

# Parasite Control On Thoroughbred Studs

Thesis submitted in accordance with the requirements  
of the University of Liverpool for the degree of Doctor  
of Philosophy by Cara Louise Hallowell-Evans.

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# Author's Declaration

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Apart from the help and advice acknowledged, this thesis represents the unaided work of the author

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This research was carried out in the Department of Infection Biology and School of Veterinary Science, University of Liverpool

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# Abstract

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## Parasite Control on Thoroughbred Studs

Cara Hallowell-Evans

Gastrointestinal parasite infections impact on the health and welfare of Thoroughbreds (TB). The parasites of concern include the strongyles; cyathostomins and *Strongylus vulgaris*, *Parascaris equorum* and *Anoplocephala perfoliata*. In addition, there is increasing recognition of liver fluke infections in horses. Life-long parasite control is needed and use of anthelmintic drugs is the major approach, but decades of intensive anthelmintic usage has promoted widespread resistance particularly in cyathostomins and *Parascaris equorum*. Intensive anthelmintic usage is defined as the administration of an anthelmintic drug at set intervals, based on the original egg reappearance period (ERP) of the pioneer product, with the aim of egg output suppression; following the recognition and widespread nature of anthelmintic resistance targeted selective treatment regimens are now being advocated. Targeted selective treatment is defined by the American Association of Equine Practitioners (AAEP) as basing treatments during high transmission periods on diagnostic testing and identification of high egg shedding individuals in order to reduce pasture contamination while leaving a parasitic population in refugia, due to the current limitations in detection whole herd moxidectin and praziquantel treatments are still advocated annually as a minimum, with frequency dependent on a risk analysis and utilisation of available assays. The aim of this project was to identify parasite control practices on UK TB studs, determine drug efficacy on a subset of TB studs and evaluate the diagnostic potential of faecal diagnostic tests.

Faecal diagnostic tests for *F. hepatica* and *A. perfoliata* were examined. A McMaster-based short method for *F. hepatica* was applied to known positive donkey (n=18) and horse (n=1) samples which were simultaneously evaluated with the standard sedimentation method. Mean egg per gram (epg) count was 21.0epg (0.2-138epg) and 31.0epg (0.4-202epg) for the short method and standard sedimentation, respectively. The centrifugal flotation (CF) method was investigated for detection of *A. perfoliata* eggs and compared to the validated double sugar flotation (DCF) test. In total 140 samples were tested. Of these, 25.7% ( $\pm 7.2\%$ , n=36/140) tested positive using CF and 22.1% ( $\pm 6.9\%$ , n=31/140) using DCF.

Nine TB studs have undergone drug efficacy testing based on their anthelmintic use, control strategies and clinical disease concerns, mebendazole was tested on one stud, pyrantel on five studs; ivermectin on three and moxidectin on two. Tests were conducted for adults and youngstock independently. The CF faecal egg count (FEC), sensitive to 1epg, was used to detect strongyle-type, *Parascaris spp* and *A. perfoliata* eggs. On studs where  $\geq 10$  animals showed  $\geq 50$ epg (strongyle), faecal egg count reduction tests (FECRT) were performed to determine resistance status. ERP was defined as when group arithmetic mean FEC post-treatment exceeded 10% of group FEC arithmetic mean pre-treatment. Larval culture and morphological identification of strongyle third-stage larvae were also performed. On stud B, youngstock [YS] (n=7) PYR efficacy was 98.5% (97.9-99.0%), ERP = 3 weeks, and 93.2% (91.9-94.3%) in mares (n=8), ERP = 4 weeks; stud D only YS (n=31) showed resistance to PYR (reduction = 58.8% (57.6-60.0%)), sensitivity to IVM (100.0% (99.9-100.0%)) but borderline efficacy to MOX (94.5% (93.9-95.1%); ERP 4 weeks); stud F showed PYR resistance (n=23 YS, n=13 mares) with 0.1% (0.0-0.7%) and 69.5% (67.0-71.8%) reductions, IVM and MOX efficacy were 100.0% (99.9-100.0%) but ERP shortened to 6

weeks (IVM) and 8 weeks (MOX) in YS; stud G YS showed PYR resistance (n=18, 67.5% (64.9-69.9%) reduction).

One stud (stud E) was referred to the project due to high levels of tapeworm-related disease in broodmares (~60-70% of mares affected per year). All stock were tested using the CF and DCF faecal methods and University of Liverpool (UofL) serum ELISA (adults, n=86; YS, n=64). For adult stock 74.4% ( $\pm 9.7\%$ , n=58/78) recorded negative *A. perfoliata* FEC using the CF method and 79.5% ( $\pm 9.0\%$ , n=62/78) using the DCF; for YS (n=48) 75.4% ( $\pm 10.8\%$ , n=46/61) were recorded as negative by both CF and DCF. Over the study period February 2015 to Jul 2016 94.7% ( $\pm 3.6\%$ , n=142/150) of all samples tested by ELISA were reported to have a moderate to high infection intensity. Highest proportion showing high infection intensity (O.D. >0.201) on ELISA occurred in June 2015 (100% of adults and YS). Despite intensive three monthly praziquantel treatments from February to September 2015, no apparent decline in O.D. values was noted for either cohort, but FEC analysis revealed fewer horses were FEC positive over time.

A questionnaire survey was conducted with, questionnaires distributed to active studs (n=184) with the option to complete by post, online or telephone. Response rate was 39 studs (21.2%). FEC had been performed by 80.6% ( $\pm 12.9\%$ , n=28/36) but 17.1% ( $\pm 12.2\%$ , n=6/36) only used FEC at suspicion of disease. 97.1% ( $\pm 5.7\%$ , n=33/34) were very concerned about resistance to worming drugs, despite this 40.0% ( $\pm 16.2\%$ , n=14/35) of studs use intensive regimens with only 27.3% ( $\pm 14.6\%$ , n=10/36) having performed a FECRT. Faeces were only removed from pasture on 53.9% ( $\pm 15.9\%$ , n=20/38) of studs with harrowing being performed annually on 23 (59.0%  $\pm 15.4\%$ , n=23/39) 60.0% ( $\pm 16.2\%$ , n=21/35) of studs use targeted selective management, however, not all of these studs correctly defined the principle of targeted selective treatment so true level of implementation is unknown.

This work has provided vital information to TB studs about drug efficacy. By evaluating current diagnostic tests for their ability to detect infection with other parasites it raises the possibility of a holistic and time-efficient approach to targeted selective parasite control. It has highlighted the crucial role diagnostics have in detecting parasitic infection and parasite-associated disease and has identified current practice on stud. Together these findings can be used to better support targeted selective treatment of parasites in TB horses and improve parasite control on studs.

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# List of Abbreviations

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%	Percentage
°C	Degree Celsius
µm	Micrometre
AAEP	American Association of Equine Practitioners
ADB	Austin Davis Biologicals Ltd
CI	Confidence Interval
CF	Centrifugal Flotation technique
CPD	Continuing Professional Development
DCF	Double-sugar Centrifugal Flotation technique
DoT	Directory of the Turf
epg	Eggs per gram
ERP	Egg Reappearance Period
ES	Excretory-Secretory
FBZ	Fenbendazole
FEC	Faecal Egg Count
FECRT	Faecal Egg Count Reduction Test
GIT	Gastrointestinal Tract
HPD	Highest Posterior Distribution
hrs/d	Hours per day
IVM	Ivermectin
LC	Larval Culture
LDL	Lower Detection Limit
MBZ	Mebendazole
ml	Millilitres
MOX	Moxidectin
OBZ	Oxbendazole
PLE	Protein-losing Enteropathy
PPP	Prepatent Period
PYR	Pyrantel
SQP	Suitably Qualified Person
TB	Thoroughbred
TBA	Thoroughbred Breeders Association
THP	Tetrahydropyrimidines
UofL	University of Liverpool
WAAVP	World Association for the Advancement of Parasitology
YS	Youngstock

# Chapter 1 - General Introduction

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## 1.1 The importance of parasite control in horses

Infection with helminth parasites has a substantial impact on the health and welfare of many groups of managed horses, within studs, training yards and the leisure horse population. Prevalence of parasite-related disease varied widely between studies, from 18% to 47%, with no chronological or demographic trends apparent and often without the type of illness specified (Lind, et al., 2007; Lloyd, et al., 2000). In the few studies where type of illness was detailed, the most common clinical signs recognised by participants were colic and ill thrift. Both of these clinical signs can have a wide range of risk factors and aetiologies leading to pathological changes that may result in disease, including a potential role for *Anoplocephala perfoliata* (Feary & Hassel, 2006; Proudman, et al., 1998). It is difficult to compare amongst studies, or use cited values, to determine disease prevalence as they rely on a combination of diagnostic modalities for confirmation and the subjective nature of personal opinion. This is further complicated by sub-clinical disease states producing non-specific syndromes and clinical signs such as ill thrift, weight loss and colic, all of which have a large list of differential diagnoses. Weight loss, a common presentation of many parasitic infestations and often a component of ill thrift, appears in excess of 50 entries of “Blackwell’s Five-Minute Veterinary Consult: Equine”, of which parasitism is only one of a wide spectrum of potential causes, from stress to inflammatory bowel disease (Lavoie & Hinchcliff, 2009). In addition, there are limitations in the reliable diagnosis and differentiation of parasitism pre-mortem, as the aetiology of clinical and subclinical disease (Andersen, et al., 2013a).

Control of parasites relies primarily on anthelmintic drugs. Consequently, the problem of anthelmintic resistance within equine parasite populations poses a real risk to the prevention of clinical and sub-clinical disease in UK horse populations. Sustainable,

evidence-based methods of parasite control have been advocated, largely driven by a need to reduce anthelmintic treatment frequency and preserve drug efficacy. The most promoted option for sustainable use of drugs exploits the high degree of over-dispersion seen in helminth infection, with the minority of grazing horses within a herd harbouring the majority of the infection. This facilitates a targeted selective deworming approach, based on identification of those horses most heavily infected, or shedding the most eggs in faeces and therefore making the greatest contribution to parasite transmission. Integration of targeted selective drug treatment with effective pasture hygiene measures is seen as the most effective means to reduce drug use and lower pasture infectivity. An important consideration is that, with reduced drug use, there is a need to perform accurate surveillance of parasitic infection and the incidence of parasite-associated clinical disease in the future if equine health and welfare is to be guaranteed in the longer term.

## **1.2 Parasites of importance**

There are a number of parasites infecting horses that are considered to be the major focus of control programs.

### **1.2.1 Cyathostomins**

Cyathostomin spp are currently the most prevalent nematodes within equid populations, particularly since intensive deworming strategies instigated in the 1960s have effectively reduced the prevalence and clinical disease from more pathogenic species, such as *Strongylus spp* (Drudge & Lyons, 1966). Cyathostomin spp are found ubiquitously in grazing horses (Bucknell, et al., 1995; Eysker, et al., 1990; Lyons, et al., 1994) and over 51 species of cyathostomin have been identified, of which 40 are thought to infect *Equus caballus*, the remainder being seen in other equids such as donkeys, mules and indigenous equid species (Lichtenfels, et al., 1998). Species recognised within the domestic horse population belong to a mixture of genera including *Cylicostephanus*, *Cyathostomum*, *Cylicocyclus*, *Coronocyclus*, *Cylicodontophorus*, with up to 17 species comprising >80% of total burden

and between two and 27 species within an individual horse (Bucknell, et al., 1995; Gawor, 1995; Mfitlodze & Hutchinson, 1990; Ogbourne, 1976; Reinemyer, et al., 1984). Little is known about individual species as they have not been studied extensively at a single species level due to extreme difficulties initiating and maintaining single species infection (Hodgkinson, et al., 2003; Love, et al., 1999). However, there have been suggestions that clinical signs and level of larval burden may be associated with presence of a particular species (Mair, 1994), although this was not subsequently supported by clinical data (Hodgkinson, et al., 2003). Different species were detected pre and post pyrantel (PYR) treatment in a faecal egg count reduction test (FECRT) followed by a larvicidal benzimidazole (BZ) treatment (five consecutive days of fenbendazole [FBZ]), potentially indicating a role for different species in the development of drug resistance (Hodgkinson, et al., 2005). Although multiple species exist, a common life cycle has been reported for the cyathostomin group of parasites.

#### ***1.2.1.1 Life Cycle of cyathostomins***

Horses become infected with cyathostomins by ingestion of infective third stage larvae (L3), from pasture. The ingested L3, enclosed within a double sheath, is exsheathed within the duodenum. The exsheathed larvae passes through the gastrointestinal tract (GIT) with ingesta to the dorsal colon, ventral colon or caecum, larvae then penetrate and encyst within intestinal mucosal or sub-mucosal layers (Eysker, et al., 1989). Encysted larvae may continue development and emerge from the mucosa as fourth stage larvae (L4) or they may hypobiose. During hypobiosis the larval development is arrested at the early L3 (EL3) and can remain in this state for up to two years before reactivation and continuation of development to L4 (Eysker, et al., 1989; Eysker, et al., 1990). The basis for this prolonged arrestment and subsequent trigger for resumption of larval development, is yet to be determined (Klei, 1991; Love, et al., 1999; Reinemyer, 1986; Uhlinger, 1991) but one possible explanation could be that climatic conditioning of L3 prior to ingestion may drive

hypobiosis and explain the primarily seasonal nature of disease (Herd, 1986; Love & Duncan, 1992). It has also been suggested, that a large luminal worm population may provide negative feedback to the mucosal population increasing the occurrence of arrested development (Gibson, 1953; Love & Duncan, 1992; Murphy & Love, 1997; Smith, 1976). The encysted population can constitute up to >90% of the total burden, with 50% of those in a hypobiotic state (Dowdall, et al., 2002).

Once late L4 stage has been reached larvae exit the cystic nodule into the intestinal lumen. Development continues within the lumen to L5 or adult stage, adhering to the mucosa of the caecum or colon. The time between ingestion of L3 from pasture and production of eggs (prepatent period, PPP) was shown to have a mean value of 53 days (range 48-62 days), although experimental trickle infection showed the mean PPP was extended to 65 days with a range of 60 to 77 days (Love & Duncan, 1992). Adults feed, mate and females produce a copious number of undifferentiated eggs which are passed onto pasture in faeces (Figure 1.1).

Eggs differentiate and form the first larval (L1) stage which hatch. This development is temperature dependant with optimal development occurring at 25°C (lower limit 7.5-10°C; optimum range 25-33°C; upper limit 38°C) and 57-65% humidity (lower limit 15-20%) (Mfitlodze & Hutchinson, 1987; Ogbourne, 1972; Rossanigo & Gruner, 1995; Rupashinge & Ogbourne, 1978). L1 can hatch from eggs within three days in optimum conditions; however, several studies have shown that this can take as long as 15-24 days in temperate zones (Duncan, 1974a; Mfitlodze & Hutchinson, 1987; Ramsey, et al., 2004). L1 feed on faecal and environmental bacteria, developing to L2, which feeds and progresses to L3 stage (Ogbourne, 1972). The L3 is completely encapsulated by the L2 and L3 sheathes and so does not feed, the double sheath provides some protection from environmental

conditions but limited energy stores are a large factor in both duration of larval survival and ability to infect a host (Ogbourne, 1972).

The temperature dependent nature of development of the free-living stages means climatic conditions play an important part in the transmission and so infectivity of pasture (Nielsen, et al., 2007; Ramsey, et al., 2004). Development of eggs to infective stages on pasture, in temperate zones, has been shown to display a biphasic pattern with the highest transmission potential between June to October, primarily July and August (Duncan, 1974a; Ramsey, et al., 2004). During temperate-zone winters, it is unlikely that eggs will develop to L3 but studies have shown survival of both L3 and unembryonated eggs over many months and so the potential for a reservoir of infection.

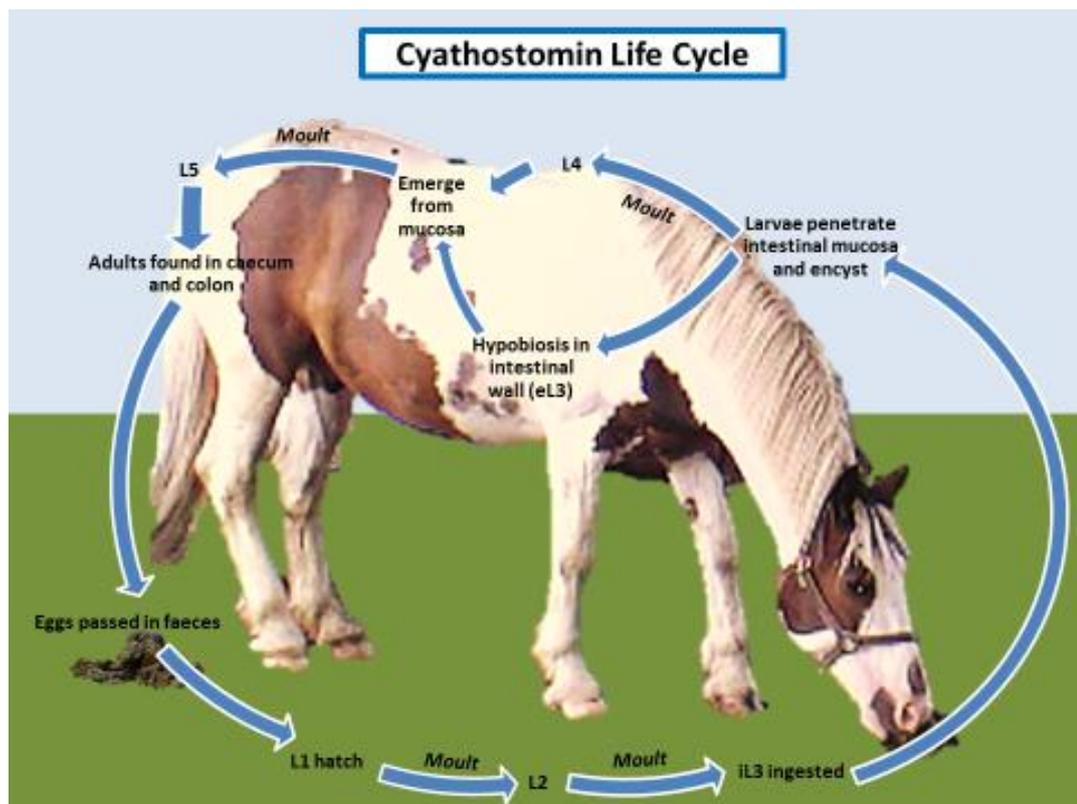


Figure 1.1 The life cycle of the cyathostomin species from ingestion to patency within the caecum and colon with and without hypobiosis at eL3 stage. Development on pasture is temperature dependant, taking a minimum of three days at optimal environmental conditions (25°C, 57-65% humidity); horses become infected by ingestion of infective third stage larvae during grazing. Following ingestion the prepatent period is six to eight weeks (Duncan, 1974a; Mfitalodze & Hutchinson, 1987; Ogbourne, 1972; Rossanigo & Gruner, 1995; Rupashinge & Ogbourne, 1978).

However, with reference to climatic conditions noted during temperate zone winters, where freeze-thaw cycles and frost are common, >1% of L3 stages will survive five cycles where a thaw lasts more than a few hours (Ober-Blobaum, 1932). In addition, larvae are able to migrate through soil and so both climatic conditions and type of soil present may also affect the viability, motility and therefore infective potential of larvae (Houston, et al., 1984).

#### ***1.2.1.2 Pathology and clinical disease due to cyathostomins***

Multiple forms of disease may occur, with larval cyathostomiasis being most severe and clinically relevant with mortality rate of up to 50%, despite intensive treatment (Bodecek, et al., 2010; Giles, et al., 1985; Love & McKeand, 1997; Proudman & Matthews, 2000).

Clinical disease is a challenge to diagnose with no consistent pathognomonic signs, characterised by a combination of non-specific clinical signs (Bodecek, et al., 2010; Love, et al., 1999). Prevalence data of diagnoses is limited to case studies, many including retrospective data due to low numbers of cases and papers relying on specific parameters not seen in all cases, such as worms in faeces.

A wide range of clinical signs can result from the mass emergence of encysted cyathostome larvae from gastrointestinal mucosa, however, up to 200,000 encysted stages can be present within a clinically healthy horse and no association has been proven between number of parasites and clinical disease (Reid, et al., 1995). This syndrome of disease occurs primarily, but not exclusively in late winter and early spring in temperate climates, in subtropical regions emergence of encysted stages, and related pathology, is seen primarily in late autumn and winter (Abbott, 1998; Feary & Hassel, 2006; Proudman & Matthews, 2000; Reid, et al., 1995). Studies have shown risk factors for disease include season, age of the horse, with those over one but less than six years of age more susceptible and recent (within the previous two weeks) adulticidal treatment especially with ivermectin (IVM) (Abbott, 1998; Bodecek, et al., 2010; Feary & Hassel, 2006; Reid, et al., 1995). The latter is

postulated to result in disease due to removal of the adult population and removing a possible negative feedback due luminal worms (Gibson, 1953; Love & Duncan, 1992; Murphy & Love, 1997; Smith, 1976). In terms of disease presentation this primarily includes protein losing enteropathy (PLE) in 93% cases, potentially leading to hypoalbuminaemia (71%) and peripheral oedema (32%) (Dowdall, et al., 2004). PLE is related to multiple separate pathologies within the intestines, there is histopathological damage to the mucosa and mucosal surface from larval emergence resulting in malabsorption and relaxation of the intercellular spaces allowing protein and other nutrients to be both absorbed in low proportions and lost during normal perfusion (Love, et al., 1999).

Diarrhoea has been emphasised by most reports as the primary clinical sign of cyathostome-related disease and, due to the non-specific nature, is thought by some to have led to an under-recognition and diagnosis of disease (Bodecek, et al., 2010; Love, et al., 1999). Multiple studies have found severe diarrhoea, both acute and chronic, to be the most common clinical presentation followed by colic and weight loss (Bodecek, et al., 2010; Peregrine, et al., 2006; Smets, et al., 1999). Mucosal irritation may lead to diarrhoea, seen in 86% of Dowdall et al study cases, through disrupted intestinal motility (Bueno, et al., 1979; Dowdall, et al., 2004; Love, et al., 1999; Ogbourne, 1978). Gross damage and subsequent reduction in gut transit time may result in malabsorption and negative energy balance shown clinically as weight loss and potential hypoglycaemia causing altered mentation and depression, noted in 64% of cases (Dowdall, et al., 2004). However, both Murphy et al (1997) and Bodecek et al (2010) noted a small proportion of cases with reduced faecal output, resulting from endotoxin resorption as a consequence of damage to the intestinal barrier. Pyrexia was seen in approximately a third of cases (32%) reported by Dowdall et al (2004), other authors (Ogbourne, 1978; Love, et al., 1999) have suggested this arises due to the horses' immune response and release of antigens from immature

larval excystment. PLE and weight loss may both be rapid and severe, and worms may be seen in faeces however, no data is available on how commonly this occurs in clinical cases.

Love et al (1999) documented that non-specific, mild non-strangulating colic may also be seen (11% of cases detailed in Dowdall et al (2004)). Although non-strangulating colic was noted by Dowdall et al (2004), previous work has implicated cyathostomes in caecocaecal intussusception and caecal tympany in addition to non-strangulating infarctions and mild non-specific medical colic (Bodecek, et al., 2010; Lyons, et al., 1994; Mair, et al., 1999; Mair & Pearson, 1995; Murphy & Love, 1997; Uhlinger, 1990). The lack of consistency in clinical presentation highlights the difficulty in diagnosis and suggests clinical signs depend on the individual horse and stage of pathology.

For colitis/typhlitis secondary to larval cyathostominosis, treatment is primarily supportive involving administration of intravenous fluids (electrolytes and colloids), antidiarrheal agents, larvicidal anthelmintics, antibiotics (namely trimethoprim potentiated sulphonamides), cimetidine, nutritional support and plasma infusions in cases of severe hypoproteinaemia (Abbott, 1998; Bodecek, et al., 2010; Mair, 1994). Anthelmintic treatment with moxidectin (MOX) needs to be used with careful consideration to prevent mass larval death within the mucosa which can result in a bolus of released antigen causing severe inflammation (Love & McKeand, 1997). In order to reduce resulting inflammation where this occurs corticosteroids are commonly used (Bodecek, et al., 2010; Love & McKeand, 1997). In cases resulting in caecal impactions or caecocaecal intussusceptions, surgical intervention in the form of coeliotomy is indicated (Bodecek, et al., 2010). As previously discussed and shown by many studies, intervention often yields poor results and prognosis is guarded (Abbott, 1998; Bodecek, et al., 2010; Giles, et al., 1985; Hodgkinson, et al., 2003; Love & McKeand, 1997; Love, et al., 1999).

### **1.2.1.3 Diagnosis of cyathostomin infection**

Definitive diagnosis is challenging as large burdens (>10,000-20,000) of cyathostomins do not necessarily result in disease, in addition, it is a prepatent disease so a negative egg count in a clinically affected horse is not unusual and L4 are not always seen in faeces of affected horses (Love, et al., 1999; Reid, et al., 1995). Haematology and biochemistry show non-specific changes, most of which are attributable to the effects of diarrhoea and the host immune response, such as neutrophilia, anaemia, mild eosinophilia, hypokalaemia and profound hypoproteinaemia and specifically hypoalbuminaemia; although the latter may be masked if only total protein levels are assessed due to hyperglobulinaemia (Bodecek, et al., 2010; Love, et al., 1999). Where surgical intervention is undertaken, diagnosis is suggested from gross appearance of the colon or caecum; biopsies primarily show oedema and eosinophilic inflammation, larvae can be identified grossly within the mucosal layer when present in high numbers. For most cases “diagnosis” is currently based on the season, clinical history and a wide variety of parameters (Giles, et al., 1985; Mair, 1994; Reilly, et al., 1993; Smets, et al., 1999).

## **1.2.2 Strongylus species (Large strongyles)**

### **1.2.2.1 Prevalence and importance of large strongyles**

The genus *Strongylus*, colloquially known as the “large strongyles” or “large redworm”, incorporates several species: *Strongylus vulgaris*, *S. edentatus*, *S. equinus* and *Tridontophorus species* (Cao, et al., 2013; Love, et al., 1999). Within the latter group, the most common are *T. brevicauda*, *T. serratus* and *T. tenuicolis* and the group as a whole are generally considered of low pathogenicity. In contrast, the most pathogenic, and well known, is *S. vulgaris*, which will be the focus of this section (Drudge & Lyons, 1966; Duncan & Pirie, 1975; Gay & Speirs, 1978). In the 1960s and 1970s, *S. vulgaris* caused a high prevalence of disease globally. Historically studies showed a prevalence of infection in Britain of 90% in yearlings and 46.6% in foals (Tolliver, et al., 1987), over 80% of Canadian horses (Slocombe, et al., 1973) and 80-100% in the USA (McCraw & Slocombe, 1976). The

threat posed by *S. vulgaris* infection lead to intensive anthelmintic dosing regimens, with administration of anthelmintics defined by the egg reappearance period (ERP), typically at six to eight week intervals, which successfully reduced its prevalence and that of associated clinical disease (DeLay, et al., 2001; Drudge & Lyons, 1966; Duncan & Love, 1991). Despite this, in 2011 a study from Sardinia found 100% horses at post-mortem examination exhibited larval-associated lesions within the cranial mesenteric artery, primarily chronic and chronic-active lesions, although sample size for this study was small (n=46) (Pilo, et al., 2011). In Denmark, where anthelmintic use is targeted due to classification of anthelmintic drugs as prescription-only medicines for vets since 1999, concerns have arisen regarding a potential re-emergence of *S. vulgaris* due to the resulting reduction in use of anthelmintics (Nielsen, et al., 2006; Nielsen, et al., 2012). Evidence from one Danish study of 42 horse farms showed a significantly increased prevalence of large strongyles in horses managed under targeted selective, diagnostic-based and intensive, non-diagnostic based anthelmintic administration regimes with prevalence of 83.3% on farms where treatments were given based on faecal egg count (FEC) diagnostics, compared to 38.9% of farms that did not target treatments based on FEC (Nielsen, et al., 2012). Routine larval culture (LC), where anthelmintic usage is targeted, has been said to have only partial implementation in Denmark and is considered to occur even less in other countries, indicating a lack of consistent monitoring of *S. vulgaris* infection, which in turn impacts on the true clinical picture and prevalence of this parasite under targeted selective treatment and prescription-only anthelmintic administration (Bracken, et al., 2012; von Samson-Himmelstjerna, et al., 2007).

Although within the UK, as a result of intensive macrocyclic lactone treatment, *S. vulgaris* is considered essentially eradicated (Herd, 1990; Love & Duncan, 1991) it is regarded as the most pathogenic equine gastrointestinal helminth, once considered the primary cause of colic, and so has significant clinical relevance. In contrast to cyathostomins, relatively few

larvae compared can result in overt disease, with severity related to number and host factors such as age and previous exposure (Drudge, et al., 1966). As severe disease results from migration of larvae, the extended PPP means that infection is not detectable until the life cycle has been completed by which time serious arterial damage may already have occurred (Nielsen, 2012b). With reduced usage of anthelmintics advocated under targeted selective programmes (Gomez & Georgi, 1991; Nielsen, et al., 2006) studies performed on horse farms in Denmark appear to show a resurgence in prevalence under this strategy where dosing intervals for anthelmintics exceeded six months, allowing for development of infection patency and so transmission (Nielsen, et al., 2012). If *S. vulgaris* is present on farm, horses not receiving anthelmintic treatment (0-500epg, “low shedding” horses) have the potential to harbour infection and it has been argued that this lack of treatment is likely to increase the prevalence within this sub-group of horses (Dopfer, et al., 2004), allowing for transmission and re-establishment of infection within herd as a whole (Nielsen, et al., 2006; Nielsen, et al., 2012). A recent Danish retrospective case-matched study demonstrated the clinical significance of increased prevalence by detection of significantly raised concentrations of antibody in 20 horses diagnosed with non-strangulating intestinal infarction (Nielsen, et al., 2016b). In the UK, routine surveillance of *S. vulgaris* is not undertaken, this is likely due to a combination of its perception as no longer being of concern and the lack of commercial availability of LC. Within UK studies performing LC, Lester et al (2013) tested 16 yards of leisure horses in Southern England and Relf et al (2012) examined 22 UK TB stud farms, both studies recording negative results. A combination of relatively low egg output and infection intensity compared to the cyathostomins where mixed infection is present, low sensitivity of LC and the current lack of surveillance there is a concern that widespread infection will become re-established and prevalent before it is detected, as has been shown in Denmark (Nielsen, et al., 2006; Nielsen, et al., 2012; Nielsen, 2012b). The development of more sensitive diagnostics, such

as the antibody serum ELISA (Nielsen, et al., 2016b), may improve chances of early detection but only if it is commercially available and widely utilised for monitoring and surveillance under targeted selective protocols.

### 1.2.2.2 Life cycle of *S. vulgaris*

As for the cyathostomins, *Strongylus spp* undergo a direct life cycle, with horses becoming infected through ingestion of infective L3 from pasture. These have developed from eggs, passed in the faeces of infected horses, which have undergone a temperature-dependent development on pasture similar to that described in section 1.2.1.1 for cyathostomins (Ogbourne, 1972; Slocombe & McCraw, 1976).

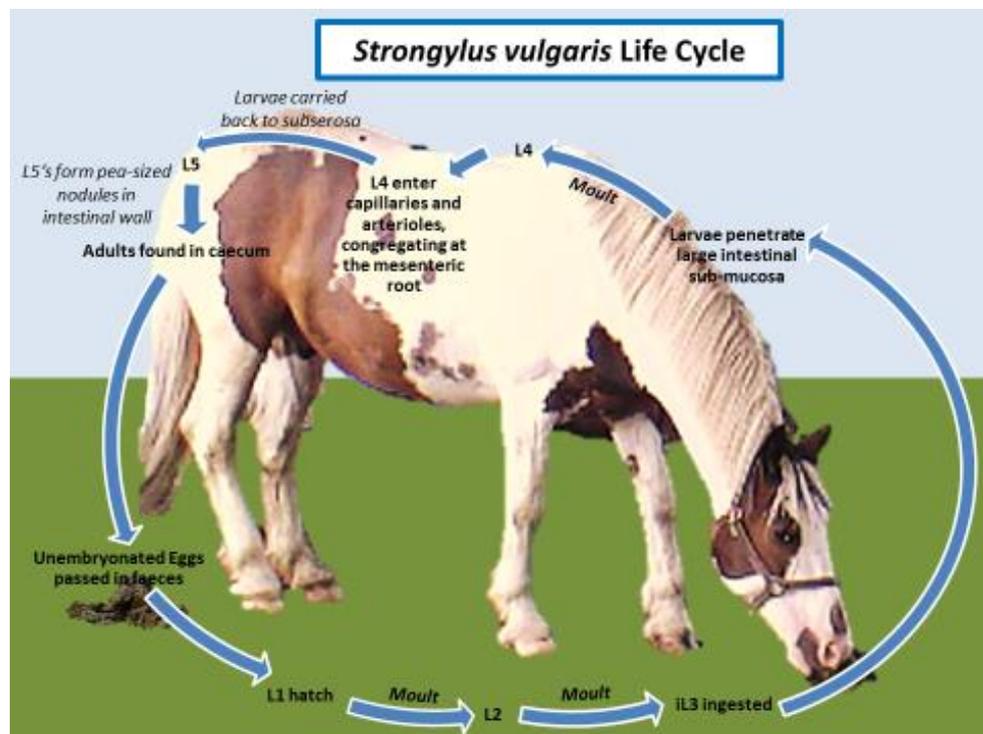


Figure 1.2 The life cycle of *Strongylus vulgaris*. Infection is acquired through ingestion of infective larvae (L3) during grazing; larvae penetrate large intestinal sub-mucosa and migrate to the mesenteric root. Following development, migration back to the intestinal wall occurs. Adult parasites are found within the caecal lumen. The prepatent period is six months; development on pasture is temperature dependant.

