al*. 2010). However, there is also evidence for geographic variation in the distribution of cyathostomin species; a study in the Ukraine by Kuzmina *et al*. (2007) found a different distribution to that commonly observed in Europe, with many of the commonly found cyathostomins absent or reduced in prevalence. The selective pressure of anthelmintic use may also have an effect on the distribution of species as reported by Traversa et. al. , and it may be the absence of a frequent worming routine that is responsible for the situation reported in the Ukraine (Kuzmina, Kharchenko *et al*. 2007). Heterogeneity in cyathostomin species distributions is important because different species of cyathostomin may have higher pathogenic potentials (Love, Murphy *et al*. 1999). Additionally different species vary in their preferred location within the gut both as larvae and adults (Reinemeyer and Herd 1986; Kuzmina, Kharchenko *et al*. 2007),

which may affect their pathogenic capabilities. However, larval cyathostominosis is not thought to be species specific (Hodgkinson, Lichtenfels *et al*. 2003).

Very few existing studies have endeavoured to speciate the cyathostomin infections found. This is for two main reasons; identification of adult worms requires necropsy of the animal, and speciation from cultured larvae is difficult due to larvae having less distinct morphological characteristics than adult worms (Gasser, Zarlenga *et al*. 2004; Kharchenko, Kuzmina *et al*. 2009). Although techniques for identification of cyathostomins utilising polymerase chain reaction (PCR) have been validated for several cyathostomin species, these methods are highly specialised and not widely available (Ionita, Howe *et al*. 2010; McWilliam, Nisbet *et al*. 2010). For these reasons cyathostomins are referred to as a single group in the majority of studies, and similarly have not been differentiated here.

1.3 Diagnosis and measurement of cyathostomin infections

1.3.1 Faecal Worm Egg Count (FWEC)

The most widely used method for diagnosing strongyle infections is the faecal worm egg count (FWEC), performed either on fresh faeces collected either from the ground after being passed by the animal or directly from the rectum. At least one gram (but usually three grams) of faeces are collected, and samples are either pooled to assess a group as a whole or, more commonly, examined individually to give a FWEC for each individual animal (Urquhart 1996). Several methods of examining faeces exist such as direct smear, or flotation methods such as direct flotation or the McMaster method (Whitlock 1939). The McMaster method is the most widely used method as it is simple to perform and gives a quantitative result. One drawback of the McMaster method as commonly used is that it has a lower egg detection limit of 50 eggs per gram (EPG), although this can easily be improved by modifying the standard method to enumerate eggs in a greater number of chambers. A second flotation method of egg enumeration is the FECPAK test (FECPAK NZ), which requires additional equipment to the McMaster procedure. Presland, Morgan *et al* (2005), assessed its use in equidae and in this study FECPAK was shown to have greater sensitivity with low egg numbers, but it has subsequently been argued that FECPAK is not inherently better than McMaster method and

that the observed improvement stems from the enumeration of a greater number of eggs (Denwood, Love *et al*. 2012). FECPAK does however have the advantage of being available as a complete kit, which can be used onsite.

It should be remembered that the number of eggs detected in the faeces is only an indicator of adult parasite numbers within the host as it does not take into account pre-patent stages, and different parasite species or individual worms may vary in fecundity. As a result, the correlation between FWEC and the number of worms present in individual animals is known to be weak (Lyons, Tolliver *et al*. 1983). Urquhart (1996) classifies an equine strongyle EPG of greater than 1000 as an indication of heavy infection, whereas 500-1000 EPG is moderate and less than 500 EPG is considered to be low. However, different classification limits are used by other authors (Uhlinger 1993). It is also important to remember that many fatal cases of larval cyathostominosis present with a FWEC of zero as the pathogenic prepatent stages are not yet producing eggs and are therefore undetectable by this method (Reid, Mair *et al*. 1995).

Another drawback of FWEC is high variability (Uhlinger 1993), resulting in a highly over-dispersed distribution of observed counts, due to inconsistent shedding by the host, and inconsistent distribution of eggs within faecal output (Vidyashankar, Kaplan *et al*. 2007). Furthermore, it is not possible to differentiate individual species of strongyles using the FWEC. These classifications are frequently desired due to the pathogenicity of some species of large strongyles, so cultures are often carried out on the faeces collected, to detect the presence of large strongyle species by larval morphology. Cultures can also be used to examine species composition of cyathostomins, but expert examination is necessary to identify cyathostomin species in their larval stages (Kharchenko, Kuzmina *et al*. 2009).

1.3.2 Pasture Larval Counts

Due to the large numbers of eggs produced by each adult female cyathostomin and the duration of the free living stages of the parasite life cycle, at any one time the majority of the parasites exist in the environment rather than in the host (Rose and Hodgson 2000). Numbers of L3 have been assessed using samples taken from pasture that has been either naturally or experimentally infected, and resultant recovery rates were lowest in the winter months in

England (Ogbourne 1973) and Scotland (Ramsey, Christley *et al*. 2004). It is thought that winters in the British Isles with freezing frost overnight and thaws in the day may have a deleterious effect on larval development and survival (Lucker 1941). In contrast, in countries where winter temperatures remain below freezing or have persistent snow cover, conditions may support the survival of strongyle free-living stages (Nielsen, Kaplan *et al*. 2007). Persistence times of 20 weeks have been observed over winter in England, whereas persistence in the summer months was lower (Ogbourne 1973). This could be due to death of larvae through desiccation in summer months (Ogbourne 1973), despite the fact that the life cycle is completed more rapidly in warm conditions (Reinemeyer 1986). Studies assessing free-living stages are notoriously variable, and it also must be noted that they merely detect the presence of L3 on pasture and do not account for those sequestered in faecal balls or for levels of true infectivity as a combination of larval challenge and viability. It is, however, clear that within the British Isles, winter alone cannot be relied upon to 'clean' pastures of strongyle larvae from one season to the next (Nielsen, Kaplan *et al*. 2007). In tropical climates with extended periods of hot dry weather, when herbage is also lost, it is likely that ground may become 'helminthically sterile' (Getachew, Trawford *et al*. 2007). However, in the temperate, wet climate of Devon, it must be assumed that either viable eggs and/or larvae can persist in the environment until the next grazing season.

1.3.3 Alternative diagnostics

Due to the shortcomings of the traditional FWEC, considerable research has been done to find improved diagnostic tests for internal parasitism. Recent investigations in the field of equine strongyles include; immunodiagnostic markers (McWilliam, Nisbet *et al*. 2010), serum protein electrophoresis (Abbott, Mellor *et al*. 2007) and reverse line blot assays (Ionita, Howe *et al*. 2010). Some of these techniques appear to be more promising than others: immunoscreening and ELISA have the potential to offer a diagnostic tool for detecting encysted larva (Dowdall, Proudman *et al*. 2004; McWilliam, Nisbet *et al*. 2010) and PCR to enable species differentiation directly from the egg stage (Ionita, Howe *et al*. 2010). At the time of writing none of these techniques are commercially available.

1.4 Control of cyathostomins in donkeys

The wild donkey evolved in areas of sparse food, leading to wild donkeys living separately from each other. It is thought that the donkeys' loud vocalizations developed to communicate with other donkeys over these large distances (Canacoo and Avornyo 1998). Increased stocking density contributes to disease transmission, including transfer of parasites (Herd 1986). The majority of intestinal parasites are transmitted via the faecal oral route, with eggs shed in the faeces developing on the pasture to larvae, which are then ingested. Equidae typically avoid areas of pasture contaminated by faeces, known as roughs (Archer 1980), when they are able to graze cleaner areas, known as lawns. Increased stocking density leads to an increased proportion of grazing on land contaminated by faeces, and thus aids the transmission of parasites.

1.4.1 Natural immunity

In horses, a reduction in faecal egg output is seen with age (Chapman, French *et al*. 2003; Becher, Mahling *et al*. 2010) and is thought to be evidence of an acquired partial immunity or tolerance (Love and Duncan 1992), perhaps through the actions of interlukin 4, T helper 2 cells, mast cells and other cytokines (Matthews, Hodgkinson *et al*. 2004). The same has not been observed in the working donkeys of Ethiopia, which appear to continue shedding high numbers of eggs throughout their lives (Getachew, Trawford *et al*. 2010). One proposed explanation for this is that the African working donkeys studied are immuno-compromised due to stress, malnutrition and/or concurrent disease and therefore unable to mount an immune response to their parasites. The same is not true for one study of Ukrainian donkeys that showed a reduced number of nematodes with age (Kuzmina, Kharchenko *et al*. 2007), and may also be assumed to be the case for donkeys in Britain, which are afforded much higher standards of care. In the UK a high FWEC in an older donkey may be a proxy for concurrent disease affecting the immune system, such as Pituitary Pars Intermedia Dysfunction which is common in older Donkeys (Grove 2008; Sprayson 2008).

The acquired immunity to cyathostomins is only partial and varies greatly between individual horses. Many mature horses have faecal egg counts of zero without receiving any

anthelmintic dosing, whereas other horses under the same conditions continue to harbour large worm burdens and pass high numbers of eggs in their faeces. In a recent study by (Becher, Mahling *et al*. 2010) no eggs were ever detected in 40.3% of 129 horses undergoing monthly faecal samples over a period of 9 consecutive months. As it is known that the majority of the worm population is contained within a minority of the hosts (Lord 2007), it is inappropriate to regard the population as homogenous. Control strategies for cyathostomin infections within a population of animals should therefore account for this heterogeneity in order to provide the optimum efficacy.

1.4.2 Anthelmintics

The anthelmintic drugs available on the market today fall into several chemical families. Those that are most commonly used in Donkeys are: macrocyclic lactones, which include the avermectins such as ivermectin and the milbemycin moxidectin; the benzimidazoles – fenbendazole, oxfendazole and mebendazole; tetrahydropyramidines such as pyrantel embonate; and the pyrazinoisoquinoline, praziquantel. Several commercial products have licences and dosing information relevant to use in donkeys but under the cascade system other available drugs can be used with justification e.g. in the face of known cyathostomin resistance to the licensed anthelmintic classes.

At standard dosage rates the majority of these products are only effective against the less pathogenic adult stages of cyathostomins, and it is a reduction in egg shedding through the removal of the adult parasite population that is the aim of anthelmintic dosing, rather than the removal of the worm itself (Kaplan and Nielsen 2010). However, moxidectin has some efficacy against encysted stages in horses (Grubbs, Amodie *et al*. 2003; Bairden, Davies *et al*. 2006) and in donkeys has been shown to suppress FWEC longer than ivermectin treatment (Trawford, Morriss *et al*. 2001). This sustained reduction in egg output may be due to better efficacy against encysted larvae, or may be explained by a longer half-life (Trawford and Getachew 2008). An alternative larvicidal regimen consists of a 5-day course of high dose (7.5mg/kg of body weight) fenbendazole (Lyons, Drudge *et al*. 1983; Duncan, Bairden *et al*. 1998).

Anthelmintic drugs first became widely available in the middle of the 20th century, and considerably reduced morbidity and improved health and performance of horses that were given the drugs (Kaplan and Nielsen 2010). They were adopted as an integral part of routine prophylactic treatment, with the recommendation to dose every six to eight weeks to ensure worms would be removed before reaching the reproductive stage, and made available to equine owners without veterinary prescription. Many owners still continue with these outdated intensive worming regimens with little or no evidence that the drug is still effective. It is felt that this ready availability often led to misuse, such as unnecessary treatment and under-dosing of animals, which has contributed to the widespread resistance to anthelmintic drugs seen today (Kaplan 2010).

1.4.3 Anthelmintic resistance

Anthelmintic resistance is said to occur when a drug previously used effectively to combat a parasite population no longer works. Dargatz, *et al*. (2000) described resistance as occurring...

> '*when a greater frequency of individuals in a population of parasites, usually affected by a dose of concentration of compound, are no longer affected'*

This includes situations where a higher dose or concentration of drug is required to remove a given proportion of a nematode population. Dargatz *et al*. (2000) also stated that anthelmintic resistance is an inherited trait. Anthelmintic resistance is traditionally monitored using the faecal egg count reduction test (FECRT), which is described later in this chapter. The reliability of this technique has been questioned, not least because of the use of arbitrary percentages of between 80 and 95% reduction in FWEC to declare resistance (Vidyashankar, Kaplan *et al*. 2007), and is only thought to be accurate when > 25 % of the parasite population present are resistant (Martin, Anderson *et al*. 1989). Laboratory assays have been proposed for detecting resistance; these include the use of an egg hatch assay, larval development assay and larval migration inhibition assay as well as molecular genetic tests. However, none are validated or widely used in equine parasites at present (Stratford, McGorum *et al*. 2011).

Widespread resistance has been found to benzimidazoles in cyathostomin populations throughout the world (Lyons, Tolliver *et al*. 1999), loss of efficacy and resistance to

tetrahydropyrimidines is growing (Coles, Eysker *et al*. 2003; Kaplan 2010), and as predicted by several authors including Sangster (1999) and Coles, Eysker *et al*. (2003) avermectin/milbemycin resistance has also more recently developed. Loss of ivermectin efficacy has been reported by the University of Kentucky (Lyons, Tolliver *et al*. 2008) and the first reported incidence of moxidectin resistance occurred at The Donkey Sanctuary Devon (Trawford, Burden *et al*. 2005). The Donkey Sanctuary Devon has also seen evidence of developing resistance to Pyrantel (Trawford and Getachew 2008) leaving very few options in chemophylaxis to prevent cyathostomin infection. The mechanisms of resistance have been linked to molecular and genetic components such as beta tubulin codons for benzimidazole resistance (von Samson-Himmelstjerna 2004), but development stems from selective pressure. It is initiated when a few individual parasites become refractory to a product and thus survive treatment. These 'survivors' then remain in the host to produce offspring. These progeny, being genetically similar to the previous generation, tend also to be resistant. As the process continues the percentage of the population with resistance increases until the product is no longer useful (Donald 1983 as cited by Wescott 1986).

A review by Wescott (1986) examined the development of resistance and identified key areas for intervention. These include using drugs of known efficacy, using regular FWEC to monitor efficacy, ensuring animals are weighed correctly to reduce the risk of under-dosing, and using an optimal dosing interval, as over exposure leads to a greater population of exposed parasites. Not complying with these recommendations can quickly lead to resistance, as demonstrated by Kaplan *et al*. (2004) where a resistant cyathostomin population was created through frequent dosing with fenbendazole in a population of horses in which this drug was known to have reduced efficacy. Wescott (1986) also recommended slow rotation of drugs or combination products because prolonged use of a single product increases selection for resistance, although it has been shown more recently that combining products with known reduced efficacy could significantly increase their efficacy and produce a high FECR (Kaplan 2010). Other recommendations by Wescott (1986) refer to the process of pasture rotation, discouraging the 'dose and move strategy', which contaminates pastures exclusively with progeny of resistant worms, and also not to rely exclusively on anthelmintics.

Despite anthelmintic resistance having been widely recognised for several decades, and being the subject of much scientific research, the situation has continued to worsen. In a recent review article Kaplan (2010) concluded that: (1) Drug resistance is more prevalent than commonly recognised, (2) the problem of anthelminitic resistance in cyathostomins is constantly worsening, and (3) anthelmintic resistance may be more severe in the United States than anywhere else in the world.

Until recently, within the UK cyathostomin parasites have been well controlled by the use of anthelmintic drugs, but over reliance and over use has led to a drop in efficacy and increased resistance to these products (Herd and Coles 1995; Traversa, Milillo *et al*. 2009). Within the companion animal and agricultural fields resistance has been found in many helminth species; however the parasites of most concern within The Donkey Sanctuary Devon (and indeed the general equine population) are cyathostomins. At The Donkey Sanctuary Devon, losses of efficacy and cases of resistance to several classes of anthelmintic have been reported (Trawford, Burden *et al*. 2005; Trawford and Getachew 2008). This includes the first report of macrocyclic lactone resistance in cyathostomins, when the efficacy of moxidectin was observed to be reduced (Trawford, Burden *et al*. 2005). These developments have left The Donkey Sanctuary Devon with limited ways to prevent infestations, resulting in a threat to the effective treatment of clinical parasitism. An alternative control strategy to chemophylaxis would help to slow the development of resistance, and hopefully preserve the efficacy of existing treatments so they can continue to be utilized in clinical cases.

1.4.4 Refugia

The concept of refugia is relatively new in the field of anthelmintic resistance. Parasites not exposed to a drug during treatment are said to be in 'refugia', and provide a reservoir of genes susceptible to anthelmintics. The concept of preserving an unexposed population in order to dilute the resistant gene pool has previously been suggested, but the importance of doing this has only recently been recognised (Van Wyk 2001). As the size of refugia increases, genetic diversity is maintained and selection towards resistance slows. There are three forms of refugia; the free living stages on the pasture, the encysted stages which are not affected by most anthelmintics and parasites in animals which do not undergo anthelmintic dosing. It has been shown that maintaining adequate refugia can slow the development of anthelmintic

resistance in sheep (Dobson, Besier *et al*. 2001) and it is the current view that equine parasite control programmes should also aim to preserve refugia (Lord 2007). Larvicidal therapies that remove the mucosal stages deplete refugia at a faster rate than those that only target adult worms. Ivermectin does not have significant efficacy against hypobiotic mucosal larvae, unlike the related compound moxidectin (Xiao, Herd *et al*. 1994; Love, Duncan *et al*. 1995). Moxidectin therefore substantially reduces refugia relative to ivermectin, which is a potential explanation for why resistance to this product emerged before ivermectin resistance (Sangster 1999).