In contrast to cyathostomins, the PPPs are significantly longer in large strongyles, ranging from six months for *S. vulgaris* to 11 months for *S. edentatus*, due to extensive somatic migration pathways. *Strongylus vulgaris* enters the submucosa of the ileum, caecum and

ventral colon; moulting to L4 stage four to five days post infection before entering the intestinal capillaries and arterioles, in particular the anterior mesenteric artery, but it may be found in any part of the arterial system (Duncan, 1973; Duncan & Pirie, 1972; Enigk, 1950; Enigk, 1969). After two to four months, larvae are swept passively, by arterial blood flow, to intestinal subserosa as L5 and form pea-sized nodules within the wall that can be seen from four months post infection (Duncan & Pirie, 1972; Enigk, 1951). After a further six to eight weeks development, they enter the intestinal lumen as adults, attach and feed within the caecum. Adult *S. vulgaris* feed by ingesting a plug of mucosa which results in microscopic ulcers at sites of attachment; maturation is completed to become sexually reproducing adults and females release eggs which are passed in faeces (Figure 1.2) (Duncan, 1974b; Duncan & Pirie, 1972).

#### ***1.2.2.3 Pathology and disease due to S. vulgaris***

Severe disease due to *S. vulgaris* infections results from the process of larval migration.

Thrombo-embolic colic as a result of verminous arteritis within the mesenteric arteries has been reported. Poynter (1969) found 86% of 43 affected horses showed lesions within the cranial mesenteric artery and 62.5% in the caecal and colic branches, which was backed up by a later study by Pilo et al (2011) where 97.8% of 46 infected horses demonstrated lesions within the ileocolic and 71.7% lacerations in the colic branches; marked fibrotic thickening of arterial walls was seen in 55.6%. Due to the disruption in haemodynamic flow by L4 and then L5, thrombus formation occurs resulting in ischemia and infarction to the corresponding segment of the intestine resulting in colic (Duncan & Pirie, 1975; McCraw & Slocombe, 1976). A recent study by Pihl et al (2017) of 12 foals from a herd where *S. vulgaris* transmission is established corroborates these findings. Pihl et al (2017) demonstrated significant changes in haemostatic indices occur as infection progresses, findings indicate that naturally acquired infection causes mild activation of coagulation, fibrinolysis and inflammation. Severity of disease relates to the number of L3 ingested and

is thought to be driven by host immunity and previous exposure, as naïve ponies show increased disease severity, with a relatively low infection intensity capable of causing severe disease (Amborski, et al., 1974; Drudge, et al., 1966; Enigk, 1950). Acute disease syndrome incorporates pyrexia, inappetance, rapid weight loss, depression, recumbancy, abdominal distress and either constipation or diarrhoea (Drudge, et al., 1966).

Minor damage due to *S. vulgaris* can also occur and causes pathological changes which may result in colitis type signs. As L3 moult to L4 a severe inflammatory reaction within the submucosa is noted which includes widespread arteritis, thrombosis and neutrophilic infiltration which extends to the muscularis and serosal layers, however this presentation is thought to be less common than in small strongyle infections (Dunn, 1969; Slocombe & McCraw, 1976). Early migration leaves behind tortuous, thread-like, fibrin tracts on arterial intima which can extend to the abdominal aorta and become overgrown with epithelium (McCraw & Slocombe, 1976). In rare cases, as with multiple parasite species, aberrant migration can cause a multitude of clinical syndromes. For *Strongylus spp* aberrant larval presence has been noted occluding the right coronary artery (Cronin & Leader, 1952); within the kidney (Mahaffey & Adam, 1963); thrombosis or embolism of the external iliac artery resulting in intermittent lameness (Wright, 1972) and, in several noted historical cases, cerebrospinal nematodiasis (Little, 1972) which was supported by experimental induction of acute verminous encephalitis by inoculating 4<sup>th</sup> and 5<sup>th</sup> stage larvae into the internal carotid artery (Little, et al., 1974). Adult parasites occupy multiple feeding sites, access nutrients by removing a plug of mucosa and create “crater-like ulcers”. This feeding and related pathology is associated with anaemia, emaciation, poor coat and performance, pyrexia and intermittent abdominal distress although the number of adult worms required to result in this sub-clinical disease form is unknown (Drudge, et al., 1966; Duncan & Pirie, 1975; Slocombe & McCraw, 1976).

Treatment is based on surgical intervention to resect sections of infarcted intestine, treatment of associated cardiovascular shock and toxicity, and efficacious larvicidal anthelmintic administration (McCraw & Slocombe, 1976). One study successfully treated 49 of 57 cases using low molecular weight dextran (dextran 70) (Greatorex, 1977). The challenges of prepatent detection of low burdens results in severe disease prior to detection of infection, reducing the chance of successful treatment. Therefore, prevention of infection with *S. vulgaris* and targeting prophylactic treatment to both larvicidal stages, for prevention of pathological damage, and adults, to reduce potential pasture infectivity is the key to reducing the clinical impact of *S. vulgaris* in horses through reducing risk of ingestion of significant numbers of larvae (McCraw & Slocombe, 1976).

#### ***1.2.2.4 Diagnosis of large strongyle infection***

As for larval cyathostomiasis, severe large strongyle-related disease is a result of prepatent infection with the stages undergoing somatic migration responsible for the pathology. It is not known what level of infection results in disease but it is thought to be relatively small numbers of infective larvae that can cause disease, making a definitive diagnosis challenging as diagnostic tests would be required to have high sensitivity in order to detect potentially significant infection. A method that is commonly advocated for routine monitoring for presence of large strongyles is to experimentally culture eggs in faeces to the L3 stage (LC) and microscopically identify larvae (Bevilaqua, et al., 1993; Russell, 1948), but, as with a FEC, this relies on the presence of patent infection. Whilst this may be useful for monitoring purposes it does not detect prepatent infection and is unsuitable for diagnosis of severe clinical disease. Other diagnostic methods that have been investigated include rectal palpation, evaluating the presence of nodular masses enclosing cranial mesenteric artery damage (Coffman & Carlson, 1971). Transrectal ultrasound was also shown to be of some diagnostic value (Wallace, et al., 1989a; Wallace, et al., 1989b; Wallace, et al., 1989c). Unfortunately, placement of the cranial mesenteric artery means

that it is not accessible per rectum in all horses and due to the risk of rectal tears rectal palpation of youngstock (YS) and small breeds is contra-indicated (Andersen, et al., 2013a). Contrast arteriography was also shown to be valuable, but due to the requirement for a general anaesthetic this is unsuitable for clinical cases (Slocombe, et al., 1977). Multiple serological parameters have been investigated for specificity, with clinical cases uniformly showing increased serum  $\alpha_2$ ,  $\beta_1$  and  $\beta_2$  globulins, with the former associated with a significant increase in IgG (T) thought to be a result of ES antigens from migrating larvae (Adeyefa, 1992; Bailey, et al., 1984a; Bailey, et al., 1984b; Kent, 1987; Patton, et al., 1978). Following these discoveries, an assay was launched by Virbac in the 1980s but on further validation by Klei (1986), IgG (T) was found to be non-specific and the assay was retracted from the commercial market. However, as colic is characterised by a non-strangulating infarction exploratory laparotomy, and subsequent resection is indicated a definitive diagnosis is often made during examination (Coffman & Carlson, 1971; McCraw & Slocombe, 1976).

Recent advances offer the most promising development for diagnosis of prepatent infection via use of a SvSXP protein, an ES protein associated with the later stage of prepatent infection, ELISA using IgG(T) (Andersen, et al., 2012; Andersen, et al., 2013b; Nielsen, et al., 2014). Antibody (IgG(T)) ELISA analysis against recombinant SvSXP protein showed a sensitivity of 73.3% with specificity of 81%, equivocal with LC for patent infection (Andersen, et al., 2013b). Caution when interpreting results in foals is required due to demonstration of *S. vulgaris* maternally-derived antibody transfer in colostrum where antibodies have a half-life of approximately three weeks and the finding of large numbers of migrating stages without seroconversion (Nielsen, et al., 2014). Some evidence of a semi-quantitative relationship between infection intensity and ELISA result was noted within this study, although the possibility of cross-reactivity with similar native SXP proteins found within cyathostomes and *P. equorum* could falsely elevate antibody titres (Andersen,

et al., 2013b). Further validation, with special consideration to identification of a minimum age of horse for which the ELISA is used, is needed for widespread application and commercial deployment.

### **1.2.3 *Parascaris spp***

#### **1.2.3.1 Prevalence and importance of *Parascaris spp***

The *Parascaris* genus, in equids, is composed of two species; *P. equorum* and *P. univalens* (van Beneden, 1883). These species are morphologically identical and cannot be differentiated unless karyotyping of germ cells prior to their first cell division is performed (Goday & Pimpinelli, 1986; Nielsen, 2016). To date, *P. equorum* has been widely regarded as the primary species infecting equids; however, studies have shown an overwhelming dominance of *P. univalens* in many countries (Bullini, et al., 1978; Nielsen, et al., 2014; Tyden, et al., 2013). Whilst this is currently not thought to be of clinical significance, parasite genus will be referred to here, rather than specific species, as no UK karyotyping has been performed such that an accurate identification to species present can be made.

*Parascaris spp* is considered ubiquitous in foals worldwide and all animals are considered to be exposed to infection by the age of eight months. Control of *Parascaris spp* is of importance as this genus can result in multiple disease forms from mild sub-clinical infections to severe life-threatening abdominal disease (Nielsen, 2016). Prevalence of infection on various continents and demographic groups has been investigated and found to be up to 80% in foals under one year of age (Armstrong, et al., 2014; Laugier, et al., 2012; Lyons & Tolliver, 2014; Relf, et al., 2013). Prevalence of patent infection in horses older than 18-24 months is normally very low to negligible in well managed horses; Relf et al (2013) found 4% yearlings, 3% two to four year olds and 1% of five to 14 year olds on UK TB farms tested positive on faecal flotation compared to 38% of foals. This is in contrast to observations in working equids where Getachew et al (2008) found a 51.1% prevalence in donkeys irrespective of age, and 16.2% of adult horses, this was suggested to be as a result

of immune compromise and associated concurrent nutritional or immunological issues. A study performed by Clayton and Duncan (1979) suggested that immunity, generally fully developed by 18-24 months of age and protective against infection and disease, may be largely age-dependant with a minimal role for magnitude of parasite exposure. The exact mechanism of this resistance has not been investigated in foals.

#### ***1.2.3.2 Life cycle of Parascaris spp***

*Parascaris spp* have a direct life cycle with foals becoming infected by ingestion of larvated eggs passed in the faeces of an infected horse. Infective eggs contain early second stage larvae and are primarily found on pasture but due to their sticky outer protein coat, can be found on the coat or udder of the dam. On ingestion this protein coat is lost in the small intestine, the second stage larvae (L2) emerge, penetrate the gut mucosa and begin a hepatotracheal migration. Mature L2 enter lymphatics or draining venules and are passively transported via vena porta flow to the liver; larvae migrate through liver and lungs for 14-17 days (Nicholls, et al., 1978). After rupturing alveolar membranes and invading the lungs, larvae are coughed up and swallowed, colonise the small intestine and mature to patency at 90-110 days post-infection (Figure 1.3) (Clayton & Duncan, 1978; Lyons, et al., 1976).

Although not specifically determined for *Parascaris spp*, ascarid spp infections in other host species have been used to draw parallels that are generally applied to *Parascaris*. Female ascarids are considered extremely fecund, with an *Ascaris lumbracoides* (human ascarid) female producing over 200,000 eggs per day (Sinniah, 1982).

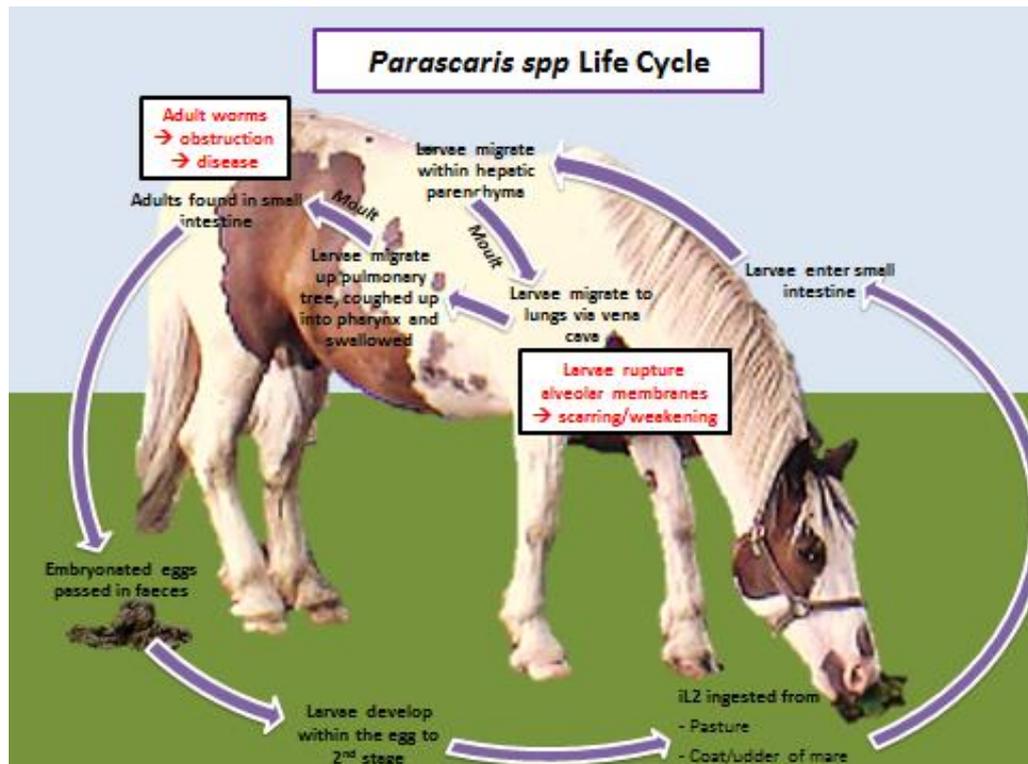


Figure 1.3 The life cycle of *Parascaris spp.* Grazing horses become infected by ingestion of the L2 within the egg; larvae enter the small intestine, penetrate the wall and migrate through the hepatic parenchyma to the vena cava. Larvae are passively carried to the lungs where they moult and penetrate the alveolar membranes before being coughed up and swallowed. Adults are then found in the small intestine. Disease can be a result of weakening of alveoli through rupture or mechanical blockage by adults within the small intestinal lumen.

In pigs, *Ascaris suum* eggs can persist for many years in the environment when compared with strongyle eggs (Roepstorff & Murrell, 1997). Whilst the epidemiology of *Parascaris spp* infection supports high fecundity and persistence of infective eggs in the environment, it is important to note that one study specifically examining *Parascaris spp* found 50% reduction in egg recovery during summer and autumn from grass within eight weeks of egg deposition into the environment and a 10% reduction in winter (Lindgren, et al., 2009). In addition, soil type has been reported to affect *Parascaris spp* egg survival and infectivity with sandy soils, and those with better drainage, showing lower retention of eggs (Ihler, 1995; Lindgren, et al., 2009).

### *1.2.3.3 Pathology and disease of *Parascaris spp* infection*

Migration through the hepatic parenchyma causes small, white, fibrotic lesions that, although sometimes evident at post-mortem, have not been associated with signs of liver disease or increased enzymes on biochemical analysis (Brown & Clayton, 1979). However, foals exposed to repeated small doses of infective eggs do not show these macroscopic lesions (Brown & Clayton, 1979; Nielsen, 2016).

Larval migration through lungs initially causes alveolar rupture and then a localised immune response and inflammation from movement through the respiratory tree which can result in varying severity of respiratory signs. Young, experimentally infected helminth-naïve reared, foals (two to four weeks of age) developed coughing and purulent or mucoid nasal discharge but, in contrast to older foals, no radiographic changes were noted (Clayton & Duncan, 1978). In experimentally infected naïve yearlings, larvae may cause clinical signs as well as radiographically detectable lesions associated with bronchopneumonia, suspected to be due to a more mature immune system; clinically characterised by coughing, serous or seromucoid nasal discharge, hyperpnoea, depression, inappetence and weight loss (Clayton & Duncan, 1978). Histologically the local immune response results in alveolitis, bronchiolitis and bronchitis, classified as a pulmonary eosinophilia (Nicholls, et al., 1978). Where larvae become trapped and die, lymphocytic nodules form in the parenchyma around the deteriorating larva; these inflammatory nodular structures generally resolve without specific treatment by 73 days post-infection (Nicholls, et al., 1978). However, the Nicholls et al (1978) and Clayton and Duncan (1978) studies examined the sequelae of experimental bolus infection of YS reared in helminth-free environments and so do not account for the effect of developing acquired immunity or trickle infection, therefore in practice pathology and associated disease may be less pronounced and clinically obvious in healthy, well managed YS on a good nutritional plane.

Adult *Parascaris spp* are large and robust, and when present in large numbers can cause blockage of the small intestine requiring surgery, which causes severe clinical disease due to small intestinal impaction and carries a guarded to poor prognosis and survival rate of 9-60% (Cribb, et al., 2006; Nielsen, et al., 2010b; Ryu, et al., 2004; Southwood, et al., 1996; Suderman, et al., 1979; Vatistas, et al., 1996; Yang, et al., 2004). This is characterised by significant pain unresponsive to medical treatment (Nielsen, et al., 2015). In addition, pre, peri and post-operative complications commonly occur due to: secondary intestinal abnormalities resulting from luminal occlusion such as intestinal volvulus and intussusception, and surgical complications including focal necrotising enteritis, intestinal adhesions or rupture leading to septic peritonitis and associated cardiovascular shock (Cribb, et al., 2006; Southwood, et al., 1996; Tatz, et al., 2012). Although prevalence of infection is high, morbidity and mortality is low, as shown by multiple studies with <0.5% of all foal laparotomies for colic at an Israeli referral centre (2002-2011) (Tatz, et al., 2012) and <0.5% of all colic surgeries performed at a Colorado referral surgical unit from 1987-1995 (Southwood, et al., 1996). Two methods of surgical treatment are commonly carried out during laparotomy: enterotomy and manual evacuation (or “milking”) of worms from small intestine to caecum and slight variations on each adapted on a case-by-case basis (Tatz, et al., 2012). Over all case studies 60% survived to discharge but only 21% survived over one year post-surgery (Cribb, et al., 2006). Tatz et al (2012) found reduced long term survival with enterotomy, and associated complications, due to the difficulties of small intestinal resection and anastomoses, compared to manual evacuation for 15 cases.

Multiple risk factors for patent infection related disease exist with the majority of cases reported to occur in horses less than one year of age, with peak incidence around weaning (five to eight months) (Cribb, et al., 2006; Nielsen, et al., 2015). However, cases have been noted in horses up to five years (Tatz, et al., 2012). Stressful periods, such as weaning and transportation, were identified as higher risk for disease occurrence. The major risk factor

for development of impactions is administration of an efficacious anthelmintic, first highlighted by Schusser et al (1988), and with three subsequent studies reporting that over 50% of cases had received PYR or IVM within 24 hours of impaction (Cribb, et al., 2006; Southwood, et al., 1996; Tatz, et al., 2012). Anthelmintic administration is considered to result in subsequent impaction due to large numbers of dead or paralysed adult ascarids being swept down the intestine, resulting in obstruction of the lower portion of the ileum (Cribb, et al., 2006; Schusser, et al., 1988). Therefore adjusting management strategies to prevent build-up of ascarids both within the environment and YS is the key to preventing both respiratory and, most importantly, gastrointestinal disease.

#### ***1.2.4 Anoplocephala perfoliata***

##### ***1.2.4.1 Prevalence and importance of Anoplocephala perfoliata***

Three cestode species infect equids: *Anoplocephala perfoliata*, *A. magna* and *Anoplocephaloides mamillana* (Bohorquez, et al., 2015). *A. perfoliata* is the most prevalent of the three cestodes and therefore is considered as the main pathogenic agent of tapeworm related colic and ileocaecal disease, with *A. magna* and *An. mammillana* infection reportedly rarely (Proudman, et al., 1998). In five to ten percent of colic cases surgery is required, causing significant risk to the horse and economic loss (White, et al., 2009). In 15% of these cases it is related to ileocaecal disease which carries a poor prognosis due to the areas inaccessibility and its role in regulation of intestinal motility (White, et al., 2009).

A total of 41 studies have been performed, using post-mortem (PM), faecal and serological methods, to examine prevalence of tapeworm infection in horses. Post mortem studies report a wide range in prevalence of *A. perfoliata* across continents; in the UK prevalence has been recorded by Owen et al (1988) as 58% (n=103), Forgarty et al (1994) found 51% of 363 horses, Morgan et al (2005) found 52% (n=81) in horses in the South-West and Pittway et al (2014) 55% of 41 horses, Pearson et al (1993) found an elevated prevalence of 80%

however this study looked at only a small sample size (n=20); in mainland Europe 4-65% has been found and 4.9-82% in USA, Canada, New Zealand and Australia (See Appendix 1A for global study data). An IgG(T) antibody ELISA test, validated against post mortem gold standard, identified increased levels of infection within YS (six months to two years of age) and older horses (>15 years of age) (Proudman, et al., 1997).

#### ***1.2.4.2 Life cycle of Anoplocephala perfoliata***

The life-cycle of *A. perfoliata* is indirect, with many species of pasture-dwelling free-living oribatid mite as an intermediate host (Figure 1.4) (Denegri, 1993). Mites are infected by ingestion of eggs passed in equine faeces and cysticercoids develop within the mite body cavity. Orbatid mites are thought to favour certain habitats and peak abundance is dependent on climatic conditions (Nielsen, 2016; van Nieuwenhuizen, et al., 1994). Horses accidentally ingest infected mites when grazing pasture, cysticercoids are released which develop to adult tapeworms in the intestine; attachment occurs predominantly around the ileocaecal valve, attaching to the intestinal mucosa by suckers on the head of the worm (scolex). As the tapeworm matures within the intestine proglottids become gravid and break off. Detached proglottids disintegrate during large intestinal transit and are passed out in the faeces, releasing eggs into the environment. PPP is approximately six to ten weeks (Proudman & Trees, 1999).

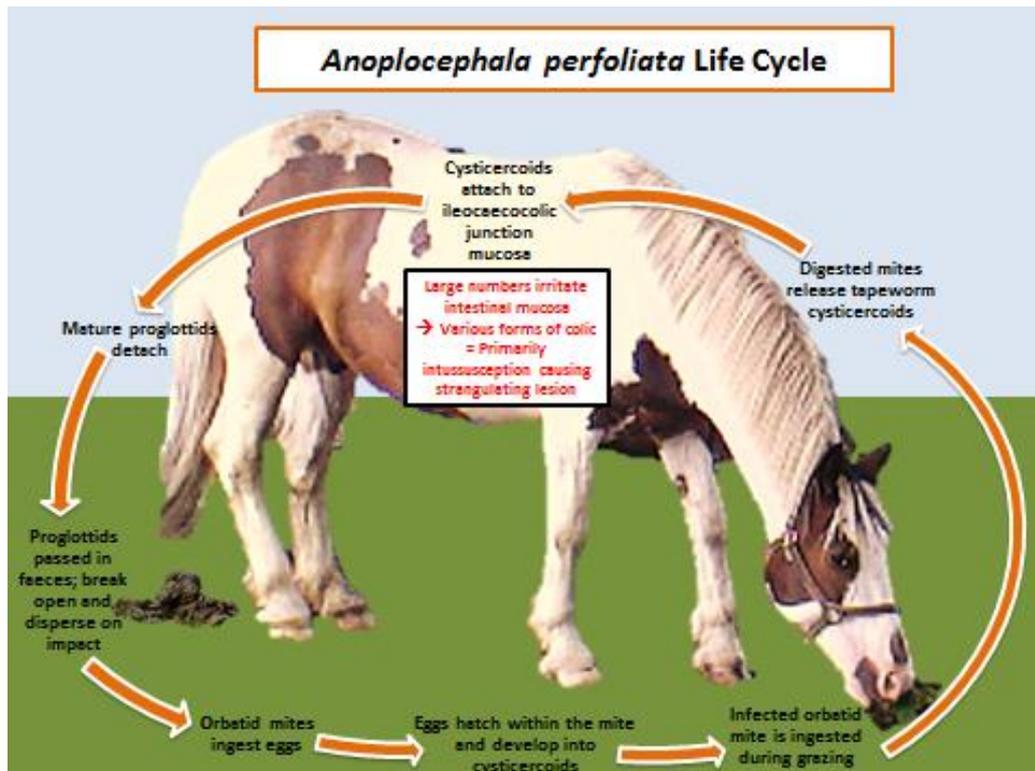


Figure 1.4 The life cycle of *A. perfoliata*. Infection is acquired through ingestion of infected oribatid mites from pasture. Mites are digested, releasing cysticercoids which pass down the intestinal tract and attach the ileocaecocolic junction, feed and develop. Mature proglottids detach and pass onto pasture in faeces, most commonly rupturing and releasing eggs during gut transit or as faecal material impacts the ground. Inflammation from attachment and feeding causes increased peristalsis, implicated in various types of colic, most strongly linked to intussusception.

#### 1.2.4.3 Clinical pathology and disease due to *A. perfoliata* infection

*A. perfoliata* attach at four primary regions of the gastrointestinal tract; Williamson et al (1997) reported that, in a study of 50 infected horses, 17% were found at the ileocaecocolic junction, 80% on the caecal wall, 1.7% terminal ileum and 0.2% ventral colon. A number of studies examining clinical risk and outcome of disease, and histopathological lesion grading of pathology, demonstrated a dose-dependent risk of pathology and associated colic (Fogarty, et al., 1994; Nielsen, 2009; Proudman & Trees, 1999; Reinemyer & Pavone, et al., 2011; Williamson, et al., 1997). Attachment causes ulceration of the caecal mucosa and in many cases this is associated with local inflammation and the development of fibrous connective tissue ranging from part to full thickness (Reinemyer & Nielsen, 2009; Williamson, et al., 1997). The development of fibrous tissue and hypertrophy of the circular

muscle layer have been suggested to mechanically constrict the ileocaecal valve reducing distensibility, affecting gut motility by neurological alterations in regional ganglia (Bain & Kelly, 1977; Beroza, et al., 1986b; Lee & Tatchell, 1964; Pavone, et al., 2010). Pavone et al (2011) found evidence of significant injury to neuronal cells and myenteric ganglia associated with moderate to high parasite burdens. It has also been proposed that the parasites excretory-secretory (ES) products modify intestinal motility, predisposing to alterations causing ileal impaction or intussusception (Proudman & Trees, 1999).

Tapeworm infection has increasingly been associated with different types of colic as new diagnostic tests are developed, specifically the anti-12/13 kDa IgG (T) serum ELISA (Proudman, et al., 1998). Ileal impaction colic, where the terminal portion of the small intestine becomes occluded and so obstructed with ingesta, is rare but potentially fatal; one study indicated that a tapeworm infected horse was 26 times more likely to suffer from this form of acute intestinal disease (Proudman, et al., 1998). Spasmodic colic, the most common form of colic, is of unknown aetiology with many causes proposed, including the presence of tapeworm infection (Proudman & Edwards, 1993; Proudman, et al., 1998; Proudman & Trees, 1999). One study of 95 spasmodic colic cases used the antibody detection ELISA to identify that 22% of these cases were associated with tapeworm infection (Proudman & Trees, 1996a). Increased incidences of caecal rupture and ileocaecal, caecocaecal and caecocolic intussusceptions have also been associated with *A. perfoliata* infection (Barclay, et al., 1982; Beroza, et al., 1986b; Owen, et al., 1989; Proudman & Edwards, 1993; Seung-ho, et al., 2001). In contrast, a study of Canadian horses failed to demonstrate an association between tapeworm burdens measured by ELISA optical density (OD) and the risk of colic (Trotz-Williams, et al., 2008). However, a later Swedish study found an association between FEC-positive horses (using a modified faecal flotation method), with horses having a 16 times higher risk of colic, although no association was found based on the median ELISA OD value (Back, et al., 2013). Similarly

several case control studies support a link between a positive FEC and non-surgical colic episodes (Beroza, et al., 1986b; Bigletti & Garbagnati, 2002; Veronesi, et al., 2009), with shedding of tapeworm eggs indicating an eight fold increased risk of spasmodic colic (Proudman, et al., 1998).

#### **1.2.4.4 Diagnosis of *A. perfoliata* infection**

Multiple methods have been described for the detection of tapeworm in horses. Validated coprological methods have variable, but poor, sensitivities (ranging from 8-61%), due to the intermittent shedding nature of the parasite, high specific gravity of eggs and relatively small number of eggs present in equine faeces (Meana, et al., 1998; Proudman & Edwards, 1992; Proudman, 2003). In addition, FEC do not correlate with infection intensity and so risk of clinical disease, as well as being extremely time consuming to perform.

Immunodiagnostic tests, specifically the antibody detection ELISA initially developed by Høglund et al (1995) using scolex antigens, followed by Proudman and Trees (1996a) assessing IgG(T) levels have mostly replaced coprological testing due to higher sensitivity and correlation of ELISA OD values with infection intensity. Unlike coprological testing, the ELISA test provides a high throughput system so, although still time consuming, it offers a more convenient way of testing large numbers of samples (Proudman, 2003). However, in the context of clinical disease results may be clouded by the persistence of horse antibody levels and potential re-exposure during grazing, reflecting a positive result for up to 26 weeks post treatment in some individuals (Proudman & Trees, 1996a).

### **1.2.5 Other helminth parasites of horses**

#### **1.2.5.1 *Fasciola hepatica***

*Fasciola hepatica* is a highly pathogenic trematode which affects multiple mammalian host species, most commonly sheep and cattle; it is found worldwide and a zoonosis and is considered a neglected tropical disease (Gonzalez, et al., 2011; Mas-Coma, 2005; Winkelhagen, et al., 2012). Due to large production losses in food-producing species, it has

become a primary focus of research (Williams & Hodgkinson, 2015). *F. hepatica* is primarily found in high-altitude tropics and temperate zones (Boray, 1999).

Fluke has long been considered of minor importance in equids with detection of infection being rare, even in endemic areas where prevalence in grazing ruminants is 100% (Nansen, et al., 1975). Prevalence of infection in donkeys, known to be a susceptible equid species, has been reported at 4% and 8.5% (by FEC), 17% (by PM) and 9.5% (by FEC and PM) in UK and Ireland (Matthews & Burden, 2013) in contrast to an Ethiopian study of working donkeys showing a higher prevalence of 40-45% (Getachew, et al., 2010b). In contrast, patent infection in horses has rarely been detected in grazing horses or achieved through experimental infection, a recent report of horses from Northern Ireland a prevalence of 9.2% of 200 horses examined was reported, within this study no sex or breed predilection was noted (Quigley et al., 2016). Outside of the UK between 0.07-60% prevalence has been reported based on FEC in horses (Sadzikowski, et al., 2009; Arias, et al., 2012).

Historically experimental infections in horses have revealed few infections reach patency, leading to the assumption that horses have an intrinsic resistance to establishment of infection within the liver (Alves, et al., 1988; Boray, 1969; Nansen, et al., 1975).

Experimental infections using oral doses of up to 800 of the infective stage (metacercariae) showed patency in an average of less than one out of ten horses (Alves, et al., 1988); in multiple studies Grelck et al (1977) found that when up to 1000 metacercariae were administered by oral or intraperitoneal routes the rate of parasite development was between 0.2-41%, with fluke only being seen in ponies concurrently infected with high strongyle burdens and no infections demonstrating eggs in faeces (Nansen, et al., 1975).

However, despite these failures to induce patent infection experimentally in horses, clinical cases have been described by several authors (Kearney, 1974; Owen, 1977). In addition, a more recent study by Palmer et al (2014) documented patent infection in 23 yearling

horses, demonstrating that infection occurs and matures under natural conditions. *Fasciola hepatica* has an indirect life cycle, where amphibious mud snails, primarily *Galba truncatula*, is the intermediate host in which multiplication and development of the parasite takes place. Eggs passed in the faeces of infected animals develop on pasture to the miracidium which penetrates the foot of the snail, forms a sporocyst which in turn produces redia. Cercariae are produced by clonal expansion of the redia and exit the snail, encysting on pasture as metacercariae which are almost immediately infective. Once within the definitive host the parasite exsheathes, penetrates the intestinal mucosa and migrates to the liver via the abdominal cavity. Juvenile fluke migrate through liver parenchyma for six to seven weeks before reaching maturity in and around the bile ducts with eggs being present in the faeces ten to twelve weeks post infection. Adult fluke have a long lifespan of up to 20 years in sheep (Figure 1.5) (Andrews, 1999; Moazeni & Ahmadi, 2016). Due to the requirement for the mud snail intermediate host the risk of infection from grazing land is dependent on climatic conditions and suitable habitats for the snail population (McCann, et al., 2010).

In ruminants, two primary syndromes occur due to parasite presence and pathology: acute fasciolosis resulting from hepatic damage due to large numbers of juvenile fluke migrating through liver parenchyma, and chronic fasciolosis caused by presence of adult fluke within the bile ducts. For ruminant disease, severity and type of disease is dependent on the infective dose and period of time over which metacercariae are ingested. However, for horses there is a lack of evidence demonstrating pathology related to fasciolosis. One abattoir study found a range of hepatic changes correlating with the presence of liver fluke from mild non-specific signs to bile duct thickening, and calcification with an increase in bile viscosity (Quigley, et al., 2016). Histopathologically, bile ducts showed periductular fibrosis and cellular infiltrate with hyperplastic changes to the biliary epithelium (Quigley, et al., 2016).

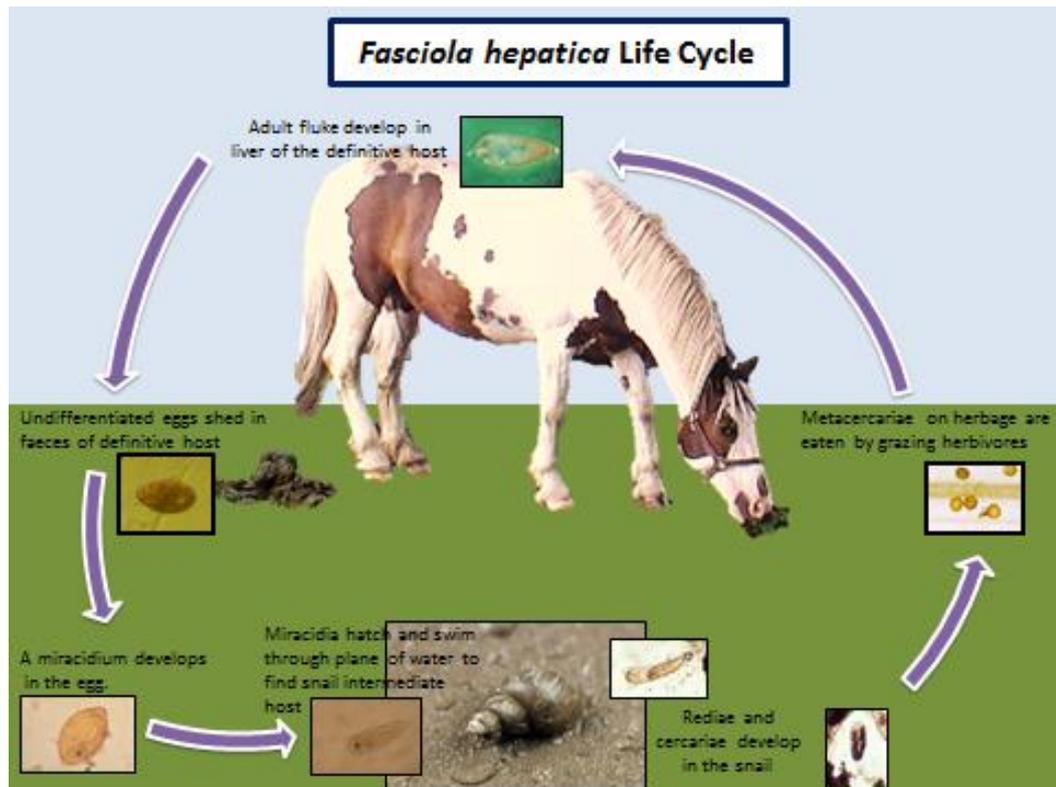


Figure 1.5 The life cycle of *Fasciola hepatica*. Undifferentiated eggs are shed from the definitive host, miracidium develop which hatch and infect a *Galba spp* mud snail where rediae and cercariae develop. Cercariae burst through the foot of the snail and encyst on grass, resulting in infective metacercariae which are ingested by the host. Juvenile fluke hatch, perforate the intestinal wall and migrate through the hepatic parenchyma to the bile ducts where adults lay eggs. (*F. hepatica* images sourced and life cycle adapted from that produced by: Professor D.J.L. Williams)

The impact of fluke infection is poorly understood in the horse and difficult to assess due to their subclinical effect despite liver disease being common and often idiopathic (Williams & Hodgkinson, 2015). Disease is considered to be caused by fibrosis contributing to cumulative liver insufficiency, leading to an impact on performance, in combination with previous or concurrent hepatic insults such as toxins (ragwort, etc.) (Pearson, 1999; Quigley, et al., 2016). Anecdotal reports have associated liver fluke infection with non-specific clinical signs such as weight loss and diarrhoea with a failure to respond to anthelmintic treatment but positive response to antilukicide administration (Quigley, et al., 2016). Owen (1977) described a range of non-specific signs, predominantly poor performance (increased fatigue and recovery times), condition loss post-partum, variable

appetite and a few cases of acute diarrhoea with urticaria in some cases. No disturbances in liver enzymes or other biochemical parameters have been noted although Owen noted slight anaemia in some cases (Owen, 1977; Quigley, et al., 2016). Quigley et al (2016) found infection was not associated with time of year, breed, age, sex, body condition, ante mortem physical examination or strongyle infection status.

#### **1.2.5.2 *Oxyuris equi***

*Oxyuris equi* is a relatively non-pathogenic parasite of equids (Reinemeyer & Nielsen, 2014). This nematode resides in the distal gastrointestinal tract where female worms travel to the rectum to deposit a single mass of 8,000-60,000 eggs within a sticky proteinaceous fluid on the perianal area (Enigk, 1949; Hasslinger, 1990; Reinemeyer & Nielsen, 2014). Deposition results in irritation and rubbing, spreading eggs onto fomites such as gateposts and stable walls. Eggs become infective in three to five days and are ingested from the environment, resulting in infection. PPP of *O. equi* is four and a half to five months (Enigk, 1949). Perianal irritation and tail rubbing are primary clinical signs of infection with *O. equi*, considered commonly to create more cosmetic concern than clinical problems, even when heavy burdens are present (Reinemeyer & Nielsen, 2014). Historical literature suggests that some pathology of the colonic mucosa, described as oedema and superficial erosion, may occur but clinical disease is not noted and adult *O. equi* are not associated with pathology other than perianal irritation (Enigk, 1949).

#### **1.2.5.3 Treatment of clinical disease due to other helminths**

Treatment of suspected *F. hepatica* related clinical disease is performed off-license using triclabendazole at a dose rate of 12mg/kg bodyweight under the veterinary medicines cascade (as this drug is not specifically licenced for horses but does hold a license for the treatment of fluke infection in other species). However, in ruminants resistance to triclabendazole is considered to be widespread in liver fluke populations, therefore if ruminants are the source of infection this may be problematic for treatment as discussed

by Williams et al (2014) and noted in Palmer et al's (2014) yearling cohort. If resistance is suspected then closantel has been used, with better efficacy than nitroxynil, under the veterinary medicines cascade (Rubilar, et al., 1988; Palmer, et al., 2014).

Control of *Oxyuris equi* is challenging, possibly due to anthelmintic resistance or their location within the distal gastrointestinal tract resulting in sub-therapeutic levels of active drug reaching the site of infection (Wolf, et al., 2014). Drug efficacy testing is challenging due to sporadic detection of eggs in both faecal samples and on peri-anal skin in addition to the influence of other aetiologies of tail rubbing (Reinemeyer & Nielsen, 2014).

### **1.3 Diagnostic Testing - Detection of patent infection**

For many nematode species, detecting eggs in the faeces of horses by means of a FEC, will give an indication of the presence of patent infection. Determining the numbers of eggs being shed, using FEC, is important when looking at the role an individual horse plays in parasite transmission as high egg shedding horses contribute most to pasture contamination. Given the utility of a FEC there has been a lot of focus on how the test should best be performed. Within this section, considerations for optimal sampling and preparation, inherent limitations of faecal testing and testing protocols will be discussed in the context of cyathostomin spp, *Strongylus spp* and *Parascaris spp*.

#### **1.3.1 Sampling and storage of faecal samples**

It has been reported that eggs are not evenly distributed within the faecal pile and it is recommended to take four to six samples from various areas around the pile (Figure 1.6) (Lester, et al., 2012; Nielsen, et al., 2010). Similarly, to avoid hatching of eggs post sampling, which can compromise FEC testing, it has been shown that samples should be stored at 4°C under anaerobic conditions (Nielsen, et al., 2010).



Figure 1.6 Multiple sampling sites around the faecal pat account for the non-uniform distribution of nematode eggs.

### 1.3.2 *Strongyle spp*

The eggs of large and small strongyles are morphologically indistinguishable and so it is not possible to differentiate eggs shed by these two groups of nematodes, meaning that FEC report only strongyle eggs (Lester & Matthews, 2014; Lichtenfels, et al., 1998). FECs lack correlation with actual worm burden in individual horses, as egg counts only take into account mature female worms which produce a variable number of eggs and do not detect encysted larvae or adult male worms which do not produce eggs (Dowdall, et al., 2002). Many FEC methodologies are utilised for detection of eggs in equine faeces (Lester & Matthews, 2014).

The highest sensitivities are demonstrated by the CF and FLOTAC techniques, with a minimum detection limit of a one egg per gram (epg) (Table 1.1), however these techniques are the least time-efficient per sample and require specialist training, reducing practicality when a large number of samples require processing (Lester & Matthews, 2014). Both techniques require a large centrifuge which, although available in research laboratories, is not commonly available or financially viable for the purpose of FEC in equine practice. An adapted version of the FLOTAC system, the mini-FLOTAC, is now commercially available and does not require a centrifuge but further validation is required

for use in equines (Lester & Matthews, 2014). The CF method has been used in a number of recent research studies investigating helminth prevalence and drug efficacy (Lester, et al., 2013; Relf, et al., 2013; Relf, et al., 2014).

Table 1.1 The relative lower detection limits (LDL), time and labour efficiency and test cost of available validated faecal diagnostic tests in horses adapted from Lester and Matthews 2014 (Lester & Matthews, 2014).

<b>Method</b>	<b>Reference</b>	<b>Lower detection limit (epg)</b>	<b>Time taken to perform (min/sample)</b>	<b>Relative Ease</b>	<b>Test Cost</b>
Centrifugal flotation (CF)	(Elsheika & Khan, 2011)	1-9epg	10-15mins	Complex	~£500/NA <sup>1</sup>
McMaster	(Ministry of Agriculture, Fisheries and Food (MAFF), 1986)	15-100epg	5-10 mins	Easy	£15-150 (slide only)/~£5-10 <sup>*2</sup>
FECPAK	Innovis Ltd (Lester & Matthews, 2014)	20epg	5-10 mins	Moderate	£760/*PD
FLOTAC	(Cringoli, et al., 2010; Cringoli, 2010)	1epg	12-15 mins	Complex	NA/*PD
Ovatec	Zoetis UK Ltd (Lester & Matthews, 2014)	+/- only	10 mins	Easy	~£1.90 per test to practice/*PD

epg – eggs per gram, \*PD – Practice dependent; NA<sup>1</sup>Not commercially available; <sup>\*2</sup>Price range estimated from Diagnosteq, University of Liverpool, and veterinary practice websites).

The McMaster and related modified techniques are a widely used method in equine practice and is a recommended test for FEC and FECRT under targeted selective guidelines published by the AAEP (Nielsen, et al., 2013) and WAAVP (Coles, et al., 1992). It is simple to do and requires only a single piece of specialist equipment (the McMaster slide). However the small sample of faecal material required, 3g compared to 10g for the CF method, has been shown to introduce error (Lester & Matthews, 2014). Modifications of the McMaster method using higher amount of faecal material and lower dilution factors (g/ml H<sub>2</sub>O) resulted in a lower multiplication factor and were more reliable with better sensitivity,

when performed on faeces spiked with a known number of *Ascaris suum* eggs (Lester & Matthews, 2014; Pereckiene, et al., 1992). More recently methods of reading a greater area of the McMaster slide have been promoted as a means to increase the detection limit, with a minimum of 15epg detected when both chambers (the grids and outside the grid) were examined (Lester & Matthews, 2014; MAFF, 1986). Precision can be further increased by duplication, however, a maximum of four chambers is recommended to balance accuracy and time-efficiency when used in practice (Lester & Matthews, 2014). Incomplete mixing of the saturated-salt and faecal suspension prior to loading of the reading slide can result in non-random distribution of eggs and either over or under estimation of the true egg (Torgerson, et al., 2012). As for the McMasters, FECPAK is not as sensitive as the CF method and has a high cost for little increase in time efficiency. Ovatec only identifies a presence or absence of eggs and due to this lack of quantification is unsuitable as a FEC method to identify 'high egg shedders' or to perform drug efficacy testing. In order to draw comparison with previous studies and in accordance with guidelines that state drug efficacy studies should be performed using the most sensitive test available, the CF technique was used for the detection of nematode eggs in this project (Kaplan & Neilsen, 2010; Vidyashankar, et al., 2012).

### **1.3.3 Differentiation of large and small strongyles – Coprological methods**

Morphologically large strongyle eggs are indistinct from cyathostomes but, as described above, given their different pathologies, identification of the proportion of strongyle infection within a horse that can be attributed to each genus, can be valuable information. There is no correlation between egg count and adult worm burden within the intestines (Andersen, et al., 2012; Bracken, et al., 2012; Duncan & Pirie, 1975; Hodgkinson, 2006; Nielsen, et al., 2012). As stated in section 1.2.2.4 one way to determine if small or large strongyle infections are present within a horse is by culturing eggs in faeces to L3; which

can then be distinguished morphologically by light microscopy (Bevilaqua, et al., 1993; Russell, 1948).

LC and morphological characterisation offers limited species identification, *S. vulgaris* L3 are the only members of the *Strongylus* family that can be positively identified (Gasser, et al., 2004; Hodgkinson, 2006; Thienpont, et al., 1986). Culture of L3 can be unreliable due to wide variation in larval survival rates and it is time consuming and labour intensive (Gasser, et al., 2004). Although moderate to high sensitivity and specificity have been recorded for coproculture (Table 1.1) the technique requires specialist training for accurate identification and differentiation of species (Bracken, et al., 2012; Nielsen, et al., 2010a; Thienpont, et al., 1986). This test has a major disadvantage in that a negative result does not necessarily rule-out *Strongylus spp* infection and a false positive rate of 25.3% has been described (Duncan, et al., 1975; Nielsen, et al., 2012). In practice, it is usual to pool faecal samples for culture with studies showing no significant difference between pooled and individually cultured sample results (Bracken, et al., 2012). Whilst this pooled technique is practical, the recommended number of individual samples used to make the pool, (typically five horses with two grams of faecal material from each individual) and the risk of false negatives due to non-homogenous distribution of eggs within the faecal pile has led to concerns about its reliability and it needs further validation (Bracken, et al., 2012; Craven, et al., 1998; Denwood, et al., 2012). Attempts to provide a practical solution to time constraints have included identification of the first 100 larvae to genus (and where possible species) and if no *S. vulgaris* larvae were found, the result was recorded as negative (Nielsen 2008); whereas a later study performed full larval enumeration to maximise the identification of *S. vulgaris*-positive horses (Nielsen, et al., 2012). The latter approach increased the test sensitivity but is impracticable for routine screening due to the workload constraints, although may have research applications.

#### 1.3.4 Differentiation of large and small strongyles – Molecular methods

Bracken et al (2012) showed that PCR amplification of DNA from strongyle eggs with *S. vulgaris* specific primers could be performed. In this study of individual horses, 12.1% showed *S. vulgaris* present by PCR compared to only 4.5% positive for *S. vulgaris* by LC; a statistically significant difference identifying PCR as a more accurate diagnostic test. However, no significant difference was observed between PCR and LC, when pooled samples were used (72% of farms were *S. vulgaris* positive using individual samples (13 farms), 61% were positive using pooled samples (11 farms) and nine farms were positive on coproculture (Bracken, et al., 2012; Nielsen, et al., 2008). This included the detection of *S. vulgaris* in Oepg horses by PCR (13.7% positive of 22.1% OEPG) compared to only 2.7% detected by LC following a Oepg count. Although, conversely horses positive by LC were not detected by PCR, possibly as a result of uneven dispersal of eggs within the faeces or variability within individual samples (Bracken, et al., 2012; Denwood, et al., 2012). Although this technique has advantages in terms of accuracy and reduced time from sampling to results, it requires specialist equipment and skills, reducing viability for veterinary practice in-house testing, unlike coproculture (Hodgkinson, 2006).

Nielsen et al (2008) developed a fluorescence-based semi-quantitative PCR assay which was able to specifically identify *S. vulgaris* eggs in faecal samples. The results of this study showed a statistically significant linear relationship between the number of eggs used and the threshold of cycle's value, giving some degree of quantification. In field evaluation it proved to be more sensitive than LC and semi-quantitative (Nielsen, et al., 2008). This was performed on four horse farms and a larger study may be of value in order to confirm the statistical significance. Storage of eggs, as for FEC, was a primary source of error in quantification, limiting potential usage in practice; in order to overcome this eggs require prompt extraction and 70% ethanol storage to prevent embryonation (Andersen, et al., 2013a; Nielsen, et al., 2012). This new assay was assessed as having potential for higher

diagnostic sensitivity compared to LC (Nielsen, et al., 2012). This is the most promising assay so far but has not been validated in wide-scale trials or made commercially available.

### 1.3.5 Diagnosis of *Anoplocephala perfoliata* infection

The intermittent shedding of tapeworm eggs, dependent on the rate of maturation of proglottids and the decomposition of egg packets within the faecal pile, makes diagnosis of tapeworm infection by coprological methods challenging (Beroza, et al., 1986; Slocombe, 1979). Multiple methods have been investigated, with high specificity, (98-100%) and a low rate of false positive results (Nilsson, et al., 1995; Proudman & Edwards, 1992; Skotarek, et al., 2010). However, the sensitivity of coprological methods was low. The McMaster method has demonstrated the lowest sensitivities of 2.8% and 8% in various studies (Meana, et al., 1998; Proudman & Edwards, 1992), double sugar centrifugation flotation (DCF) technique has demonstrated the highest sensitivity at 61% (Rehbein, et al., 2011; Tomczuk, et al., 2014). The sensitivity of DCF was increased to 92% by removal of horses with low parasitic burdens from statistical analysis by Proudman and Edwards (1992), leading to suggestions it could be used to detect horses with high burdens which may be at higher risk of clinical disease. The FLOTAC method was investigated for use with *A. perfoliata* and results show potentially increased sensitivity and specificity in a single small study, however more validation is required before conclusions can be drawn (Chlastakova, et al., 2009). However, diagnostic sensitivity and specificity is highly influenced by the prevalence of *A. perfoliata* in the herds being tested (Nielsen, 2016).

Many authors have evaluated factors affecting the sensitivity and specificity of faecal tests for tapeworms. Rehbein et al (2011) suggested that the choice of flotation medium, sucrose versus sodium chloride and zinc sulphate solutions, significantly affected diagnostic performance with the intermediate specific gravity solution, sucrose (SG = 1.26) showing the best results. Sensitivity has also been shown to vary depending on the time of year, with lowest values of 40% obtained April to June and highest of 75% seen January to March

(Tomczuk, et al., 2015). Corroborating this seasonally increased prevalence, studies have demonstrated higher numbers of mature tapeworms seen on necropsy during the 1<sup>st</sup> quarter of the year, suggesting that in temperate climates tapeworm detection may be more effective if undertaken January to March. Regardless of time of year, multiple studies have found that burdens of >20 tapeworm are ~90% likely to provide a positive coprological result, this does however leave around 10% of horses with a high burden which would remain undetected (Kjaer, et al., 2007; Meana, et al., 1998; Proudman & Edwards, 1992). Although a positive egg count may reliably detect infection in more heavily infected animals, as for other parasitic species, the number of eggs is not indicative of actual burden (Meana, et al., 1998; Nilsson, et al., 1995; Proudman & Edwards, 1992). One way shown to increase the detection rate of tapeworm infection is post-treatment coprological analysis, with various studies demonstrating higher egg counts and percentage positive animals 18-24 hours post-treatment, suggested to be due to dead tapeworm segment degradation and release into the intestinal lumen (Elsener & Villeneuve, 2011; Hearn & Hearn, 1995; Nielsen, 2016; Sanada, et al., 2009; Slocombe, 2004). Despite advances in adaptation of coprological techniques to optimise performance and useful diagnostic potential, these methods are time consuming and, for increased sensitivity, require specialised equipment such as a centrifuge.

Serological methods to detect antibodies to tapeworm infection have been investigated and subsequently made commercially available. A study in slaughtered horses, detecting *A. perfoliata* antibodies using a scolex antigen, showed a sensitivity of 70% and specificity of 95%; the degree of detection was highly correlated with the parasite burden and risk of pathological lesions (Hoglund, et al., 1995). This study demonstrated that serological methods could be used to show the degree of exposure within a cohort of horses but this ELISA was not made commercially available. Proudman and Trees (1996a, 1996b) produced the first widely available serological test detecting antibodies to *A. perfoliata* excretory-

secretory (ES) antigens, showing sensitivity and specificity of 68% and 95% respectively. Validation showed statistically significant positive correlation with tapeworm burden, with correlation coefficients of 0.54-0.63 indicating information about the level of exposure to tapeworm within herds of horses could be detected (Hoglund, et al., 1995; Kjaer, et al., 2007; Proudman & Trees, 1996a). This assay has been available via the Diagnosteq Laboratory at University of Liverpool (UofL), with modified versions also available through Beaufort Cottage Laboratories (Rossdale Veterinary Surgeons, Newmarket, UK) and Austin Davis Biologicals (ADB), utilised to determine the timing and number of anti-cestode treatments administered at a herd level (Nielsen, 2016). In addition a modified version using detection of antibodies to tapeworm infection in saliva, Equisal™ (Austin Davis Biologicals Ltd), making it more accessible financially to owners, is now commercially available and initial validation work shows sensitivity and specificity of over 80% (Lightbody, et al., 2016). The application of an ELISA for determination of individual horse tapeworm infection and burden is not considered reliable due to the persistence of antibody levels for up to five months following treatment (Kjaer, et al., 2007; Proudman & Trees, 1996a); in addition, other studies have reported high proportions of false positive results and cross-reactivity with another equine tapeworm, *A. magna*, which has a low to negligible clinical impact (Abbott, et al., 2009; Back, et al., 2013; Bohorquez, et al., 2012). A coprological antigen ELISA developed by Kania & Reinemeyer (2005) was utilised in a recent Canadian study (Skotarek, et al., 2010) which reported a sensitivity and specificity of 74% and 92% respectively, correlation to worm intensity was deemed to be significant and so this test may have future potential as a diagnostic method for individual horse diagnosis.

### **1.3.6 Diagnosis of *Parascaris spp***

To detect prepatent infection a single study has been performed using western blot to detect *Parascaris spp* ES products and found IgG (T) antibodies to multiple excretory-secretory antigens in naturally infected foals. However, passive transfer of colostral

antibodies from the mare precludes practical use of a crude larval ES-product for the development of a diagnostic western blot based test (Burk, et al., 2014; Burk, et al., 2016). A similar challenge has been encountered in the attempt to develop a diagnostic serological assay, because it is complicated by the ubiquitous prevalence of the parasite in YS, meaning that the clinical value of a qualitative test is negligible (Vollger, et al., 2012; Burk, et al., 2014). As patent stage disease is of primary concern, diagnosis of this stage will be focussed on here.

Using the centrifugal flotation (CF) technique FEC can be used to detect ascarid eggs, down to a sensitivity of one epg; this confirms the presence of adult female worms with a positive predictive value of 0.95 and negative predictive value of 0.66 (Nielsen, et al., 2010b; Relf, et al., 2013). However, FEC does not detect male worm burden and there is no linear relationship between EPG and number of adult worms present resulting in lack of quantitative measurement of burden. In addition, mares and foals practice coprophagy, which raises the possibility of false positives for an estimated 5% of samples (Nielsen, et al., 2010b; Nielsen, et al., 2015). Around weaning, where highest degree of risk for clinical disease exists and significant immunity is developing, it has been shown that a large adult worm burden may be present in the absence of a positive FEC result, suggesting a false negative rate of ~30% (Nielsen, et al., 2015). This diagnostic limitation of FEC at peak disease risk period has led to development of a novel ultrasound detection method, which grades the clarity of image and presence of worms within the intestinal lumen (Nielsen, et al., 2015). Although this can detect presence of adult burden and some degree of clinical risk it is only based upon visualisation of parasites within specific portions of the intestine and the entirety of the small intestine cannot be evaluated. In addition, it requires an experienced operator and often, in order to obtain diagnostic quality images, for the foal to be sedated which may not be possible, financially viable or realistic on a regular screening basis. This technique grades "severity" using a cut-off value of ten worms for "high risk"

foals, although there is currently no evidence relating to the number of adult worms in the small intestine with the risk of impaction and colic. Nielsen et al (2015) investigated financial viability and only under conditions of low veterinary pricing and high disease status of the farm is it potentially worthwhile for stud farms, with the high usage of anthelmintics within YS this is therefore unlikely to be viable for routine screening. This technique has the advantage of potentially providing estimation of clinical risk, so that intensive monitoring can be implemented prior to anthelmintic administration or around weaning for high risk foals, in order to decrease the time to diagnosis and treatment. However, for all of these methodologies, definitive diagnosis of surgical colic aetiology on admission to a hospital facility is unlikely to improve survival rate.

### **1.3.7 Diagnosis of other helminth parasites of horses**

Definitive diagnosis of fluke infection for horses relies on FEC, which has a low sensitivity only detecting 60-70% of patent infections in addition to the fact that horses lack of a gall bladder which results in lower egg shedding (Owen, 1977). Post-mortem or the recently developed recombinant ES mutant CL1 antibody CL1 ELISA, were found to have a specificity of 95.6% but low sensitivity of 42.1% (Quigley, et al., 2016). Another ELISA test, fluke 2.9kDa antigen ELISA, has higher sensitivity and specificity (Arias, et al., 2012). Coproantigen ELISA, used in ruminants, has proven unsuitable for use in the horse, detecting 9% of infected horses compared to FEC (Palmer, et al., 2014). Studies have also investigated increased serum glutamate dehydrogenase (GLDH) and gamma glutamyl transferase (gGT), biochemical markers of hepatic insult, as diagnostic tools (Soule, et al., 1989). gGT is of limited use for hepatopathy diagnosis or prognosis due to multiple potential sources including the pancreas and kidneys; in addition increases in this enzyme may be seen in the absence of detectable liver histopathology (Durham, et al., 2003). However, gGT has been noted to remain raised during periods of potential reparative processes and where biliary hyperplasia is seen. GLDH, although derived from the liver, has

only moderate specificity for liver disease and is raised even from mild hepatic insults (Durham, et al., 2003; The Liphook Equine Hospital, 2012). In contrast to gGT however GLDH has a short half-life and so an association with the degree of currently active hepatic insult has been suggested (Durham, et al., 2003; The Liphook Equine Hospital, 2012). Due to the lack of evidence for a clearly defined clinical syndrome, controversy over the clinical impact of infection and the rate at which patency is reached, diagnostic testing and definitive diagnosis is challenging.

Diagnosis of *Oxyuris equi* is made by observation of female worms protruding to lay eggs; expulsion of females in faeces or found during rectal examination; occasionally noted on faecal flotation if samples are collected per rectum or, most commonly, using the sticky-tape test on the peri-anal skin (Reinemeyer & Nielsen, 2014).

## **1.4 Control of Parasites**

### **1.4.1 Anthelmintic control**

Anthelmintic administration is considered the cornerstone of control strategies and treatment of parasitic infection in horses. Three classes of anthelmintics are licensed for the treatment and control of parasites of horses (Table 1.2). Note that there are no flukicides licensed for use against *Fasciola hepatica* infection in equines.

#### **1.4.1.1 Benzimidazoles**

BZ's were the first drug class licensed for use in horses; they consist of oral preparations of FBZ, Mebendazole (MBZ) and Thiabendazole. Studies found that either 7.5mg/kg or 10mg/kg given daily for five consecutive days reduced early L3 stages of cyathostomins by 91.5% and 98%, and late L3 burden by 99.4% and 96% respectively (DiPietro, et al., 1997; Duncan, et al., 1998). BZ drugs selectively bind to parasitic  $\beta$ -tubulin subunits, interfering with microtubule formation which prevents glucose uptake and starves the parasite (Friedman & Platzer, 1979). This class, in particular FBZ, has a large safety margin due to its poor absorption into systemic circulation from the gastrointestinal tract. Dosing interval,

defined by ERP was six to eight weeks when the drug was first licensed (Fort Dodge Animal Health, 2008).

Table 1.2 The anti-parasitic activity and licensed dose rate for each drug licensed for use in the horse. Data is taken from the pioneer product for each active drug

<b>Parasite</b>	<b>Stage</b>	<b>FBZ</b> (7.5mg/kg)	<b>PYR</b> (19mg/kg)	<b>IVM</b> (0.2mg/kg)	<b>MOX</b> (0.4mg/kg)	<b>PRAZQ</b> (2.5mg/kg)
<i>Cyathostomin</i> spp	L3/4 encysted	*5day course				
	L3 hypobiosed	*5 day course				
	Luminal larvae					
	Adult					
<i>Strongylus vulgaris</i>	Migrating larvae					
	Adult					
<i>Parascaris</i> spp	Immature					
	Adult					
<i>Dictyocaulus arnfeldi</i>	Inhibited L4					
	Adult					
<i>Oxyuris equi</i>	Inhibited L4					
	Adult					
<i>Trichostrongylus axei</i>	Adult					
<i>Habronema</i> spp	Adult					
<i>Onchocerca</i> spp	Microfilariae					
<i>Strongyloides westeri</i>	Immature					
	Adult					
<i>Gastrophilus</i> spp	Oral stages					
	Gastric stages					
<i>Anoplocephala perfoliata</i>	Adult		*38mg/kg			
<i>Anoplocephala magna</i>	Adult					
<i>Paranoplocephala mammillana</i>	Adult					

FBZ – Fenbendazole; PYR – Pyrantel embonate; IVM – Ivermectin; MOX – Moxidectin; PRAZQ – Praziquantel. [Source Veterinary Medicine Directorate datasheets for Panacur oral paste®, Strongid-P™, Eqvalan®, Equest®, Equitape®.]

#### 1.4.1.2 Tetrahydropyrimidines - Pyrantel

Tetrahydropyrimidines (THP), namely PYR, were the second class to be licensed for equine use. Two preparations are licensed – pyrantel pamoate, for daily in-feed administration, and pyrantel embonate, given as a single oral dose. THP works by mimicking the action of

acetylcholine at neuromuscular junctions, binding to nicotinic Ach receptors causing spastic paralysis (Nielsen & Reinemeyer, 2013). PYR is licensed for the treatment of adult cyathostomins, including BZ-resistant (BZ-R) strains; adult *Strongylus spp*; adult *Parascaris spp* and, at double dose, *A. perfoliata*. Morantel, a less commonly used THP, has an additional license for the treatment of luminal cyathostomin larvae. Dosing interval of PYR embonate when first released was four to eight weeks (Fort Dodge Animal Health, 2008).

#### **1.4.1.3 Macrocyclic lactones**

Macrocyclic lactones (ML) are the most recent class of anthelmintic drugs licensed for use in horses and include the avermectins, abamectin and IVM, and a milbemycin, MOX. IVM has been shown to reduce pharyngeal pumping in *Haemonchus contortus*, but pharyngeal activity remains unchanged in the presence of MOX (Paiement, et al., 1999) and cause paralysis of both body muscles and the uterus, leading to failure of movement and egg laying (Yates, et al., 2003) through targeting of glutamate and GABA-gated chloride channels (Cully, et al., 1994; Holden-Dye & Walker, 1990). Other factor suggesting differing modes of action between MOX and IVM is the effect of p-glycoproteins, transmembrane pumps which reduce the uptake of lipophilic compounds from the GIT to enhance elimination (Cobb & Boeckh, 2009). MOX has been shown to be a poor P-gp substrate compared to IVM and other avermectins (Lespine, et al., 2007). Both MOX and IVM are lipophilic, resulting in selective concentration in the fat and liver of the host (Canga, et al., 2009). The lipophilic nature of MOX, 100 times greater than that of IVM, selectively concentrates in the hosts' body fat for slow release into the bloodstream, resulting in greater persistence of activity (Schumacher & Taintor, 2008; Zulalian, et al., 1997). Studies have shown terminal half-life of IVM (Pérez, et al., 1999) and MOX (Cobb & Boeckh, 2009) to be 4.25 days and 23.11 days respectively. MOX is not licensed for use in young foals and emaciated individuals due to the lack of body fat and consequent risk of toxicity which can produce severe neurological signs (Khan, et al., 2002; Muller, et al., 2005). Dosing intervals

when first licensed were eight weeks for IVM and 90 days for MOX, defined by the ERP when first licensed (Fort Dodge Animal Health, 2008; Perez, et al., 2001).