1.4.5 Faecal Egg Count Reduction Test (FECRT)

The presence of anthelmintic resistance *in vivo* is traditionally determined through the FECRT procedure (Coles, Bauer *et al*. 1992). This is based on performing a FWEC before and 10-14 days after anthelmintic dosing and determining the percentage reduction in egg shedding (Stratford, McGorum *et al*. 2011).

$$\text{FECR}(\%) = \frac{(\text{Baseline FWEC} - \text{14 days post treatment FWEC}) \times 100}{\text{Baseline FWEC}}$$

(Stratford, McGorum *et al*. 2011)

However, as stated previously the FWEC is a relatively poor measure of parasite numbers and contains many sources of variability, thus making FECRT inherently unreliable as a measure of the proportion of worms removed. It has been shown that in sheep at least 25% of the worm population must be resistant in order for resistance to be detected through a FECRT (Martin, Anderson *et al*. 1989). Despite the imperfections of the FECRT, the results are generally accepted and only recently have computationally intensive methods of analysing FWEC data been suggested to improve the accuracy of detecting anthelmintic resistance (Vidyashankar, Kaplan *et al*. 2007; Denwood, Reid *et al*. 2010). The traditional FECRT analysis proposed by Coles Bauer *et al*. (1992) and advocated by the World Association of the Advancement of Veterinary Parasitology (WAAVP), calculates the empirical mean and variance before and after anthelmintic dosing and determines the mean reduction and 95% confidence intervals for the true reduction using these figures. With anthelmintic resistance receiving increasing attention there is a need to standardise the statistical methods used to

assess FECRT (Denwood, Reid *et al*. 2009; Denwood, Reid *et al*. 2010). The non-parametric bootstrapping approach suggested by Vidyashankar, Kaplan *et al*. (2007) is more robust than the WAAVP method as it does not make assumptions about the underlying distribution of the data, but can run into difficulties when dealing with small numbers of samples, which is often the case with equine data. Computationally intensive parametric methods, such as the MCMC technique advocated by Denwood *et al*. (2009), fit the data to a distribution by making informed assumptions about the true distribution of the data. This method consistently out performs other methods and can be applied to sample sizes less that 50, making it the more appropriate choice for equine FECRT (Denwood, Reid *et al*. 2009; Denwood, Reid *et al*. 2010).

1.4.6 Pasture hygiene

Various methods have been recommended to reduce the numbers of infective L3 on pasture and thus limit re-infection of hosts; these include removing faeces either manually or with a mechanical sweeper or mechanical vacuum, grazing with alternate hosts (e.g. sheep), and cropping the pasture for hay or silage (Herd 1986). The purpose of this is to expose the larva on the pasture leading to more rapid desiccation, and also to keep equidae away from infective pasture at times when larval numbers are high (Herd 1986). However, these recommendations are largely based on theoretical reasoning, and these techniques have not been extensively validated for either donkeys or horses. It should also be remembered that pasture hygiene also depletes one of the potential sources of refugia.

Kuzmina *et al*. (2007) found comparatively low levels of infection in donkeys from the "Askania-Nova" Biosphere reserve in the Ukraine, where a long-standing pasture management system is in place. Herd (1986) and Herd *et al*. (1993) found that removing faeces twice a week provided control of equine cyathostomins and ascarids in horses, as well as increasing grazing area. The increase in grazing area is important both aesthetically and in terms of parasite control as it has been shown that donkeys with access to better food resources have lower strongyle egg counts than those on limited grazing (Wells, Krecek *et al*. 1998). However, Matthee, Krecek *et al*. (2002) found that monthly faeces removal alone did not significantly reduce numbers of L3 on the pasture, and Becher Mahling *et al*. (2010) found that pasture hygiene did not significantly reduce FWEC.

Manual removal of faeces from pasture is laborious and time consuming, but it has been speculated that mechanical sweepers may act to break up and spread some faeces rather than removing all faecal material, and may therefore increase pasture larval infectivity. However, since faecal balls offer protection from the environment (Enigk 1934 as cited by Nielsen, Kaplan *et al.* 2007), exposing free-living stages may also reduce their survival. Larvae can develop quickly and leave the faecal mound, travelling distances of up to 30cm when aided by rain splash (English 1979). Therefore even when faeces are removed, if it is not done frequently enough then infective larvae will not be prevented from contaminating the pasture. This may be the reason for the lack of significant results in several studies on faeces removal (e.g. Matthee Krecеck *et al*. 2002), which removed faeces only once a month and did not monitor rainfall, although a lack of statistical power of the study is also a possible explanation. The strategies of pre-grazing with sheep and faeces removal, as mentioned above, are currently practiced on some of the pastures at The Donkey Sanctuary Devon (Trawford and Getachew 2008).

1.4.7 Targeted Selected Therapy (TST)

It has long been acknowledged that traditional interval dosing regimens contribute to the development of anthelmintic resistance. Combining the principle that many horses are able to naturally maintain low faecal egg counts together with the concept of maintaining refugia has led to development of 'Targeted Selective Treatment' (TST) methods (Duncan and Love 1991; Gomez and Georgi 1991). This approach aims to be sustainable and works on the idea of dosing the right animal with the right drug at the right time (Kaplan and Nielsen 2010) i.e rather than blanket treatment of animals, only animals shedding high numbers of eggs are dosed. A recent trial using this technique in horses has proved successful in reducing pasture contamination, the number of anthelmintic doses used, and consequently the selection pressure for resistance (Becher, Mahling *et al*. 2010).

One possible TST approach is as follows: Regular FWEC are taken to distinguish animals with high egg shedding, which require dosing, and those with lower counts, which do not. Those animals, which do not undergo dosing, contribute to the maintenance of adequate

refugia. In horses the reduced number of doses necessary can offset the cost of performing multiple FWEC (unpublished work by the University of Georgia cited by Kaplan and Nielsen 2010), but the same may not be true in donkeys which are smaller and so require less costly doses i.e. less anthelmintic drug but the same laboratory cost per FWEC test. By targeting the animals with high FWEC the level of contamination on the pasture is limited, while maintaining refugia in other forms.

An important part of a TST is being able to identify which animals it is necessary to treat. Equine parasitologists have expressed the consensus opinion that in the horse a limit of 200-500 eggs per gram should be used as the cut-off for the administration of treatment (Uhlinger 1993). It is known that FWEC is not necessarily directly related to actual number of luminal worms, and Nielsen, Baptiste *et al*. (2010) found that FWEC of greater than 500 EPG bore no strong correlation to adult worm numbers. However it is recognised that an output of less than 200 EPG is unlikely to cause ill effects (Kaplan 2010). Krecek, Gurthie *et al*. (1994) used a cut-off of over 300 EPG to determine which horse to dose, whereas Becher, Mahling *et al*. (2010) used 250 EPG. However it is important to note that a FWEC of over 1000 was very common in healthy Venezuelan wild horses (Perez, Garcia *et al*. 2010), in South African donkeys the average count is 5000 EPG (Matthee *et al* 1998 unpublished as cited by Krecek and Waller 2006), and in Ethiopia the majority of donkeys have been shown to have an EPG of over 1000 (Getachew, Feseha *et al*. 2008). As mentioned above the current estimates were based on expert opinion rather than evidence derived from clinical studies, and could therefore be inappropriately low, especially for donkeys where cyathostomins are possibly better tolerated than in horses. Additionally due to the innate variability of FWEC, a single high result may not truly represent a high worm burden in that individual thus clouding decision making in TST (Denwood, Reid *et al*. 2009).

Due to the fact parasite transmission is seasonal, the timing of TST is also highly important. The majority of parasites can be controlled with as few as two doses of anthelmintic per year, however in some animals doses twice during the parasite transmission season may not be sufficient to control cyathostomins (Kaplan and Nielsen 2010). When considering the interval between doses of anthelmintic it is important to consider the egg reappearance period (ERP) characteristic of specific classes of anthelmintic - since only adult stages are killed by most

drugs, leaving encysted larvae within the host, the intestines can be rapidly repopulated. The ERP of BZ drugs and Pyrantel are usually only 6 weeks and 4 weeks respectively, although avermectin class drugs are associated with a longer ERP (Kaplan 2010). It can therefore be deduced that FWEC performed as frequently as once a month may be necessary at times of year when larval transmission is high, however the optimal interval has not been determined (Becher, Mahling *et al*. 2010). Once a TST plan has been implemented, due to the speculated consistency of shedding in an individual, low or high egg count animals will be identified (Becher, Mahling *et al*. 2010) and thus the frequency of FWEC needed will decrease. It may also be beneficial to administer seasonal treatments to the entire population to target parasites other than cyathostomins, for example large strongyles and tapeworms.

1.5 Study Objectives

Much of the current insight into parasite control in donkeys is often deduced from studies conducted outside of the UK, or is extrapolated from studies conducted in horses and ponies (Trawford and Getachew 2008). Because of the influences of climate on the epidemiology of cyathostomins, and the substantial differences between donkeys and horses in terms of disease susceptibility (Krecek and Guthrie 1999), this means that the available information relating to donkey cyathostomins within the UK is deficient in key areas. The Donkey Sanctuary Devon is also unusual compared to most equid groups in that a very large number of animals are grazed during the spring and summer together on communal grazing, which further limits the applicability of studies performed in the traditionally smaller groups of horses and ponies in the UK. The atypical demography of the animals in terms of age and health status is a further complication.

The aim of this study was to carry out a large-scale examination of TST and pasture management on a donkey population, in order to work towards the development of "best practice" for donkey parasite control at The Donkey Sanctuary Devon. The specific objectives were:

1. To evaluate the effectiveness of two methods of faeces removal relative to the current method of control, by comparisons of donkey FWEC and pasture larval counts between groups using a robust statistical method.

2. To compare the required rate of anthelmintic dosing between groups managed using different pasture hygiene protocols and FWEC dosing thresholds, and relate this to the estimated efficacy of the same anthelmintic at the end of the grazing period.

The progression of this thesis towards these goals is as follows. Firstly a description of the study format is presented, including numbers and types of animals involved across the farms of The Donkey Sanctuary Devon. This is followed by a quantitative assessment of the effects of group level pasture hygiene method and dosing threshold, along with individual animal age and sex, on observed donkey FWEC. Finally, the pasture larval counts, dosing rates and observed anthelmintic efficacy at the end of the grazing seasons are compared between groups. The results are discussed with particular emphasis on practical recommendations for future cyathostomin control strategies at the Donkey Sanctuary Devon.

Materials and Methods

2.1 Introduction

A total of 684 animals were involved in this study. This included 266 female donkeys (DKF), 401 gelded male donkeys (DKG), one donkey stallion (DKS); one female horse (HOF), two gelded horses (HOG); eight mules (MUF, N=5 and MUG, N=3); and five ponies (PYF, N=4 and PYG, N=1). These animals were in closed groups distributed across the four neighbouring farms that constitute The Donkey Sanctuary Devon: Brookfield (BF), Paccombe (PC), Trow (TR) and Slade House Farm (SHF), shown in Figures 2.1-2.4. The farms were in close proximity and therefore exposed to similar climatic conditions, limiting the influence of environmental factors and features. The animals were divided into 15 grazing groups named according to the fields or barns in which they were kept, listed in Table 2.1, and these groups were assigned to one of four trials. Each of the groups within a trial were matched as closely as possible for animal ages, management practices and stocking density, to minimise environmental factors within each trial. Ideally stocking density is calculated as the total weight of donkey per square meter, however numbers of animals per unit can be used, provided the animals within the trial are of roughly similar weight. In the sanctuary where fields are of a set size and herbage quality varied, an approximate match of stocking density was used. The groups were chosen partly to match grazing type, to ensure that dry matter availability was approximately the same across the groups within each trial. An approximate estimation of stocking density can be found in Appendix 2.

Within each of trials 1-3 the groups were assigned to one of three management strategies: No removal of faeces (No removal); twice weekly removal of faeces manually using hand held shovels (Manual) or twice weekly removal of faeces using an automated pasture sweeper

(Terra-Vacc, Sweeper, Terra-Vac Ltd, Suffolk, UK). Animals in these groups were not treated with anthelmintic except in cases of clinical disease or demonstration of a FWEC in excess of 2000 EPG. FWEC was monitored on a four weekly basis to determine the need for anthelmintic dosing. Trial 4 was different, as the group size was much larger and the pastures involved did not allow easy removal of faeces. In these groups no removal of faeces took place and different cut-offs for TST of 300, 600 or 1000 EPG were implemented. These animals also underwent a FWEC at 4 weekly (28 day) intervals. When FWEC of individual donkeys exceeded the threshold for anthelmintic dosing, the specific animals were dosed by Donkey Sanctuary Devon personnel using Pyrantel embonate at 19mg/kg body weight, as measured by in house weigh scales.

During the winter months the donkeys at The Donkey Sanctuary Devon are maintained full time in large custom designed barns with deep litter bedding and concrete yard exercise areas. The study period began when the Donkeys were turned out to pasture in late April 2010. Initially the SHF Buffalo group was assigned manual faeces removal and SHF Shelter 4 automatic faeces removal. However, due to an unrelated disease outbreak within SHF Shelter 4 in early May use of the automatic sweeper was stopped due to potential disease spread as it moved between groups. Thus SHF Shelter 4 became a manual removal group and SHF Buffalo an automatic group as of 19th May 2010.

Table 2.1 The distribution of Donkeys and other animals by group and trial with information on management. BF=Brookfield, PC=Paccombe, SHF=Slade House Farm, TR=Trow. DKG=Donkey Gelding, DKF=Donkey Female, DKS=Donkey Stallion, HOG=Horse Gelding, HOF=Horse Female, MUG=Mule Gelding, MUF=Mule Female, PYG=Pony Gelding, PYF=Pony Female.

Trial	Group/ Field Name	Starting No. of Donkeys	Mean Age (Years)	Other animals	Faeces Removal Plan Post May 19th	Dosing Threshold (EPG)	Manage-ment
1	BF New Barn Grannies	40 (DKG-31 DKF-9)	28	HOG-1 MUF-1 MUG-2	Manual	2000	Set Stocked. Older animals with poor dentition. Fed chop
	PC Cherry Barn	48 (DKG-17 DKF31)	30	HOF-1	No Removal	2000	
	TR Rennies Left	33 (DKG-12 DKF-21)	29	0	Automated	2000	
	TR Naughty Face	16 (DKS-1 DKG-7 DKF-8)	29	PYF-1 PYG-1	Automated	2000	
2	BF Middle Barn	33 (DKG-19 DKF-14)	16	0	No Removal	2000	Partially strip grazed (otherwise as below)
	TR Rennies Right	65 (DKG-25 DKF-40)	23	0	Automated	2000	Set stocked. Broad age range, no dental problems
	SHF EST Cottage	17 (DKG-16 DKF-1)	12	0	Manual	2000	
	SHF E&A Shelter	16 (DKG-12 DKF-4)	18	0	Manual	2000	
3	SHF Buffalo	27 (DKG-16 DKF-11)	18	PYF-1	Automated	2000	Strip Grazed. Broad age range, No particular dental problems
	BF Shelter 4	32 (DKG-32)	10	0	No Removal	2000	
	SHF Shelter 4	23 (DKG-11 DKF-12)	23	0	Manual	2000	
4	PC Spring Barn	82 (DKG-57 DKF-25)	22	0	No Removal	1000	Set stocked, general donkeys
	PC Elephant Barn	117 (DKG-65 DKF-52	27	MUF-2 HOG-1	No Removal	600	
	BF Jackward Barn	54 (DKG-38 DKF–16)	16	MUF-2 MUG-1 PYF-2	No Removal	300	
	BF Joseph Pickering	66 (DKG-44 DKF-22)	17	0	No Removal	300	

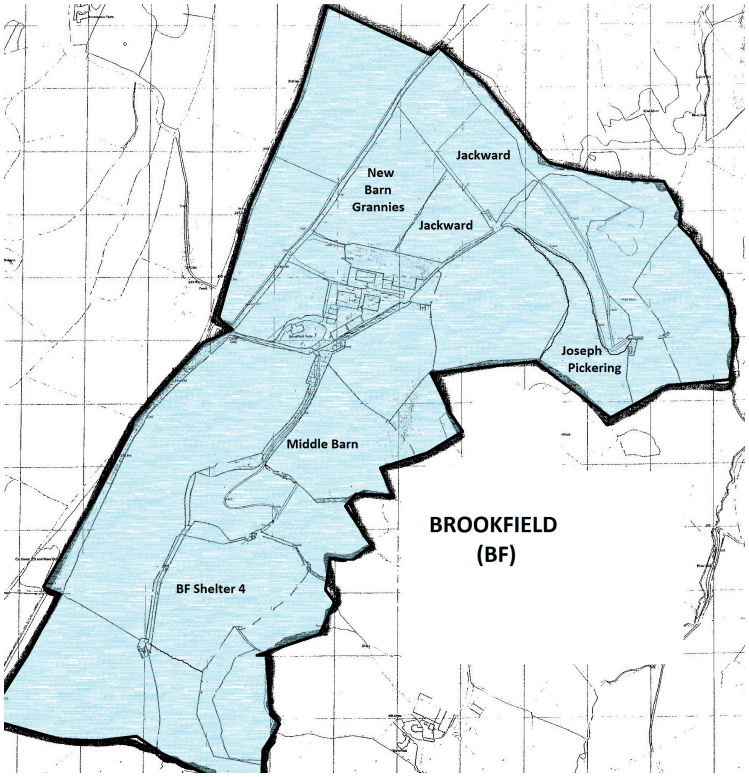

Figure 2.1 (a) Diagrammatic representation of Brookfield Farm (BF). Each small square represents 100m