#### ***1.4.1.4 Praziquantel***

Praziquantel (PRAZ), an isoquinolone, is licensed for the treatment of the adult stage of all three major tapeworm spp as a single dose of 2.5mg/kg. This drug's mode of action involves absorption through the tegument, causing tetanic paralysis and intense focal vacuolisation and disintegration of the parasites' tegument resulting in metabolic disturbances, contraction of tegumental muscle and paralysis (Elsheikha, et al., 2011). The disintegration of tegument results in the parasite becoming vulnerable to the immune system and digestive secretions and enzymes (Elsheikha, et al., 2011). PRAZ is rapidly absorbed and distributed within the host's organs, being metabolised rapidly by the liver. Dosing is recommended every six months, often administered as a combination product including IVM or MOX (Source: Veterinary Medicines Directorate).

#### ***1.4.1.5 Other anthelmintics for use in horses***

Several novel anthelmintics licensed in ruminant spp have been investigated for use in equids with little success. A cyclooctadepsipeptide (CODs), emodepside; with a mode of action that is still unclear was used in an in vitro test on cyathostomin spp. looking at the effects of the drug on egg hatch and larval development; however, Tandon et al (2004) and Pook et al (2002) both used the Drenchrite assay and reported conflicting results regarding its reliability for cyathostomins. Another study using the larval motility inhibition assay showed emodepside to be twice as potent as levamisole but much less effective than IVM (Schurmann, et al., 2007). A license has been issued for canine and feline hookworm and ascarid control in small animals but no further equine work has been performed (Nielsen & Reinemeyer, 2013). There has been some success in ruminants using the novel aminoacetonitril derivatives (AADs), such as monepantel, which is now commercially marketed in Europe, New Zealand and Australia for multi-drug resistant nematodes of

sheep. AADs cause hypercontraction of the parasitic body wall resulting in spasmodic contraction of the anterior pharynx, leading to inability to feed and death (Kaminsky, et al., 2008). AADs are well tolerated and of low toxicity in mammalian host species despite this, a product for use in horses is thought to be unlikely to be developed, potentially due to the economic viability of development and licensing (Kaminsky, et al., 2008; Matthews, et al., 2012). Spiroindoles, an acetylcholine antagonist which causes flaccid paralysis and death of nematodes, is also marketed as a combination product (with abamectin) in New Zealand and Australia for sheep (Little, et al., 2010; Matthews, et al., 2012). However, studies have been performed in equids and this compound is highly toxic, therefore further development is not possible.

## **1.5 Anthelmintic Resistance**

### **1.5.1 Definition of anthelmintic resistance**

Anthelmintic resistance (AR) genes are thought to be present within genetically diverse parasite populations or occur as a result of natural genetic mutation. They are heritable traits that allow the worm to survive drug treatment and so pass AR genes on to the next generation (Sangster, 1999). Anthelmintic treatment provides an environment which allows the resistant parasite to survive and multiply (Bowman, et al., 2005). Resistance has been demonstrated to be heritable, identified by an increase in the proportion of the population carrying a gene identified to be linked with resistance; these are either genetic, direct changes to specific genes, or epigenetic changes, methylation of genes or promoter regions which change the expression in response to the presence of the drug (Fojo, 2007; Prichard, 2007). Resistance is not reversible, even when the selection pressure is removed for a prolonged period of time (over ten years) (Lyons, et al., 2007; von Samson-Himmelstjerna, 2012).

### **1.5.2 Factors that are thought to facilitate the development of AR**

Several factors have been identified which may facilitate the development of AR, the most significant of these being incorrect dosing, particularly under-dosing, of animals and frequent use of anthelmintics resulting in high frequency of exposure of a parasite populations to a given drug, leading to high selection pressure and drug treatments that do not consider leaving a large proportion of the parasite population not exposed to drug (termed 'in refugia') (van Wyk, 2001).

#### ***1.5.2.1 Under-dosing***

Under-dosing is considered very important to the selection of drug resistance (Nielsen, et al., 2006; van Wyk, 2001). Many studies have examined if, and how, individual horse weight is determined prior to drug administration. In most studies, dose was determined based on individual weights (mean 70%, median 80%, range 14-100%) with use of weigh-bridge the least common in all studies, possibly due to reduced access and expense of equipment; by far the most common method across the majority of studies was estimation "by eye" (Lendal, et al., 1998; Lind, et al., 2007; Relf, et al., 2012; Stratford, et al., 2013; von Samson-Himmelstjerna, et al., 2009). In order to investigate the accuracy of by eye measurement of weight by both vets and owners several studies have been performed with Johnson et al (1989) finding that owners and vets were likely to significantly underestimate the weight of horses when using visual appraisal; this is supported by a large scale study of 600 horses by Ellis and Hollands (1998) demonstrating lowest accuracy of by-eye technique regardless of breed, age or height.

#### ***1.5.2.2 Parasite refugia***

The term refugia refers to the proportion of the parasite population which is left unexposed to drug treatment, therefore escaping the selection pressure (van Wyk, 2001). This includes parasite stages on pasture, parasites in untreated hosts and has been considered to include parasite stages not targeted by the specific anthelmintic administered, such as encysted cyathostomin stages. This lack of treatment of a proportion

of the population provides a pool of anthelmintic-susceptible genotypes which aid in dilution of resistant alleles within the parasite population, slowing the development of resistance (van Wyk, 2001). Sangster (2001) has suggested that the larvicidal and persistent activity of MOX reduces potential refugia populations and may increase selection pressure. There is currently no evidence to support this hypothesis specifically for MOX but Reinemyer et al (2003) demonstrated that the larvicial FBZ dosage intensified AR selection in cyathostomes. Reinemyer et al (2003) performed FECRT on two groups of male horses using MOX and FBZ, recording FECRT of 99.6% and 100% for MOX and 92.9% and 85.6% with FBZ; larvicidal doses of both drugs were administered at +90 days followed by administration of an adulticidal dose of FBZ at day +234. Reduction within the MOX group was 45.2%, compared to the FBZ group which recorded a reduction of -60.7%.

#### ***1.5.2.3 “Dose and move”***

Few studies have investigated the prevalence of “dose and move”, where horses are given an anthelmintic prior to being moved to “clean” pasture. This practice was transferred from ruminant management, strongly advocated from the early 1900s and regained popularity and backing from parasitologists worldwide in the 1980s and 90s as a method to reduce the risk of anthelmintic resistance development (van Wyk, 2001). However, this practice is now considered to substantially contribute to selection pressure as “clean” pasture with no parasite refugia is contaminated with progeny of resistant worms if AR survivors are present in treated horses when they are moved. Studies recording occurrence of dose and move estimate it to be approximately 25% (range 19% - 33%) of establishments spanning studies from 1998 to 2012 (Lendal, et al., 1998; Matthee, et al., 2002; Relf, et al., 2012).

#### ***1.5.2.4 Frequency of Anthelmintic treatment***

The YS group (one to three years) are widely documented to be the highest egg shedding cohort; necessitating more frequent treatment (Relf, et al., 2013). However, more frequent treatment increases selection pressure and reduces refugia (van Wyk, 2001). Several

studies identify that YS treatment frequency was higher than adults (Bolwell, et al., 2015; Hinney, et al., 2011; Lind, et al., 2007; Matthee, et al., 2002; Robert, et al., 2015; von Samson-Himmelstjerna, et al., 2009). Lind et al (2007) delineated responses by establishment type and demonstrated a larger difference in YS versus adult treatment number per year on stud farms compared to other types of farm such as livery yards, trotting stables, riding schools and small private yards. However, the definition of YS varied from under three years to less than five years making clear conclusions and comparisons of treatment frequency difficult. Whether the increased treatment frequency of YS in particular has an effect on the rate of development of AR is unknown.

Studies examining drug usage show a high dependence on MLs, often as PRAZ combination products. Allison et al (2011) showed 39% of 574 UK leisure horse respondents used MOX in the previous 12 months and 34% IVM; which was corroborated by Stratford et al (2013) with MOX comprising 43% of all treatments used on 92% of yards surveyed and IVM 33% of all treatments, used on 71% of yards of 193 UK leisure horse respondents. Hinney et al (2011) demonstrated MLs were most commonly used with 26% of 235 German horse farms stating they were the sole drug class utilised, and in Bolwell et al (2015) in New Zealand TB and SB studs 91% of 136 participants stated a specific drug detailed use of MLs +/- PRAZ, although within this study abamectin-PRAZ combination was most common (77% of treatments), with adult and YS treatment frequencies of four and six per year respectively. Frequency of administration varied between studies with Lester et al (2013) describing the majority of respondents administering an ML anthelmintic every two to six months; Stratford et al (2013) reporting administration of an anthelmintic to be most common either every 13-15 weeks (22% participants) or every four to six months (20%); in Sweden similar results found by Hinney et al (2011) in Brandenburg, Germany (one to seven treatments per year in adults, two to 12 in YS). These surveys demonstrate a widespread, high level of reliance on MLs for parasite control in horses.

### 1.5.3 Detecting anthelmintic resistance

Anthelmintic resistance has currently only been detected in cyathostomin and *Parascaris* spp, therefore only detection of resistance in these parasites will be discussed (Kaplan, 2004). High prevalence of resistance to triclabendazole, for the treatment of *F. hepatica*, has been documented in ruminants but due to the low egg output, apparent infrequency of infection and lack of license of TBZ for use in horses, no specific studies have been performed; although one study from Australia indicated that infection of horses with triclabendazole resistant fluke populations had occurred (Palmer, et al., 2014). Many in-vitro methods for early detection of AR have been investigated, such as the egg hatch and larval development assays (Ihler & Bjorn, 1996), larval motility (Schurmann, et al., 2007) and feeding inhibition tests (Alvarez-Sanchez, et al., 2005), but these methods require nematode species-specific standardisation and further work to determine the impact of the results generated (Matthews, et al., 2012). None are currently considered to be a reliable or practical alternative to the FECRT and so only the latter will be discussed in depth here (Matthews, et al., 2012).

#### 1.5.3.1 Faecal Egg Count Reduction Test

The primary method recommended as the gold standard and used for in vivo assessment of drug efficacy is the FECRT. This is generally performed for strongyle parasites and involves measuring the FEC at day of treatment and 14-17 days post-treatment and calculating the percentage reduction in egg count; a technique adapted from ruminants (Coles, et al., 2006; Lester & Matthews, 2014; Nielsen, et al., 2013; Stratford, et al., 2013). For *Parascaris* spp drug efficacy, specific guidelines are not standardised (Matthews, et al., 2012). The FECRT technique relies on faecal egg output and has some limitations: a) FEC are highly variable within individuals; b) epg does not share a linear relationship with adult stage parasite burden present or prepatent stage numbers; c) a FEC cannot differentiate cyathostomin and *Strongylus* spp;; d) there is non-uniform distribution of eggs within faeces; e) there is an effect of sampling and storage of faeces prior to testing and f) the

sensitivity of the tests used (Denwood, et al., 2010; Denwood, et al., 2012; Kaplan, et al., 2010; Lester, et al., 2013; Leveke, et al., 2011; Matthews, et al.; 2012; Nielsen, et al., 2010; Pook, et al., 2002; Relf, et al., 2013; Stratford, et al., 2013; Vidyashankar, et al., 2012; Wood, et al., 2012). In order to maximise the use of the FECRT a number of guidelines have been developed. FECRT is recommended for use at a population level in order to account for individual variation in epg counts, with a minimum of six to ten horses used (Nielsen, et al., 2013). In order to be eligible for inclusion, each horse must reach a threshold pre-treatment epg, however, these criteria vary slightly between studies using high sensitivity methods such as the CF. Relf et al (2014) used a  $\geq 40$ epg cut-off, with Stratford et al (2013) and Lester et al (2014) setting inclusion at  $\geq 50$ epg, both using the CF technique, highlighting an important area where further standardisation is required. FECRT percentages for susceptibility/resistance have been set (Coles, 2006; Nielsen, et al., 2013); lower confidence limits were calculated for inclusion in order to account for errors within results due to daily variability and testing errors (Vidyashankar, et al., 2007; Vidyashankar, et al., 2012). Several methods for analysing FECRT data have been investigated in order to increase accuracy in interpretation: the WAAVP/AAEP method previously described (Coles, et al., 1992); percentage change in arcsine transformed proportional reductions and nonparametric bootstrapping (Vidyashankar, et al., 2007; Vidyashankar, et al., 2012). Further work and clarification needs to be performed in order to determine the most appropriate method, taking into account the additional operator computational skill and requirements (Lester & Matthews, 2014). However, the WAAVP/AAEP calculation guidelines are used in practice for the majority of published studies and the FECRT test is currently the best available for determination of drug efficacy (other than critical testing which is not viable for routine surveillance of horse populations) and can be considered the method of choice (Kuzmina & Kharchenko, 2008). Current recommended guidelines for

interpretation of group average FECRT percentages, and lower confidence intervals, for each drug class can be seen in Table 1.3.

Table 1.3 The reduction percentage cut-off values indicating susceptibility for each drug class and the lower confidence intervals where duplication of test is recommended prior to resistance status being diagnosed (Vidyashankar, et al., 2007; Nielsen, et al., 2013)

<b>Drug class</b>	<b>Cut-off value for susceptible result</b> (Nielsen, et al., 2013)	<b>Lower confidence limits</b> (Vidyashankar, et al., 2007)
Benzimidazoles	>90% reduction	<80% reduction
Tetrahydropyrimidines (Pyrantel)	>90% reduction	<80% reduction
Macrocyclic lactones	>95% reduction	<90% reduction

### **1.5.3.2 Egg reappearance period**

The ERP, when eggs first start to appear in faeces following anthelmintic treatment, is thought to provide an early indicator of a lack of sensitivity to anthelmintics and a shift towards resistance status (Sangster, 2001). Again it relies on FEC and so has the same limitations regarding sources of error and variation, but perhaps the most pertinent issue with ERP is that there is no standardisation or world-wide consensus on definition, with two main interpretations found within studies -

- A. The first week where a positive egg count is observed (Dudeney, et al., 2008; Lyons, et al., 2008; Molento, et al., 2008)
- B. The time after treatment at which the mean of the individual counts is  $\geq 10\%$  of pre-treatment FEC (Boersema, et al., 1996; Borgsteede, et al., 1993; Jacobs, et al., 1995; Larson, et al., 2011; Mercier, et al., 2001; Tarigo-Martinie, et al., 2001; von Samson-Himmelstjerna, et al., 2007).

Definition B is considered a more conservative estimation which relates to the distribution of FEC data prior to treatment, so giving a more accurate measurement of anthelmintic

sensitivity at a population level (Matthews, 2014). Due to the differences in definition it is difficult to compare results across studies and further investigation is required in order to standardise the procedure, definition and interpretation of results.

#### **1.5.4 Prevalence of Anthelmintic resistance in equine parasites**

##### **1.5.4.1 *Cyathostomin spp***

Populations of cyathostomins showing resistance to BZ and PYR, in addition to multi-drug resistant strains, have been documented by FECRT across Europe and the USA, as seen in Table 1.4. Benzimidazole resistance is considered common in many areas, with >80-85% of farms showing <85% BZ efficacy by FECRT in the UK (Lester, et al., 2013; Relf, et al., 2014 ), USA (Chapman, et al., 2003), Sweden (Osterman Lind, et al., 2007) and France (Traversa, et al., 2012) on both TB and non-TB establishments. Benzimidazole resistance has also been shown in encysted stages following a five day course of FBZ (Chandler, et al., 2000). However, oxbendazole (OBZ) may still be effective in some benzimidazole resistant populations (Chapman, et al., 2003). Mutations at the  $\beta$ -tubulin isotype-1 codon 167 and codon 200 loci can confer resistance, especially for OBZ (Demeulenaere, et al., 1997; Hodgkinson, et al., 2001; Hodgkinson, et al., 2005). In areas such as the Ukraine, where due to economic factors, anthelmintics have not been as widely available for as long as in the UK and USA, a lower prevalence of BZ-R has been recorded, with less than ten percent of farms demonstrating resistance by FECRT (Kuzmina & Kharchenko, 2008). With respect to resistance to PYR, 40-50% of farms in Europe and the USA show evidence of PYR-R in cyathostome populations when evaluated by FECRT (Table 1.4). Multidrug resistant strains, where FBZ/OBZ and PYR lack efficacy, have been reported in several studies (Molento, et al., 2012; Tandon & Kaplan, 2004; Traversa, et al., 2009; Traversa, et al., 2012).

Macrocyclic lactones are, currently, the most frequently used anthelmintic in both YS and adults cohorts across all establishment types (Bolwell, et al., 2015; Hinney, et al., 2011; Ireland, et al., 2013; Lendal, et al., 1998; Lind, et al., 2007; Lloyd, et al., 2000; Matthee, et

al., 2002; Relf, et al., 2012; Stratford, et al., 2013). Resistance, as determined by FECRT, has not yet been commonly detected with efficacies of >95% documented worldwide (Comer, et al., 2006; Klei, et al., 2001; Osterman Lind, et al., 2007; Pook, et al., 2002; Tandon & Kaplan, 2004; Traversa, et al., 2007; von Samson-Himmelstjerna, et al., 2007). A study in Brazil using yearlings, reported an efficacy of 87% following IVM treatment but given the variability of egg counts and higher egg shedding in young animals, remains equivocal as to its interpretation of resistance (Molento, et al., 2012). An increasing number of reports of reduced ERP following treatment with IVM or MOX have been noted in studies from Germany (von Samson-Himmelstjerna, et al., 2007), Sweden (Traversa, et al., 2007), Brazil (Molento, et al., 2008), USA (Lyons, et al., 2008) and the UK (in donkeys) (Trawford, et al., 2005) whilst other studies found no reduction (Larson, et al., 2011; Osterman Lind, et al., 2007). One study suggests that reduced ERP following IVM treatment may be due to a lack of susceptibility, or increased tolerance, to the anthelmintic of fourth stage luminal larvae, therefore resulting in a shorter period before recovery of the adult population and egg shedding (Lyons, et al., 2009).

#### **1.5.4.2 *Parascaris spp***

FECRT studies from Europe and North America have shown ML resistance in ascarid populations with one of the first UK case studies showing the death of a high value TB foal due to treatment failure (Stoneham & Coles, 2006)(Table 1.4); highlighting the concern of AR in ascarids for stud owners. However, another issue highlighted with these studies is the lack of validation of the FECRT for use with *Parascaris spp*. A critical test was performed by Kaplan, using a suspected IVM-resistant isolate of *P. equorum*, the FEC of IVM-treated foals compared to untreated foals were not significantly reduced at two weeks post-treatment and necropsy showed only a 22% decrease in actual worm burden (Kaplan, et al., 2006). Studies examining PYR efficacy by FECRT have found resistance present in approximately 50% of populations studied (see Table 1.4). Although the Kaplan study showed IVM-R

strains of *Parascaris*, FBZ showed good reduction in egg counts (1.35epg post-treatment average in FBZ-treated group compared to 281epg in untreated control group) and adult burdens (4.3 adults in FBZ-treated group, 115.7 adults in untreated control group) leading FBZ to become the treatment of choice, although the intensive regime used in the study was not considered necessary by the authors (Kaplan, et al., 2006; Reinemyer, 2012). In addition to increasing reports of ML and PYR failure by FECRT, Matthews et al reported anecdotal evidence of BZ-R in UK ascarid populations (Matthews, 2014).

Table 1.4 A summary of the published studies of resistance in cyathostomin and *Parascaris spp* detailing drug class and method used for determination of resistance.

Parasite	Benzimidazoles	Pyrantel	Ivermectin	Moxidectin	References
Cyathostomin spp	+++  Faecal Egg Count Reduction Test (FECRT) Worldwide	+  FECRT 50% Europe 40% USA 50% UK	+?  (Shortened ERP)  ERP Testing Controlled efficacy study ERP ranges - 4weeks (IVM); 5+weeks (MOX)		Chandler, et al., 2000; Hodgkinson, et al., 2005; Lester, et al., 2013; Relf, et al., 2014; Rossano, et al., 2010; Lyons, et al., 1999; Traversa, et al., 2007; Molento, et al., 2008; von Samson-Himmelstjerna, 2012; Lyons, et al., 2009; Molento, et al., 2012
<i>P. equorum</i>	?  Anecdotal	+  Controlled efficacy study + FECRT USA	+++  FECRT UK, USA, Denmark, Germany, Italy, Sweden, Canada	++  FECRT North America Netherlands	<b>IVM &amp; MOX</b> - Boersema, et al., 2002; Slocombe, et al., 2006. IVM - Hearn, et al., 2003; Stoneham, et al., 2006; Craig, et al., 2007; Schougaard, et al., 2007; von Samson-Himmelstjerna, et al. 2007; Veronesi, et al., 2009; Lindgren, et al., 2008. <b>IVM &amp; PYR</b> - Reinemyer, 2012 <b>BZ</b> - Matthews, 2014 – Matthews, unpublished)

Resistance is reported as: ? = anecdotal reports; + = various contrasting reports showing a range of both susceptible and resistant populations; ++ = limited number of studies, FECRT only – no critical studies performed; +++ = large numbers of studies demonstrating corroborating evidence

## 1.6 Control of equine parasites

### 1.6.1 Interval/Intensive Treatment programmes

During the 1960's disease caused by *S. vulgaris* was the primary concern and led to the recommendation of anthelmintic (BZ) administration every six to eight weeks in order to completely suppress egg output and reduce prevalence (Drudge & Lyons, 1966). This regime of interval dosing was effective in reducing the incidence of *Strongylus spp* associated disease (Lyons, et al., 1999). As a consequence of intensive treatment, high selection pressure for drug resistance was applied to both the cyathostomins and *Parascaris spp*, resulting in AR. Questionnaire studies show the vast majority (>85%:range 88-100%) of participants from all types of equine establishments used interval dosing programmes (Lind, et al., 2007; Lloyd, et al., 2000; von Samson-Himmelstjerna, et al., 2009). However, this trend has continued through to studies as recently as 2015. In 2012, Relf et al (2012) found 88% of 61 TB studs still reported using anthelmintics intensively, as did studies from New Zealand (Bolwell, et al., 2015) with 80% dry mares on TB (n=90/136) and Standardbred (SB) (n=46/136) farms, and 94.7% of 26 Italian mixed equine establishments (von Samson-Himmelstjerna, et al., 2009), respectively using interval dosing. Similarly, a 2015 American study performed exclusively on TB stud farms (n=112) showed similar 67.9% used a rotational intensive anthelmintic-based control programme (Robert, et al., 2015). In contrast a 2013 study surveying 193 UK leisure and competition horse owners, showed that only 15% still used intensive programmes with 44% classing themselves as using strategic and 40% as targeted selective regimens (Stratford, et al., 2013).

### 1.6.2 Targeted strategic and targeted selective Treatment

Alternatives to interval dosing regimens are strategic (also known as targeted and targeted strategic) and targeted selective treatment.

Targeted strategic treatment where anthelmintic use is reduced through application of epidemiological principles and drug treatment at key times of year. In contrast, targeted selective treatment is an evidence-based protocol employing diagnostic testing to guide anthelmintic use. The success of both of these approaches rely on selecting drugs which are active against the species and stage of parasite present, considering the time of year the horse is being treated, ensuring preservation of parasite refugia, determination of drug efficacy on a farm-by-farm basis and incorporating an element of pasture management. The primary difference between these regimes is the use of diagnostic testing to determine treatment for targeted selective regimes. This places increased emphasis on an evidence-based approach to parasite control and pasture management in order to increase refugia and reduce the rate of development of resistance. Targeted selective treatment is the current gold standard and so will be discussed in more detail here.

Nematode burdens are highly aggregated within most host species including equines, commonly quoted as 20-30% of horses contributing 80% of the total herd egg excretion (Anderson & May, 1978; Crofton, 1971; Shaw & Dobson, 1995; Shaw, 1998), commonly referred to as the 80:20 rule. This overdispersion is most apparent when looking at a herd of well-managed horses (Calabrese, et al., 2011; Lester, et al., 2013; Relf, et al., 2013). This distribution is due to the horses acquired immunity which, although for strongyles is not fully protective, is thought to prevent the establishment of substantial worm burdens in some individuals (Monahan, et al., 1997). In adult horses, this is seen as consistently low egg count for individuals comprising the majority of the population, with a minority remaining high shedders despite regular treatment (Nielsen, et al., 2006). Recent studies by Relf et al (2013) and Lester et al (2013) showed that 11% of 1221 horses and 15% of 928 horses, respectively excreted 80% of eggs detected by FEC. However when comparing YS (under three years old) to adult cohorts this negative binomial distribution is shifted to the right, with an increased proportion of high shedders and overall increased group mean in

YS populations (Relf, et al., 2013). This age group is at higher risk of increased burdens and clinical disease. This overdispersion of egg shedding provides a logical rationale for controlling transmission through the targeted selective use of anthelmintics, by treating only high egg shedding horses (Sangster, 2003).

Targeted selective strategies, only currently recommended in adult horses (above three years old) due to YS risk of disease from increased burdens, utilise the high degree of parasite aggregation in order to administer anthelmintics only to horses which require treatment, reducing clinical disease risk in the herd by reduction of pasture contamination and subsequent levels of larvae ingested during grazing (Nielsen, et al., 2013). Given that cyathostomins are the primary target of these control programmes and that there is no diagnostic for larval cyathostomins, targeted selective treatment based on FEC is only encouraged during high transmission periods; April to September in the UK. High egg shedding horses are classed as those contributing significantly to pasture contamination, shedding >200-500epg and a need to treat is based on this cut-off value (Larson, et al., 2011; Nielsen, et al., 2013; Uhlinger, 1993). In addition to reducing the use of anthelmintics, and so reducing drug selection pressure, this leaves a proportion of the herd untreated who may be shedding low levels of eggs. This untreated proportion increases parasite refugia, an important consideration in slowing the development of drug resistance (Kaplan & Neilsen, 2010).

Given the importance of administering efficacious drugs and relying on fewer drug treatments each year, FECRT are recommended annually or biannually to ensure an effective drug is being used in targeted selective treatment programmes. BZs at a single dose are likely to be ineffective due to drug resistance, so during high transmission periods PYR can be used to kill adult parasites and reduce egg output (if tested and found to be effective) in order to preserve MLs. Currently MOX is, due to the lack of commercially

available diagnostics for prepatent cyathostomin infection, recommended in the UK to be administered to all animals in autumn/winter in order to reduce mucosal burden and prevent disease (Nielsen, et al., 2013).

Regarding tapeworm control, no negative binomial distribution has been noted, egg output is irregular, techniques have low sensitivity with a high rate of false negatives, and so annual or biannual treatment is recommended based on either serological or salivary antibody detection ELISA tests to determine if a herd has been exposed to infection, as previously discussed in section 1.3.5. As reliability of ELISA testing for individual diagnosis is currently debated, with individual results thought to overestimate occurrence due to cross-reactivity, persistence of antibody following clearance of infection and high proportion of false positives “blanket” or whole herd treatment is advocated, although frequency can be guided using diagnostic results (Nielsen, 2016).

#### ***1.6.2.1 Uptake of targeted selective treatment protocols and diagnostic-based treatment***

Studies published in 2002 reported that 59% of Irish farms (O'Meara & Mulcahy, 2002) and 81% of South African TB stud farms (Matthee, et al., 2002) used FEC, although of the 59%, 16% conducted FEC annually and only 12% of Irish and 11% of SA respondents performed FEC more than once yearly prior to anthelmintic administration. A 2007 Swedish study (Lind, et al., 2007) found 32% used FEC but only 1% of this group performed diagnostics prior to anthelmintic administration, whilst 21% of German farms (Hinney, et al., 2011) reported that FEC results had a “significant influence on deworming decisions”. When considering only TB stud farms, >50% of 61 UK farms (Relf, et al., 2012), 30.4% of Kentucky stud farms (Robert, et al., 2015) and 22% of New Zealand studs (Bolwell, et al., 2015) used FEC, but of those 0%, ~30% and 64%, respectively utilised them for routine testing. In the UK 24% of TB studs (Relf, et al., 2012) and 53.8% of New Zealand TB and SB studs (Bolwell, et al., 2015) performed diagnostics due to “signs of disease” rather than using FEC as a

routine test. This demonstrates a misunderstanding of the use and interpretation of FEC as severe strongyle-type disease results from prepatent, non-egg producing stages, which are undetectable by currently available diagnostics including coprology (Love, et al., 1999).

## **1.7 Pasture management**

Frequent and effective pasture management is recognised as one of the most important factors in reducing egg counts within a herd, lowering the number of infective larval nematodes on pasture and thereby reducing the requirement for anthelmintic administration (Herd, 1986; Krecek & Waller, 2006; Matthee & McGeoch, 2004; Relf, et al., 2013; Schumacher & Taintor, 2008).

### **1.7.1 Pasture hygiene**

Faecal removal, manually or mechanically, is key to effective pasture management, but the timing of this is critical to obtaining maximal reduction in pasture larval burden. Rapid removal of faecal material prevents the dissemination of faecal balls, reducing the dispersal of eggs into the environment. When performed within the time taken for eggs to develop to the infective stage (seven to ten days for strongyle and ascarid eggs), transmission can be significantly decreased, although when rainfall is high contamination is inevitable even when weekly pasture cleaning is implemented, due to mechanical disruption of faecal balls (Nielsen & Reinemeyer, 2013). A regular meticulous approach to lifting faeces from pasture can reduce larval numbers 18 fold and is four times more effective than anthelmintics alone, with the consequent reduction in egg count vastly reducing the need for treatment within high transmission periods (in temperate zones April-September) (Nielsen & Reinemeyer, 2013).

Manual faecal removal has been shown to be equivocal to hoover and sweeper mechanical methods for reduction of pasture larval burdens, as measured by FEC in a population of donkeys (Corbett, et al., 2014). Although pasture larval counts showed that pasture sweepers may partially disperse faecal material, potentially reducing this techniques

benefit (Corbett, et al., 2014). However, this method shows large variability in results in addition to the effect of weather conditions, with rainfall and temperature highly influencing migration from the faecal pat, and grass plucking only representing the proportion (Corbett, et al., 2014; English, 1979; Larson, 1999). Although some sources advise that weekly removal may be adequate to significantly reduce transmission, Corbett et al (2014) found that the largest difference was obtained when pasture cleaning was conducted twice weekly. Studies examining pasture-grooming practices on farms showed that although faeces were removed it was performed infrequently and with a wide variance between farms. Only one study investigated how faeces are removed in practice, examining only practices on TB stud farms, showing a majority used mechanical means (Relf, et al., 2012). Methods which remove faeces from pasture have a larger impact on reduction of pasture infectivity for strongyles (Corbett, et al., 2014) but potentially increase *Parascaris spp* infection levels, as can manual removal, as the eggs of this species remain in the soil for decades (Relf, et al., 2013).

Harrowing, where faecal matter is broken up and dispersed across the paddock area, was extremely popular with TB stud questionnaire participants both in the UK (Relf, et al., 2012) and New Zealand (Bolwell, et al., 2015). The suitability of this practice is related to the climatic conditions of the geographical location and, where appropriate, season within which it is undertaken. Where high environmental temperatures are reached, harrowing can disperse larvae across the surface and encourage desiccation, however, in temperate climates such as the UK and New Zealand average temperatures do not reach sufficient levels for the period of time required for this to occur (Reinemyer, 2012). Removal of “roughs”, areas of tall grass growth, during harrowing or cropping aid reduction of pasture larval burden by removal of protective habitats for the development of larvae which prevent desiccation (Herd & Willardson, 1985).

### **1.7.2 Pasture rotation**

Rotation of groups of horses around pasture operates on the principle that consistently low shedding adult horses, graze paddocks previously occupied by higher shedding younger stock. This practice was adapted from that described in sheep by (Leathwick, et al., 2008). Rotational grazing was identified as the most significant factor in reducing likelihood of a positive strongyle egg count, with studs rotating groups at least monthly, being 18 times less likely to have positive egg counts (Relf, et al., 2013).

### **1.7.3 Ruminant co-grazing/rotational grazing**

One of the major methods used is mixed (sometimes termed “cross”) grazing, where ruminants are used in order to reduce relative stocking density by decreasing numbers of infective strongyle larvae on pasture through ingestion, as ruminant species are not susceptible to infection (Nielsen, et al., 2006). A range of 17% to 69% of farms within all surveys, conducted between 2000 and 2015 on both TB and mixed breed establishments (livery yards, non-TB stud farms), utilised co-grazing as a pasture management technique (Bolwell, et al., 2015; Hinney, et al., 2011; Lendal, et al., 1998; Lind, et al., 2007; Lloyd, et al., 2000; Matthee, et al., 2002; Relf, et al., 2012; Stratford, et al., 2013; von Samson-Himmelstjerna, et al., 2009). Within those studies specifically considering TB stud farms (Table 1.5), high numbers of participants used mixed grazing and over 50% of those surveyed stated they removed faeces from pasture in the UK (Relf, et al., 2013) and South African (Matthee, et al., 2002) studies, the most important method in reducing egg counts within a grazing herd.

Table 1.5 The TB stud farm specific values for various pasture management strategies.

<b>Study (year)/ Grazing management method</b>	<b>Matthee et al (2002) (n=110)</b>	<b>Relf et al (2012) (n=61)</b>	<b>Bolwell et al (2015) (*n=90/139)</b>
<b>Ruminant co-grazing?</b>	49%	49% (53% performing this annually)	69%*
<b>Removal of faeces from pasture</b>	61%	75%	~36%*
<b>Most common frequency</b>	NS	49% differs seasonally	A few times per year (~18%)*
<b>Method - Manual or machine</b>	NS	61% machine	NS*
<b>Chain-harrowing</b>	NS	~95% (45% monthly, 42% annually) *harrow/clip one category	~97% (~45% a few times/yr.)
<b>Removal of roughs</b>	NS	*harrow/clip one category – see above	NS
<b>Group rotation to "clean" pasture</b>	80%	~80% (48% monthly)	-Non-breeding season - 56% -Breeding season - YS 71%, Wet mares 68%, dry mares 60%; ~50% "a few times/yr."

NS – data values not stated/investigated. \*TBSS-TB farm specific values separated from mixed establishment values.

## 1.8 The Thoroughbred industry

The Thoroughbred (TB) industry comprises two branches, national hunt (NH) or jump racing and flat racing. An important consideration when comparing these two branches is the average working lifespan of horses, within NH horses are retained on training yards longer, competing well into their teens in contrast to flat racing where most horses careers are complete by five to six years of age and successful animals may be sent back to stud before the age of five. This is important when considering progression through management systems (Figure 1.7). Only flat racing will be discussed here, as studs involved in this project breed for the flat racing sector.

Foaling typically occurs in winter due to the desired foaling date of January 1<sup>st</sup>. During the initial two trimesters of pregnancy mares are usually managed extensively, especially as this occurs over the summer months. Pregnant mares are usually kept in separate groups to barren mares and other equines on site. Approximately four to six weeks pre-parturition mares are brought-in to the farms foaling unit with turnout restricted or removed, in order for them to be more closely observed for signs of parturition. IVM or MOX is administered by some studs around two week's prepartum. This is a common practice worldwide, based on a report that it reduced the lactogenic transmission of *Strongyloides westeri* to foals (Ludwig, et al., 1983). This was originally thought to have been effective in reducing prevalence of *S. westeri*, with studies showing 1.5-6.0% of foals positive (Lyons, et al., 1993; Lyons & Tolliver, 2004; Lyons, et al., 2006) on coprological examination compared to historical levels of ~90% within the same geographical area (Lyons, et al., 1973; Todd, et al., 1949). More recently two studies have shown an increased prevalence of 15% and 28-33%, respectively, but the reason for this apparent increase is not known (Lyons & Tolliver, 2014a; Lyons & Tolliver, 2014b). Treatment of this parasite, despite the development of immunity by four to six months of age, is stated by some to reduce parasite-related neonatal diarrhoea and poor weight gain (Lucena, et al., 2012). On many stud farms

treatment is only given where *S. westeri* has been reported, or more commonly where there is a suspicion of disease.

Newborn foals-at-foot are generally kept in small nursery paddocks, which are reused for this purpose year on year, with intensive pasture management for the first few weeks of life before being turned out with other mares and foals-at-foot. Anthelmintic treatment until the age of six months (minimum), when weaning occurs, is intensive, often beginning around eight weeks of age. Treatment before this point for ascarids or for *S. westeri*, in the absence of clinical disease, is not recommended (Reinemeyer & Nielsen, 2016). The exact timing of weaning varies depending on maturity, condition and target parameters such as weight gain and general form as assessed by the stud manager, but typically occurs between four to six months. Weaning involves a short period of stabling until initial stress has gone, usually in pairs to reduce stress as much as possible. Foals are either prepared and sold at foal sales or kept on “home” premises, usually if development has not reached targets or if the stud intends to retain them long-term. Age at point of sale varies with higher prices often achieved for yearlings without more recognisable pedigree, with surplus foals in good condition holding desirable pedigrees more suited to early sale due to lack of monetary depreciation regardless of race performance as potential breeding animals. From weaning, intensive anthelmintic regimes are maintained for the lifetime of the horses on the majority of stud farms, as previously discussed in section 1.6.1. (Comer, et al., 2006; Earle, et al., 2002; Relf, et al., 2012).

Summer of the second grazing season in preparation for yearling sales (October - December) begins. YS are turned away to mature prior to transfer for training either on home premises or outsourced depending on farm facilities and space. At 18-24 month old, YS are transferred to training yards, most often in France, Germany, USA, UK or Ireland. In the event that the owner, or trainer, considers the horse unprepared for sale as a yearling,

it can still be sold on as a two year old. Two year olds are backed and trained or actively raced in novice classes (often sold under “Horses in Training” section at sales).

Alternatively, sales are held in spring following the yearling session in the previous autumn; all horses to be sold are “breezed”, or galloped as if racing, over two furlongs and the time recorded, this is often used by buyers in order to assess future performance and the decision of whether to purchase the youngster.

Within training yards, smaller establishments are more likely to allow grazing than larger ones (Earle, et al., 2002). Where given, grazing is usually allowed for five to 60 minutes after exercise but varies with the time of year and is more limited for flat racers in training than National Hunt (Earle, et al., 2002). Pasture hygiene measures are carried out at high frequency, with Comer et al (2006) finding 35% removing faeces every day or every other day and 41% between cohort rotations. Earle et al (2002) recorded similar results with 39% of farms removing faeces from pasture weekly. Despite restricted access to grazing and intensive pasture hygiene measures on a majority of training yards intensive treatment protocols are performed with 40% of yards in a 2002 study dosing every four to six weeks, primarily with IVM or PYR, although some had used unlicensed injectable or pour-on products (Earle, et al., 2002). It is important to note however that data from these studies were collected prior to the marketing of MOX, the follow-up study in 2006 showed that MOX was being preferentially used by a majority of training yards (Coles, et al., 2006). Quarantine protocols at flat racing yards were deemed more stringent than for NH with the majority administering an anthelmintic, preferentially IVM-based, and keeping new arrivals in quarantine for seven days or more (Comer, et al., 2006; Earle, et al., 2002). In 2002, 54% of trainers had seen signs of parasite-related disease, with weight loss (87%), worms in faeces (64%) and colic (47%) being most common (Earle, et al., 2002).

If while racing the trainer, or vet, feels the horse is in need of rest, either due to waning performance or in order to recover from illness/injury, they are transferred to establishments which undertake rest and rehabilitation (R&R). This may include turn-out, gentle exercise (such as a horsewalker), lunging, hydrotherapy and remedial farriery. Many smaller studs and training yards also offer this service in addition to their primary line of business to broaden service provision and increase financial viability of their business. There are also “consignors” who act as an intermediary for buying and selling high value stock, connecting studs/yards to trainers and those experienced in sales preparation and race training. These individuals are often experienced trainers or breeders and will buy in as well as helping clients to sell on, they will often advise clients on which sales to aim for when considering specific horses (e.g. foal, yearling, in-training, breeze-up). When horses transfer to these contracted third parties parasite control and individual establishment owners/managers decide management strategies.

Horses with good pedigrees or who have been successful within racing (who have close relatives such as siblings who have achieved highly) are transferred back to the home premises, or a stud where the owner boards them, for those owned by syndicates, for breeding or sold at bloodstock sales.

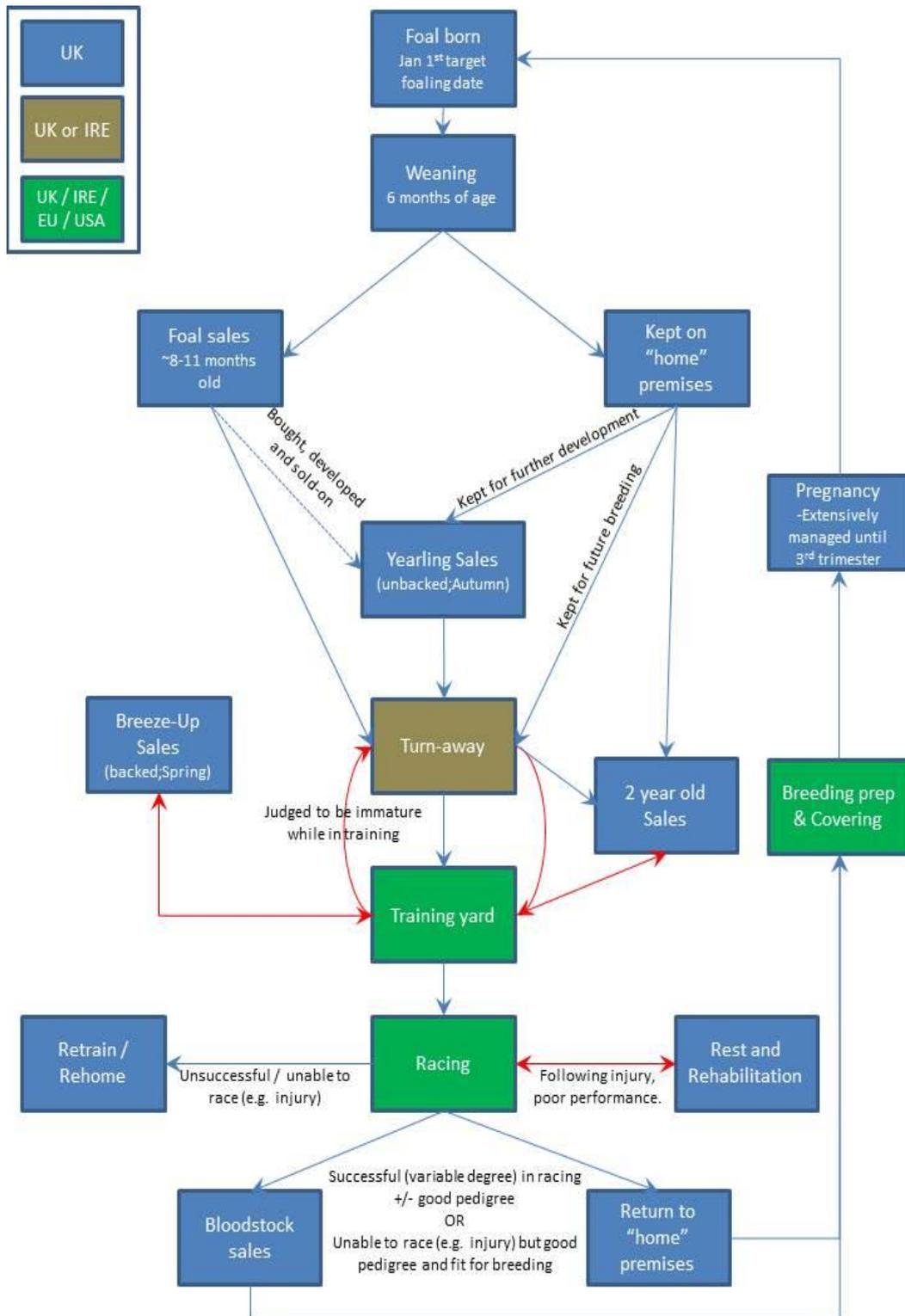


Figure 1.7 Diagrammatic description of potential routes of progression of an individual horse through the flat-race TB industry. Broodmares at stud are “nominated” for covering by the stallion of choice and foals born are given 1<sup>st</sup> January birthday for the year of birth. Foals will be kept following weaning or sold at foal sales; those retained may be sold at yearling sales, later at breeze-up sale following backing or turned away before and/or after breaking to saddle at a training yard. Following the conclusion of racing career stock may be rehomed, sold or returned to home premises for broodmare/stallion duties.

## 1.9 Aims and objectives

The aims of this project are to:

1. Establish drug efficacy or drug resistance within nematode species of clinical importance on TB studs in the UK and assess the prevalence of *Strongylus spp* on stud. This will in turn provide bespoke worming programs based on each stud farms management practices, drug usage and clinical concerns.
2. Evaluate practical diagnostic methods currently available to detect parasitic infection on TB studs
3. Capture current parasite control practices on UK TB studs with particular focus on current pasture management strategies, administration of anthelmintics and use of targeted selective regimes.
4. Investigate the importance of tapeworm-associated disease on a TB stud

# Chapter 2 - Materials and methods

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This materials and methods chapter describes the common methods of faecal sample collection, faecal analysis and egg count methodologies that were used throughout the thesis. Where specific materials and methods were used, or variations of those methods described here were employed, details are provided within each chapter.

## 2.1 Faecal sample collection

Faecal samples less than four hours old were collected from the ground from individual horses' resident on each stud/site. A detailed sampling protocol was provided (Appendix 2A) instructing collection of faeces from five or more sites around a freshly voided faecal pile. The stud owner or manager was responsible for assigning collection duties, for larger studs (studs A, C, D, F) general yard staff were responsible for collection and labelling of samples; labelling and collection of samples was the responsibility of the stud owner/managers of studs B and G and stud E samples were collected by the referring veterinary surgeon. The sample was instructed to be placed into a zip-lock sealed bag, all air expelled, the bag was sealed and shipped immediately to Department of Infection Biology, UofL. All samples were processed within five days of collection (except Stud A, see below) and data logged on data capture forms prior to being digitally stored on a secure university server (M: drive). The date samples were taken and processed was recorded, along with horse identification number (ID); for those samples where no date of sampling was provided, this was estimated based on accompanying samples and shipment date. Samples were grouped based on age: 1) foals (under one year); 2) YS (above 12 months to three years old) and 3) adults (above three years old). For LC and faecal analysis, samples were sub-grouped based on geographical location and, where details were available, to the field location. Samples were stratified in this way to allow results to be analysed separately for each age group and grazing group. Samples were analysed from individual horses for

FEC; grouped according to age strata and stud overall for FECRT and pooled by grazing group for LC.

Samples were taken and shipped the same day or early next day by 24 hr courier service for all studs, with the exception of Stud A, which conducted its own FEC analysis in-house prior to shipping. Stud A homogenised ~500g faecal samples and stored them at 4°C prior to shipping. Due to the time taken for sample processing for the on-site laboratory testing and shipment time to our laboratory, samples were tested approximately seven to ten days after collection.

## **2.2 Centrifugal Flotation faecal egg count methodology**

A modification of the salt flotation method (Christie & Jackson, 1982). Sensitive to one egg, known as the centrifugation flotation (CF) method was used (Elsheika & Khan, 2011). All samples were analysed in duplicate. Ten grams of faeces was weighed out and 100ml (10ml per gram) tap water added. The solution was mixed until homogenous and a 10ml sub-sample was removed and passed through a tea-strainer (~750-800µm) to remove large fibrous debris. Material left in the sieve was rinsed with 5ml of tap water and the remaining liquid pressed from the sieve using a wooden tongue depressor.

The solute was decanted into flexible (Beckmann Coulter, 16mm x 102mm) 17ml centrifuge tubes and centrifuged at 203g (953rpm) for two minutes and the supernatant removed to leave pelleted particulate matter in the base of the tube. 10ml of saturated sodium chloride solution (Specific Gravity=1.02) was added and the tube slowly inverted until the pelleted material completely resuspended. If pellet material did not resuspend with gentle inversion, the top of the tube was occluded and a vortex used to break down the compacted material. This was then re-centrifuged for two minutes at 203g (953rpm). The top of the tube was occluded using haemostatic forceps approximately 1cm below the level of the meniscus, the portion containing the eggs, and was decanted into a counting cuvette

(Brand Tech Methacrylate (PMMA) 3ml cuvette, Cole-Palmer scientific). The top, occluded, portion of the tube was then rinsed with saturated sodium chloride solution and washings transferred into the cuvette. The cuvette was topped up with saturated sodium chloride if needed and the internal space within the lid filled until two positive menisci were formed (to prevent air becoming trapped, altering the reading surface area and causing eggs to be pushed out of the reading frame, creating false negative or low results). The lid was placed on the cuvette which was then rested on its side, reading surface up, for five minutes before examination, to allow eggs to float up to the reading surface and larger sediment to settle to the bottom.

The cuvette was examined under a compound microscope at x10 magnification and initially scanned to approximate the number of eggs within the reading frame and determine the best method of counting. Then a Miller's square (7x7mm grid, 21mm diameter; NE57, Electron Microscopy Sciences) eyepiece graticule (Figure 2.1) was used as follows: a) for egg counts approximating 50-100epg, the entirety of the large x3 square (including the area of the smaller square) was used; b) for counts estimated to be above 100epg the small x9 square was used and c) for counts below 50epg full enumeration of the entire reading frame, without use of the graticule, was performed. Two passes equidistant from the centreline and extreme edge of the cuvettes reading frame were made, with the inside corners lined up horizontally with the most lateral point of the reading frames' triangular indicator as a starting point to ensure identical, repeatable and comparable examination of all cuvettes (Figure 2.2). Eggs fully within the boundaries of the square were counted, those laid across the Miller square border were discounted. Cumulative count of both passes were multiplied by the appropriate factor, determined by the method used (a[x3], b[x9] or c[x1] as detailed previously). Egg counts were completed in duplicate for all samples and an arithmetic mean taken for final epg in order to further reduce errors.

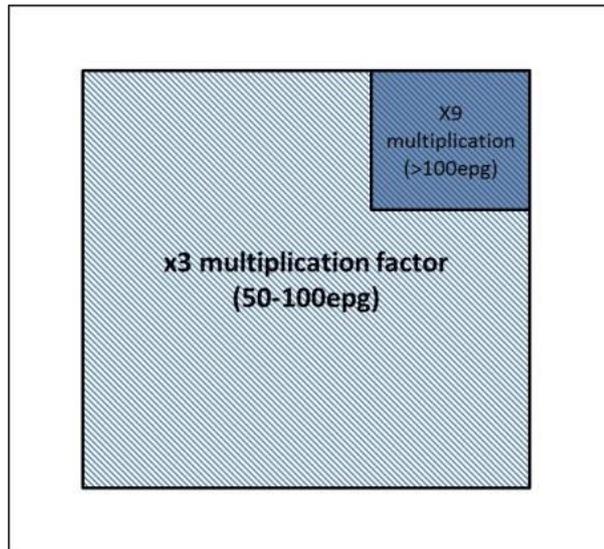


Figure 2.1 The Miller (7x7mm grid) Square eyepiece graticule showing both reading frames, conditions for use and multiplication factors.

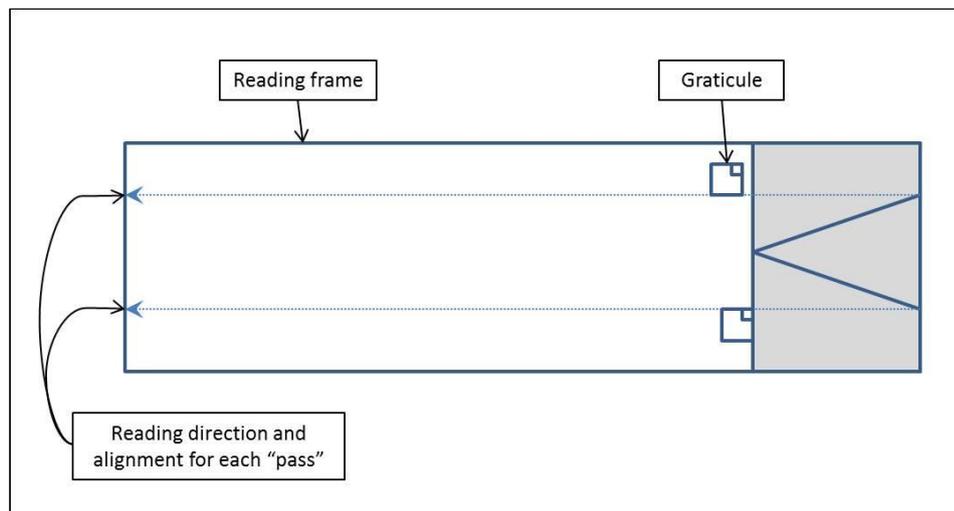


Figure 2.2 The reading frame of the cuvette and alignment of the Miller (7x7mm grid) Square eyepiece graticule for readings of 500-100epg and >100epg for both reading passes. The direction of reading for both passes is also shown as the square is realigned with the marker triangle prior to each pass to ensure accuracy, repeatability and consistency between all samples.

Parasite eggs were identified as: strongyle (incorporating cyathostomin and *Strongylus spp*), *Parascaris spp*, *S. westerii*, *A. perfoliata* and *O. equi* (see Figure 2.3). The CF method is not currently validated for *O. equi*, *S. westerii* or *A. perfoliata* but counts of eggs observed were recorded in order to report accurate findings to the participating studs and their veterinary surgeons, for later interpretation and potential action. In support of this approach, this technique has been used in previous studies to detect presence of both of

these parasites (Relf, et al., 2013). Due to the anatomical predilection site of *O. equi* within the host, found within the rectum, and the method of dispersing eggs, by dispersal onto peri-anal skin, the CF method is not the most appropriate test. For *S. westerii* faecal testing is most appropriate but as foals develop immunity by four months of age, the age at which most studs begin or are able to sample foals easily (usually at weaning when separated from the dam) these findings were reported but were very uncommon and it was recommended to stud managers to consult their primary veterinary surgeon. *A. perfoliata* eggs are shed sporadically in egg packets from adult tapeworm within the colon, making faecal testing unreliable, with heavily infected horses potentially recording repeat negative egg counts. However, as a positive result is a definite indication of infection, any positive samples were highlighted and further testing or treatment recommended to the studs veterinary surgeon and stud manager.

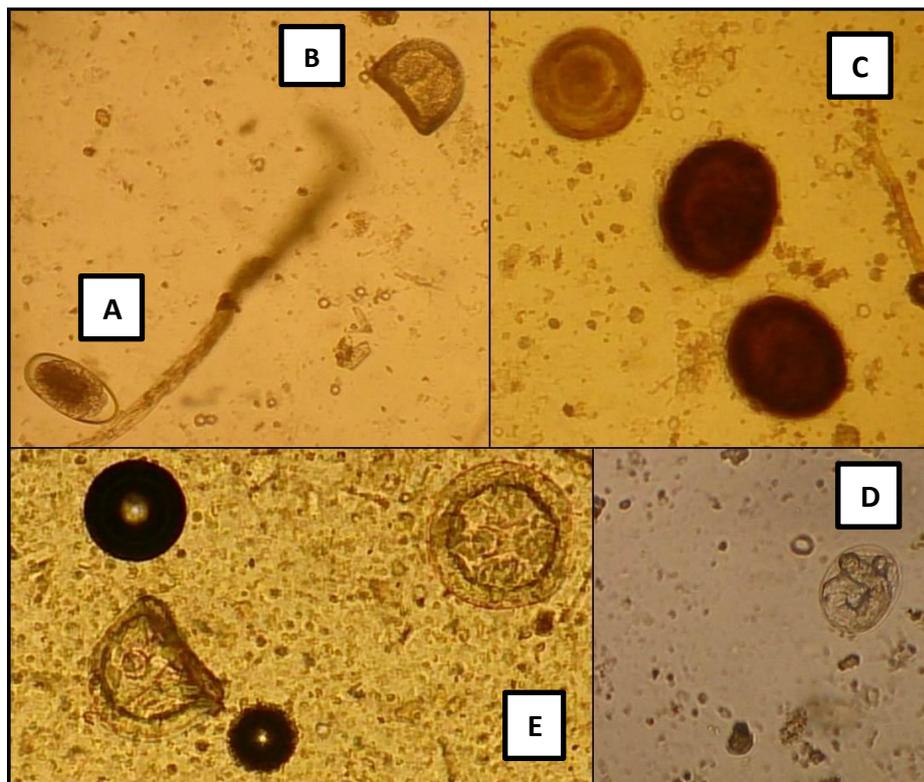


Figure 2.3 Species of egg identified within faecal samples using the CF technique. A). Strongyle egg (70-90µm long), B) *A. perfoliata* (40-50µm diameter), C) *Parascaris spp* (90-100µm diameter), D) *S. westerii* (55 x 30µm), E) *A. perfoliata* shown presenting in both lateral and ventrodorsal planes.

### 2.3 Faecal egg count reduction tests

Faecal egg count reduction tests (FECRT) were performed as described in Relf et al (2014). Essentially a minimum criteria for inclusion in FECRT was ten or more horses recording FEC of  $\geq 50$ epg. Samples from horses within the FECRT study were taken and shipped as described in Section 2.1. Pre-screening faecal samples were obtained from as many horses as possible on each stud pre-treatment. Horses with a strongyle FEC of  $\geq 50$ epg, as shown by the CF method, were included and the anthelmintic under test administered *per os* on Day 0 at the following dose rates: FBZ 7.5mg/kg, PYR 19mg/kg, IVM 0.2mg/kg and MOX 0.4mg/kg as per manufacturers recommendations. Horses were wormed based on weight, using the strategy normally employed by the stud farm, this included weighbridge, weight taping and by eye estimation. Studs were requested to dose based on individual weights rather than group averages or maximums. Each individual was treated by the stud staff with post-dosing monitoring, consisting of more intensive observation for 12-24 hours, conducted to ensure product was ingested and post-dosing colic would be detected if it occurred. This was not consistently employed across studs as they and/or their veterinary surgeons did not feel it was necessary; stud E employed post-dosing observation most stringently due to the clinical colic issues present on stud.

Following administration of the worming product, horses were managed normally until +14 - +17 days post-treatment when they were re-sampled. Post-treatment samples were again tested by CF method. After collection of data from both time points, group arithmetic mean by age group was calculated and for all horses included per stud and the reduction percentage calculated. The following equation was used to calculate FECRT:

$$\text{FECRT} = \left( \frac{(\text{Pre-treatment EPG} - \text{Post-treatment EPG})}{\text{Pre-treatment EPG}} \right) * 100$$

From these results the group averages, by age and stud, were compared using currently accepted resistance thresholds and lower confidence limits as seen in Table 2.1 (Lester, et

al., 2013; Stratford, et al., 2013; Relf, et al., 2014; Vidyashankar, et al., 2007; Vidyashankar, et al., 2012). The populations of parasites on the stud were then termed to be resistant, susceptible or inconclusive (if reduction levels fell within the 95% confidence interval) to the drug under test. The results were presented to the stud manager and their veterinary surgeon for discussion. Where possible, FECRT were performed in duplicate per drug class, age group and stud, to ensure accurate interpretation. Where a product was used and resistance detected during testing, or where all horses tested still exceeded the 50epg testing threshold, a rescue treatment strategy was employed in order to limit pasture contamination. IVM was used as a rescue treatment in the event of PYR or BZ failure to reduce egg shedding. If IVM was required to be given as a rescue treatment, the +14 day post-treatment FEC from the initial failed treatment was used as a day 0 count and all horses treated were re-tested at +14 - +17 days post IVM treatment, in order to determine efficacy of IVM (see Figure 2.4). Where possible a second, independent efficacy test for IVM was repeated.

Table 2.1 Currently accepted mean FECRT efficacy thresholds and 95% lower confidence limits (LCL) for each drug class (Lester, et al., 2013; Stratford, et al., 2013; Relf, et al., 2014; Vidyashankar, et al., 2007; Vidyashankar, et al., 2012).

<b>Drug class</b>	<b>Arithmetic mean FECRT efficacy threshold</b>	<b>95% lower confidence limit (LCL)</b>
Benzimidazoles & Tetrahydropyrimidine	>90%	80%
Macrocyclic lactones	>95%	90%

## **2.4 Egg reappearance period**

ERP was defined as when post-treatment group arithmetic mean exceeded ten percent of the pre-treatment group arithmetic mean, as reported in Relf et al., (2014).

Horses that showed FECRT >90-95% were re-sampled at 14 day intervals post-treatment until the above ERP criteria was fulfilled. Samples from horses within the ERP study were taken and shipped as described in Section 2.1.

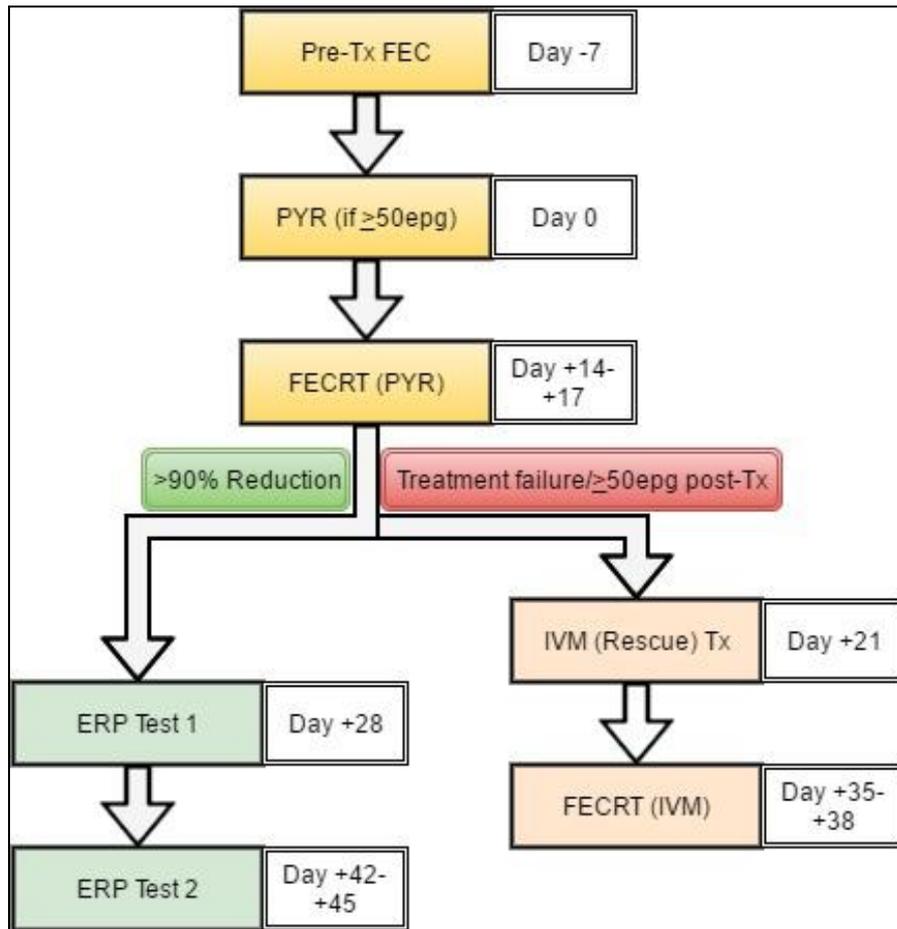


Figure 2.4 Flow diagram demonstrating efficacy testing within all age groups on all studs. FECRT and ERP testing protocol was initiated if percentage reduction in strongyle egg count showed a susceptible parasite population (>90% for PYR and BZs, >95% for MLs) and adequate reduction; Rescue protocol was initiated if strongyle FECRT showed a resistant population and/or egg counts remained above test criteria threshold (>50epg).

## 2.5 Larval Culture

Following homogenisation of the faecal sample and removal of sample for FEC testing, ten gram subsamples were taken from individual horses within the same age group, co-grazing group or geographical location. For each LC, faecal samples from ten individual horses were pooled to give a total culture weight of 100g. The faecal material was mixed and then reformed into a ball and holes were punched in the top half of the bag to allow airflow during incubation. Cultures were incubated for 14-21 days at 20-22°C and checked every three days for signs of desiccation. In the event of excessive drying 5ml of tap water per 10g of faeces was added to the bag.

To harvest larvae, bags were filled with tepid tap water until the faecal ball was completely submerged and left to stand for four hours at RT to encourage larvae to migrate out of the faecal ball. The water was then poured through the Baermann filter apparatus, allowing the water to filter through under gravity and larvae and any larger fibrous material was retained on the filter. A jar was then filled with tepid tap water and the Baermann apparatus was placed within the neck of the jar so the filter paper of the apparatus rested in contact with the water without flooding (Figure 2.5).