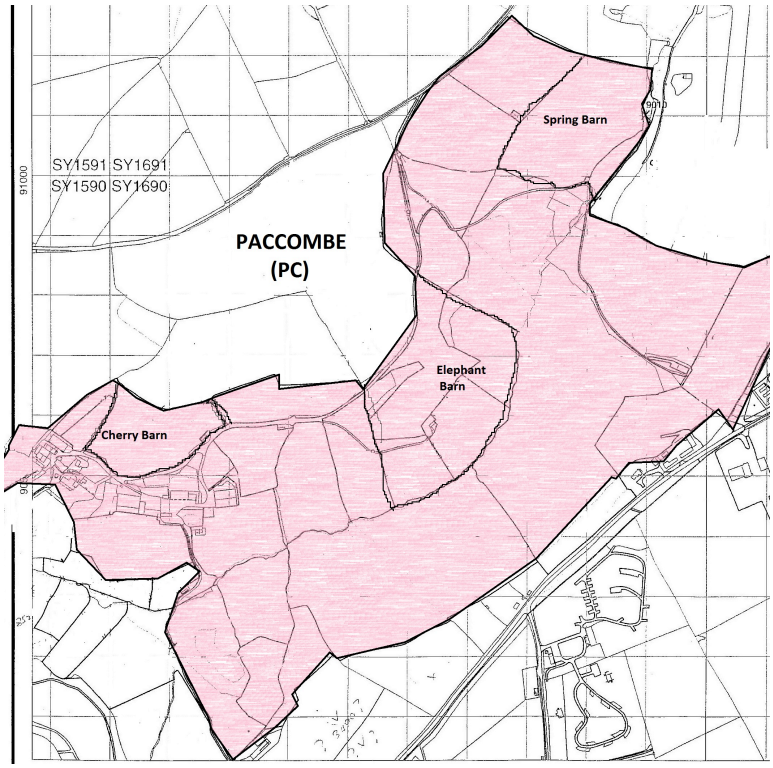

Figure 2.1 (b) Diagrammatic representation of Paccombe Farm (PC). Each small square represents 100m

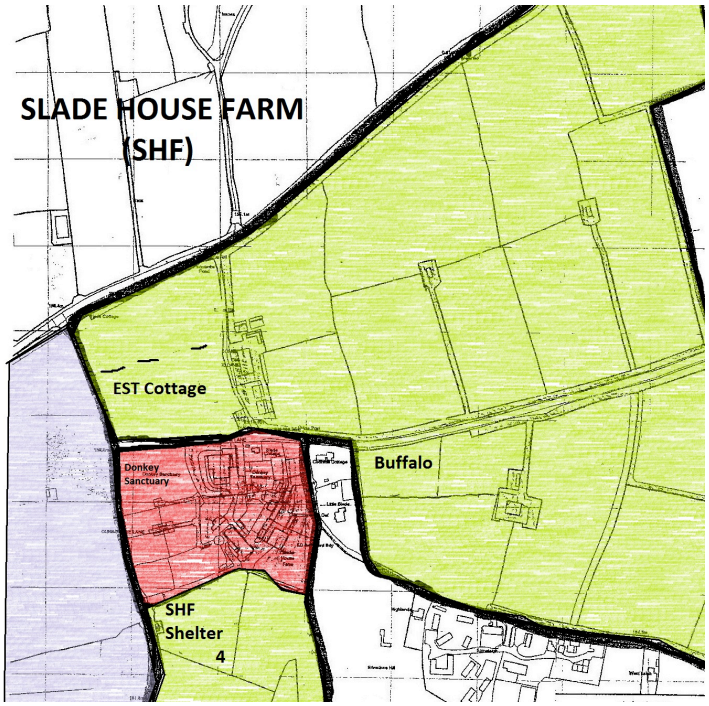

Figure 2.1 (c) Diagrammatic representation of Slade House Farm (SHF). The red area represents the 'visitors centre' part of The Donkey Sanctuary Devon. Each small square represents 100m

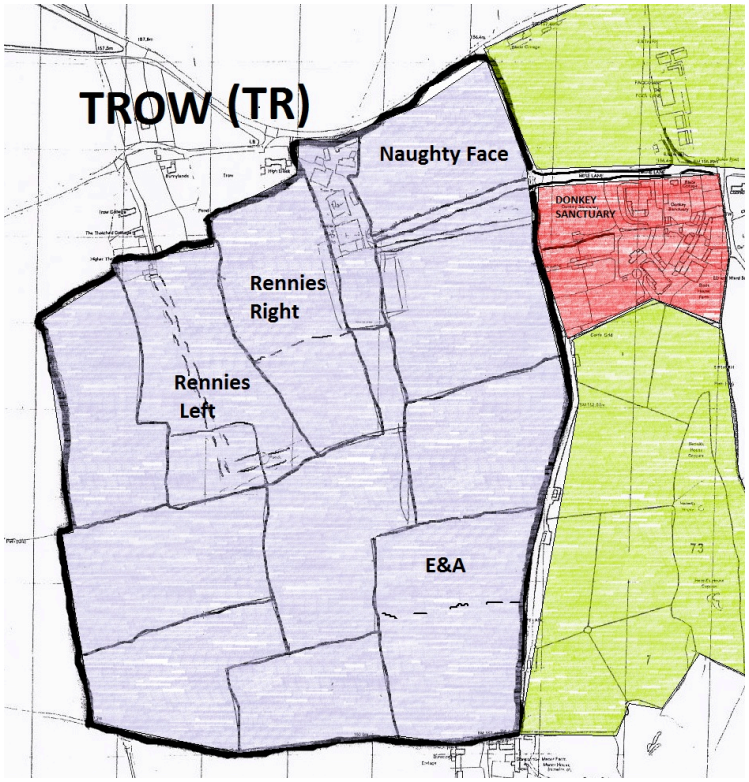

Figure 2.1 (d) Diagrammatic representation of Trow Farm (TR). The red area represents the 'visitors centre' part of The Donkey aSanctuary Devon. Each small square represents 100m

2.2 Targeted Selective Treatments

Throughout the study if any animal had a FWEC exceeding the dosing threshold designated to its group, (300 EPG for BF Jackward and BF Joseph Pickering, 600 EPG for PC Elephant Barn, 1000 EPG for PC Spring Barn, and 2000 EPG for the remaining groups,) targeted selective treatment was instigated. Oral dosing, with Pyrantel embonate at a dose rate of 19mg/kg, was administered to the animals meeting the targeted treatment criteria immediately following the FWEC results. This was done by trained staff from the veterinary team at The Donkey Sanctuary Devon.

2.3 Faecal Worm Egg Counts

Each animal in the 4 trials had a 4-weekly FWEC performed using the modified McMaster Salt Flotation Technique described below. The McMaster method was chosen over other methods of egg enumeration such as FECPAK (Presland, Morgan *et al*. 2005) as The Donkey Sanctuary Devon laboratory contained all the necessary equipment and staff were already trained to carry out the process. Animals in the same group were sampled at 28-day intervals throughout the study, however each group was sampled on a different week of the month and/or day of the week to suit laboratory availability. The samples were taken at approximately the same time of day, (i.e. morning/afternoon/evening) and were analysed within 24 hours of collection. Samples were collected from freshly passed faeces, rather than directly from the rectum whenever possible. A small number of The Donkey Sanctuary Devon's own research staff were involved in sample collection and the results were recorded by Dr Faith Burden, in a central Excel (Microsoft Corporation) spreadsheet, along with the date taken, name, identification number, age, group and trial. All faecal samples were analysed by the in house laboratory at The Donkey Sanctuary Devon, by their own laboratory technicians using the method described below.

2.3.1 McMaster method for FWEC

The modified McMaster salt flotation technique, adapted from a protocol used by the School of Veterinary Medicine, University of Glasgow, is as follows:

1. Three grams of faeces are weighed and added to 42ml of tap water.
2. After mechanical homogenisation, the suspension is poured through a 250-micron aperture sieve and the filtrate collected.
3. After thorough mixing 15ml of the filtration is transferred to a centrifuge tube and spun for 5 minutes at 3000rpm.
4. The supernatant is discarded and the remaining faecal pellet broken up using a whirl mixer.
5. The tube is then filled to its former level with saturated sodium chloride solution, mixed by inverting slowly six times and a sufficient amount of the suspension to fill both chambers is transferred to a McMaster slide.
6. The preparation is then examined using the x25 objective of a microscope, the number of eggs present in both chambers counted and the total number of eggs recorded in a spreadsheet.
7. The number of eggs recorded can then be multiplied by 50 to give the number of eggs per gram (EPG)

2.4 Pasture larval counts

Throughout the study period, pasture samples were collected from the paddocks being grazed by the different groups to test for the presence of strongyle L3 larvae. These were collected on a rotating, weekly basis, so that each grazing area was sampled every 4 weeks. These samples were collected by one of two Donkey Sanctuary staff and then sent by post to the School of Veterinary Medicine at the University of Glasgow to be processed on site, by the author, in the parasitology laboratory. The analysis carried out uses techniques similar to those described by Parfitt (1955) and Jorgensen (1975) for the recovery of gastrointestinal and respiratory tract nematode larvae respectively. However, the method has been modified from the previously used techniques by the replacement of a hand-operated washing machine with a 1.5Kg capacity, electrically powered, automatic washing machine (Good ideas mini washing machine XPB15-2318, Tensor Marketing Ltd. Darlington). The larvae were counted by Mr James McGoldrick who was blinded to the study design.

2.4.1 Herbage Analysis

Pasture larval recovery method, as used by the School of Veterinary Medicine, University of Glasgow, is as follows:

1. Herbage samples are collected into a plastic bag by crossing the paddock diagonally four times. Fifty evenly spaced stops are made along each route and at each stop four plucks of grass (the amount that can be grasped between thumb and forefinger) are taken giving a total of 400 plucks per plot. These samples were then posted, by next day delivery to the Author at the University of Glasgow.
2. The bag containing the grass is weighed then the herbage removed from the bag and placed into a 1.5Kg capacity, electrically powered, automatic washing machine (Mini Washing Machine XPB15-2318, Good Ideas, Tensor Marketing Ltd. Darlington). Four litres of lukewarm water are added, in the case of large samples more water may be required to ensure the herbage is sufficiently submerged. The machine is then set for approximately four minutes; during this time it revolves mixing the grass sample with the lukewarm water.
3. The water is then released from the washing machine through the out pipe and collected through a course sieve in a 10litre bucket. The sieve will collect any large pieces of debris such as grass blades that are passed through the pipe.
4. The remaining herbage is removed from the drum of the washing machine. As it is lifted from the drum as much fluid as possible is recovered by squeezing. This fluid should fall into the drum and be collected though the out pipe with the rest of the water.
5. The grass is then spread on a tray and dried in a warm airing cupboard or an incubator at 70°C. When thoroughly dry the herbage is weighed again and its dry weight used in the final calculation of numbers of larvae per kilogram of dried herbage (L3/kdh).

$$\text{L3/kg of dried herbage} = \frac{\text{Number of infective larvae in the sample}}{\text{Dry weight of pasture}} \times 1000$$

6. The washings contained in the bucket are filtered through a 38-micron sieve and the material retained by the sieve processed by Baermannisation for the recovery of L3

nematodes. For the Baermann technique, the larval suspension is drawn through a coarse filter paper (Whatmans Grade 113, 18.5cms) using a Buchner funnel and vacuum pump.

7. A single milk filter (Maxa Milk filters, A. McCaskie, Stirling) is put on top of the retained material, the combination is inverted and placed on a Baermann filter funnel filled with tepid water.
8. After a minimum of six hours, 10ml of fluid are withdrawn and the larvae in 1ml differentiated and counted under microscopy. Only equine nematode larvae were recorded for this study.

2.4.2 Spring Pasture Samples

In the spring following the study, April 2011, a secondary set of samples was taken from each of the paddocks to assess the numbers of larvae which had over wintered and give an indication of the current infectivity of the pasture before turnout. These were processed at the University of Glasgow

2.5 Faecal Egg Count Reduction Test

Following the final round of FWEC performed in December 2010 a FECRT was also undertaken on a subset of donkeys from across all the farms at The Donkey Sanctuary Devon. A total of 131 animals were faecal sampled (Brookfield 29, Slade 17, Paccombe 47, Trow 37). An in–house FERCT was performed by repeating the FWEC 14 days after the initial count and subsequent oral dosing with Pyrantel emboanate at a dose rate of 19mg/kg. This FECRT was carried out using the same modified McMasters technique outlined above (Coles, Bauer *et al*. 1992). Ideally the second FWEC should be 0 indicating a near 100% efficacy of the anthelmintic.

$$\text{FERC}(\%) = \frac{(\text{Baseline FWEC} - \text{14 days post treatment FWEC}) \times 100}{\text{Baseline FWEC}}$$

(Stratford, McGorum *et al*. 2011)

2.6 Statistical Methods

The main output variable from the study was FWEC, which are known to be inherently variable and follow a highly aggregated count distribution. Several methods of statistical analysis have been proposed to analyse such data (Vidyashankar, Kaplan *et al*. 2007; Denwood, Stear *et al*. 2008; Denwood, Reid *et al*. 2010) - all are computationally intensive statistical methods, which are required to generate appropriate confidence intervals. Simple nonparametric tests such as Wilcoxon and Spearman Rank correlation were used in the primary qualitative presentation of results. However an autoregressive analysis using Baysian Markov chain Monte Carlo (MCMC) was used for statistical modelling of the data. These methods have the advantage of estimating full posterior distributions for all the parameters and allow appropriate 95% confidence intervals to be obtained. The model was run from the statistical package R (R Development Core Team, 2009) using JAGS (Plummer 2008) through the runjags package (Denwood 2008). Minimally informative priors were used and each model was run to convergence, trace plots checked to confirm convergence, and the deviance information criterion (DIC) calculated to assess model fit. A similar method was used to analyse the FECRT data set, which has been previously shown to perform better than other methods under most conditions (Denwood, Reid *et al*. 2009). A generalised linear mixed model (GLMM) was used to analyse the pasture larval count data. Further details of the precise statistical methods used for each analysis are given in the following chapters.

Evaluating the effectiveness of pasture hygiene and TST using FWEC

3.1 Introduction

The FWEC is the most commonly used method for quantifying strongyle infections, but is known to be a poor indicator of adult parasite numbers (Lyons, Tolliver *et al*. 1983). A FWEC can be thought of as indicator of subsequent pasture infectivity as well as a diagnostic indicator of parasitic disease. FWEC are also known to be highly variable (Uhlinger 1993) with sources of variability stemming from, amongst other sources, inconsistent shedding within the host and inconsistent distribution of eggs within the faecal output (Vidyashankar, Kaplan *et al*. 2007). Pasture infectivity and subsequent parasite numbers can be reduced through pasture hygiene methods such as regular faeces removal either by manual removal or by an automated pasture sweeper (see Chapter 1.4.6). Another method of control is the use of Targeted Selective Treatment (TST), only treating those animals with a FWEC above a designated 'cut-off' level (see Chapter 1.4.7). The effectiveness of parasite control methods was assessed by analysis of the FWEC of the donkeys over the course of the study using parametric methods which have previously been applied to the examination of FWEC and FECRT (advocated by Denwood 2008, Denwood, Reid *et al*. 2009 and further validated by the same authors in 2010).

3.2 Materials and methods

Each of the donkeys within the 15 groups in the four trials was sampled at four weekly intervals (see Appendix 1 for a full sampling schedule) from late April 2010 to November 2010. These samples were analysed on-site in the parasitology lab at The Donkey Sanctuary Devon, using the McMaster method described in Chapter 2.2. This resulted in six or seven sets of FWEC data within each trial.

In addition to the seasonal patterns of FWEC, comparisons of the initial FWEC of the donkeys to that of the other equidae in the study are presented below and compared using Wilcoxon rank tests. The FWEC of male donkeys was compared to those of females and the influence of age was examined using a Spearman Rank Correlation. This preliminary analysis was made using the first FWEC of each donkey to avoid confounding factors introduced once the parasite control methods were underway, as factors such as age and gender were not rigorously matched for when the groups were compiled.

To overcome the inherent variability and obtain appropriate 95% confidence intervals for parameters of interest, the use of computationally intensive parametric methods is often necessary to analyse FWEC data. An autoregressive analysis using Bayesian Markov chain Monte Carlo (MCMC) statistical methods were used to assess the true impact of the pasture control methods on the egg output of the Donkeys within the study. The structure of the model was as follows:

- The number of observed eggs (EPG/50) was assumed to follow a Poisson distribution, with log mean following a normal distribution with mean equal to the log of the true FWEC of that animal at that time ($Mean_{at}$)
- $Mean_{at}$ for the first observed time point was modeled as a linear regression with common intercept, random effect of animal, and fixed effects of age and sex
- $Mean_{at}$ for the subsequent time points was modeled as a linear regression based on the previous time point plus a random effect of month, random effect of animal within month, and fixed effects of pasture control method and treatment (where treatment was applied to that animal after the previous month's FWEC)

From this model, estimates of pasture control method effect, along with treatment, sex and age effects can be made. The model was run with and without the effects of sex and age and model fit compared using deviance information criterion (DIC) (Spiegelhalter, Best *et al*. 2002). A similar model was also used to assess the effects of the different dosing thresholds for TST within trial 4, with the fixed effects of pasture control methods in the first model being replaced with fixed effects of dosing thresholds.

The model was run using JAGS (Plummer 2008) from R using the runjags package (Denwood 2008). For each model run, convergence was assessed using the Gelman-Rubin statistic with 2 chains, and run length assessed using the Raftery and Lewis diagnostic. All trace plots were also assessed for convergence by eye.

3.3 Results

No animal developed clinical signs of parasitism during the period of the study.

3.3.1 Mean FWEC

In total each group went through either six or seven rounds of testing. The pattern of mean FWEC across the 2010-grazing season for all groups across the four trials are shown in Figure 3.1a, sorted by pasture management strategy. The presence of a peak egg output during the summer months was noted in all of the groups. Trial 4, which had a different study design to the other three trials, based on a TST approach, contains a smaller peak and maintains a lower average than the other trials.

Within trials 1-3 each of the three pasture treatments, automated removal of faeces (Automated), manual removal (Manual) and No Removal were applied to the individual groups. The Manual removal groups (Figure 3.1b; top right) had a lower mean FWEC compared to those groups that underwent Automated (top left) and No Removal (bottom left). A maximum value of less than 900 EPG was seen for Manual removal whereas counts of over

1200 EPG can be seen for Automated removal, and mean FWEC as high as 1500 EPG where faeces where not removed. Mean FWEC was on average lower for trial 4 pastures on which there were lower dosing thresholds.

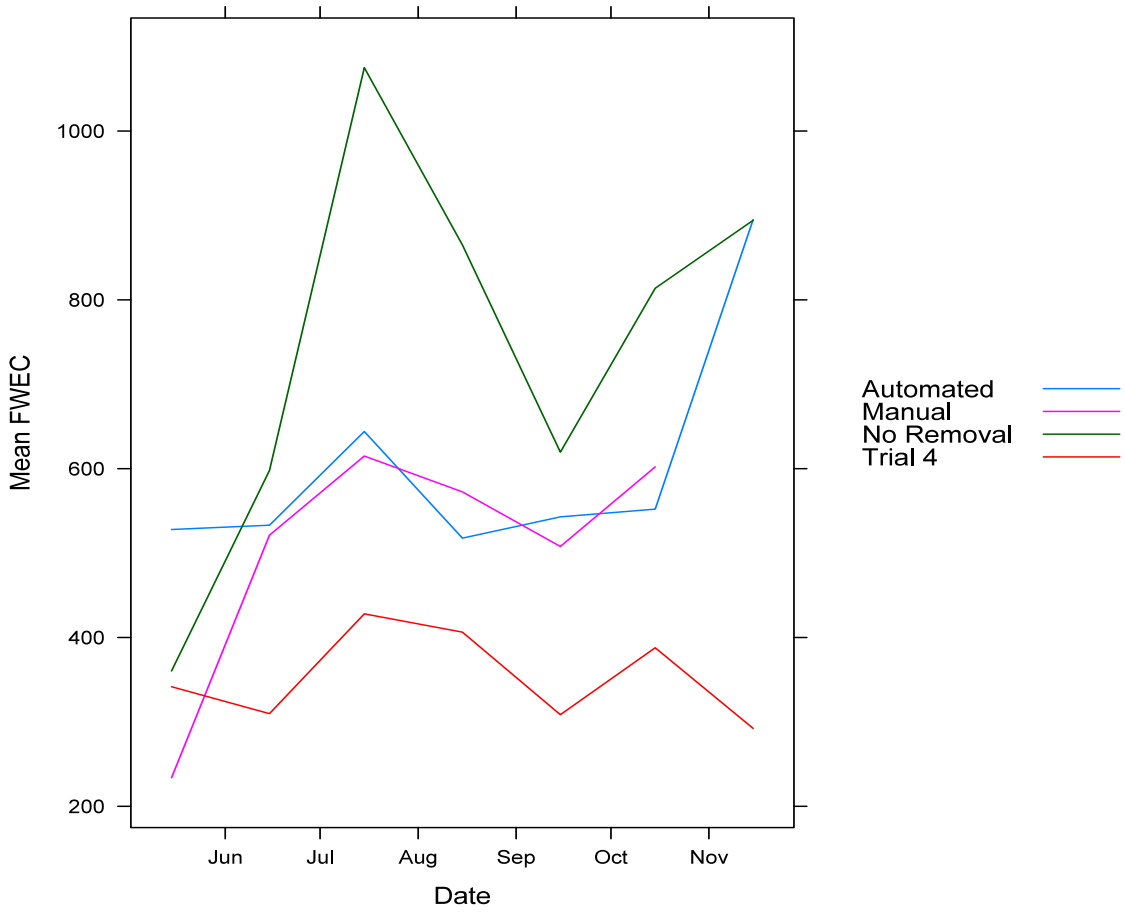

Figure 3.1(a) Mean FWEC (EPG) taken from 667 donkeys over a 6 month period in 2010 at The Donkey Sanctuary Devon. The pink and blue lines represent the pastures on which faeces were removed twice a week by hand or automated sweeper, respectively. The green line shows the pastures on which no removal of faeces took place and the red represents trial 4, which was under a different management strategy of lower varied treatment thresholds and no faeces removal.

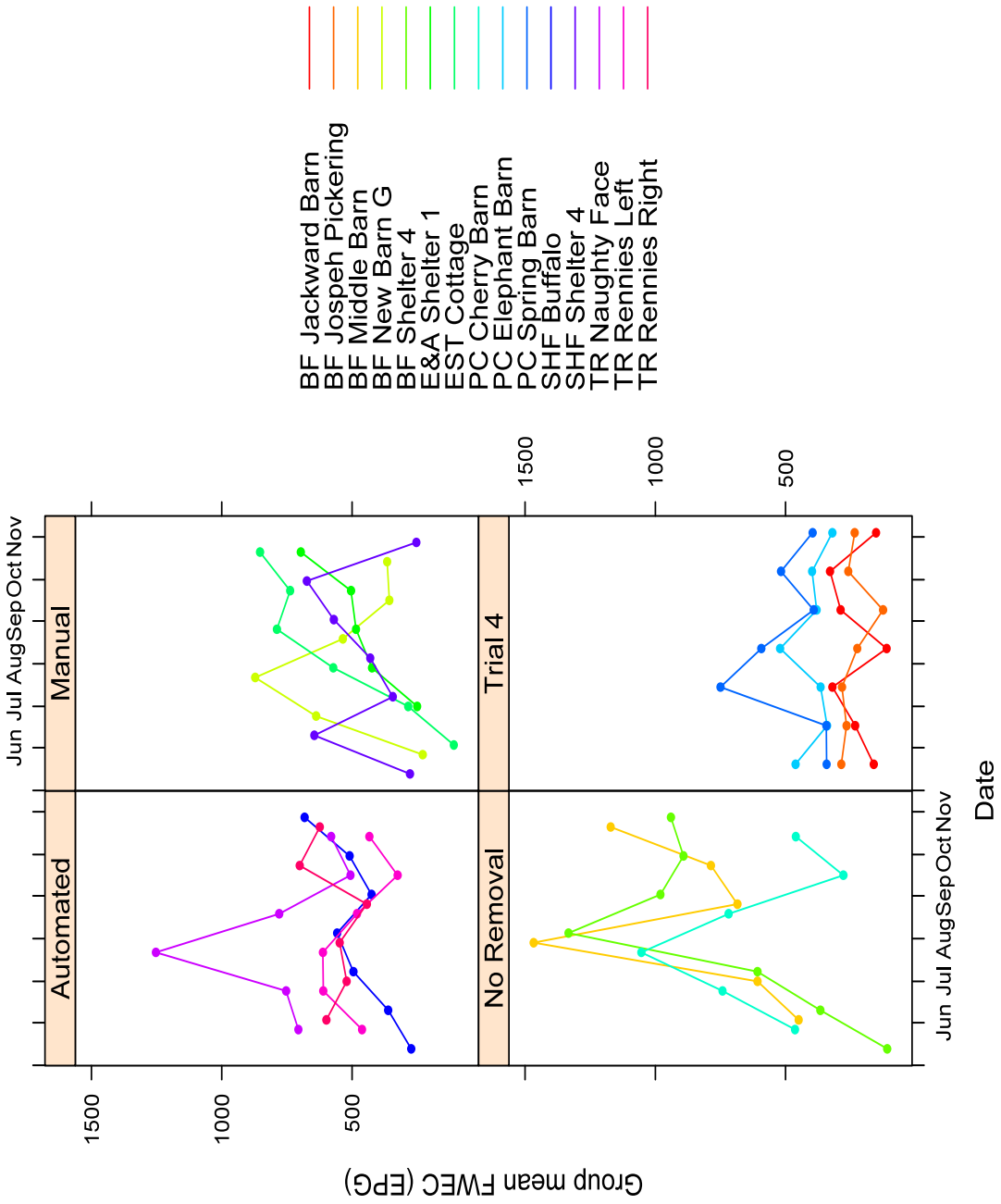

Figure 3.1 (b). The mean FWEC (in EPG) for each of the individual groups/fields across the grazing season divided by pasture management strategy. The No Removal group contains some of the highest FWEC whereas Manual removal is lower. Trial 4 which operated a lower TST threshold contains lower FWEC.

3.3.2 Breed, Age and Gender analysis

Breed

The study consisted mostly of donkeys (n=667) but in addition there were 16 other non-donkey equidae housed within the study groups: namely three horses, eight mules and five ponies. Box plots comparing the mean FWEC of donkeys and other equidae at the beginning of the study are shown in Figure 3.2. Using a Wilcoxon rank sum test Donkeys were not significantly different from ponies (p=0.3413) but were almost significantly different to mules (p=0.07937) and horses (p=0.0545). Due to the small numbers, the non-donkey equidae were removed from the rest of the analysis.

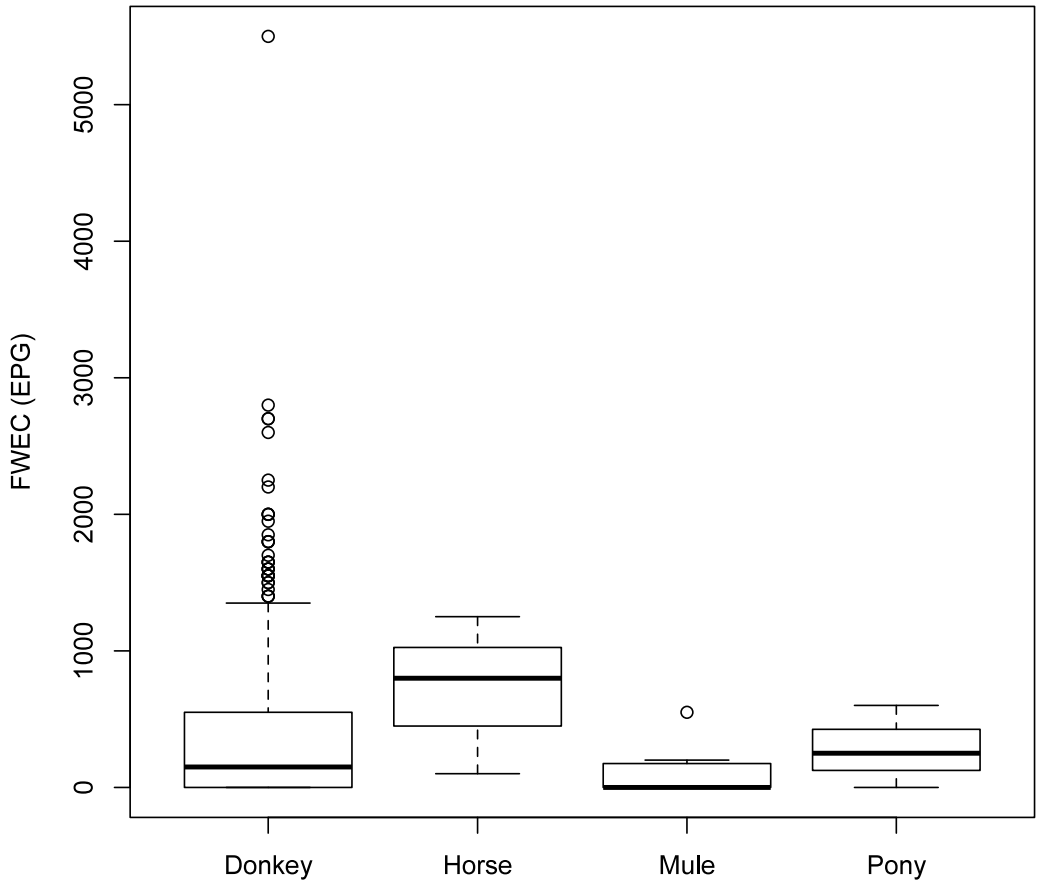

Figure 3.2: Comparison of FWEC between donkeys, mules, horse and ponies collected from the Donkey Sanctuary Devon at the start of the study in Spring 2010. Numbers of animals are as follows: Donkeys (n= 588), Horse (n=3), Mule (n=9), Pony (n=2).

Age

When the FWEC from the individual rounds of testing were compared to age using a Spearman Rank Correlation there was a significant correlation within the first round of FWEC testing ($p<0.001$), shown in Figure 3.3 below. However, when average FWEC across the grazing reason was compared to age this significance was lost ($p= 0.2474$) see Figure 3.4 below.

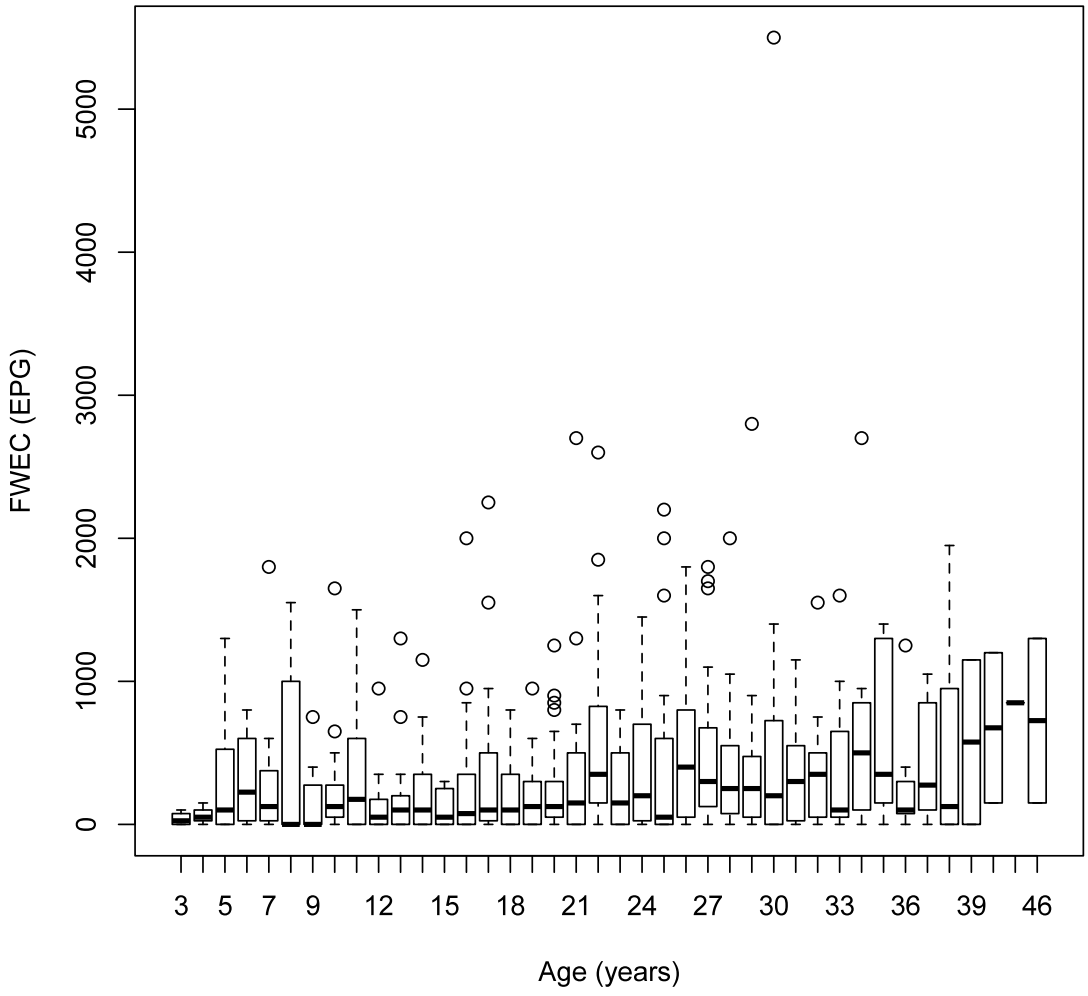

Figure 3.3: The relationship between the FWEC and age of 588 donkeys in the Donkey Sanctuary Devon, with FWEC collected at the first round of testing in Spring 2010.

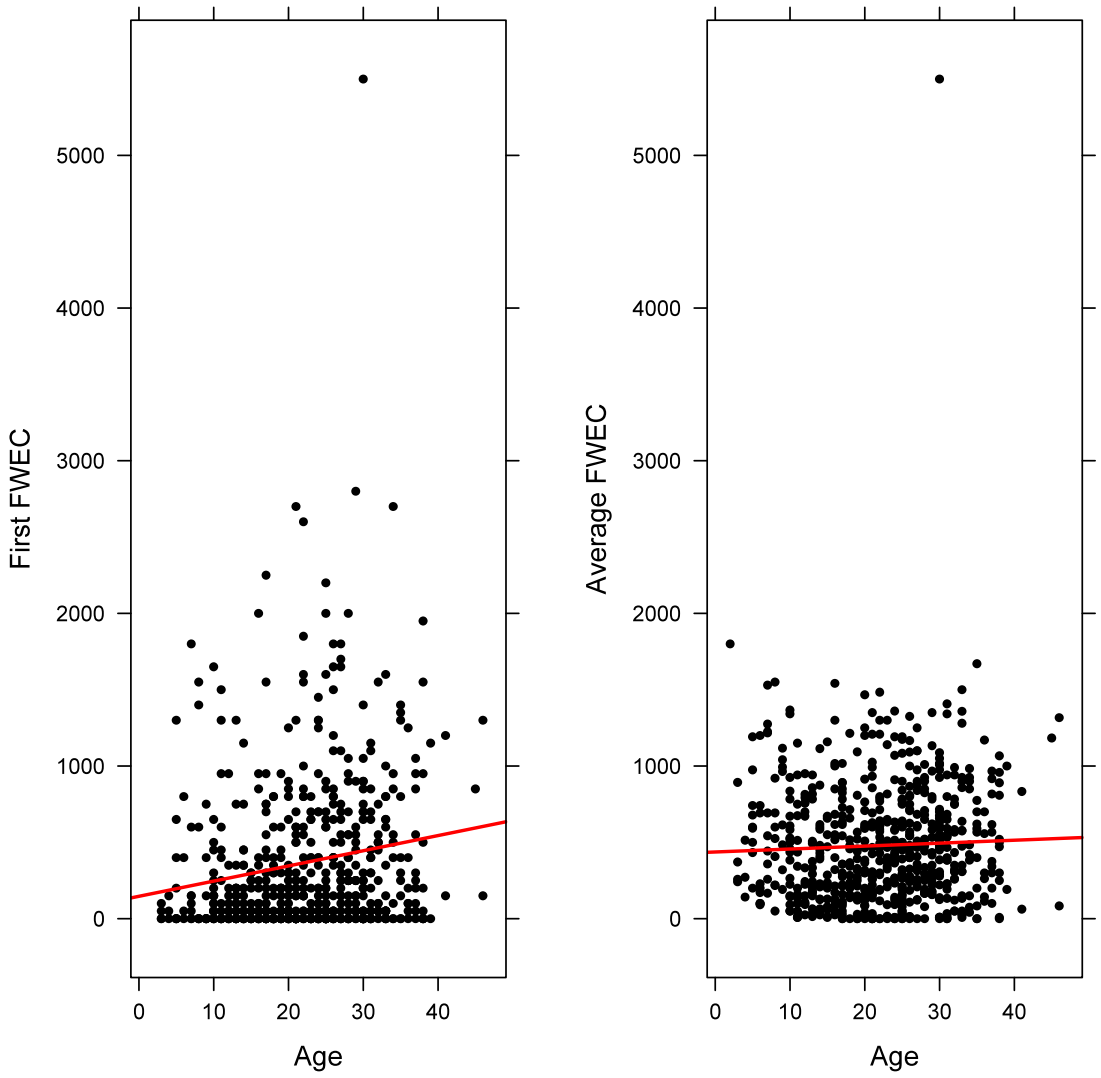

Figure 3.4 Initial FWEC by age (in years) for the first spring FWEC (Left) taken at the start of the study and the average FWEC across the whole season (Right). The line of best fit is shown in red, correlation was lower for the FWEC averaged across the season.