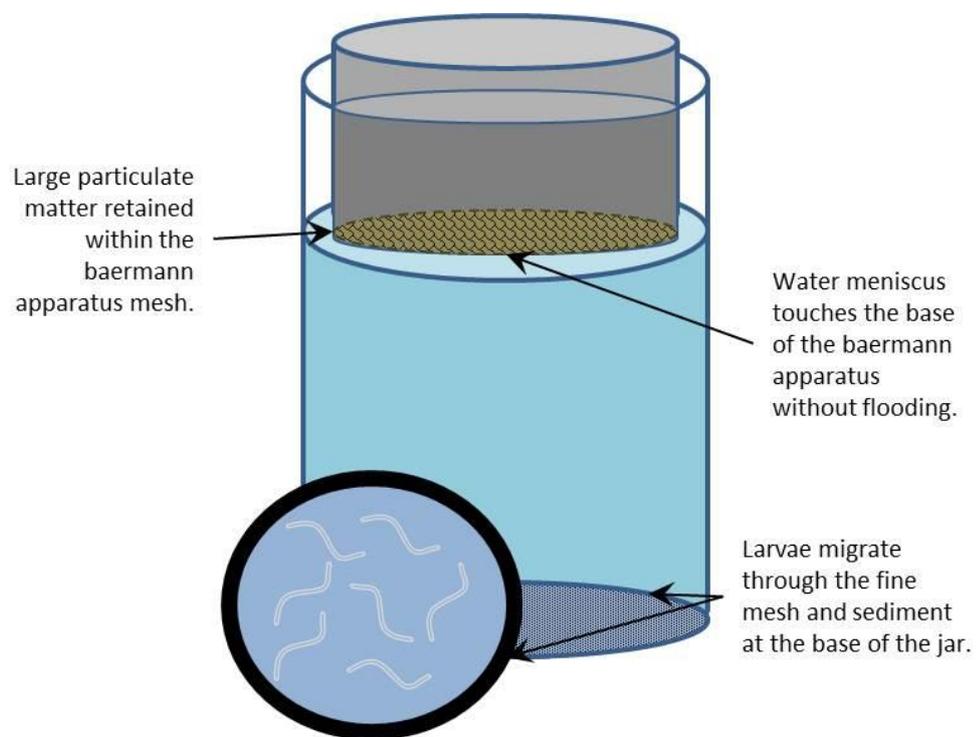


Figure 2.5 The Baermann and larval harvesting apparatus showing meniscus-filter contact and sedimentation of larvae.

This was left to stand overnight at RT to allow migration and sedimentation of larvae into the jar, at which point the filter apparatus was removed, tapped firmly to dislodge any remaining larvae and suspended above the jar using wooden tongue depressors for 30 minutes before complete removal. Larvae were allowed to settle for 1hr before supernatant was removed using a suction hose, leaving the bottom 2cm of water containing the sedimented larvae in the base of the jar. Larvae were then transferred to a

25cm<sup>3</sup> tissue culture flask and the jar washings added to the flask to maximise retrieval. The flask was made up to half full with fresh tap water and refrigerated at 4°C prior to larval examination and enumeration. Every four weeks larvae were allowed to sediment in the flask and the water was replaced with fresh tap water, with care taken to avoid removal of larvae. Full enumeration was performed by removal of excess water and transfer of flask contents (including rinsing of the flask inner surfaces to dislodge any remaining larvae) into a grid-marked petri-dish. Dishes were examined in a grid search pattern on a dissection microscope for all larvae to be individually examined and counted. Any larvae which required further examination were transferred to a slide for detailed observation under higher power on a compound microscope.

## 2.6 Larval differentiation and species identification

Species identification was performed by observation of morphological characteristics: the first step was to differentiate strongyle species from free-living larvae. The L3 stages of strongyle species are completely enclosed within the L2 sheath, the cuticle crinkles in the centre third of the larvae during typical sigmoid flexion movement and they may exhibit a long tail as part of the caudal end of the sheath. In cyathostomins, eight triangular gut cells can be visualised in fresh samples but in older samples gut cell differentiation is lost as larvae begin to degrade. There may be a notch after the 5<sup>th</sup> gut cell, after which definition of more caudal cells is lost, this notch itself is diagnostic for identification even if gut cells cannot be enumerated. Large strongyles are easily differentiated when seen in close proximity to cyathostomin L3 due to their increased size in combination with complete sheath enclosure and a long flagellate tail. When seen alone, or with free-living nematodes, differentiation was more difficult and removal for more detailed analysis was necessary. *Strongylus spp* have  $\geq 20$  gut cells. From keys available it is possible to differentiate the various species, however due to time constraints only differentiation of strongyles to genus (*Strongylus*) was performed (Figures 2.6 & 2.7). Larvae of free living nematodes were

present but, due to their wide range in size and features, they were readily distinguished from cyathostomin and *Strongylus spp* L3. Following enumeration, larvae were transferred back to flasks and stored as previous described.

<b>Box 54-1 Key to Identification of Nematode Larvae from Equine Feces</b>	
1. Esophagus with obvious midlevel constriction (rhabditiform). Esophagus without such a constriction.	Free-living trematodes 2
2. Body not enclosed in sheath; tip of tail has V notch. Body not enclosed in sheath.	<i>Strongyloides westeri</i> 3
3. Containing fewer than 16 distinct gut cells. Body with more than 16 gut cells.	Cyathostomes 4
4. Body with 16 gut cells. Body with more than 16 gut cells.	5 7
5. Sheath tail is short and rounded. Sheath is long and whiplike.	<i>Trichostrongylus axei</i> 6
6. Very large larvae with well-defined triangular cells. Medium-sized larvae with rectangular gut cells. Long, thin, larvae; poorly defined gut cells; small trilobed process on posterior end.	<i>Oesophagostomum spp.</i> <i>Posteriostrongylus spp.</i> <i>Strongylus equinus</i>
7. Larvae with 28 to 32 well-defined gut cells. Larvae with 18 to 20 gut cells.	<i>Strongylus vulgaris</i> 8
8. Broad larvae, medium length, with well-defined gut cells. Small, slender larvae with blunt tail and poorly defined gut cells.	<i>Triodontophorus spp.</i> <i>Strongylus edentatus</i>

Figure 2.6 An identification key for the species identification of third stage infective larvae of equids. Taken from Equine Infectious Disease, 2013 (Sellen & Long, 2013).

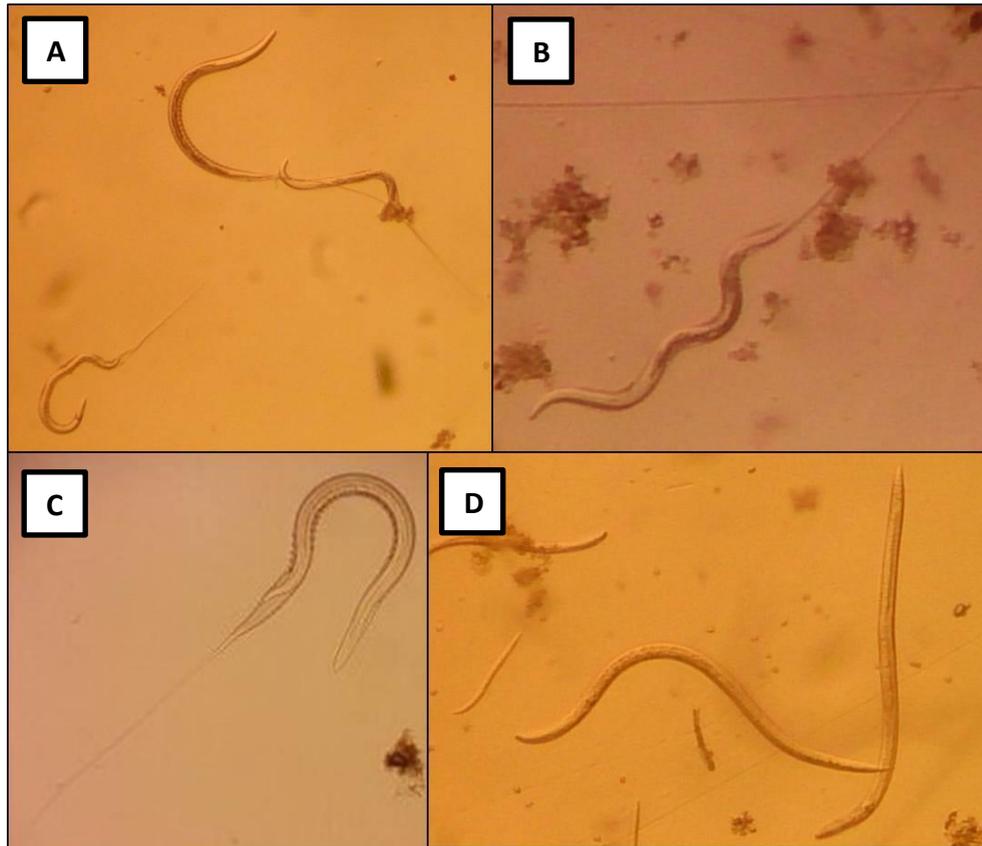


Figure 2.7 Large and small strongyles and free-living nematodes found in culture. A) two cyathostomin spp 3<sup>rd</sup> stage larvae (L3) and a *Strongylus spp* L3, the gross difference in size when seen side by side is demonstrated in addition to larger, darker and more prominent gut cells in *Strongylus spp*; B) a closer image of a *Strongylus spp* L3 with prominent of gut cells C), a cyathostomin L3. D) a range of free-living nematodes commonly found in culture.

## 2.7 *Fasciola hepatica* sedimentation of faeces

### 2.7.1 Short fluke egg sedimentation method

In order to provide a quick and readily accessible practical test for detection of *F. hepatica* eggs (Figure 2.8) in faeces, a rapid method was trialled and compared with the traditional method described in 2.7.2. 3g of faeces were weighed out and 42ml of saturated sodium chloride solution was added, the solution was then homogenised with glass beads. The homogenate was poured through a tea-strainer sieve into a beaker (at this point a small volume can be withdrawn for a McMaster nematode egg count if required). The solute was passed through a 180 µm aperture sieve into a conical flask and left to sediment for three minutes, which was long enough for eggs to settle out but not long enough to allow particulate matter to settle and make reading the sample difficult. Once the solution was

left to sediment, the solute was siphoned off with care not to disturb the sediment where eggs were located. One drop of methylene blue stain was added to the sediment to provide contrast with *Fasciola spp* eggs, which were observed as yellow-gold in colour and did not take up the stain. The base of a petri dish was marked, by drawing or scoring, with a vertical grid pattern. The stained sediment was then transferred into the petri dish with sufficient water to create an even layer covering the whole of the reading surface and allowed to sit for 60-90 seconds, allowing eggs and larger debris to settle to the bottom. The entire sample was then examined under a dissection microscope using the grid lines as a guide. Eggs were counted and the following equation used to calculate eggs per gram within the sample –

$$EPG = \frac{\# \text{ eggs observed}}{\text{Total volume of faeces examined (3g)}}$$

All short method sedimentation tests were performed in duplicate and final egg found by calculating the arithmetic mean of duplicate counts.

### **2.7.2 Traditional fluke egg sedimentation method**

10g of faecal material was placed into a 500ml beaker with 500ml of tap water and mixed thoroughly. The faecal solution was poured through a stack of sieves: 700µm, 150µm and 38µm aperture diameter, to remove all fibrous debris and leave only eggs and particulate matter trapped on the 35µm sieve. The sieves were rinsed with 500-800ml of tap water. Fibrous material from the top sieve was agitated under running water to release any trapped eggs. This was repeated until the water exiting the base of the sieve ran clear. The second sieve was then washed until the water ran clear, to leave all fine debris and eggs in the bottom (35µm) sieve. Contents of the 35µm sieve were washed, using running water, to one edge and decanted into a beaker. These washings were topped up to approximately 500ml with tap water and stood for four minutes to allow sedimentation of eggs. The top 400ml of supernatant was poured off taking care to avoid disturbing the sediment

containing eggs and the beaker refilled with tap water. This process was repeated until the top 400ml of water was completely clear with no floating particulates, opacity or discolouration. The supernatant was then gradually poured off and discarded to leave 50ml of water and sediment. This sediment was then washed into a grid-marked petri dish, two to three drops of methylene blue was added and the number of eggs counted under a dissection microscope on medium (X 2.5-3) magnification using a grid-sweep counting pattern (Figure 2.8). During reading, a plastic pipette tip was used to redistribute pockets of sediment to disturb clumps, to ensure there were no eggs escaping detection at the edges of the plate. An egg count was obtained using the following equation –

$$EPG = \frac{\# \text{ eggs observed}}{\text{Total volume faeces examined (10g)}}$$

Results were recorded and samples analysed in duplicate. In order to produce a final egg value the arithmetic mean of the duplicate values was calculated.



Figure 2.8 A *Fasciola hepatica* egg (lower right corner) with stained faecal debris and crystalline particulate matter. *Fasciola spp* eggs are 130-145µm length, 70-90µm wide, exhibiting a regular ellipse shape with thin shell and operculum at one pole. Granular yellow-brown contents fill the whole of the inside of the egg.

## 2.8 Double Sugar Flotation (DCF) for *Anoplocephala perfoliata* eggs

The method used for the detection of *Anoplocephala perfoliata* eggs was as optimised by

Rehbein et al (2011) by adaptation of the Cornell-Wisconsin CF Technique described by

Egwang and Slocombe (1982) and initially evaluated for equine *A. perfoliata* faecal analysis

by Hearn and Hearn (1995). The faecal sample was mixed and a 15g sub-sample was removed and placed into a labelled bag and 40ml of tap water was added and the sample mixed thoroughly until the consistency of slurry. The faecal slurry was tipped through a tea-strainer and retained fibrous matter pressed with a wooden tongue depressor to extrude as much water as possible. The filtrate was immediately poured into two conical-bottomed 30ml centrifuge tubes and centrifuged at 400 x g for ten min. The supernatant was removed with a vacuum line or pipette with care not to disturb the pellet at the base of the tube. The sediment was re-suspended in a small volume of saturated sugar solution using a vortex and transferred to a 15ml conical-bottomed centrifuge tube. Additional saturated sugar solution was added until a slight positive meniscus was formed and a 22mm x 22mm glass coverslip was placed onto the top. Centrifuge tubes, with coverslips, were placed into the swinging-bucket centrifuge at 22 x g for ten minutes. The coverslip was then removed, with care to retain any attached solution, and placed onto a glass microscopy slide. Both sub-samples per individual were examined by placing both coverslips onto one slide. Slides were examined using a compound microscope at x40 or x100 in a grid-sweep pattern and only *Anoplocephala spp* eggs were recorded.

Due to the sporadic nature of tapeworm egg shedding a positive/negative result was considered sufficient but in order to better compare CF and DCF methodologies an epg was calculated for both techniques. Epg was calculated using the following formula –

$$epg = \frac{\left( \frac{\#eggs\ per\ slide\ [2\ coverslips]}{\#replicates} \right)}{Volume\ of\ faeces\ (15g)}$$

This was performed in duplicate for each individual for accuracy (i.e. four coverslips per individual). In order to produce an epg value for comparison with the CF technique, the arithmetic mean of the duplicate results was taken.

## 2.9 Data analysis

Descriptive statistics were performed using Microsoft Excel (2010). Pre-treatment FEC,  $k$  aggregation parameters were calculated using the online Wessa software ([http://www.wessa.net/rwasp\\_fitdistrnegbin.wasp](http://www.wessa.net/rwasp_fitdistrnegbin.wasp)). Graphical representations of pre-treatment egg counts and questionnaire data were performed using Microsoft Excel (2016). For FECRT the arithmetic mean of pre-treatment and post-treatment counts, for stock meeting the test inclusion criteria (pre-treatment result of  $\geq 50$ epg) were used in order to calculate reduction percentages and results were graphed using R statistical package (version 3.1.3) and Microsoft Excel (2016). Where FECRT were carried out studs were analysed individually and the results performed for both mixed age group (individual studs overall results) and for separate YS and adult cohorts per stud. FECRT results were analysed using “eggCounts” online software from the University of Zurich (<http://www.math.uzh.ch/as/index.php?id=eggCounts>) (Wang & Paul, 2017) in order to calculate 95% confidence intervals for means, and mode values with their corresponding highest posterior density intervals (HPD); arithmetic means and FECR percentages were also examined in Microsoft Excel (2016). Percentages for FECRT and questionnaire data were rounded to the nearest integer. LC results were organised, and analysed, on an individual stud basis with horses grouped according to grazing cohort only.

# Chapter 3 – Improving Diagnostics

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## 3.1 Introduction

The ability to accurately detect a particular species of parasite at each stage of its development and ideally give an indication of the level of parasitic infection is a key aim of diagnostic parasitology. As discussed in Chapter 1 the focus of parasite control strategies in horses is targeted selective parasite control with anthelmintics; where a given drug is administered based on the result of a diagnostic test.

There are many diagnostic tests available for a range of equine gastrointestinal parasites, the majority of which rely on analysis of faecal or serum samples. The primary parasites that are the focus of diagnostic tests include the strongyle species (cyathostomins and *Strongylus spp*), *Parascaris equorum* and *Anoplocephala perfoliata*. The tests that are available for each of these parasites have a defined sensitivity and specificity which is an important consideration when interpreting the results and making decisions on anthelmintic use. Other important considerations are the additional limitations including errors introduced during collection and storage of samples (Nielsen, et al., 2010; Lester, et al., 2012; Lester & Matthews, 2014).

### 3.1.1 Detection of nematode infection in horses

The detection of prepatent stages limits the clinical usage of many diagnostic tests during disease or in order to quantify the risk of disease to an individual animal (Andersen, et al., 2013a). The latter is of key importance when disease states result from non-egg producing stages of the parasite such as encysted cyathostome burden, migrating *Strongylus spp* larvae, migrating visceral stage of *F. hepatica* and adult ascarid burden post-immunity development in weanling foals. Within this chapter evaluation of diagnostic tests for faecal detection of patent stages of *A. perfoliata* and *F. hepatica* in equids will be discussed.

The strongyles have been the focus of many recent research efforts to develop accurate and sensitive diagnostics tests. A range of tests with differing sensitivities, termed lower detection limits (LDL) for FEC, are validated for use in equines (summarised in Chapter 1, Table 1.1) (Lester & Matthews, 2014). Most of these tests rely on analysis of eggs in faeces. The McMaster method is one of the most commonly available within equine practice, having an LDL of between 15-100epg (Ministry of Agriculture, Fisheries and Food (MAFF), 1986); FECPAK demonstrates an LDL of 20epg (Lester & Matthews, 2014); FLOTAC and CF methods (Cringoli, 2010; Elsheika & Khan, 2011) both have an LDL of 1epg, with CF increasing to 9epg if the maximum multiplication factor for CF (x 9) is used (Lester & Matthews, 2014). Similarly, FEC are used to detect *Parascaris spp* eggs but their ability to detect the level of infection is compromised due to the fecundity of adult worms and development of acquired immunity resulting in reduced egg output with potentially high burdens of adult worms still within the intestinal lumen (Nielsen, et al., 2010b).

### **3.1.2 Detection of *Anoplocephala perfoliata* infection in horses**

The potential for *A. perfoliata* to cause disease has resulted in considerable research efforts over the last two decades (Lightbody, et al., 2016; Proudman & Trees, 1996a; Rehbein, et al., 2011). Post-mortem detection of adult tapeworms at the ileocaecal junction is the gold standard for tapeworm detection and enumeration and has been used for the validation of existing diagnostics, to assign a specificity and sensitivity. FEC methods for *A. perfoliata* have been widely used to diagnose infection in the live horse. The McMaster method, when investigated in multiple studies demonstrated the lowest sensitivities, 3.4% and 8% (Meana, et al., 1998; Tomczuk, et al., 2014), whilst the sedimentation method demonstrated a sensitivity of 10.3% (Tomczuk, et al., 2014); Williamson et al (1998) showed wide variation depending on infection intensity, a sensitivity of 5.6% at an infection intensity of one to nine parasites but up to 50% sensitivity with infection intensities of 40+ worms. When all infection intensities were taken into account (n=40 horses) an overall

sensitivity of 22.5% was found (Williamson, et al., 1998). Two methods of centrifugation, resuspension and flotation with saturated sucrose demonstrated sensitivities of 25% and 37.5% respectively (n=40, infection intensity 1-40+ worms) (Williamson, et al., 1998). The DCF technique has the highest sensitivity shown for detection of tapeworm eggs in faeces, with Proudman and Edwards (1992) finding sensitivity to be 61%. Subsequent studies showed a sensitivity of 54% using the same test, with lowest likelihood of detecting infection in horses with burdens of less than 100 tapeworms (Ihler, et al., 1995; Meana, et al., 1998; Nilsson, et al., 1995). This was reported to increase to 92% when individuals with low burdens, defined as  $\leq 20$  parasites, were excluded from analysis (Proudman & Edwards, 1992), indicating that egg detection is of greater diagnostic value in horses with higher worm burdens (Rehbein, et al., 2011).

The Cornell-Wisconsin technique has been investigated pre and post treatment to determine optimal timing for testing; studies found a 32% sensitivity and 98% specificity when analysing pre-treatment samples (Hearn & Hearn, 1995) which increases to 65-100% when testing approximately 24 hours post-treatment with an effective product such as PYR (38mg/Kg) (Hearn & Hearn, 1995; Slocombe, 2004). However, in the study reporting up to 100% sensitivity, all horses were known positives (Slocombe, 2004) but findings were corroborated by Elsner et al (2011). While this observation may be of use in determination of prevalence of infection and exposure, it is not suitable for treatment decision making. Sensitive, specific and consistent detection of tapeworm eggs in faeces is compromised by intermittent shedding of mature proglottids from parasites within the gastrointestinal tract. Additionally, as for all FEC methods, only patent infection is detected and results do not reflect a quantitative estimate of worm burden. FEC are considered of most use in a herd setting in order to increase the chance of detection of tapeworm egg shedding and so determine presence of tapeworm on farm (Matthews, et al., 2004; Proudman, et al., 1992).

The limitation of coprological analysis was the driver behind development of a commercial diagnostic method to detect anti-tapeworm antibody in serum. Proudman et al (1996) found anti-12/13kDa IgG(T) subtype antibody production to be directly proportional to infection intensity with statistically significant differences between age ranges (Proudman & Trees, 1996a). The study demonstrated a peak in antibody titres in animals between six months and two years of age, which then declined from three to 15 years of age followed by an increased titre in older age ranges (Proudman, et al., 1997). This triphasic pattern is proposed to be a result of age-dependent exposure to infective challenge or a degree of acquired immunity (Proudman, et al., 1997; Woolhouse, 1993). A Swedish study also provided evidence that horses or age groups spending maximum time on pasture have the highest exposure and mean worm burdens with maximum force of infection presented by year-round turnout (Hoglund, et al., 1995). Multiple isotypes of IgG are produced within the immune system, the distribution of which is thought to potentially become significant when results are interpreted at a population level (Proudman, et al., 1997; Traversa, et al., 2008). Traversa et al (2008) highlighted that the variability in antibody response and detection limits imposed by the test, can both limit the sensitivity and result in a lack of discrimination between false and true positive results, resulting in underestimation of *A. perfoliata* infection. In the interests of making diagnostics more accessible to horse owners and reducing the need for invasive tests, recently a commercially available tapeworm diagnostic test (EquiSal™, ADB) was developed to detect the 12/13kDa antigen in saliva (Lightbody, et al., 2016) and it is thought that this may be able to detect recent exposure as levels of IgG(T) decline more rapidly in saliva, although this remains to be demonstrated.

Other, novel approaches include coproantigen testing and nested PCR based methods to detect parasitic DNA within equine faecal samples, specifically the Internal Transcribed Spacer 2 (ITS) region, originally described by (Drogemuller, et al., 2004). The nested PCR approach tested by Traversa et al (2007) and Skotarek et al (2010) demonstrated a 74%

sensitivity with 92% specificity, potentially indicating some advantage in detection of active infection by removal of confounding factors which can impair serological test results such as the persistence of antibodies post-treatment, individual variability and rate of decay of antibodies documented by Høglund, et al., (1995) and Proudman and Trees (1996b). However, the PCR method is currently not quantitative and so, unlike serological methods, will not indicate the magnitude of infection and so potential risk of disease (Traversa, et al., 2008; Skotarek, et al., 2010). Neither method has proved to significantly improve sensitivity or specificity over commercially available tests; are laborious, require a prohibitive degree of specialist equipment or skill and/or are prohibitively expensive for commercial viability (Wilford, 2016).

### **3.1.3 Detection of *Fasciola hepatica* infection in horses**

Diagnosis of disease due to *F. hepatica* in ruminants and other predisposed species, such as donkeys and camelids, is most commonly based on clinical signs, season, local climate, topography and suitability of the grazing environment for mud snail habitats and diagnostic tests. In ruminants, diagnostic testing relies on the sedimentation FEC as the pre-mortem gold standard, but includes antibody-detection ELISA on serum and milk to detect herd/flock level exposure (Salimi-Bejestani, et al., 2005). More recently a coproantigen ELISA showed 80% sensitivity in both sheep and cattle, however in horses this was reduced to 9% when compared to faecal sedimentation (Palmer, et al., 2014).

In an area of Spain known to have a high prevalence of *F. hepatica* infection (28-65% prevalence in ruminants) an early study detecting IgG, using an indirect 2.9kDa ELISA in 536 horses, showed 60% of horses tested positive to the recombinant surface protein (FhrAPS), with higher seroprevalence within mares (67%) compared to foals (12%). Seroconversion patterns seen within tested horses demonstrated a significant positive correlation between seroprevalence and the age of horses, posited to be due to increased risk of exposure with age in endemic areas (Arias, et al., 2012). As no patent infections were detected by faecal

sedimentation, and this study was not able to utilise post-mortem examination, results are not conclusive and require more robust analysis (Arias, et al., 2012). Work on production of a sensitive and specific serological test for detection of infection in equids is currently the research focus of both The UofL and University College Dublin (Williams & Hodgkinson, 2015).

#### **3.1.4 The need for practical, accessible diagnostics for equine parasites**

The targeted selective control of parasites in horses is considered the way forward

(Matthews 2014). For this to be implemented effectively, diagnostic tests that are practical and accessible to studs and horse owners are vital tools to guide anthelmintic treatment decisions. Faecal egg counts are carried out on studs and are considered a diagnostic test that can be effectively and efficiently carried out without the need for specialist equipment. Strongyle egg counts can rapidly be carried out using existing protocols but detection of tapeworm and liver fluke eggs involve the much more laborious DCF and faecal sedimentation methodologies, also requiring a large time commitment and specific equipment. A faster screening protocol for tapeworm and patent liver fluke infection in horses would be advantageous, ideally as a method which allows multiple parasite eggs to be detected using the same procedure.

The recent availability of a saliva-based tapeworm diagnostic, EquiSal™, is an example of a test which is less invasive, cheaper and can be carried out directly by horse owners. The UofL routinely tests for tapeworm infection with the antibody detection ELISA (Diagnosteq, UofL) and have had >15years experience of interpreting tapeworm ELISA results. It is important that the new EquiSal™ diagnostic is subject to independent evaluation as follows: a) compare validation of EquiSal™ with the UofL tapeworm ELISA by testing serum samples used for internal validation of EquiSal™; b) compare the performance of both tests in the horse population using a cohort of leisure and competition horses and c) identify

how the two tests compare in a clinical context. Given the time constraints of this project it was only possible to complete testing for the first objective.

In conclusion the overall aims of this chapter were to:

1. Evaluate the CF method, currently used for the detection of strongyle eggs, for its ability to simultaneously detect tapeworm eggs and compare this with the DCF method currently used for detecting tapeworm eggs in faeces.
2. Evaluate the McMaster method, utilised at the Moredun Research Institute to detect liver fluke eggs in ruminant faeces, for its ability to detect liver fluke eggs in the faeces of equids and compare this with the standard sedimentation method.
3. To compare validation of EquiSal™ with the UofL tapeworm ELISA by testing 137 serum samples held by ADB, with our UofL tapeworm ELISA. These samples are from horses enumerated for levels of tapeworm infection post-mortem (the gold standard) and were used for validation of EquiSal™ prior to its launch as a commercial test.

## **3.2 Materials and Methods**

### **3.2.1 Faecal sample collection**

For the *F. hepatica* diagnostics all faecal samples, except one, were collected from donkeys at the Donkey Sanctuary, UK and sent to the UofL as described in section 2.1. The final sample was a faecal sample submitted to the UofL Diagnosteq equine parasitology diagnostic service. The Donkey Sanctuary provided 18 faecal samples known to be positive for *F. hepatica* eggs based on faecal sedimentation and UofL provided one sample from a horse via the Diagnosteq service, all of which were used to evaluate the McMaster method for its ability to detect liver fluke eggs. For the evaluation of *A. perfoliata* eggs in faeces samples from a TB stud (n=140) were used. All samples were collected ante-mortem.

### **3.2.2 Detection of *Anoplocephala perfoliata* eggs in faeces**

Samples were processed by the centrifugation flotation (CF) method as described in section 2.2 and used 10g of faeces. Eggs were visualised on a Miller square eyepiece graticule as described in section 2.2. Egg counts were completed in duplicate for all samples and an arithmetic mean taken for final egg.

### **3.2.3 Detection of *Fasciola hepatica* eggs in faeces**

Faecal samples from Donkeys were collected and tested as part of the routine parasite surveillance carried out by the Donkey sanctuary, which involved the sedimentation test for the detection of *F. hepatica* eggs. Those samples that were positive for *F. hepatica* eggs were then shipped in rectal gloves with air expelled, packed within airtight Biobottles to the UofL where they were refrigerated and tested within seven days of arrival. The date of sampling was not included with these faecal samples but all were refrigerated soon after collection until time of postage. At the UofL each sample was subjected to testing by both of the following protocols: a) from each sample the McMaster method typically used for the detection of nematode eggs, using 3g of faeces and 42ml of saturated sodium chloride, was carried out as detailed in section 2.7.1. For the purpose of detecting liver fluke eggs, 6g was used to allow for duplicate McMaster tests to be performed and b) from each sample a 20g faecal sample was used to perform duplicate traditional liver fluke sedimentation tests, as described in section 2.7.2. To calculate the epg, a mean of the duplicate tests was calculated.

### **3.2.4 Comparison of the UofL tapeworm ELISA and EquiSal™ tests**

Ethical approval for the tapeworm study was obtained from the Veterinary Research Ethics Committee and conducted under approval VREC361. Serum samples used by ADB (n=137) to develop and validate the EquiSal™ test (known as 130AD) were tested with our UofL tapeworm ELISA. Whilst it was also intended to compare the performance of EquiSal™ with the UofL tapeworm ELISA in the general horse population, the number of samples required for statistical analysis and the practicalities of generating those samples meant that this

work is still ongoing, so is not reported here. Similarly, to identify how the EquiSal™ and the UofL tapeworm ELISA compare in a clinical context, cases referred to the Philip Leverhulme Equine Hospital, UofL, as suspected colic cases and routinely tested for tapeworm infection on admission by UofL ELISA, were simultaneously subjected to EquiSal™. This work is also currently underway and will not be reported here.

### 3.2.5 Data analysis

Descriptive statistics were performed using Microsoft Excel (2010). Graphical representations of egg counts were performed using Microsoft Excel (2010). Paired serum ELISA O.D. values for the Diagnosteq and Equisal™ tests were analysed using calculation of the Pearson correlation co-efficient (r) using the equation seen in figure 3.1.

$$r = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2} \sqrt{\sum_i (y_i - \bar{y})^2}}$$

Figure 3.1. Equation used for calculation of the Pearson correlation co-efficient, for measurement of linear correlation of two variables (x, UofL ELISA OD values, and y, ADB OD values), by online software available at <http://www.socscistatistics.com/tests>.

From this value, the co-efficient of determination (r<sup>2</sup>) was calculated and a P-value generated with a significance of <0.05 set. These calculations were performed using online software available through Social Science Statistics (<http://www.socscistatistics.com/tests/pearson/>). Graphing of paired values from the two tests under comparison was performed using Microsoft Excel (2016).

## 3.3 Results

### 3.3.1 Comparison of faecal analysis for the detection of *Anoplocephala perfoliata* eggs

In total 140 (n=78 samples from adults and n=62 YS samples) samples were tested from one stud between February 2015 and July 2016. Samples were collected at six time points, winter, spring, summer and autumn 2015 and spring and summer 2016 (Table 3.1). Of the

140, 36 (25.7%  $\pm$ 7.2%) of samples tested positive by the CF method and 31 (22.1%  $\pm$ 6.9%) tested positive by the DCF method. The CF method was capable of detecting more samples as FEC positive, due to the absence of a gold standard test statistical significance was not established. The lowest number of faecal samples for which a positive result was recorded by either method was for samples collected in June and September 2015. The greatest proportion of animals testing positive was in February 2015 in both the adult and young stock. This trend was observed in samples collected in April 2016, although lower numbers of samples were tested (Table 3.1).

Table 3.1 The total number of faecal samples testing positive by centrifugation flotation (CF) method and Double Centrifugation Flotation method (DCF) in adult stock and YS.