Gender

Figure 3.5 shows a boxplot of the initial spring FWEC for gelded males (DKG), females (DKF), excluding the single stallion donkey (DKS). There was a significant difference in FWEC between DKF and DKG (Wilcoxon p-value = 0.009) with females shedding on average 432 EPG (524 EPG when averaged across the study) and males shedding on average 321 EPG (445 EPG when averaged across the study). The single stallion donkey was removed from the rest of the analysis.

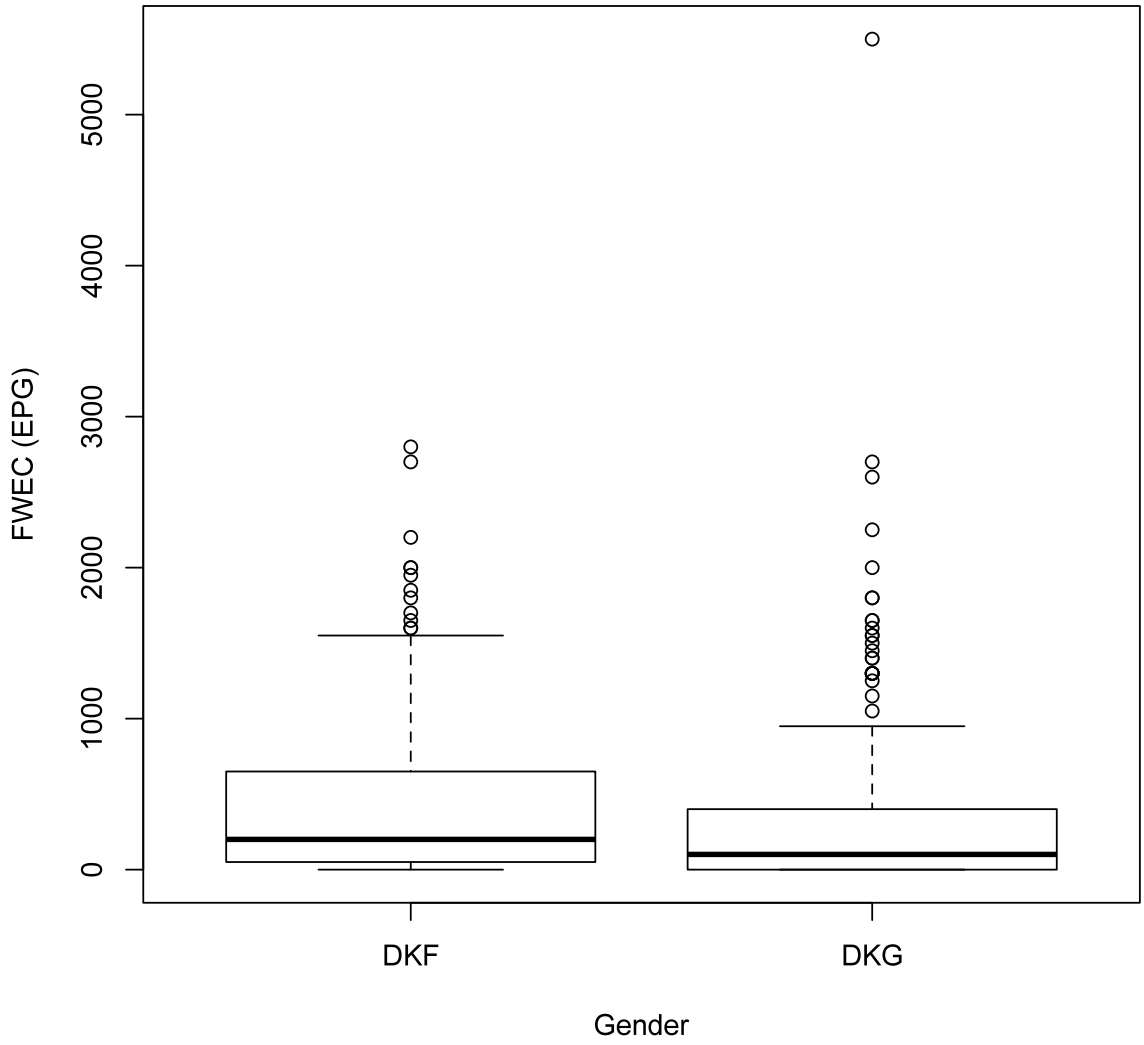

Figure 3.5: FWEC for the different Genders for at the start of the study (Spring 2010). Female donkeys (DKF) n=241, shed an average of 432 EPG in the first month of the study, where as male donkeys (DKG) n=341 which shed an average of 321 EPG.

3.3.3 Modelling for Trials 1-3

The effect of the different pasture treatments was assessed using an autoregressive Bayesian MCMC model for data from trials 1-3. The model fit was marginally better with inclusion of both age and sex effects (DIC=12318.7) compared to age only (DIC=12319.1), sex only (DIC=12319.7) and neither age or sex (DIC=12319.2) models. Posteriors for common parameters did not differ to a great extent between models, so only results for the best fitting model are presented.

Median and 95% confidence interval estimates for the fixed effects are shown in Table 3.1. The 95% CI for age (linear effect) and sex (gelding relative to female) include 0 at the extremes of the intervals, indicating a borderline significant effect of each. The dosing effect does not overlap zero, which shows a significant effect of anthelmintic dosing on FWEC.

Table 3.1: Fixed effect estimated from autoregressive Bayesian MCMC analysis on FWEC data from trials 1-3

Fixed Effect	Lower 95	Median	Upper 95
Dosing	-3.291	-2.983	-2.681
Gender (DKG vs DKF)	-0.660	-0.332	0.002
Age	-0.005	0.015	0.035

Median and 95% confidence interval estimates for the variance of the random effects is shown in Table 3.2. The most important source of variance was differences between individual animals, with sample (associated with over dispersion of FWEC) the next most important. There was comparatively little difference between fields (after taking into account pasture management type) and months.

Table 3.2: Variance parameter estimates for random effects from autoregressive Bayesian MCMC analysis on FWEC data from trials 1-3

	Lower 95	Median	Upper 95
Sample	0.196	0.264	0.358
Animal	1.402	1.725	2.158
Field	0.016	0.093	1.426
Month	0.026	0.082	1.458

The full posterior for effect of the three pasture control methods relative to each of the others is shown in Figure 3.6. The 95% CI for Automated compared to Manual removal was -0.099 to 0.078, i.e. there was not a significant difference between these two methods of removal. However, Automated removal was significantly different to No removal, with 95% confidence intervals of -0.313 -0.147; as was Manual removal compared to No removal (95% CI -0.313 -0.125). So Manual and Automated removal although not significantly different to each other were both significantly different to No Removal.

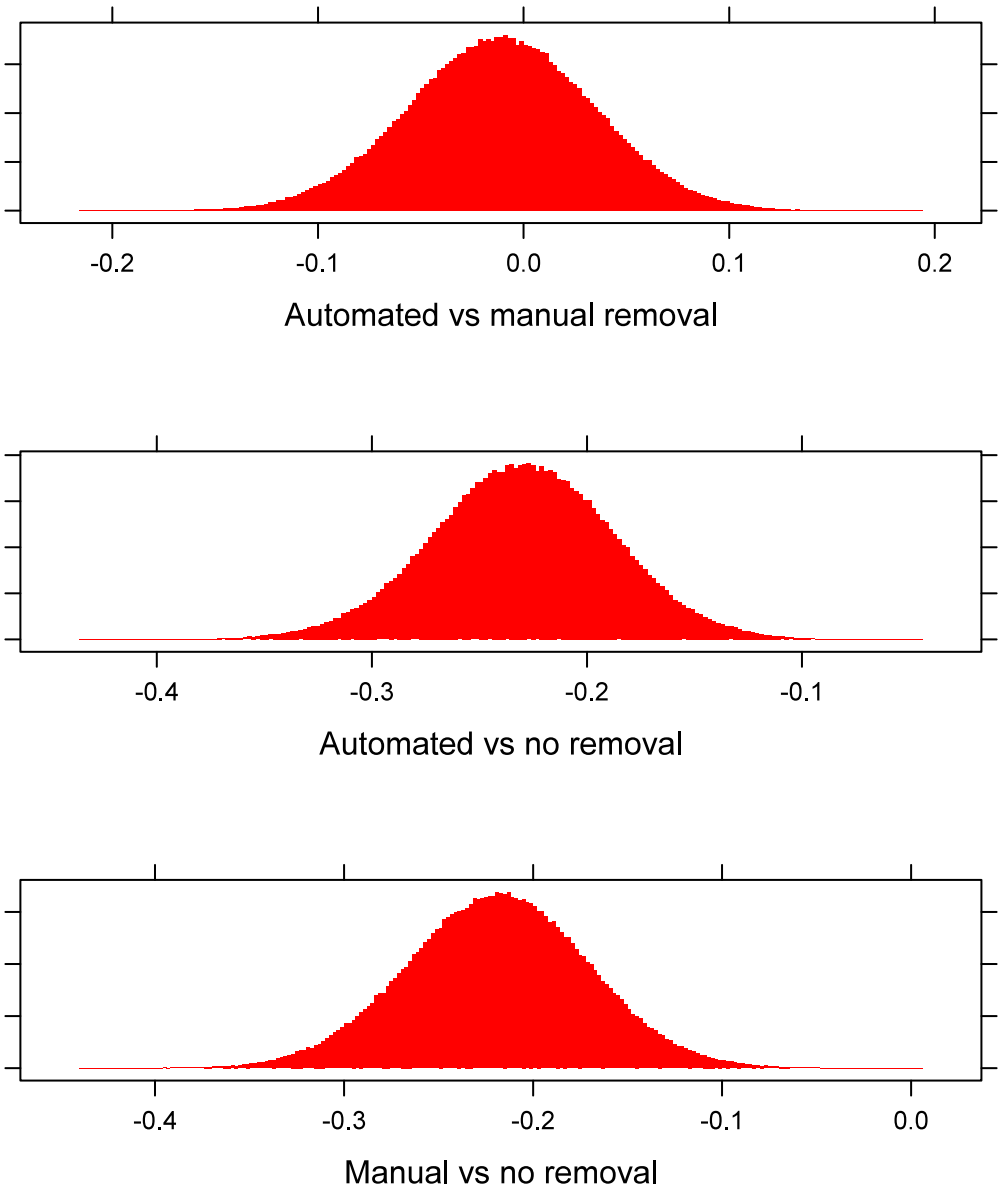

Figure 3.6: Full posterior distributions for the effect on donkey FWEC of three pasture control methods relative to each other. A 95% confidence interval spanning zero is representative of no significant difference.

3.3.4 Modelling for Trial 4

Data from trial 4 were analysed using a separate autoregressive model to evaluate the effects of the different treatment thresholds. Again, the model fit when including both age and sex effects (DIC=10420.2) was marginally better than the model fit with age only (DIC=10421.0), sex only (DIC=10423.0) and neither age nor sex (DIC=10420.7). Posteriors for common parameters did not differ to a great extent between models, so only results for the best fitting model are presented.

Median and 95% confidence interval estimates for the fixed effects are shown in Table 3.3. As for the trials 1-3 results, the 95% CI for age (linear effect) just includes 0, indicating a borderline significant effect, however gender does not have a significant effect for the trial 4 data. As with trials 1-3 anthelmintic dosing does have a significant effect, however this effect is less pronounced within trial 4.

Table 3.3: Fixed effect estimates from autoregressive Bayesian MCMC analysis for FWEC data from trial 4.

	Lower 95	Median	Upper 95
Dosing	-2.694	-2.384	-2.080
Gender (DKG vs DKF)	-0.517	-0.167	0.190
Age	-0.005	0.020	0.046

Median and 95% confidence interval estimates for the variance of the random effects are shown in Table 3.4. The most important source of variance was differences between individual animals, with sample (associated with over dispersion of FWEC) the next most important. There was also a substantial difference between fields (after taking into account pasture management type), and comparatively little difference between months.

Table 3.4: Variance parameter estimates for random effects from autoregressive Bayesian MCMC analysis of FWEC data from trial 4

	Lower 95	Median	Upper 95
Sample	0.227	0.367	0.589
Animal	1.388	1.767	2.330
Field	0.036	0.250	40.864
Month	0.039	0.133	2.804

The full posteriors for the effects of each treatment threshold relative to the other treatment thresholds are given in Figure 3.7. After accounting for the direct effects of treatment it was found that there was a significant effect on the monthly change in log FWEC of increasing the dosing threshold from 300 EPG to 600 EPG (95% CI 0.0133 0.232), and from 300 EPG to 1000 EPG (95% CI 0.106 0.358), but not from 600 EPG to 1000 EPG (95% CI -0.015 0.232).

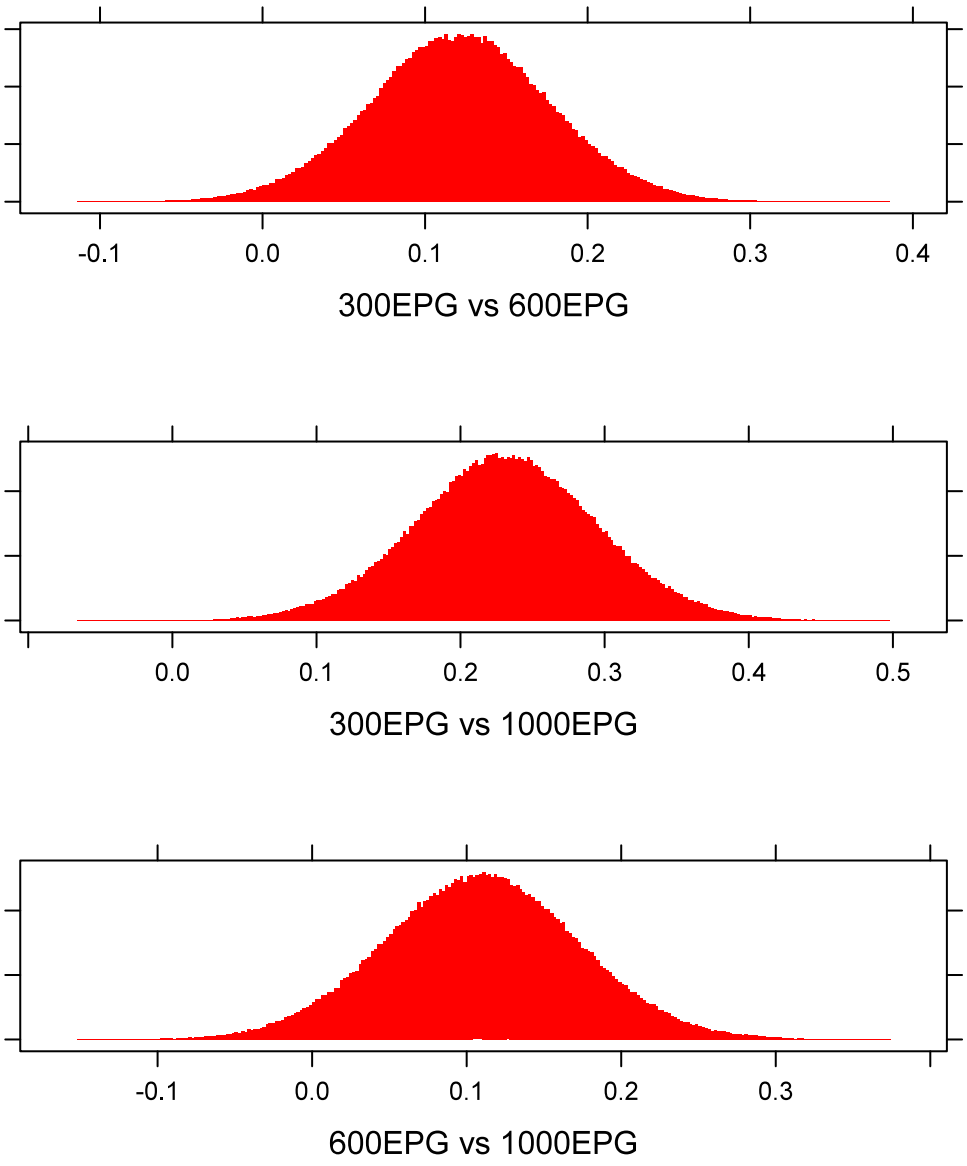

Figure 3.7: Full posterior distributions for the effect on FWEC of different TST dosing thresholds methods relative to each other. A 95% confidence interval spanning zero is representative of no significant difference.

3.4 Discussion

The qualitative results presented in this chapter show a clear trend towards higher group mean FWEC in the donkeys grazed on pastures with no faeces removal compared to donkeys grazed using both automated (mechanical) and manual methods of pasture hygiene. This difference was particularly evident during July and August, where a peak in mean FWEC of over 1000 EPG is observed in all three no removal groups and only one of the eight pasture hygiene groups. A quantitative analysis of these data using computationally intensive Bayesian statistical methods confirmed the statistical significance of the qualitative trends observed. The median estimate for the monthly additive effect of pasture hygiene compared to no removal of faeces was in the region of -0.25 on the natural log scale, which translates to a 22% reduction in individual animal FWEC after accounting for the direct effects of anthelmintic dosing. This result supports the findings of Herd (1986) that pasture hygiene can be successfully used as a method of cyathostomin control. The effect of automated relative to manual faeces removal was close to zero, indicating that there is little evidence to suggest that one method is superior to the other based on FWEC – although it is interesting to note that the only faeces removal group in which a group mean FWEC of over 1000 EPG was observed was an automated removal group.

While effective, removal of faeces from pasture may not always be an option – as is the case the groups of donkeys that were examined as part of trial 4. For these animals, there was a significant positive increase in FWEC associated with increasing the dosing threshold from 300 EPG to 600 EPG, and from 300 EPG to 1000 EPG, after controlling for the direct effect of anthelmintic dosing. This indicates that allowing donkeys to contribute a greater amount of pasture contamination before dosing results in a higher infection pressure on the group, which is as expected. Interestingly, the reduction in FWEC associated with decreasing the dosing threshold from 1000 EPG to 300 EPG is very similar to that obtained by initiating pasture hygiene, which provides an easily interpretable indication of the usefulness of pasture hygiene methods. It was also notable that the estimated direct effect of dosing (the change in FWEC of that animal between immediately pre-dosing and 4 weeks later) was significantly stronger for the trials 1-3 model than in the trial 4 model – indicating a lower efficacy of the same drug in the trial 4 animals. This issue will be explored further in the following chapter.

Although the primary aim of the FWEC study was to evaluate the effect of treatment

threshold and pasture hygiene method, inference can also be made on individual characteristics such as breed, age and sex, as well as some estimates of the variability structure of FWEC in donkeys. Based on qualitative analysis of individual animal FWEC data it appears that that horses have higher FWEC than donkeys, and mules have lower FWEC. These differences were not statistically significant, almost certainly due to the small numbers of non-donkeys available, but were potentially large enough to be of relevance to cyathostomin control – especially when co-grazing horses with donkeys or mules. This could be due to acquired immunity, genetic susceptibility (Love and Duncan 1992) or species specific cyathostomin populations (Lichtenfels, Kharchenko *et al*. 1998) and raises the question as to whether co-grazing donkeys with horses raises their exposure to cyathostomins to a greater level than if they were grazed solely with co-specifics or mules. There was also a clear correlation between increasing donkey age and the FWEC at the beginning and end of the grazing season; although interestingly this correlation was lost for the typically higher mid season FWEC and the season mean FWEC. This may be because during the middle of the grazing season there is a strong infection pressure due to large numbers of infective L3 on pasture that effectively swamps the variation in baseline FWEC between individuals due to factors such as age and sex. The median estimate for age effect was also positive for both modeling datasets, although for each the lower 95% confidence interval was just below zero indicating that the effect was not significant for either the trials 1-3 data or the trial 4 data in isolation. In any case, this finding is not consistent with previous studies that found a negative association between age and FWEC in horses (Chapman, French *et al*. 2003; Becher, Mahling *et al*. 2010), indicating a potentially important difference between the two species.

An effect of sex on FWEC was also found. From the descriptive analysis of FWEC, the mean FWEC of donkey geldings was found to be lower than the mean FWEC of females, for both the first month FWEC and season average FWEC. This finding was also borne out by the quantitative analysis with a lower FWEC in geldings from the trials 1-3 model, although the 95% confidence interval for sex effect from the trial 4 model was much larger, possibly because all trial 4 groups had higher numbers of DKG than DKF.

The autoregressive model was also able to make some inference on the variance structure of the FWEC data. A large amount of variation was observed between individual donkeys, even after accounting for common factors of age, sex and management group. The variance due to sampling, which accounts for the non-random distribution of nematode eggs within faeces as

well as any small variation in laboratory technique, was also substantial – with a median estimate that was higher than that for the variance between fields. These findings were consistent between the trials 1-3 and trial 4 datasets (although there was more variation between fields in trial 4), and with a similar study conducted in horses (Denwood, Love *et al*. 2012). These results highlight the importance of random variation in FWEC within an animal, resulting in the previously reported poor repeatability of the method (Vidyashankar, Kaplan *et al*. 2007; Denwood, Reid *et al*. 2010).

Several different sections of staff from The Donkey Sanctuary Devon were involved in the data collection and laboratory analysis. No rigorous blinding took place and twice weekly faeces removal would have been visible to all staff on the site. Those who collected the faecal samples would have been most familiar with the study, however this is unlikely to introduce biases as they were limited to collecting freshly passed faeces, had little knowledge of the egg counting process and passed samples onto the lab for analysis. The laboratory staff performing the egg counts would have been most oblivious to the study structure, limiting bias in this area. Dr Faith Burden kept the database and recorded the results, and was fully acquainted with the study design and structure and so was a possible source of bias, but this is considered to be unlikely.

Secondary methods of evaluating pasture hygiene and TST

4.1 Introduction

While the main part of this study was involved with monthly FWEC collected from individual donkeys, other data were also collected in the form of pasture larval counts and measures of anthelmintic efficacy at the end of the grazing season. As part of the TST protocol described in Chapter 2, the faeces of each donkey were sampled on a four-week basis, according to group and trial, and any animals with FWEC exceeding the predetermined cut-off levels (300 EPG for BF Jackward and BF Joseph Pickering, 600 EPG for PC Elephant Barn, 1000 EPG for PC Spring Barn, and 2000 EPG for the remaining groups,) were dosed with Pyrantel embonate at a rate of 19mg/kg. The aim of TST is to dose only the animals shedding high numbers of eggs, reducing pasture contamination while limiting anthelmintic exposure to the whole group and maintaining refugia in untreated animals.

Within trials 1-3 it can be hypothesized that many high FWEC, resulting in a high number of anthelmintic doses being administered, is a reflection of poor cyathostomin control. Conversely low and zero (negative) FWEC potentially indicate good control, whether through good pasture cleanliness, good immunity within an animal or effective anthelmintic dosing. Therefore, the proportion of animals with negative FWEC could also be considered as an indirect measure of the efficacy of cyathostomin control within a group. The pasture larval counts, described in Chapter 2, are also an indirect measure of pasture hygiene. This chapter is concerned with evaluating these secondary measures of the effectiveness of the pasture control programs.

4.2 Study Design

4.2.1 Proportion of animals given targeted strategic treatment

Multiple high FWEC will result in frequent use of anthelmintic doses. Repeated dosing is known to promote resistance (Wescott 1986; Kaplan, Matthews *et al*. 2004). Therefore the percentages of animals treated were compared with the efficacy calculated by the FECRT analysis, with the expectation that those groups where frequent dosing occurred would have lower efficacy than those groups where dosing was minimal.

4.2.2 Pasture Larval Counts

As well as measuring FWEC of the animals in the study, grass samples were taken from the individual pastures grazed, using the method described in Chapter 2. From these samples the pasture larval counts (PLC), in larvae per kilogram of dry herbage, were calculated. Theoretically PLC's are a reflection of the infectivity of the pasture at the time of sampling but there are no past studies of their use in donkeys and they are often regarded as somewhat variable and unreliable. However, their use was advocated here as they are non-invasive and ultimately it is the suppression of pasture L3 that is the goal of pasture control.

4.2.3 Faecal Egg Count Reduction Tests

Faecal Egg Count Reduction Tests (FECRT) have often been used to assess drug efficacy (Coles, Bauer *et al*. 1992), but have also come under criticism for high variability and subsequent unreliability when analysed using inappropriate statistical methods (Vidyashankar, Kaplan *et al*. 2007; Denwood, Reid *et al*. 2009; Denwood, Reid *et al*. 2010). This has led to the development of computationally intensive statistical techniques to help validate and improve their usefulness. At the end of the study period a FECRT was carried out on a proportion of The Donkey Sanctuary Devon subjects (see Table 4.1 and Appendix). The data from this FECRT were analysed using the MCMC method described by Denwood, Reid *et al*. (2009) to measure the effectiveness of the Pyrantel embonate doses administered and to monitor for resistance.

4.3 Materials and Methods

4.3.1 Analysis of anthelmintic usage

Donkeys were dosed with Pyrantel embonate in response to a single individual FWEC exceeding the pre-designated cut-off (see Chapter 2). Within trials 1-3 this threshold was 2000 EPG; trial 4 had varied cut off points of 1000, 600 and 300 EPG and FWEC from this trial were therefore analysed separately. The proportions of animals either dosed or with FWEC of zero within each round are summarised to allow comparison between the 3 treatment groups.

4.3.2 Pasture analysis

The pasture larval analysis results are displayed as a line graph (Figure 4.5), along with the corresponding FWEC from the same period of time. A generalised linear mixed model (GLMM) with log-link Poisson response, management strategy as fixed effects and field and week of sampling as random effects along with a random effect of observation to allow over dispersion between Poison observations, was used to analyse the PLC data from trials 1-3. trial 4 data were analysed using a similar model with treatment threshold instead of management strategy as fixed effects, and the random effect of field removed due to the three thresholds overlapping almost totally with the four fields.

4.3.3 Faecal Egg Count Reduction Test

At the end of the study a FWEC was carried out on a cohort of donkeys from within seven of the groups in the study (Table 4.1). For each individual, two pre-treatment samples and three post treatment samples were taken from the same faecal pile. The data were analysed using the FECRT analysis functions of the bayescount R package (Denwood 2012), as advocated by Denwood, Reid *et al*. 2009. The groups and number of animals selected was as follows:

Table 4.1: Number of animals from each group within the FERCT carried out at the end of the study (Nov/ Dec 2010).

Field	Number of Donkeys used for FECRT	Total Donkeys
BF Middle Barn (Trial 2, No removal)	15	33
BF New Barn Grannies (Trial 1, Manual)	14	40
PC Cherry Barn (Trial 1, No Removal)	18	48
PC Spring Barn (Trial 4, 1000EPG)	29	82
SHF Buffalo (Trial 3, Automated)	13	27
SHF Shelter 4 (Trial 3, Manual)	5	23
TR Rennies R (Trial 2, Automated)	37	65

4.4 Results

4.4.1 Anthelmintic doses administered and FWEC of zero

Trials 1-3

The proportions of donkeys treated within trial 1-3 of the study are shown in Figure 4.1. Due to laboratory availability the subjects were sampled on a 4-week rotational basis resulting in seven rounds of testing, roughly equivalent to monthly sampling. A relatively high proportion of animals in the groups with no faeces removal exceeded 2000 EPG, and underwent subsequent anthelmintic dosing. Manual removal of faeces, in contrast resulted in the administration of significantly less anthelmintic doses ($p=0.05$) when compared to no removal. There was no significant difference between automated faeces removal and no removal ($p= 0.23$).

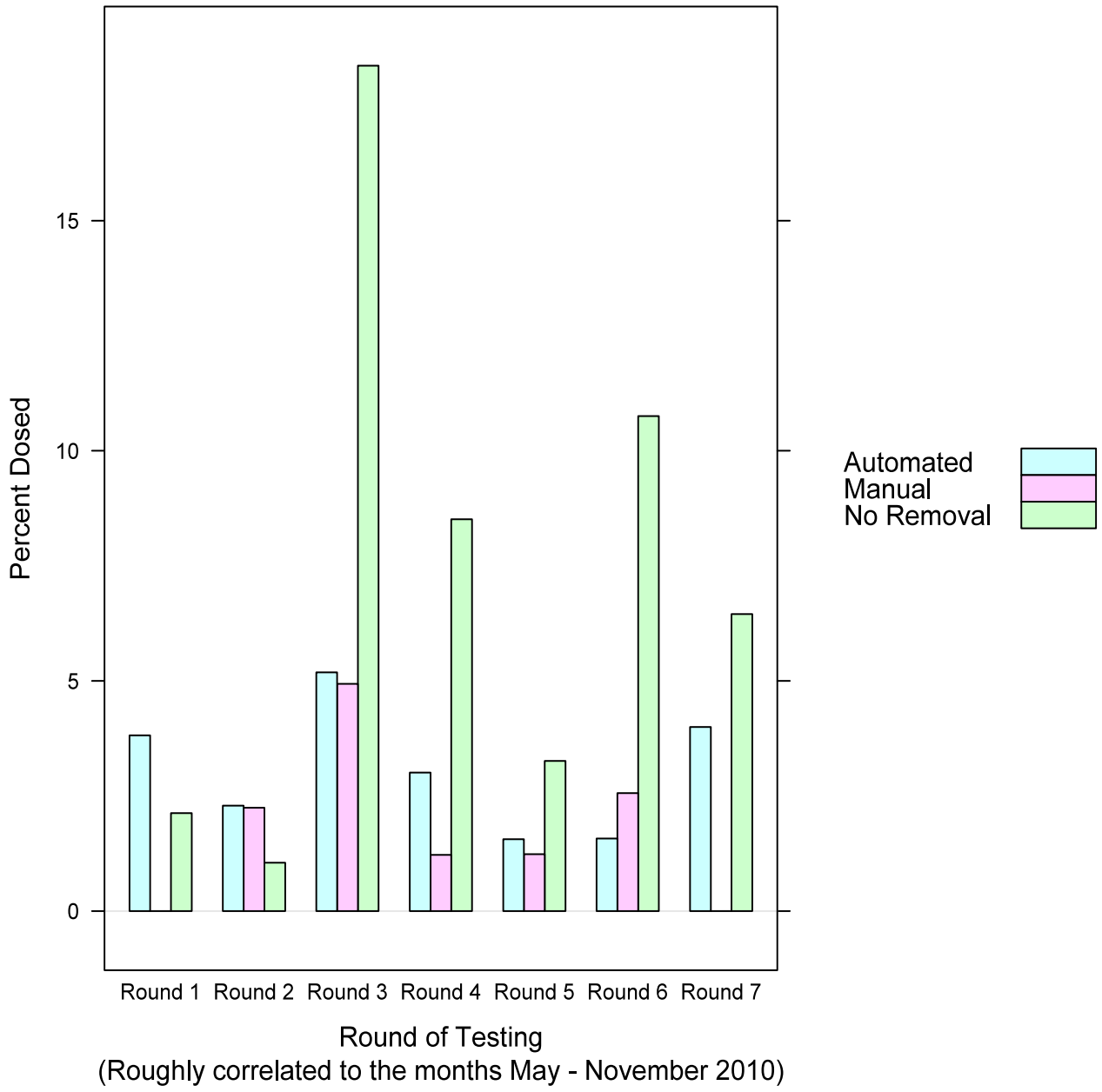

Figure 4.1: The proportion of animals dosed in each round of testing, combined for all groups within each management strategy. The no faeces removal groups shown in green are PC Cherry Barn, BF Middle Barn and BF Shelter 4. Manual removal, shown in pink represents the four groups BF New Barn Grannies, SHF EST Cottage, SHF E&A Shelter and SHF Shelter 4. The automated removal groups shown in blue are TR Rennies Right and Left, TR naughty face and SHF Buffalo.

When the proportions of donkeys with negative FWEC were compared the differences were less pronounced. Figure 4.2 illustrates the proportions of donkeys with FWEC of zero, within the Manual removal group nearly 50% of the subjects had negative FWEC by the end of the study, despite, as shown in Figure 4.1, having the lowest dosing rates.

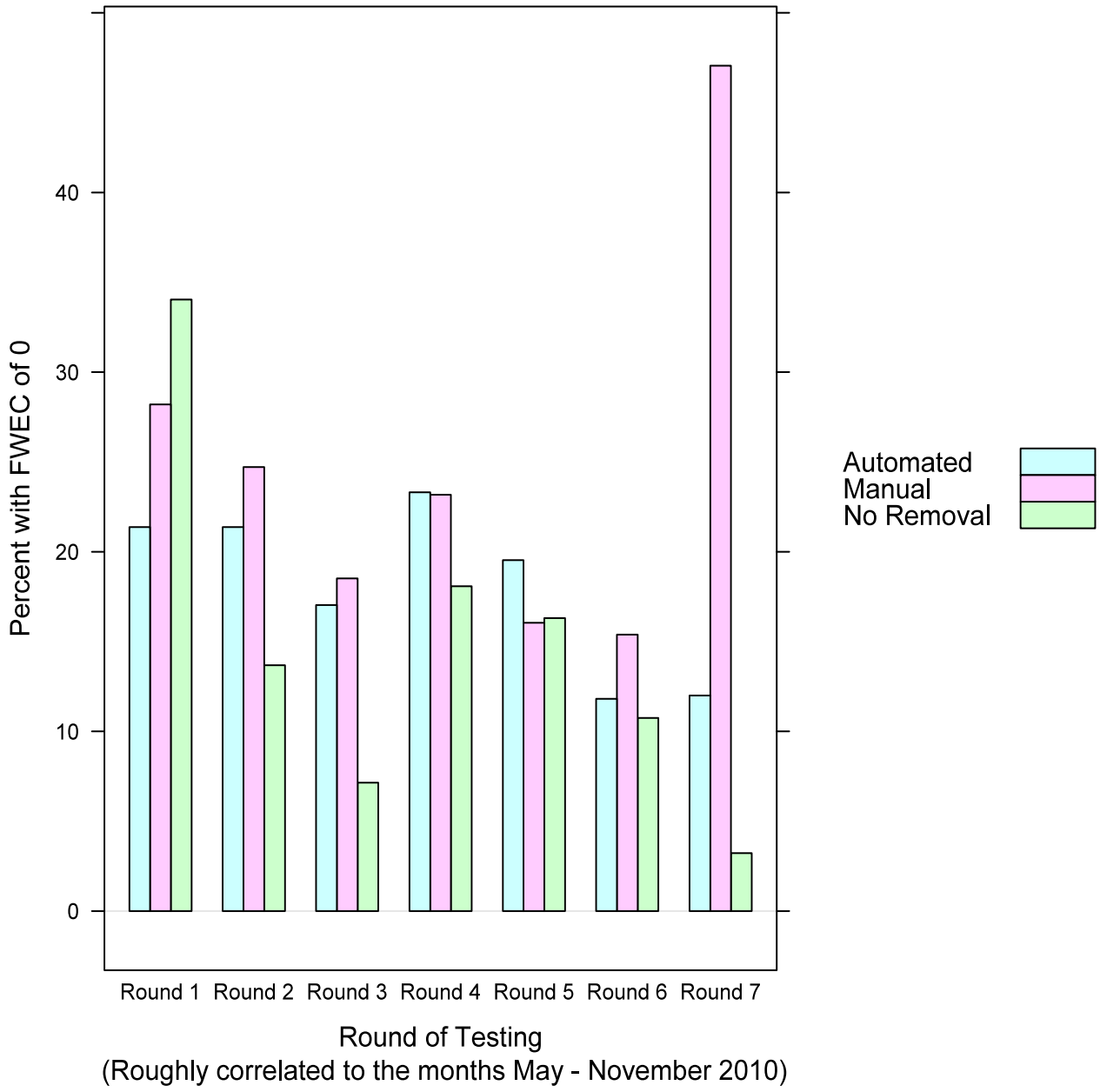

Figure 4.2: The proportion of animals with FWEC of zero in each round of testing, combined for all groups within each management strategy. The no faeces removal groups shown green are PC Cherry Barn, BF Middle Barn and BF Shelter 4. Manual removal shown in pink represents the four groups BF New Barn Grannies, SHF EST Cottage, SHF E&A Shelter and SHF Shelter 4. The automated removal groups shown in blue are TR Rennies Right and Left, TR naughty face and SHF Buffalo.