<b>ADULT STOCK</b>							
	<b>Feb 2015</b>	<b>Apr 2015</b>	<b>Jun 2015</b>	<b>Sep 2015</b>	<b>Apr 2016</b>	<b>Jul 2016</b>	<b>Total +ve</b>
CF +ve	7	3	1	1	5	3	20
DCF +ve	5	3	0	1	3	3	15
Total no. tested	12	12	15	15	10	14	78
% +ve by CF ( $\pm$ 95%CI)	58.3 (27.9)	25 ( $\pm$ 24.5)	6.7 ( $\pm$ 12.6)	6.7 ( $\pm$ 12.6)	50 ( $\pm$ 31.0)	21.4 ( $\pm$ 21.5)	25.6 ( $\pm$ 9.7)
% +ve by DCF ( $\pm$ 95%CI)	41.7 ( $\pm$ 27.9)	25 ( $\pm$ 24.5)	-	6.7 ( $\pm$ 12.6)	30 ( $\pm$ 28.4)	21.4 ( $\pm$ 21.5)	19.2 ( $\pm$ 8.7)

<b>YOUNGSTOCK</b>							
	<b>Feb 2015</b>	<b>Apr 2015</b>	<b>Jun 2015</b>	<b>Sep 2015</b>	<b>Apr 2016</b>	<b>Jul 2016</b>	<b>Total +ve</b>
CF +ve	11	2	1	0	1	1	16
DCF +ve	12	2	0	0	1	1	16
Total no. tested	18	18	13	8	2	3	62
% +ve by CF ( $\pm$ 95% CI)	61.1 ( $\pm$ 22.5)	11.1 ( $\pm$ 14.5)	7.7 ( $\pm$ 14.5)	-	50 ( $\pm$ 69.3)	33.3 ( $\pm$ 53.3)	25.8 ( $\pm$ 10.9)
% +ve by DCF ( $\pm$ 95%CI)	66.7 ( $\pm$ 21.8)	11.1 ( $\pm$ 14.5)	-	-	50 ( $\pm$ 69.3)	33.3 ( $\pm$ 53.3)	25.8 ( $\pm$ 10.9)

Egg counts of one or more were classed as positive (+ve)

Table 3.2 The number of individual faecal samples testing positive by both CF and DCF; positive by CF but negative by DCF; positive by DCF but negative by CF or negative by both tests of 140 samples screened.

% ( $\pm$ 95% CI, n/140)	CF +ve	CF -ve
DCF +ve	17.9 ( $\pm$ 6.3, n=25)	4.3 ( $\pm$ 3.4, n=6)
DCF -ve	7.9 ( $\pm$ 4.5, n=11)	70 ( $\pm$ 7.6, n=98)

Although the tests differed in their ability to detect positive samples overall (25.7% [ $\pm$ 7.2%, n=36/140] for CF vs 22.1% [ $\pm$ 6.9%, n=31/140] for DCF) the two methods showed the same trend at individual time points in terms of being able to detect eggs in faeces in both adults and YS and at many time points they recorded the same number of samples positive, for example April 2015, September 2015 and July 2016 (Table 3.1). For all individual samples over all time points, 17.9% ( $\pm$ 6.3%, n=25/140) tested positive by both methods, CF detected 11 positive samples (7.9%  $\pm$ 4.5%) which were negative by DCF and DCF detected six positive samples (4.3%  $\pm$ 3.4%) which were negative on CF; 70.0% ( $\pm$ 7.6%, n=98/140) of samples were negative by both methods (Table 3.2).

### 3.3.2 Comparison of faecal analysis for the detection of *F. hepatica* eggs

All 19 samples tested positive for liver fluke eggs by both the standard sedimentation protocol and by the McMaster 'short method' (100.0% agreement) and the same trend was observed for each method with the same value showing the lowest and highest egg; sample number 18 recorded the lowest and sample number 16 the highest value, when either method was used (see Figure 3.2). With the exception of sample 13 all samples were consistently categorized into the <50epg or >50epg using both methods. The mean egg value for the short method was 21.0epg (median 8.2epg) and a minimum count of 0.2epg and maximum of 138.0epg was detected. For the standard sedimentation method the mean value was 31.0epg (median 10.8epg) and a minimum count of 0.4epg and maximum of 202.0epg was detected.

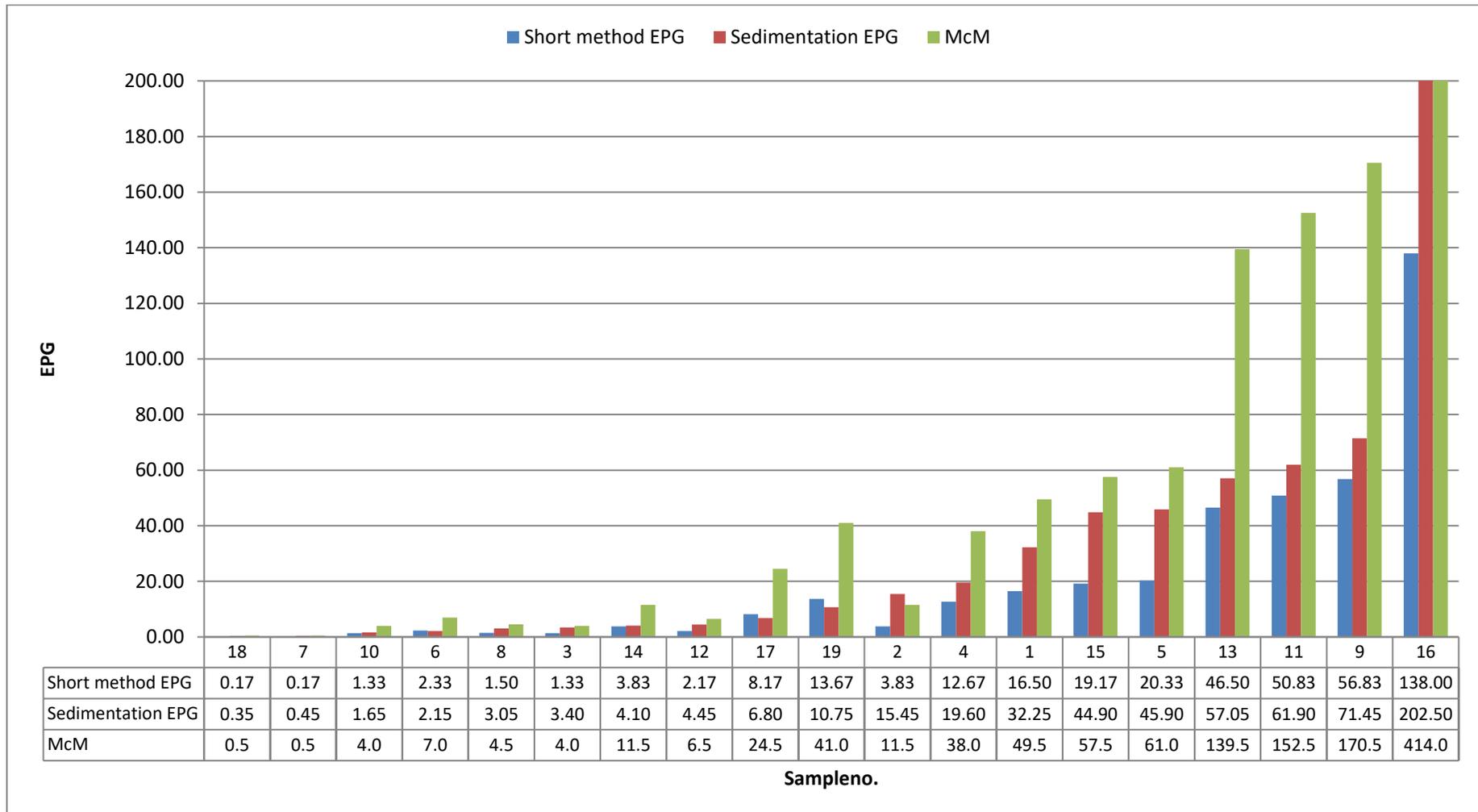


Figure 3.2. A bar chart showing eggs per gram for the short testing method (blue bars) and the standard testing method (red bars) for each of 19 samples (labelled 1 to 19). The McMaster result for strongyle eggs that were detected from the same test are also shown (green bars).

Given that the McMaster test was used to simultaneously provide a FEC for strongyle eggs these were also recorded for each equid (see Figure 3.2). The mean value was 63.1 epg (median 24.5) for strongyle-type eggs. Those donkeys (and one horse) recording the highest epg count for *F. hepatica* eggs were the same individuals that recorded high strongyle egg counts (Figure 3.2) which suggests that the same few animals are responsible for shedding the highest numbers of both trematode and nematode eggs.

### 3.3.2 Comparison of serum and salivary analysis for the detection of *A. perfoliata* antibodies by ELISA

The 137 serum samples from horses with enumerated post mortem burdens of tapeworm, and which were subsequently used to validate the Equisal™ saliva test, were tested by two ELISA methods; UofL ELISA and ADB ELISA. The correlation coefficient ( $r$ ) was found to be 0.8, with a coefficient of determination ( $r^2$ ) of 0.7, indicating a strong linear correlation (where  $r=1$  indicates a perfect positive correlation). The P value (significance set at  $<0.05$ ) calculated from these values was  $<0.00001$ , indicating a statistically significant result.

Figure 3.3 shows ADB ELISA values plotted against the UofL Diagnosteq ELISA O.D. values.

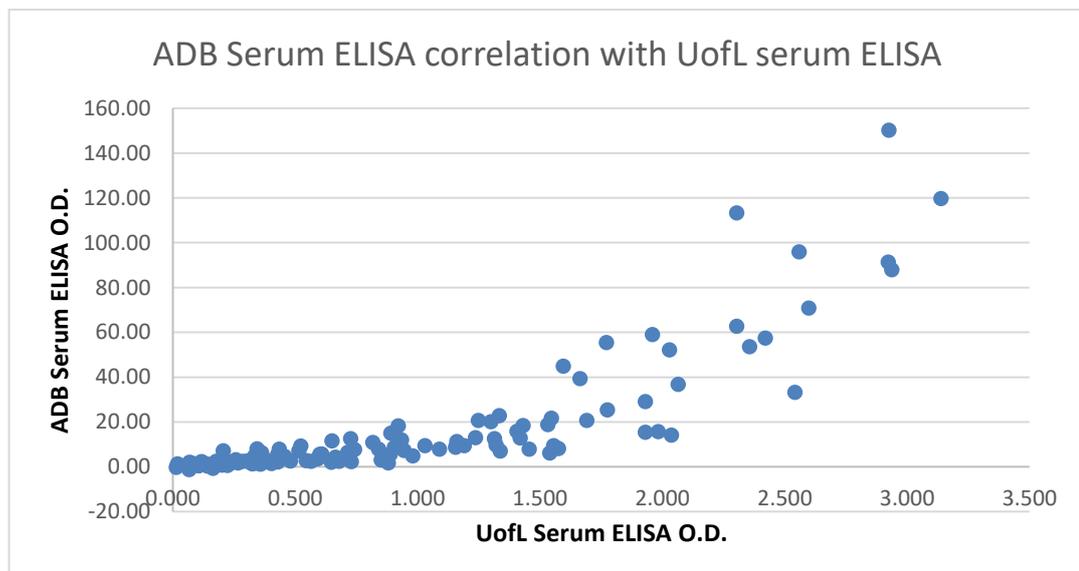


Figure 3.3. ADB serum ELISA O.D. results for 137 paired samples, for which tapeworm were enumerated on post mortem, correlated with corresponding UofL serum ELISA O.D. values.

### 3.4 Discussion

Two faecal screening methods that take a shorter time to process (CF for detection of tapeworm eggs) and capable of detecting both nematode and trematode eggs (McMaster + short method of fluke egg detection) have been tested against currently validated methods for the detection of *A. perfoliata* (DCF method (Rehbein, et al., 2011)) and *F. hepatica* (Standard sedimentation method (Anderson, et al., 1999; Happich & Boray, 1969)) eggs in faeces. The ideal approach to validating these two methods would have been against a post mortem gold standard but for practical reasons this was not possible. However, in the absence of a gold standard we conducted this evaluation with faecal samples from two cohorts of individuals that were known to be fluke positive (donkeys at the Donkey Sanctuary, Sidmouth UK) or where tapeworm infection was known to be present (Stud E). For both aspects of this study, the number of available positive samples, lack of post mortem data to given definitive gold standard results and compliance with sample collection resulted in low sample sizes which limited the statistical analysis. This initial study does however potentially indicate that these methods can be used as low cost, practical, time and resource efficient parasite screen in general practice, compared to currently utilised methods.

#### 3.4.1 *Anoplocephala perfoliata* diagnostic testing

*Anoplocephala perfoliata* infection is common but challenging to detect due to intermittent shedding and low sensitivity of currently available diagnostic techniques (Proudman & Edwards, 1992). Serological tests have demonstrated an improvement in the sensitivity of detection of exposure through quantification of anti-12/13kDa IgG(T) titres at point of testing. Until recently this method of testing required blood sampling and therefore a veterinary surgeon visit for sample collection which can be costly with consequent reduced uptake by owners (Proudman & Trees, 1996a). This consideration is important when considering the number of stock on TB farms and the challenges of housing and bringing in

all stock for sampling at specific points of year when both YS and adults may be at pasture. The saliva test, EquiSal™ aims to increase uptake of diagnostic testing by removing the cost of veterinary visits, with owners able to collect and ship samples themselves. This method has demonstrated good sensitivity and specificity compared to other serological methods and results from paired post-mortem enumerations (Lightbody, et al., 2016) and our data here suggests a strong positive correlation with regards to serum used to develop the EquiSal™ when compared with the UofL serum ELISA. This is shown by the high correlation co-efficient of 0.83, where a perfect positive correlation gives a result of one. In addition, the resulting P value shows statistical significance in the positive correlation when  $P < 0.05$  is classed as significant (data value  $< 0.00001$ ) (Figure 3.3). Further independent validation is underway at the UofL and it will be interesting to observe the outcome of this analysis. However, one potential area of concern in owner utilisation of this novel diagnostic method is the potential knowledge gap amongst owners in interpretation of test results and subsequent implementation of changes in practice. This is partially addressed through interpretation provided with EquiSal™ results regarding treatment which is delivered directly to the client, there is however limited opportunity for further discussion of results and potential issues with integration into an overall parasite control plan.

Additional sources of information on worming practice and utilisation of diagnostic tests are those holding the “Suitably Qualified Person” (SQP) qualification. SQPs provide a good opportunity for face-to-face discussion of parasite control practice and drug usage for the client with bespoke advice based on individual stock demographics and management practices. It is therefore important to ensure that the information given to clients is up to date and accurate with continual advancement in diagnostic and best practice protocols. This requires the provision, and uptake, of accessible and widely available parasitology continuing professional development (CPD) for EQM module qualified SQPs as part of annual CPD requirements (Animal Medicines Training Regulatory Authority Ltd (AMTRA)).

The DCF method is messy, laborious, requires large pieces of expensive laboratory equipment and has a low sensitivity, it is not commonly financially viable for practices to run (Proudman & Edwards, 1992). The CF technique, although more time consuming and requiring more equipment than the commonly used McMaster test, has high (one to nine epg LDL) sensitivity when detecting strongyle and ascarid eggs and is able to detect *Strongyloides westerii*, providing a wide spectrum of detection and surveillance within a single test (Relf, et al., 2013). The data presented from this initial study suggests that the CF technique may also be able to detect tapeworm eggs with a similar sensitivity to DCF. The ability to conduct a single test to screen for all common parasite species, albeit with common limitations of faecal testing for cestode species (i.e. sporadic shedding, non-uniform dispersal through the faecal pile and detection of only patent infection) makes it efficient and potentially offers greater value for money for the client. As for DCF, it does have the disadvantage of requiring specialist laboratory equipment, resulting in lack of financial viability for some practices, however as many commercial laboratories do not routinely offer DCF testing this is a single test which could provide a rapid screen at low time and resource cost. In combination with herd level ELISA testing, results could be utilised to monitor exposure, shedding and prevalence of infection to support an evidence based treatment approach. Tapeworm infection, and the population density of the intermediate host whose peak abundance is climate dependant, has been shown to be seasonal in terms of the disease caused and the increased burden of infection seen, in the UK and temperate zones of Europe (Nielsen, 2016; Tomczuk, et al., 2015; van Nieuwenhuizen, et al., 1994). This seasonality needs to be considered when engaging with diagnostics, with the important caveat that a negative result cannot be said to indicate a negative tapeworm burden. In reality, horse owners were reported to strategically administer anthelmintics in spring and autumn in order to combat burdens accumulated during the grazing season without engaging with diagnostics (Tomczuk, et al., 2015),

however these diagnostics must be encouraged to ensure judicious use of drugs in future (Matthews, 2014).

### **3.4.2 *Fasciola hepatica* diagnostic testing**

Within targeted selective regimes, pasture management is of key importance including co-grazing of ruminant stock to reduce pasture burdens of parasites, such as L3 of strongyle species, by ingestion by non-susceptible host species. The rise in anthelmintic resistance and prevalence of *F. hepatica* infection is currently an increasing concern in ruminants (Williams, et al., 2014). These factors, in combination with recent prevalence studies in horses demonstrate that infection of horses with liver fluke is not only possible but more common than previously considered, with prevalence from FEC of 0.07% in Polish horses to 60% in a Northern Spanish cohort of horses (Sadzikowski, et al., 2009; Arias, et al., 2012). Co-grazing donkeys with horses increases the risk of infection in the horse population and poses a threat for establishment of infection in horses with anthelmintic resistant strains, as demonstrated in a 2014 Australian study (Palmer, et al., 2014). Screening of horses potentially at high risk of exposure needs to be carefully considered. Triclabendazole is the primary drug of choice within ruminants due to its high degree of action on both adult and juvenile stages (Boray, et al., 1983), due to increased prevalence in cattle and sheep drug usage has increased, leading to selection for resistant populations which are now widely documented in ruminants (Fairweather, 2005; Fairweather, 2011). The primary concern regarding equine populations is the potential lack of education and awareness of risk posed, especially when considering co-grazing of ruminants is a common pasture management practice on stud farms. However, before we can accurately advise stud owners on the level of surveillance, concern and risk posed by *F. hepatica* to equine populations we first need further research and evidence into the true risk of infection posed, prevalence of patency of infection and characterisation of clinical disease (Raftery, et al., 2015).

Donkeys are known to harbour patent liver fluke infection, with a documented prevalence of 44.4% in coprologically sampled donkeys and 41.9% in those examined at post-mortem in an Ethiopian study (Getachew et al 2010b). Their ability to shed high epg counts relative to horses (10-960epg) has been noted in studies performed in Ethiopia and within the UK through the Donkey Sanctuary (Getachew, et al., 2010b; Williams & Hodgkinson, 2015). In contrast, in horses it has been shown that patent infections are not common with multiple studies recording positive serological values without corresponding positive FEC results, few studies where positive FEC results were obtained did not describe results quantitatively (Arias, et al., 2012; Quigley, et al., 2016). Given that horses are thought to exhibit a lower level of patency compared to donkeys and cattle, a screening test would need to detect a range of egg counts with minimum equivalent sensitivity to traditional sedimentation methods, documented as 60% sensitive when analysing cattle faecal samples for presence of *Fasciola spp* eggs (Charlier, et al., 2014). Initial results from this study indicate that the short method of fluke egg detection, from McMasters preparation, may detect as few as 0.17epg (an average epg recorded from seeing one egg in two x 3g duplicate McMaster preparation), making it possible to detect low egg shedding in horses. In the samples tested, a wide range of egg counts was found using the standard sedimentation method, including low <10epg and moderate/high >50epg; whilst the short method consistently recorded lower epg counts all samples also recorded positive results using the McMaster technique, with the same trend observed (Figure 3.2). Further testing using larger sample sizes is required, in addition to paired gold-standard post-mortem test data, for validation and evaluation of sensitivity and specificity.

Traditional sedimentation methods are laborious and time consuming, requiring a moderate amount of equipment, highlighting the need for a fast but sensitive faecal screening method. McMaster egg counts are commonly offered in-house by equine and mixed practices as a fast and moderately sensitive (15-100epg LDL) test for strongyle and

ascaris eggs; with consequent increased uptake of targeted selective protocols (Lester & Matthews, 2014). A test which offers increased monitoring and surveillance of liver fluke infection in horses as well as nematode infection, for minimal additional time investment, would be advantageous. This may become all the more pertinent given that in donkeys those individuals seen to shed the highest number of strongyle eggs were also shedding the highest number of liver fluke eggs (see Figure 3.2) and suggests some increased susceptibility to co-infection.

As for all faecal parasite detection methods, the limitation is the presence of patent infection and shedding of eggs into faeces at the time of sampling. Given the success of serological testing for *A. perfoliata*, production of a sensitive and specific serological test for detection of liver fluke infection in equids is now underway (Williams & Hodgkinson, 2015) and is offered on a pre-validated basis by the UofL and it may eventually provide a sensitive and specific alternative to reliance on patent infection detection and give an indication of herd level exposure in future (Williams & Hodgkinson, 2015). Similarly, an antibody detection ELISA for larval stage antigens of cyathostomins is in the advanced stages of development (Mitchell, et al., 2016). The success of the EquiSal™ test in delivering this serological test in a non-invasive, user friendly format promises much for the future of antibody-based detection of parasitic infection in horses. In reality it is widely acknowledged that faecal-based methods still remain practical, cost-effective routes to target drug treatments and reduce parasite transmission and associated disease in horses.

# Chapter 4 – Distribution and drug resistance status of parasites on Thoroughbred Studs

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## 4.1 Introduction

Given the significant clinical impact intestinal nematodes of horses can have within breeding populations intensive anthelmintic treatment regimens were introduced in the 1960s. Treatment at intervals defined by the ERP for the drug was advocated in order to suppress strongyle egg shedding and reduce the prevalence of large strongyle species in particular; due to their high pathogenic potential (Drudge & Lyons, 1966; Drudge, et al., 1966). These intensive drug treatment regimens were effective in reducing the prevalence of *S. vulgaris* and related disease, but resulted in a high selection pressure for anthelmintic resistance in cyathostomins (Boxell, et al., 2004; Lyons, et al., 1999). Drug resistance was first detected in the BZ class of drugs and is now a worldwide problem where anthelmintics have been widely available, with PYR resistance seen in some parts of the world and reduction in ERP post ML treatment noted in many countries (Table 1.4, Chapter 1). This resistance is of particular concern as no new drug classes are under development for use in equines in the foreseeable future.

Cyathostomins are now recognised as the most prevalent parasite of horses and have become the focus of recent treatment strategies (Herd, et al., 1981; Lyons, et al., 1999). Comer et al (2006) identified that 84% of horses on TB studs were being treated even when their FEC were low, identifying an over-reliance on anthelmintic control on TB studs and raising concerns about drug resistance. Subsequently a number of studies have identified anthelmintic resistance in TB horse populations, primarily in small strongyle populations (Lyons, et al., 2011; Relf, et al., 2014) and resistance is also observed in the general horse population (Rossano, et al., 2010; Traversa, et al., 2009; Tzelos, et al., 2017).

A recent review collated our knowledge of anthelmintic resistance in cyathostomins, based on either FECRT and shortened ERP values (Matthews, 2014). Resistance of strongyle parasites to the BZ class based on FECRT is considered extensive (Lester, et al., 2013; Stratford, et al., 2013b). Traversa et al (2009) found that, in the case of PYR, strongyle FECRT indicated that resistance was present on 25% of yards tested (n=102) in Italy, Germany and the UK, studies in the USA show approximately 50% of premises with PYR resistance (Kaplan, et al., 2004). Whilst for IVM, Traversa et al (2009) showed only one Italian stud demonstrating resistance when tested by FECRT. When shortened ERP was considered as a measure of the early stages of resistance (Sangster, 2001), reduced efficacy to MOX has been reported (Geurden, et al., 2014; Lyons, et al., 2011; Relf, et al., 2014; Rossano, et al., 2010; van Doorn, et al., 2014; von Samson-Himmelstjerna, et al., 2007) but based on FECRT, UK parasite populations appear to have 100% efficacy at two weeks post treatment for the ML class of drugs (Lester, et al., 2013; Relf, et al., 2014). However, Brazil have recorded a single premises where strongyles show resistance to MOX using FECRT (Molento, et al., 2008).

Current best practice involves an evidence-based approach, using diagnostics, to target use of anthelmintics, thereby reducing drug selection pressure and maintaining a population of parasites in refugia so that the development of drug resistance can be slowed. This is possible due to over dispersion of nematode infections within the host where >80% of egg shedding is from <20% of the individuals (Anderson & May, 1978; Calabrese, et al., 2011; Crofton, 1971; Lester, et al., 2013; Relf, et al., 2013; Shaw & Dobson, 1995; Shaw, 1998). The approach that is advocated is to use FEC to target anthelmintic treatment to those horses within the herd that are shedding high numbers of eggs (high egg-shedders) and who contribute the most to pasture contamination. This strategy is most effective when carried out during the peak period of transmission, typically April-September in the UK (Tzelos & Matthews, 2016). Profiles of egg shedding in TB mares in Kentucky indicated that

repeated sampling and FEC analysis of strongyle egg shedding from individual horses did not produce significantly different results over a three week period, identifying that a single strongyle FEC is indicative of the level of egg shedding for a particular horse (Lyons, et al., 2012). However, it is important to note the presence of variability in counts from the same individual, this is especially pronounced at lower egg (Nielsen, et al., 2006).

A number of studies from Denmark, where targeted-type regimes have been practiced for a long time, have noted potential re-emergence of large strongyles due to reduced treatment of low egg count individuals and limited ML usage. Nielsen et al (2012) found a prevalence of 15.4% in individual horses and 83.5% prevalence on farms managed under selective routines compared to 7.7% of individuals and 38.9% of farms using interval programmes. As cyathostome and *Strongylus spp* are unable to be differentiated on FEC, until recently, LC was the only pre-mortem method available (Lester & Matthews, 2014; Lichtenfels, et al., 1998), a real-time PCR has been developed and initial validation has demonstrated a significantly higher proportion of positives within the test population compared to LC (Kaspar, et al., 2017). Molecular testing has been investigated with favourable results, as discussed in Chapters 1 and 3 and used as an adjunct to LC by Tzelos et al (2017), but none are yet commercially available.

Targeted selective treatment regimens have been adopted successfully in some horse sectors, for example with leisure horses (Lester, et al., 2013b; Stratford, et al., 2013), whereas in other horse enterprises, such as TB stud farms, they have had a poorer uptake (Relf, et al., 2013; Robert, et al., 2015). There are several important factors to consider when implementing a targeted selective treatment regime, many of which have been the focus of much debate within the equine parasitology community. These include the choice of diagnostic test and its LDL (as discussed in Chapter 3) and defining what threshold of FEC should be used for recommendation of treatment. It is also important to consider all the

parasites that can infect horses and how the age or grazing habits of horses may influence their susceptibility to infection. Due to the high prevalence of drug resistance in populations of cyathostomin spp and *Parascaris spp*, it is important to establish that the drugs used in targeted selective treatment programmes are effective at reducing egg shedding, by means of a FECRT. Finally, drug treatment is considered only one component of parasite control and effective pasture management strategies have been shown to reduce overall pasture infectivity and infective larvae ingested by the grazing cohort in some studies (Corbett, et al., 2014; Relf, et al., 2013).

The aims of this chapter were to:

1. Engage with TB studs to capture their current parasite control practice and to develop bespoke test-and-treat protocols on stud
2. Carry out detailed analysis of the distribution of parasitic infection on stud in foal, YS and adult cohorts by means of FEC.
3. Evaluate the efficacy of anthelmintics against strongyle infection on stud by means of a FECRT and ERP.
4. Detect if large strongyle infection is present on stud using a LC assay.

## 4.2 Methods

### 4.2.1 Recruitment of studs

From Jan 2013 to October 2016, faecal samples were examined from ten studs (Studs A to J). Participating premises were selected through a number of routes: stud A was already involved in parasite surveillance with the Moredun Research Institute (MRI); additional studs were approached using indirect (advertisement through related third parties such as MRI and Diagnosteq) and direct (letter/email) methods. The latter group were identified through Directory of the Turf (DoT) and a total of 60 studs were selected based on the DoT listing order for invitation by letter and, where available, email. Stud B responded following

advertisement through a seminar conducted at a Thoroughbred Breeders Associate (TBA) meeting; studs C, G and J responded to letter invitations, with studs D, E, F, I and H being referred for participation by their veterinary surgeon due to concerns regarding anthelmintic usage or clinical disease. Once a stud had been contacted to confirm their interest in the study an invitation letter, questionnaire on current practices including information on key areas (Table 4.1) and consent form was sent to the stud owner/manager by post.

Stud A was a large stud (>400 horses); the other studs (studs B, C, D, E, F, G, H, I, J) participating in the project were of variable size (Table 4.2). A total of 3130 faecal samples were collected with repeated sampling for FECRT performed according to the needs of each stud. All studs were located within England (Table 4.2). Equines included in the study, resident on farm at the time of testing and having access to grazing, were categorised according to age (under one, one to three and over three years old). The term “yearling” refers to those horses born in the year prior to sampling; “foal” refers to those born within the year of sampling, including weanlings.

#### **4.2.2 Questionnaire to capture current practice on stud**

The project aimed to evaluate the effectiveness of current practice on stud in controlling parasitic infection and where necessary, to promote best practice parasite management. To do this effectively we needed to know the age of their stock, whether they engaged with diagnostics, the timing and type of drug administered and pasture management practices (Table 4.1). Additionally, we aimed to provide studs with information of herd-level egg shedding and evaluate drug efficacy by means of a FECRT. This required detailed information to be gathered from each stud by means of a questionnaire that was divided into 13 sections and consisted of a maximum of 88 questions. The questionnaire was a modified version of that used by Relf et al (2012). The questionnaire was conducted

electronically and was analysed descriptively. The key questions posed by the questionnaire are shown in Table 4.1. See Appendix 4A for a copy of the full questionnaire.

### **4.2.3 Parasitological methods**

Stud staff were asked to collect freshly voided faeces (less than four hours old) from identified individuals and store them in sealable plastic bags, being careful to expel all the air to reduce larval development. They were sent by post to the UofL and processed within 48 hrs of collection (or seven days for stud A), as detailed in Chapter 2. The CF technique was used for FEC analysis and carried out as described in Chapter 2. For FECRT drug was administered at the recommended dose by stud managers with weight estimation performed prior to treatment using protocols typically employed by each stud. FECRT were essentially carried out as described in Chapter 2, with FECRT conducted only when ten or more horses recorded counts of  $\geq 50$ epg.

FECRT was calculated based on group mean at Day 0 and Day 14-17 post treatment and interpreted using defined lower confidence levels as described in Chapter 2. Strongyle FEC were analysed post treatment at Day 14 and those negative at Day 14 were re-tested every two weeks until 12 weeks post treatment or the defined ERP criteria were met: i.e. the group arithmetic mean FEC exceeded ten percent of the group arithmetic mean FEC at Day 0 (see Chapter 2). To determine the presence of large and small strongyle species, samples were stratified by age and grouped into cohorts of ten co-grazing animals, then faeces were pooled per cohort and subjected to LC as described in Chapter 2. A total of 300 LCs were performed.

Table 4.1 Questions posed and data captured from studs engaging with FEC and FECRT

Questions posed	Data captured
Stud information Equine movements	Basic operating information for stud e.g. veterinary contact, stock residing on stud Average duration of stay, parasite related pre-arrival checks, de-worming and grazing policies for visiting mares, if quarantined
Quarantine protocol for visiting stock/returning stock	Level of quarantine which the stud owner/manager felt necessary for visiting stock, Quarantine location and details of parasitological protocols used within quarantine and/or reintegration of returning stock.
Grazing and Pasture Management	Current grazing and pasture management strategies: total grazing acreage, whether residents and visitors are co-grazed, grazing management including use and method of faecal removal and grazing access hours per day.
Anthelmintic (wormer) administration and advice	Source of de-worming advice and drug purchase, including the most important factor in product purchase. Involvement of the veterinary surgeon in parasite control strategy. Drug choice and anthelmintic supplier.
Administration and use of anthelmintics	Use and frequency of use of anthelmintics and diagnostics, basis on which decision for treatment was made, person responsible for drug administration, knowledge of drug action against parasites and stages, how dosage is calculated and most recent treatment.
Resistance to anthelmintics	The level of concern about anthelmintic resistance and specific drugs/products and parasites involved, whether resistance was perceived or confirmed with diagnostics.
Foal and youngstock (YS) de-worming practices	Which parasites are of concern for foals and youngstock, age at first treatment, drug choice and frequency of drug administration during the first year of life.
Use of Faecal Egg Counts (FEC)	Use on FEC on stud, frequency of FEC testing, FEC service provider, treatment threshold and opinion on how important FECs have been for parasite control measures. For those participants stating they have had FECRT performed, this section asked which age group/s and drug/s were tested including testing date, whether eggs were seen post-treatment and which age group/s and drug/s were tested including testing date.
Use of Targeted selective de-worming strategies	Information on parasite control and their interpretation of a targeted selective strategy.