Trial 4

Trial 4 was analyzed separately to the other three trials. Figure 4.3 illustrates the numbers of doses administered across the 4 groups within trial 4. It can be seen that the proportions dosed

are far higher than in Figure 4.1 due to the lower dosing thresholds, and that the Brookfield groups (BF), with the lowest threshold have some of the highest levels of doses administered.

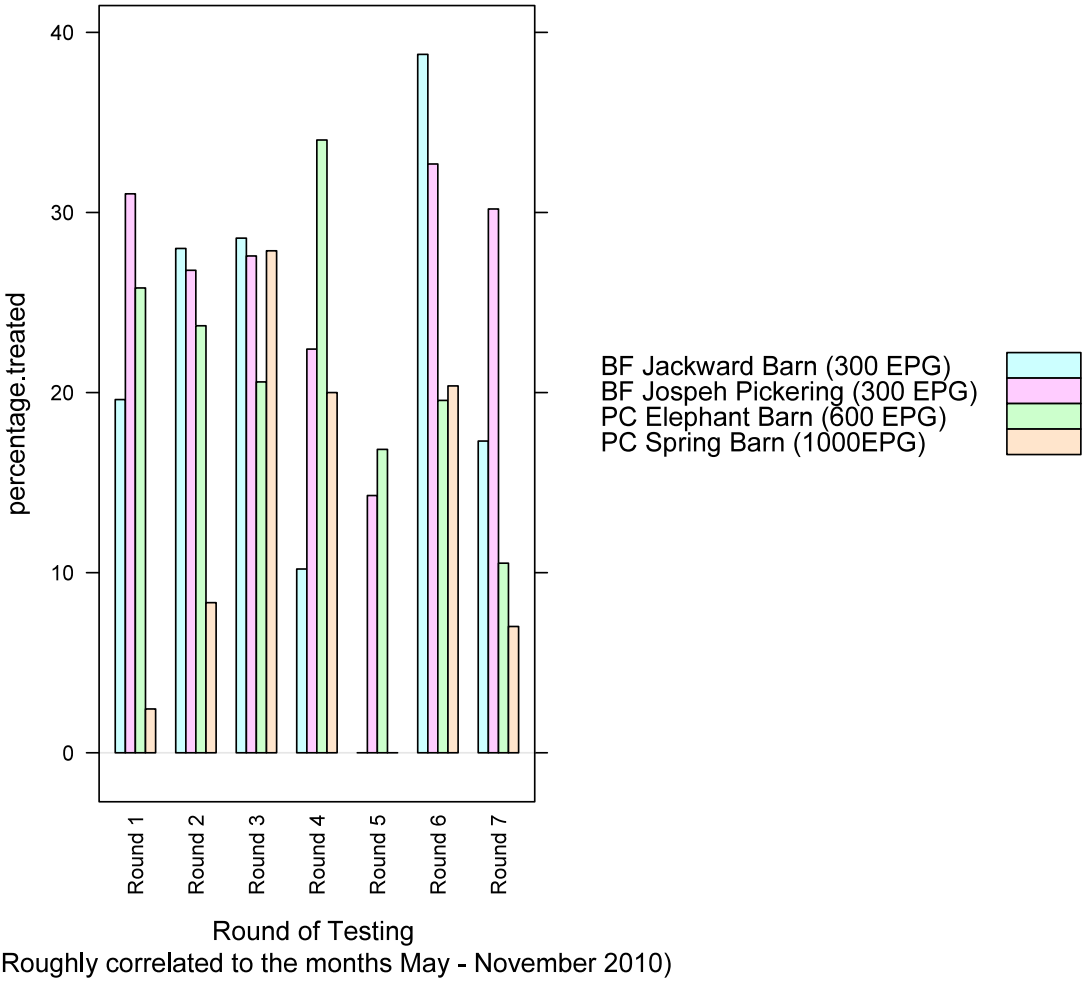

Figure 4.3 The proportion of animals exceeding the designated FWEC cut-off (and therefore dosed) for each group within trial 4. PC Spring barn, represented by the orange bar, had the highest dosing threshold and frequently had the lowest levels of dosing.

Figure 4.4 shows the proportions of negative FWEC within trial 4; once again the values are higher than those of the other three trials displayed in Figure 4.2. The Brookfield (BF) groups with the highest numbers of doses also show the highest proportions of negative FWEC.

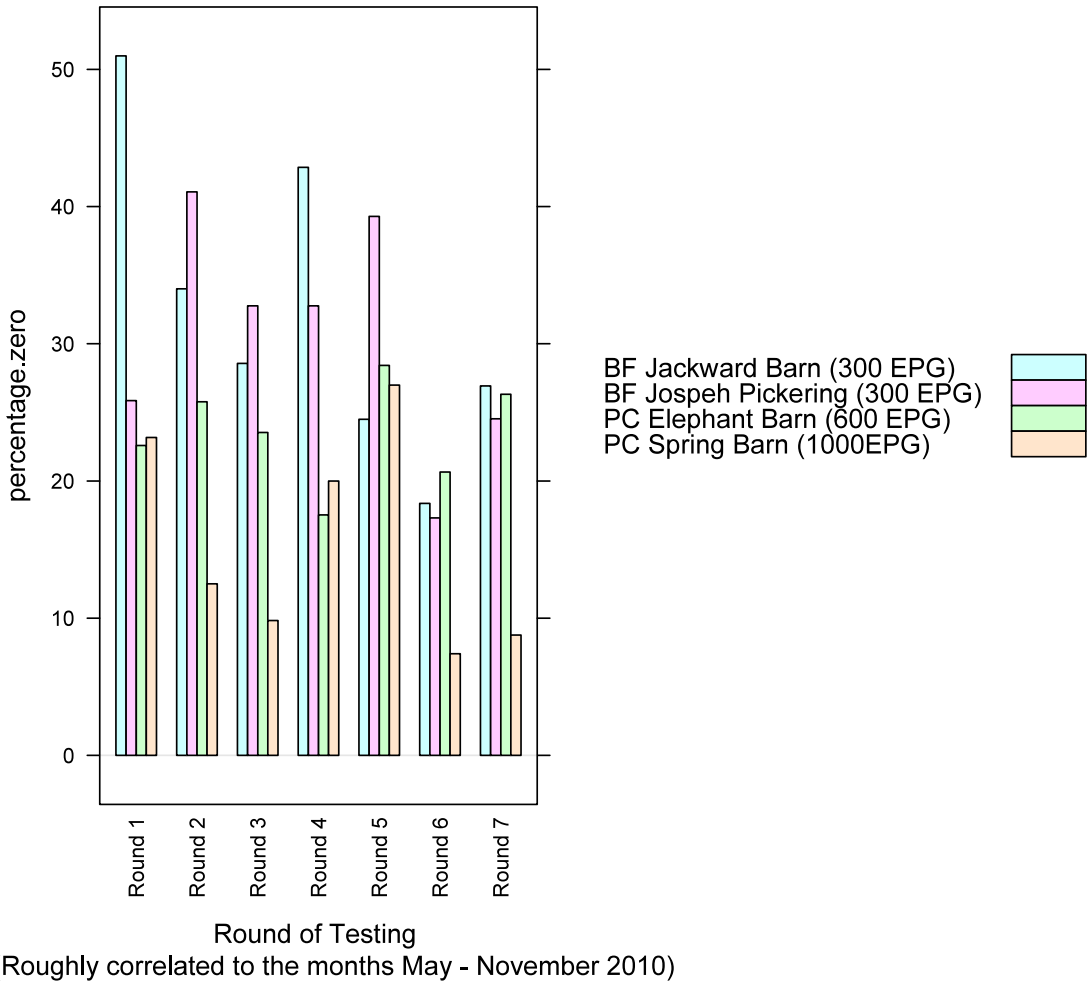

Figure 4.4: The proportion of animals with a FWEC of zero for each group within trial 4. The BF groups, shown in pink and blue, frequently had the highest proportions of animals with negative FWEC, whereas PC Spring Barn with a 1000EPG cut off had the lowest proportions of animals with a negative FWEC.

4.4.2 Pasture larval counts

Only cyathostomin L3 were recovered from the pastures at The Donkey Sanctuary Devon, pictured in Image 4.1 (a and b). The trends in pasture L3 counts (PLC) are shown in Figure 4.5 along with the FWEC of the donkeys on the pasture in the same time period for comparison. Those fields in which Manual removal of faeces occurred had the lowest PLC. A spring PLC carried out in 2011 (March 30th – April 19th) showed no larvae on all but one pasture, BF Middle Barn, a group that underwent no removal of faeces during the study period. This pasture had 115 larvae per kilogram of dry herbage.

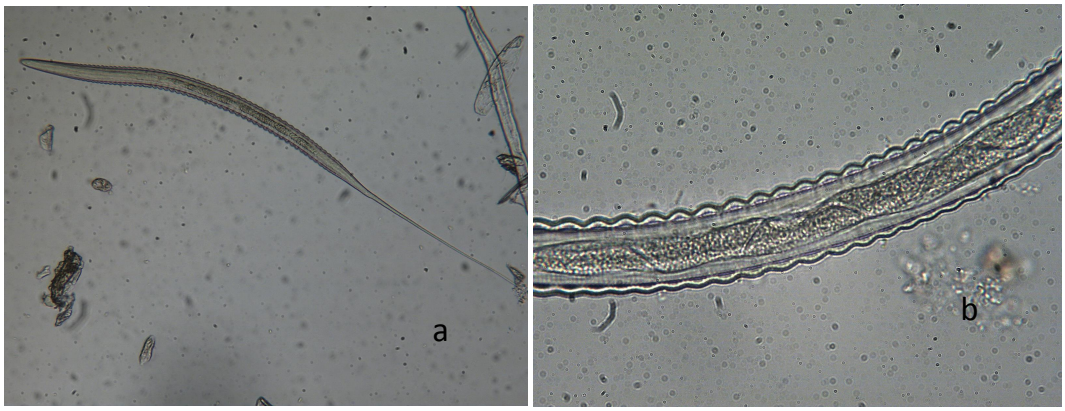

Image 4.1 (a): x100 L3 larvae recovered from pasture sample 23 taken from Jackward Barn on 14.06.2010. (b): Intestinal cells of L3 larva shown in Image 4.1 (a), shown at x400

For trials 1-3, pasture larval counts were consistently low in all Manual removal paddocks, but more variable in paddocks with Automated faeces removal. Manual removal of faeces had a significant effect on the pasture larval counts observed relative to no removal ($p=0.003$; mean estimate -6.0), but automated removal of faeces did not ($p=0.659$; mean estimate -0.8). The variance estimates for the random effects were 13.2 for observation level variance (over-dispersion of Poisson counts), 6.8 for week and 1.9 for field indicating a substantial variability between observed PLC and a greater degree of variability over time than between fields.

There was no significant effect of treatment threshold used on the pasture larval count for trial 4 paddocks, either for a threshold of 600 vs 300 EPG ($p=0.82$; mean estimate=0.37) or 1000 vs 300 EPG ($p=0.40$; mean estimate=1.43), although the paddock with the highest threshold value also had the highest observed larval count (PC Spring Barn). Estimates of variance parameters were similar to those for trials 1-3 data, with estimates of 11.4 for observation level variance and 3.2 for variance over time. Due to the variable nature of pasture larval counts these results should probably be regarded as suggestive rather than conclusive.

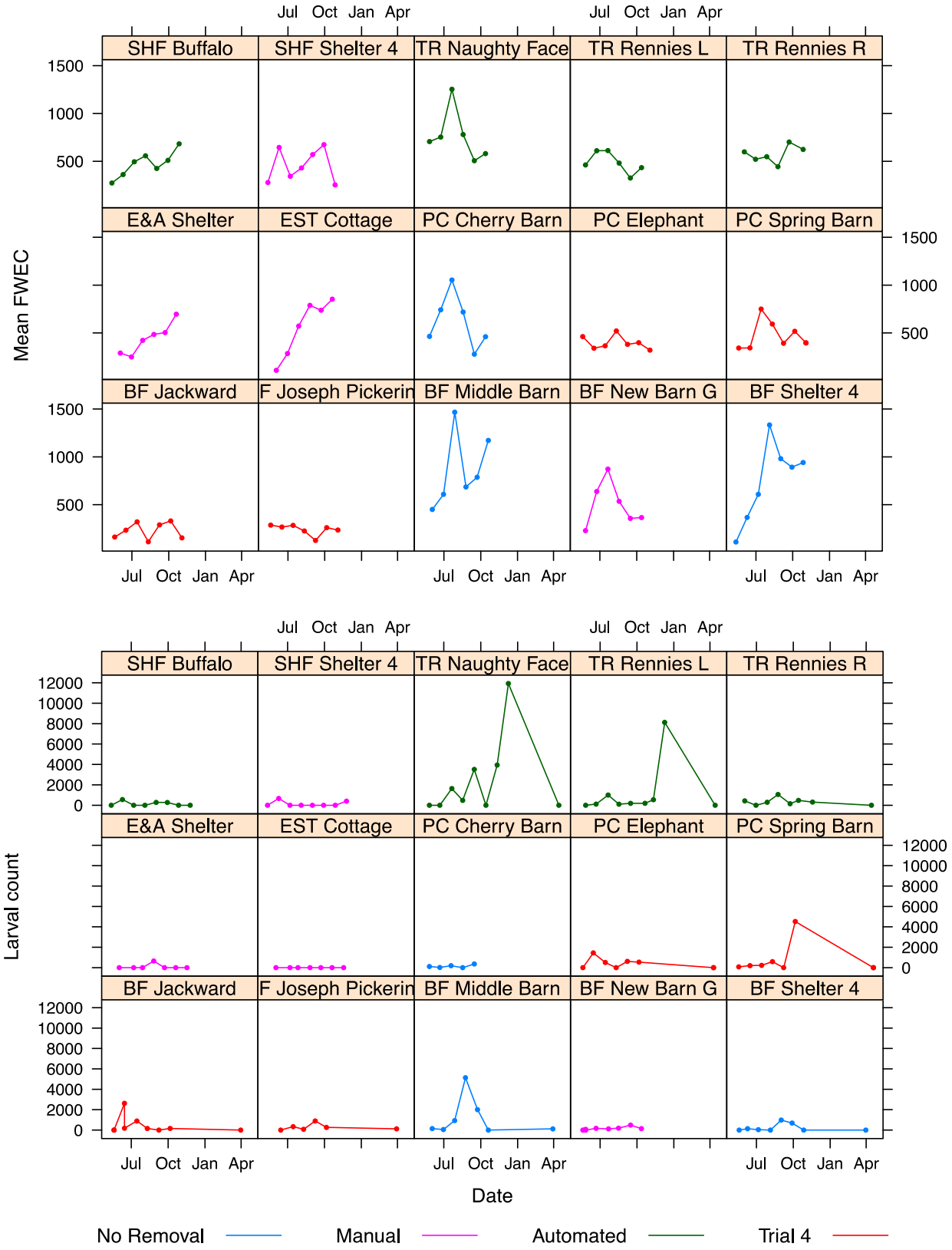

Figure 4.5: Set of two graphs displaying monthly mean FWEC (top) and pasture larval counts (bottom) of each of the groups sampled.

4.4.3 Faecal Egg Count Reduction Test

The results of the FECRT data analysis are presented in Table 4.2. Pyrantel embonate has a lower efficacy limit 95% reduction in FWEC, therefore for those groups in which the upper confidence interval (CI) is below 95% there was evidence of anthelmintic resistance, whereas those with a lower CI above 95% demonstrate susceptibility to the drug.

Table 4.2 Results of Bayesian MCMC analysis on the FECRT data.

Group	Pasture Treatment	Lower 95% CI	Median estimate	Upper 95% CI
BF Middle Barn	No Removal	89.5	94.8	98
BF New Barn Grannies	Manual	96.1	97.5	98.2
PC Cherry Barn	No Removal	48.9	71.9	87
PC Spring Barn	Trial 4	81.7	89.1	94.1
SHF Buffalo	Automated	-566.3	94.1	98.2
SHF Shelter 4	No Removal	-1845.5	86.3	98.2
TR Rennies Right	Automated	89.3	93.7	96.6

From Table 4.2 it is evident that resistance is present within the PC Cherry Barn population, which was a No Removal group that underwent more frequent anthelmintic dosing than the other groups in trials 1-3; and on PC Spring barn, which was the only group to be sampled out of trial 4. BF Middle Barn, another No Removal group appears to have some reduction in efficacy, with the Bayesian MCMC analysis results concluding that the data indicated a 53% chance of the cyathostomin population being resistant (where resistance is defined as a true FWEC reduction of less than 95%). BF New Barn G, which was part of the Manual removal treatment group and underwent minimal dosing throughout the study, had an efficacy of above the 95% threshold for resistance. The negative values for the lower 95% confidence intervals for the SHF Buffalo and Shelter 4 groups indicate that within these groups the true FWEC may have increased following treatment.

4.5 Discussion

It has previously been reported that frequent dosing with anthelmintic in groups of equidae can contribute to a build-up of resistant nematodes, resulting in reduced efficacy of the drug used (Wescott 1986; Kaplan, Matthews *et al*. 2004). It is therefore interesting to examine the

effects of pasture hygiene method and dosing threshold on the number of anthelmintic doses required, and the drug efficacy evaluated at the end of the grazing season.

For the trials 1-3 data, using a dosing threshold of 2000 EPG, manual removal of faeces significantly reduced the amount of anthelmintic doses administered compared to no removal groups. This represents not only a cost saving in terms of drug usage and improved welfare in terms of reduced handling of animals, but could also slow the development of resistance (Wescott 1986; Kaplan, Matthews *et al*. 2004) and preserve drug efficacy. There was a slightly higher proportion of animals dosed in automated removal groups compared to manual removal groups, but still a reduction compared to no removal groups. Manual removal of faeces also led to the highest proportions of negative FWEC in comparison to Automated removal and No Removal despite minimal anthelmintic dosing. Within trial 4, lower dosing thresholds led to more frequent dosing of animals and consequently higher numbers of negative FWEC within the Brookfield (BF) groups.

The PLC data collected were highly variable, with some pastures recording only zero or low PLC at each round of sampling, and some pastures recording occasional very high PLC interspersed with low or zero PLC. This reflects the poor sensitivity of the technique, and supports the consensus view that a single low PLC is not a reliable indicator that a pasture is free from infective larvae. Qualitatively, the manual removal groups appear to have lower PLC than the no removal and trial 4 groups, with the samples taken from automated removal groups at the end of the grazing season showing unexpectedly high PLC. No significant effect of treatment threshold was found for the trial 4 data, although the higher dosing threshold of 1000 EPG may have contributed to higher PLCs at PC Spring Barn compared to the rest of trial 4. The lower PLC in manual removal groups was found to be significant using a mixed effects model, which corroborates the analyses of the FWEC, but no significant effect was found for automated faeces removal relative to no removal. One potential explanation for the discrepancy between manual and automated removal of faeces in terms of PLC is that the mechanical pasture sweeper may have dispersed a proportion of the faeces across the pasture, leaving a number of L3 larvae that were detectable on PLC. However, due to the highly variable nature of PLC these results should be interpreted with caution.

No base line of Pyrantel embonates efficacy within each of the groups at the Donkey Sanctuary Devon was established at the start of the study; only the guideline of 95% optimum

efficacy was used to assess a reduction in efficacy. Some donkeys may have had recent exposure to Pyrantel, but this would have been consistent within farm. As there is a FERCT treatment group within each of the four farms (BF, PC, TR and SHF), this means that although efficacy cannot be compared between farms, we can safely compare efficacy between trials on the same farm. The only group from trial 4 to be included in the FERCT analysis, which underwent more frequent dosing than the other groups from trials 1-3, showed a reduced efficacy to the drug Pyrantel embonate by the end of the study. Although the repeatability of this finding within trial 4 cannot be assessed due to the lack of replicate fields in which a FECRT was performed, the reduction in efficacy indicates that a combination of no pasture hygiene measures and relatively low TST thresholds may contribute to the development of anthelmintic resistance. The PC Cherry Barn group, which did not have pasture hygiene measures and also had a higher rate of dosing that those groups with faeces removal, also showed reduced efficacy. Furthermore, the highest efficacy reported was in the New Barn G group where manual removal of faeces and a TST threshold of 2000 EPG resulted in a lower rate of anthelmintic use. The fact that such differences are evident after only a single grazing season supports the findings of Kaplan (2004) that repeated dosing can quickly promote resistance, especially in a short-life cycle parasite such as cyathostomins, and demonstrates the urgent need to reduce anthelmintic use at The Donkey Sanctuary Devon.

The proportions dosed and with negative FWEC are secondary data derived from the results recorded by Dr Faith Burden, therefore are subject to the possible biases discussed at the end of the end of Chapter 3. The data from the PLC were collected by one of two individuals, one of who had full knowledge of the study. These results are more likely to be subject to some bias as field size and herbage type may limit collecting abilities, for example one of the collectors was male and with larger hands may have collected larger samples, or gathered more soil with the sample. However, laboratory examination of the larvae from these samples was blinded and reported per kilogram of dry herbage.

General Discussion

Anthelmintic resistance in cyathostomins has been recognised across the world (Kaplan 2010), and at The Donkeys Sanctuary Devon resistance to several classes of anthelmintic drug has already been identified (Trawford and Getachew 2008). This limits the Sanctuary's options in treating clinical cases of parasitism to the potential detriment of animal welfare. Current resistance problems, both at the Sanctuary and elsewhere, have stemmed from the over use of anthelmintics as a prophylactic measure (Kaplan 2010; Kaplan and Nielsen 2010). Available without prescription, this often leads to misuse and unnecessary dosing, speeding the development of resistance (Wescott 1986; Kaplan and Nielsen 2010). In Denmark anthelmintics are only available under a veterinary prescription in a bid to curb their unnecessary use and maintain efficacy. Evaluation of sustainable methods of parasite control is essential in order to reduce the reliance on anthelmintics and preserve the efficacy of the existing drugs licensed for use in equine species.

Much of the information on cyathostomins in donkeys is derived from studies conducted in Southern temperate regions where differences in climate make comparisons to the UK difficult (Reinemeyer 1986; Nielsen, Kaplan *et al*. 2007), or extrapolated from studies conducted with horses and ponies (Trawford and Getachew 2008). Within The Donkey Sanctuary Devon study population there were 16 non-donkey equidae, which showed substantial differences in FWEC between donkeys, horses, ponies and mules. Although these differences were not significant, possibly due to the small numbers of non-donkeys involved, it seems likely that some difference exists between equine species. This is further supported by the higher typical FWEC, and possible better tolerance of cyathostomins found in donkeys (Getachew, Feseha *et al*. 2008), and the difference in cyathostomin species found between

equidae (Lichtenfels, Kharchenko *et al*. 1998; Kuzmina, Kharchenko *et al*. 2007). Although this does not provide sufficient evidence to warrant discouraging co-grazing, it certainly advocates the development of separate worming protocols for different species, even if grazed together. Although the greatest source of variation was between individual donkeys trends were also identified, there was a consistent positive effect of increasing age on FWEC found in our data, and a tendency for geldings to have lower FWEC than female donkeys. Few other studies have distinguished between males and females in their analysis and is it not stated if the donkeys involved in these studies were gelded. This leaves speculation that perhaps hormonal influences or gender related feeding behaviours may lead to these differences and further investigation should be considered. Although the effects of age and gender are relatively small, they are large enough to warrant consideration in TST protocols.

The theory behind pasture hygiene as a method of nematode parasite control is that by removing faeces from the pasture the infection pressure on grazing animals is reduced, and consequently the build-up of the pathogenic stages of cyathostomins is prevented. Previous evaluation of the effectiveness of pasture hygiene in ponies and horses has produced mixed results (Herd 1986; Herd 1993; Becher, Mahling *et al*. 2010), but it has been part of a long standing control program in the Ukraine (Kuzmina, Kharchenko *et al*. 2007) where the control system affords low use of anthelmintic drugs, resistance is low, and parasite species demography was different to that commonly seen. Within the current study the available groups were managed using either no faeces removal, or faeces removal either manually or by automated pasture sweeper twice weekly. When these methods were compared using both a descriptive statistical analysis and using a computationally intensive statistical method previously described (Denwood, Stear *et al*. 2008), significantly lower FWEC were seen in those animals grazing pastures where faeces were removed. No difference was observed between groups managed using manual and automated faeces removal, however when comparing the number of doses of anthelmintic used (i.e. the number of animals exceeding the TST threshold of 2000 EPG) manual removal led to significantly fewer doses of anthelmintic being administered compared to the automated groups. In addition, larger proportions of the animals in the manual removal groups maintained a FWEC of zero EPG throughout the study. The reduced levels of anthelmintic used is highly important, not only because it reduces the financial cost of treatment and potential welfare cost of handling donkeys in order to administer doses, but also because it reduces the levels of exposure to anthelmintic which helps to maintain efficacy (Van Wyk 2001; Kaplan, Matthews *et al*. 2004;

Kaplan and Nielsen 2010). There was also a recognisable difference in pasture larval counts between faeces removal strategies, which was not brought out using FWEC alone. Those fields, which underwent manual removal, had the lowest PLC, presumably because manual removal of faeces is more effective at reducing the number of larvae on the pasture. The higher average PLC and greater variability within the automated removal groups relative to the manual removal groups could relate to the argument that pasture sweepers serve to break up and spread faeces, rather than lift it, especially when wet. However, the lower FWEC in the automated group compared to the no removal group demonstrates that this still results in a net reduction in the level of parasitism, despite not being as effective at reducing transmission of cyathostomin larvae onto the pasture when compared with the manual removal of faeces.

While pasture hygiene methods have been shown to be effective in controlling cyathostomins, it is not always possible for practical reasons to remove faeces from pasture. This is the case for those groups in trial 4 of this study, predominantly due to the steep slopes and uneven ground in the fields where these animals are grazed. In such circumstances, control of cyathostomin burdens may continue to rely on TST based on individual FWEC, although the most appropriate threshold to use for donkeys has not previously been determined. Based on the findings presented, increasing the dosing threshold from 300 EPG through 600 EPG to 1000 EPG is associated with an increase in FWEC after accounting for the direct effects of dosing, although this has to be balanced against the increased development of resistance. Based on the patterns of PLC and group mean FWEC observed between trial 4 groups, and the fact that there were no reports of increased incidence of verminous colic or other presentation of cyathostominosis, we would advocate using a higher dosing threshold to preserve efficacy. In fact, there were no observed ill effects even with a TST intervention threshold of 2000 EPG for the no removal groups in trials 1-3, which is far higher than that advocated by the literature for horses (Uhlinger 1993; Urquhart 1996). This supports previous findings that donkeys are able to support relatively high nematode burdens with few ill effects (Krecek and Waller 2006; Getachew, Trawford *et al*. 2007; Getachew, Feseha *et al*. 2008). Other studies recommend an autumn or twice yearly anthelmintic dose (Kuzmina, Kharchenko *et al*. 2007; Kaplan and Nielsen 2010) in order to protect against the pathogenic effects of large strongyles and other parasites, and in the interests of animal welfare this should continue to be advocated. The current study was only conducted over one grazing season and therefore there is no follow up information available on the long-term effects of

harbouring heavier worm burdens, and due to the risk of mass larval emergence in the spring autumn dosing should be practiced. There is substantially less concern regarding resistance in large strongyles compared to cyathostomins; however in order to further reduce development of resistance in the existing cyathostomin population rotation or combination of products is an option (Wescott 1986).

The current study is limited by the fact it was conducted over only one grazing season, in a year with unusually heavy rainfall following a winter with heavy snow. These environmental factors could influence the findings, and so future studies of a similar nature are essential to confirm the current findings and limit the influence of climatic factors. It may also be pertinent to explore the use of different thresholds for different species and age groups due to the observed differences in FWEC. There is considerable potential merit in expanding the findings from this study that age, sex and breed impact on FWEC so that decisions on parasite control based on signalment along with individual history of clinical disease, FWEC and dosing, combined with individual characteristics such as age and sex. Such an approach is a natural extension to the current TST program, which would improve the targeting of anthelmintic doses to consistent high shedding animals and those animals at particular risk of clinical disease, and should provide effective control of cyathostomins with a requirement for fewer, more selective, anthelmintic doses. This would result in prolonged anthelmintic efficacy both through the direct effect of reduced treatment, but also by increasing refugia population of worms in animals that maintain moderate parasitism with no ill effects.

The main objective of pasture control at The Donkey Sanctuary Devon is to reduce the reliance on anthelmintics as part of routine cyathostomin control, due to the development of resistance in several different classes of anthelmintic drug (Trawford and Getachew 2008). With BZ resistance common worldwide (Lyons, Tolliver *et al*. 1999), and the first report of Moxidectin resistance occurring at the Donkey Sanctuary Devon (Trawford, Burden *et al*. 2005), resistance is one of the primary concerns within the Donkey Sanctuary Devons's parasite control program. Factors contributing to the development of resistance include using drugs which have known resistance, under-dosing, and not using an optimal dosing interval, as over-exposure leads to a greater population of exposed parasites (Wescott 1986). The use of manual removal combined with a TST threshold of 2000 EPG significantly reduced the number of treatments administered, which in turn helped maintain the efficacy of the

anthelmintic drug. Based on this, the strategy of pasture management combined with a targeted selective treatment protocol can be strongly supported as a means of reducing pasture contamination, subsequently lowering FWEC, and reducing reliance on anthelmintics at The Donkey Sanctuary Devon. There are potentially some advantages to using manual removal over automated removal of faeces; however in cases where manual removal is not possible automated removal is preferable to no removal of faeces. On pastures where faeces removal is not possible, a TST based management with a dosing threshold of 1000 EPG is justifiable as providing adequate control of cyathostomins with substantially reduced reliance on anthelmintic compared to lower dosing thresholds.

The Donkey Sanctuary Devon is a unique environment differing from many other equine units or farming situations as performance and production are not desired outcomes of husbandry practices. In fact, animal welfare is the sole objective in caring for the sanctuary's residents. In this respect the results of the current study may not be directly applicability to commercial equine units, or even other charity situations which tend to house more horses and ponies than donkeys. However, it does contribute to an on going body of work investigating sustainable parasite control and the statistical methods appropriate to assess it (Denwood 2008; Denwood, Reid *et al*. 2009; Denwood, Reid *et al*. 2010; Denwood, Love *et al*. 2012). This study provides information on donkey FWEC in the UK, where donkeys are primarily leisure rather than working animals, and therefore under different physiological conditions than the animals used in previous studies based in countries such as Ethiopia (Getachew, Feseha *et al*. 2008). Further extension of this work could usefully include work at The Donkey Sanctuary Devon in subsequent grazing seasons. Work to further validate and demonstrate the repeatability of PLC, and similar studies conducted on other equine units housing a more evenly distributed mix of equidae, would also be beneficial.

Appendix 1

Dates of sampling for FWEC, PLC and FERCT

Trial	Group/Field Name	Dates FWEC samples collected	Dates PLC Samples Collected
1	BF New Barn Grannies PC Cherry Barn TR Rennies Left TR Naughty Face	25/05/2010 23/06/2010 21/07/2010 17/08/2010 15/09/2010 12/10/2010	19/05/2010 (BF New Barn G) 26/05/2010 21/06/2010 21/07/2010 17/08/2010 15/09/2010 12/10/2010 (New barn G & Naughty face) 21/10/2010 (Rennies L Only) 11/10/2010 (Rennies L & Naughty face) 9/12/2010(Rennies L & Naughty face) 14/04/2011(Rennies L & Naughty Face)
2	BF Middle Barn TR Rennies Right SHF EST Cottage SHF E&A Shelter	02/06/2010 30/06/2010 28/07/2010 24/08/2010 23/09/2010 20/10/2010	1/06/2010 30/06/2010 (Middle Barn & Rennies R) 07/07/2010 (SHF EST and E&A) 28/07/2010 25/08/2010 23/09/2010 14/10/2010 (Rennies R only) 20/10/2010(Excluding Rennes R) 18/11/2010 (Excluding Middle Barn) 03/03/2011 (Middle Barn) 14/04/2011 (Rennies R)
3	SHF Buffalo BF Shelter 4 SHF Shelter 4	12/05/2010 11/06/2010 06/07/2010 03/08/2010 01/09/2010 28/09/2010 26/10/2010	12/05/2010 (SHF pastures only) 19/05/2010 (BF Shelter 4) 9/06/2010 07/07/2010 04/08/2010 01/09/2010 29/09/200 27/10/2010 25/11/2010 (SHF pastures only) 31/03/2011 (BF Shelter 4)
4	PC Spring Barn PC Elephant Barn BF Jackward Barn BF Joseph Pickering	19/05/2010 15/06/2010 14/07/2010 10/08/2010 08/09/2010 05/10/2010 03/11/2010	19/05/2010 (Excluding BF Joseph Pickering) 14/06/2010 15/07/2010 10/08/2010 07/09/2010 06/10/2010 30/03/2011 (Joseph Pickering) 10/04/2011(Jackward and Elephant) 19/04/2011 (Spring Barn)

	Farm	Pre-Treatment	Post-Treatment
FECRT Dates	Brook Field BF	09/11/2010	24/11/2010
	Paccombe (PC)	15/11/2010	29/11/2010
	Slade (SHF)	25/11/2010	08/12/2010
	Trow (TR)	23/11/2010	07/12/2010

Appendix 2

Approximate stocking densities

Approximate stocking density [number of animals per 100 square meters] for each of the fields involved in the study. Stocking rates were designed to be approximately equal after adjustment for the variable herbage quality within the fields.

Trial	Group/Field Name	Approximate Stocking Density (animals per 100 square meters)
1	BF New Barn Grannies	17.6
	PC Cherry Barn	21.3
	TR Rennies Left	16.5
	TR Naughty Face	9
2	BF Middle Barn	11
	TR Rennies Right	26
	SHF EST Cottage	11.6
	SHF E&A Shelter	8
3	SHF Buffalo	18.6
	BF Shelter 4	10.6
	SHF Shelter 4	23
4	PC Spring Barn	15.8
	PC Elephant Barn	20
	BF Jackward Barn	23.6
	BF Joseph Pickering	26.4

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