### 4.2.3 Data analysis

Descriptive statistics for FEC and questionnaire results were performed using Microsoft Excel (2016). Pre-treatment FEC, *k* aggregation parameters were calculated using online Wessa software ([http://www.wessa.net/rwasp\\_fitdistrnegbin.wasp](http://www.wessa.net/rwasp_fitdistrnegbin.wasp)). Pre-treatment egg count data analysis, 95% confidence intervals and graph production was performed using Microsoft Excel (2016). Prevalence of each parasite species based on FEC analysis using data from all studs was calculated as follows:

$$\left( \frac{\text{No. FEC positive samples for given parasite spp}}{\text{Total no. samples tested}} \right) \times 100$$

For each stud, herd level prevalence was determined using:

$$\left( \frac{\text{No. individuals FEC positive } [ > 1\text{epg}] \text{ for given parasite spp}}{\text{Total no. samples tested}} \right) \times 100$$

For FECRT the arithmetic mean of pre-treatment and post-treatment counts, for stock meeting the test inclusion criteria (pre-treatment result of  $\geq 50$ epg), were used in order to calculate reduction percentages and results were graphed using Microsoft Excel (2016). Where FECRT were carried out, studs were analysed individually and the results performed for both mixed age group (individual studs overall results) and for separate YS and adult cohorts per stud. “eggCounts” online software was used to calculate mean and 95% confidence intervals, modes and associated HPD interval values (Wang & Paul, 2017). This software utilises a hierarchical model that accounts for sampling variability and between-animal variation (Wang, et al., 2017). Mean and 95% confidence intervals are displayed graphically with mode and HPD values presented to aid interpretation as it has been shown that these values show smaller bias and better coverage in simulation studies performed by the authors of the “eggCounts” programme (Wang, et al., 2017). Percentages for FECRT were rounded to the nearest integer. LC results were organised, and analysed, on an individual stud basis with horses grouped according to grazing cohort only.

## 4.3 Results

### 4.3.1 Descriptive data of studs and parasite control practice

Stud farms within the study were located in various counties and geographical areas. Studs A,C and G were located in the Eastern county of Cambridgeshire; studs E and F in the South-East in Surrey and East Sussex respectively; stud D in Hampshire to the South; stud H in Dorset in the South-West and stud B in Merseyside to the North-West of England (Figure 4.1)

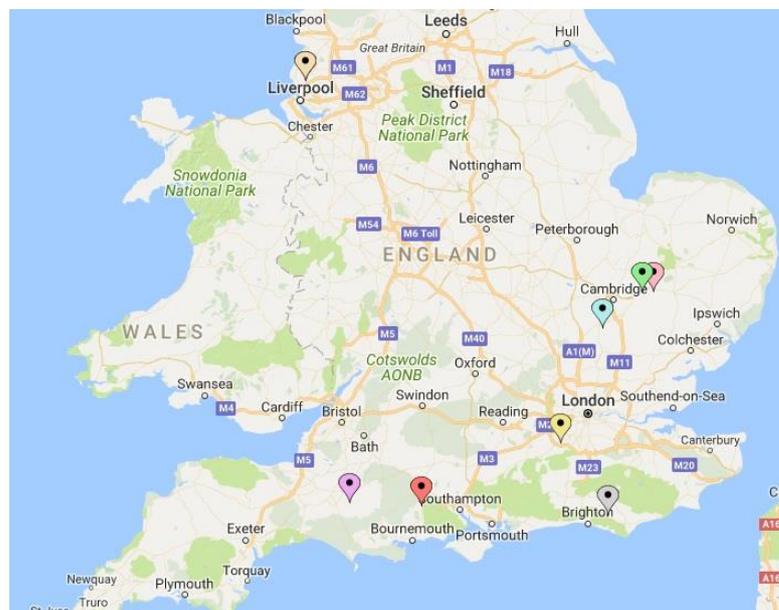


Figure 4.1. Geographical mapping of all participant studs A-H showing all, except for stud B, are located within the South-East and South of England. Where studs had multiple sites only the primary stud is shown. Studs have been mapped based on general location in order to preserve confidentiality. mapping software available at <https://www.mapcustomizer.com/>).

#### 4.3.1.1 Stud A

Stud A is a large private TB stud comprised of multiple ( $n=7$ ) UK sites with each site overseen by individual stud managers who set management protocols for stock under their care. The UK herd is comprised of eight to ten stallions, one teaser, approximately 200-220 broodmares and foster mares with 100-140 foals and 60-80 YS. There is a high degree of movement of stock within and between studs with sites accepting visiting mares for covering, primarily on a walk-in basis, but some board and a minority (10-30) remain past foaling. Parasite control on one Irish site is inconsistent, with little pasture management

practiced and frequent drug treatments employed. Within their quarantine protocol, egg counts and routine swabs for infectious disease were performed. The complexity of a stud of this size and its associated activities is shown in Figure 4.2.

Grazing management varied depending on stud manager, topography, stock requirements and specific issues. Core sites A & B are 379 and 145 acres respectively, all mare paddocks were swept or vacuumed weekly to monthly; nursery paddocks and stallion paddocks (separate from other grazing areas) had faeces removed manually daily, in addition to sweeping or vacuuming. Horses were co-grazed or rotationally grazed with sheep. Site C is 1583 acres; had a history of harrowing at irregular intervals but had recently moved to sweeping with very occasional Hoovering. Site D, 894 acres, had a combination of regular manual and mechanical pasture management. Irish sites had no faecal removal performed. These sites were solely inhabited by YS which primarily lived out, except in severe weather. Here, stock was co/rotationally grazed with cattle at a stocking density of ten acres per cohort of six; all ruminant co-grazers were required to be treated for *Fasciola hepatica* prior to turn-out on all sites. The stud carried out FEC of all stock at a central in-house laboratory using CF method, with an LDL of one to nine epg (Chapter 2). For adult stock, all anthelmintic treatments, except for a single annual MOX and twice yearly cestode treatments, were based on FEC diagnostic testing using a treatment threshold of  $\geq 200$ epg. FEC was performed in April, June, August and October with IVM most commonly used when the threshold was exceeded.

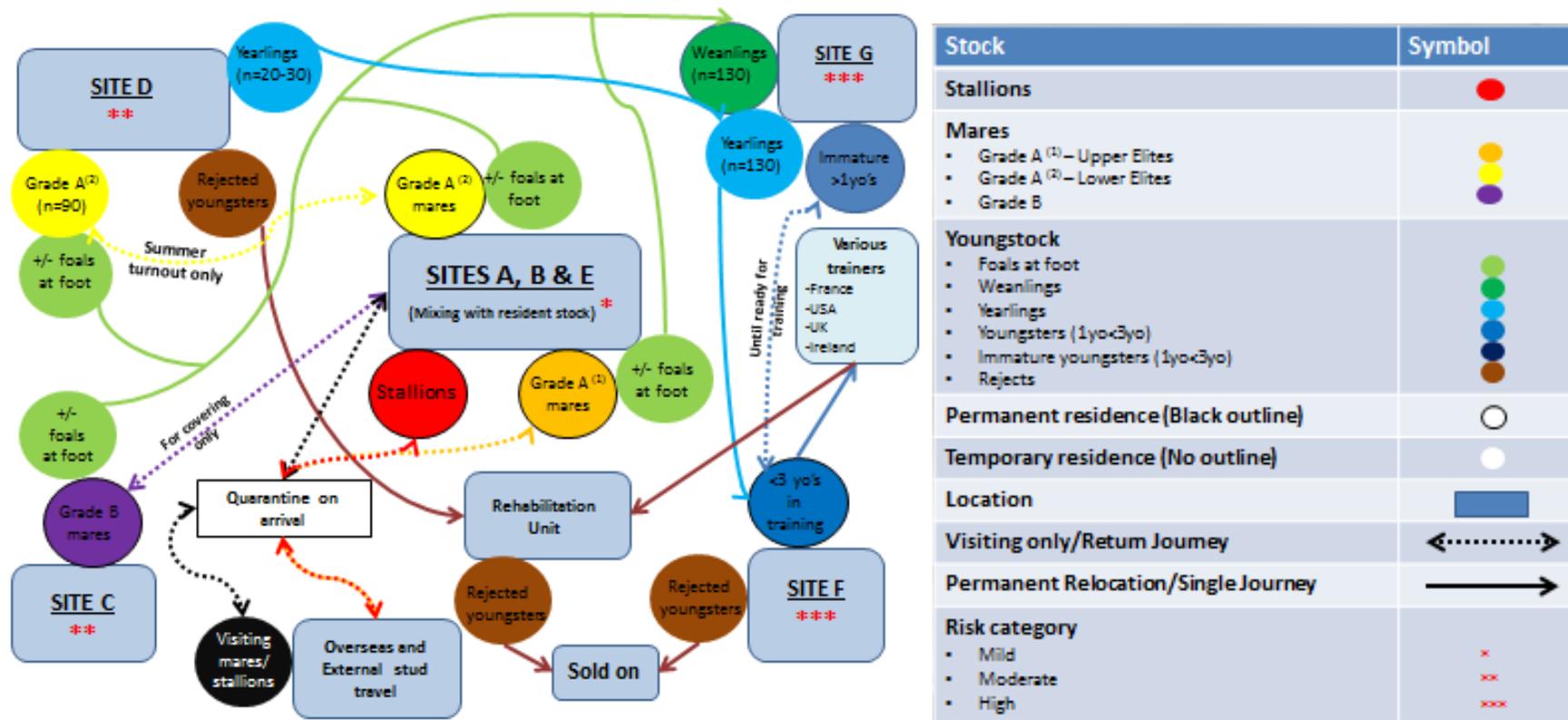


Figure 4.2 Horse movement within stud A, a large stud farm. A key is provided for interpretation. Stallions and teasers, when in the UK, were kept permanently within core sites (A&B). A1 (n~40) were elite mares kept primarily at core sites when in the UK, but which travel abroad and while resident on other studs parasite control regimes were under the control of the destination stud owner/manager. A2 mares (n~90) were lower-elite and were maintained between core sites and summer grazing pasture (site C). Site C was shared with ~20-30 yearlings and spelling horses, but groups were segregated in separate pastures and rarely rotate. Horses spelling were relocated to a rehabilitation facility which was not involved in the study. B mares (n~90) were the lower performance group whose primary residence was outside of the core units (A and B). These mares travelled to core sites for covering only, with summer grazing and foaling completed on the site of primary residence. No YS cohorts were present on these core sites.

For YS (one to three year olds) egg counts were performed as described above but with most individuals being treated regardless of the result. Within a typical year six to seven treatments were used, for example MOX-PRAZ combination in November, June and October, a MOX only product in April and August, FBZ in December with February treatment based on FEC with a treatment threshold of  $\geq 50$ epg. This regime used MOX at a four to six week interval. In terms of disease, there were two recorded cases of larval cyathostomiasis in the previous five years in YS and ascarids were raised as a concern at site C.

#### ***4.3.1.2 Studs B, C, D, E, F, G, H, I, J***

On studs C, D, E, G and J >90% of stud farm activity was breeding of TBs for racing, whilst on stud B ~70% were TB foals bred for racing and resale with the remaining 30% comprised of sports horses and ponies. Stud H was a mixed farm of TB and sports horses present on farm for breaking and training for eventing purposes. Five studs, C, D, F, G and H, accepted visiting horses (stud D stated duration of stay at 60 days and stud H retained horses until four years of age) and all studs stated that a three month drug treatment history was important. No studs requested the disease history of origin sites or pre-arrival FEC. All studs travelled mares and/or stallions to other stud farms for breeding and boarding purposes (Table 4.2).

Studs C, D, E, F and G quarantined mares for at least seven days (D, F and G operated daytime turnout with overnight stabling) whilst stud H did not operate quarantine. Only stud C operated quarantine FEC with result-led drug treatment decisions. The data gathered on grazing practices on each stud is summarised in Table 4.3. Grazing management showed that all but stud H used ruminant grazing at least once per year and all studs rested land, most often on a yearly basis (Table 4.4).

Table 4.2 Details for establishments B, C, D, E, F, G, H, I, J

Stud	Region	Stock					Acreage	Routine FEC performed	Visiting horses accepted?	Home stock visiting other premises?	Quarantine practiced
		M	YS	F	S	C/T					
B	North-West	8	8	2	1	0	20	No	No	Yes <sup>1A</sup>	£No, unless turned out <sup>Y</sup>
C	Multiple	92	30-53*	62	3	3	1200	Yes, every 2 weeks for foals, quarterly for mares ( <i>Ovatec</i> , >5epg threshold)	Yes "50/yr <sup>C</sup> .	Yes <sup>2C</sup>	£Yes, >7days <sup>W</sup> \$Yes, 7 days <sup>Y</sup>
D	South England	80	35	0	4	3	600	Yes, adults biannually (100epg threshold)	Yes "90/yr <sup>B</sup> .	Yes <sup>1B</sup>	£Yes, 7 days <sup>Z</sup> \$No <sup>W</sup>
E	South-East	25	10	18	0	3	500	Yes, suspicion of disease	No	Yes <sup>1B</sup>	£\$Yes, >7days <sup>ND</sup>
F	South-East	32	28	0	0	4	340	Yes, adults biannually (>0epg threshold)	Yes "10/yr <sup>C</sup> .	Yes <sup>1B</sup>	£\$Yes, >7days <sup>Y</sup>
G	East Anglia	40	20	20	2	1	200	No	Yes "25/yr <sup>C</sup> .	Yes <sup>1B</sup>	£Yes, >7days <sup>Z</sup> \$Yes, >7days <sup>W</sup>
H	West Midlands	3	7	3	0	2	20	Yes, suspicion of disease	Yes "2-5/yr <sup>C</sup> .	ND	No
J	South-West	19	7	6	0	4, 4 <sup>^</sup>	150	No	No	ND	ND

M = Broodmares, YS = Youngstock, F = Foals, S = Stallions, C/T = companion/teasers, \*number varied so approximation only, ^ Geldings, " = average no. visiting. <sup>1</sup>Mares only, <sup>2</sup>Mares and stallions. <sup>A</sup>Walk-in, <sup>B</sup>2-6months/Stud season, <sup>C</sup>Variable duration. £=Visiting horses, \$=Returning horses. <sup>W</sup>=anthelmintic never administered on arrival/return, <sup>X</sup>=anthelmintic administered if needed (FEC-based), <sup>Y</sup>=anthelmintic administered to all on arrival/return, <sup>Z</sup>=anthelmintic administration dependant on drug treatment history ND = information not disclosed.

In terms of parasite associated disease two studs declared parasite-related disease: Stud C described over ten cases in the preceding two years of parasite-related colic, which was diagnosed by a veterinary surgeon from ascarid impaction; additional cases were thought by staff and veterinary opinion to be parasite related. Stud E has had multiple cases of colic deemed tapeworm-related by veterinary opinion and this stud was referred to us by a veterinarian due to concerns over tapeworm infection (see Chapter 6).

Table 4.3 Grazing time for stock on studs B-J per season

<b>Stud</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>	<b>J</b>
<b>Season</b>	<b>(hr/day)</b>							
Spring	6-10	6-10	16-24	16-24	11-15	6-10	16-24	16-24
Summer	16-24	16-24	16-24	16-24	16-24	16-24	16-24	16-24
Autumn	6-10	16-24	16-24	16-24	16-24	16-24	6-10	11-15
Winter	0	6-10	11-15	6-10	1-5	6-10	0	1-5

In terms of drug choice and purchase, most studs stated that advice involved their veterinary surgeon and this was the most influential factor when choosing which product to use, but anthelmintics were purchased elsewhere, primarily online. The exception was stud D who felt that no advice was needed and stud H who obtained advice from feed shops, drug representatives and articles within equine publications. Stud owner/managers knowledge of the spectrum of activity was investigated (figure 4.3). Studs D, F, H and J stated they would use IVM or an IVM combination product for treatment of encysted cyathostomin burden, with F not utilising MOX for this purpose at all. D and G stated that they believed that MOX could be used for *F. hepatica*, with stud G also proposing the use of IVM. All studs correctly identified PRAZ and PRAZ combination products for the treatment of tapeworm.

		Active ingredient													
		Moxidectin / Praziquantel		Moxidectin		Ivermectin / Praziquantel		Ivermectin		Pyrantel		Benzimidazoles		Praziquantel	
Parasite & life stage	Small Strongyle Adults	C	G	C	G	C	G	C	G	C	G	C	G	C	G
		D	H	D	H	D	H	D	H	D	H	D	H	D	H
		F	J	F	J	F	J	F	J	F	J	F	J	F	J
	Small Strongyle Encysted larvae	C	G	C	G	C	G	C	G	C	G	C	G	C	G
		D	H	D	H	D	H	D	H	D	H	D	H	D	H
		F	J	F	J	F	J	F	J	F	J	F	J	F	J
	Large Strongyle Adults	C	G	C	G	C	G	C	G	C	G	C	G	C	G
		D	H	D	H	D	H	D	H	D	H	D	H	D	H
		F	J	F	J	F	J	F	J	F	J	F	J	F	J
	Large Strongyle Larvae	C	G	C	G	C	G	C	G	C	G	C	G	C	G
		D	H	D	H	D	H	D	H	D	H	D	H	D	H
		F	J	F	J	F	J	F	J	F	J	F	J	F	J
	Roundworm Adults	C	G	C	G	C	G	C	G	C	G	C	G	C	G
		D	H	D	H	D	H	D	H	D	H	D	H	D	H
		F	J	F	J	F	J	F	J	F	J	F	J	F	J
	Roundworm Larvae	C	G	C	G	C	G	C	G	C	G	C	G	C	G
		D	H	D	H	D	H	D	H	D	H	D	H	D	H
		F	J	F	J	F	J	F	J	F	J	F	J	F	J
	Tapeworm	C	G	C	G	C	G	C	G	C	G	C	G	C	G
		D	H	D	H	D	H	D	H	D	H	D	H	D	H
		F	J	F	J	F	J	F	J	F	J	F	J	F	J
	Liver Fluke	C	G	C	G	C	G	C	G	C	G	C	G	C	G
		D	H	D	H	D	H	D	H	D	H	D	H	D	H
		F	J	F	J	F	J	F	J	F	J	F	J	F	J

Figure 4.3 Knowledge of drug spectrum of activity and awareness of drug resistance as reported by stud owners and managers. Green indicates when knowledge was correct, red indicates incorrect answer and orange indicates a correct answer but lack of awareness of drug resistance for this drug:parasite combination. Un-shaded areas indicate no knowledge was provided. Studs are indicated by letters B-J. Common names for parasites were used throughout the questionnaire (roundworm refers to *Parascaris spp*; small strongyles, cyathostomin spp; large strongyles, *Strongylus spp*; tapeworm, *Anoplocephala perfoliata*; liver fluke, *Fasciola hepatica*) (Appendix 4A).

In terms of anthelmintic administration, adult horses were given drugs quarterly with or without FEC analysis. Studs B, C and H kept horses stabled for approximately seven days, one to two days and less than one day post-treatment, respectively; only stud B moved horses to clean pasture post treatment. Studs considered spring, autumn and foaling the most important periods for anthelmintic treatment. Drugs were rotated on stud B (biannually), stud C (infrequently), stud F (quarterly) and stud, G (every treatment).

Table 4.4 The pasture management practices performed on each stud and frequency of their implementation

	<b>Stud</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>	<b>J</b>
<b>Practice</b>									
<b>Ruminant grazing</b>	Monthly	Occasionally	Yearly	Monthly	Occasionally	Occasionally	Never	Occasionally	
<b>Harrow</b>	NS	Monthly	Yearly	NS	Occasionally	Occasionally	Yearly	Monthly	
<b>+/- clip</b>									
<b>Rested</b>	Yearly	Yearly	Monthly	Yearly	Occasionally	Occasionally	Occasionally	Yearly	
<b>Group rotation</b>	Yearly	Yearly	Monthly	Yearly	Occasionally	Occasionally	Occasionally	Monthly	
<b>Done?</b>	Yes	Yes/No	Yes	Yes	Yes	Yes/No	Yes	Yes	
<b>Frequency</b>	Fortnightly	Nursery – weekly Others – never	Nursery - daily/weekly Others – seasonal	Weekly	Occasionally	Nursery - weekly Others - never	Daily	Occasionally	
<b>Manual or Mechanical ?</b>	Manual	Manual Mechanical	Manual Mechanical	Mechanical	Manual Mechanical	Manual Mechanical	Manual Mechanical	Mechanical	
<b>Machine used?</b>	NA	Terravac	Nicholson Paddock Sweeper	NS	Paddock sweeper	Paddock sweeper	Paddock sweeper	Suffolk Paddock Sweeper	

NA: Not applicable; NS: Not specified

On studs A, B and C, administration of drugs was the responsibility of members of management (head girls, barn leader, and stud owners) with studs D, E, F, H and G distributing this duty among general staff. Dosage per horse was calculated “by eye” on studs A, B and C; studs E, F and G administered one tube/packet per horse; a single stud (H) used weight tapes and the remaining stud (D) dosed all based on the average weight within that cohort.

Studs considered parasite control for foals distinct from other age groups. Studs B, D, E, F, and J administered the first anthelmintic treatment at three to four weeks and studs C, G and H began treatment at five to six weeks, birth to two weeks and ten to twelve weeks, respectively. A range of drug classes were used in foals on all studs where information was available. In the year prior to the study, as seen in Table 4.5, foals on studs B and G were treated with all drug classes, including ML-PRAZ combination products; stud C use predominantly BZ treatments (n=3) with single PYR, MOX and MOX-PRAZ treatments, and studs D and E relied heavily on IVM, administering three treatments per year, with D also including a MOX and MOX-combination treatment and E supplementing with three PYR doses. Stud F administered more than three IVM treatments to foals per year with an additional two IVM-PRAZ treatments.

Only two studs, D and F, considered YS separately to adults when planning anthelmintic treatment regimens. For both YS and adults across all studs, IVM and MOX +/- PRAZ were heavily relied upon, with PYR and BZ class drugs used only on studs E (PYR) and J (PYR and BZs). The number of treatments per year stated by studs indicated that drugs were administered at shorter than recommended intervals. Three studs considered that they were using targeted selective treatment strategies, studs C, D and E; all other studs considered they were following an interval programme.

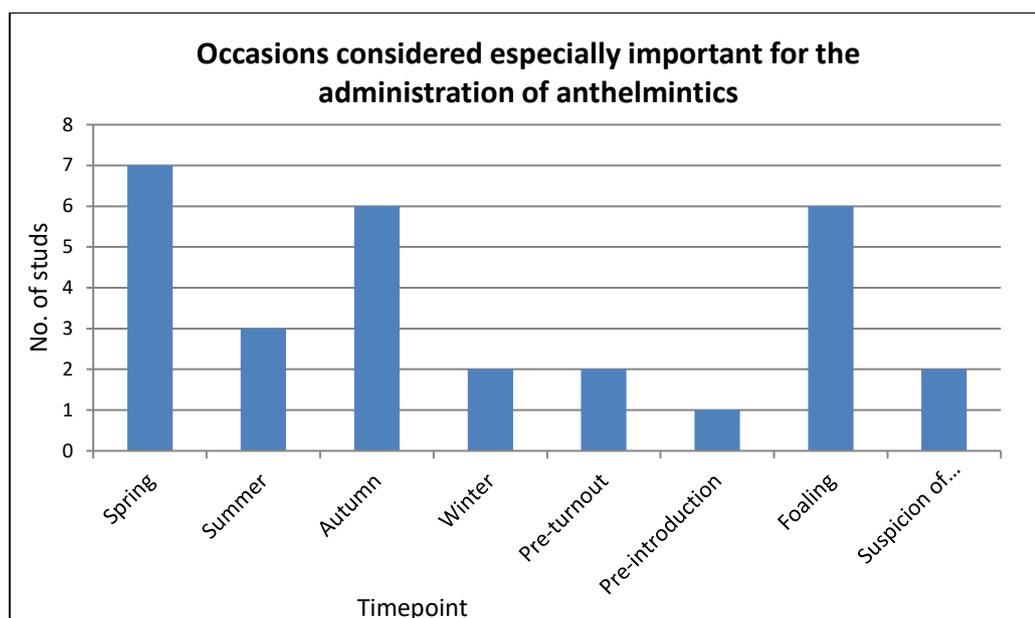


Figure 4.4 Time-points which stud managers considered important for the administration of anthelmintics.

The use of FEC for each stud is shown in Table 4.2. With the exception of stud C, the FEC testing was undertaken by their veterinary surgeon with a postal/email report or face to face discussion of the need for treatment. The treatment threshold for studs E and H was not disclosed, stud D used a 100epg treatment threshold as recommended by their veterinary surgeon, Stud C had an on-site laboratory which used Ovatec, where a treatment threshold of over five was used. Only stud F treated all horses, even when recording an epg count of above zero epg.

Table 4.5 The product active ingredients and number of treatments administered to foals within their first year of life on each stud.

Stud	Active ingredient					
	BZ	PYR	IVM	IVM-Praz	MOX	MOX-Praz
B	1	1	1	1	1	NU
C	3	1	NU	NU	1	1
D	NU	NU	3	NU	1	2
E	NU	3	3	NU	NU	NU
F	NU	NU	>3	2	NU	NU
G	1	2	2	2	1	1

BZ = benzimidazole, PYR = pyrantel, IVM = ivermectin. Praz = Praziquantel, MOX = moxidectin, NU: Not used

All studs expressed concerns about anthelmintic resistance. Two studs had performed FECRT previously. Stud D had confirmed resistance to FBZ, determined by FECRT. Stud C had previously tested MOX in foals and YS with eggs seen in post-treatment samples.

#### **4.3.2 Proportion of samples testing positive for a given parasite species based on faecal egg count analysis**

In total, 3130 samples were tested during the project, with samples taken from different age groups: total number of foals (23.0%, n=720), YS (25.3%, n=793) and adults (n=51.7%, n=1617) (Figure 4.5). In order to compare with previous studies reporting parasite prevalence we calculated an overall prevalence of strongyles, irrespective of age group, based on the proportion (%) of the 3130 samples that tested positive for one or more strongyle eggs. Overall 58.5% ( $\pm 1.7\%$ , n=1832/3130) horses were positive for strongyle eggs (Table 4.6, Figure 4.5). The proportion of strongyle FEC positive horses was also calculated by age group was also calculated. From all studs (A-H), a total of 1617 samples from adult stock were tested for strongyle eggs and an overall prevalence of 53.3% ( $\pm 2.4\%$ , n=862/1617) was found. YS samples were analysed with a prevalence of 85.0% ( $\pm 2.5\%$ , n=674/793) and prevalence in foals was 41.1% ( $\pm 3.6\%$ , n=296/720) (Figure 4.5).

For *Parascaris spp*, all samples were analysed for presence of eggs and an overall prevalence of 6.5% ( $\pm 0.9\%$ , n=204/3130) was found across all studs and age groups (Table 4.6, Figure 4.5). Within adult stock a prevalence of 0.6% ( $\pm 0.4\%$ , n=9/1617) was found; 6.6% ( $\pm 1.7\%$ , n=52/793) of YS samples and 19.9% ( $\pm 2.9\%$ , n=143/720) of foal samples demonstrated a positive (>0epg) count (Figure 4.5). For *Anoplocephala perfoliata* 3.9% ( $\pm 0.7\%$ , n=121/3130) of all samples tested were FEC positive (Table 4.6, Figure 4.5). Within distinct age groups 4.9% ( $\pm 1.1\%$ , n=80/1617) adults, 5.0% ( $\pm 1.5\%$ , n=40/793) YS and 0.1% ( $\pm 0.3\%$ , n=1/720) foals were positive on CF FEC (Figure 4.5). As this technique is not quantitative (see Chapter 2) mean epg values were not calculated.

At the stud level, the proportion of the eight studs where at least one individual tested positive for a strongyle egg, a *Parascaris spp* and an *A. perfoliata* egg was 100.0% (n=8/8), 75.0% (n=6/8) and 100.0% (n=8/8), respectively (Table 4.6).

Table 4.6 The proportion (%) of samples and studs testing positive for strongyles, *Parascaris spp* and tapeworm by CF (all samples tested) in results reported here compared to those of Relf *et al.* (2013)

Proportion +ve (%)	Results reported here			Relf <i>et al.</i> , 2013		
	Strongyle	<i>Parascaris spp</i>	Tapeworm	Strongyle	<i>P. equorum</i>	Tapeworm
Samples	58.5 (±1.7%, n=1832 /3130)	6.5 (±0.9%, n=204 /3130)	3.9 (±0.7%, n=121 /3130)	56 (±2.8%, n= 684 /1221)	9 (±1.7%, n=100 /1109)	4 (±1.5%, n=27 /672)
Studs	100 (n=8/8)	75 (n=8/8)	100 (n=8/8)	100 (n=22/22)	50 (n=11/22)	41 (n=9/22)

n = no samples testing positive/total number of samples tested

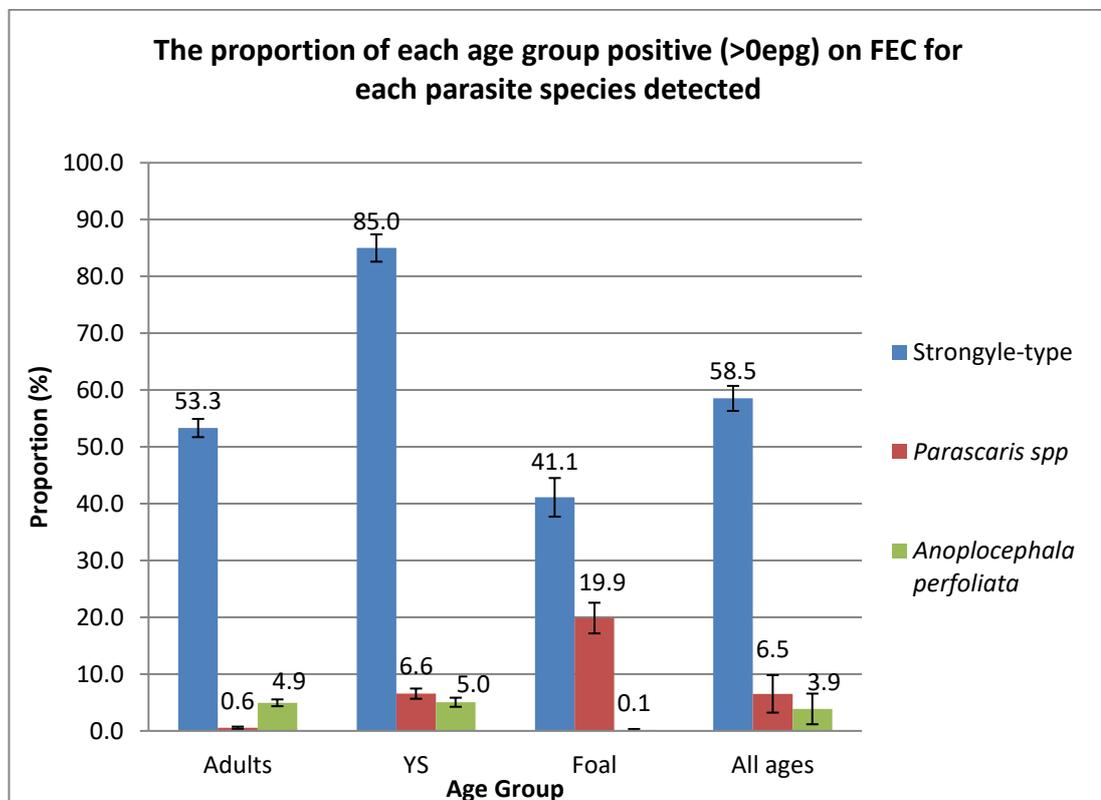


Figure 4.5 The proportion (%) of horses testing positive for strongyle, *Parascaris spp* and tapeworm (*A. perfoliata*) eggs within each age cohort and as a proportion of all samples tested, regardless of age with 95% confidence intervals.

### 4.3.3 Faecal egg count analysis: distribution of strongyle and *Parascaris spp* FECs

For strongyle FEC, the arithmetic mean for all horses tested was 73.6epg (range 0-2736epg; S.D. 3.6) and distribution was noted to be highly over-dispersed; 12.9% ( $\pm 1.2\%$ ,  $n=403/3130$ ) of individuals excreted 80.0% of the total eggs. The  $k$  aggregation parameter for all samples tested was 0.1 (S.D. 7.3). A high degree of over-dispersion was also seen within individual age groups. Strongyle mean and aggregation varied between age groups with adults recording an arithmetic mean of 39.5epg (range 0-1800epg; S.D. 2.8) and aggregation parameter of 0.1 (S.D. 0.005); YS 193.2epg (range 0-2736epg; S.D. 12.1) and aggregation of 0.3 (S.D. 0.01) and foals recorded a mean count of 18.7epg (range 0-1498.5epg; S.D. 2.3) with aggregation parameter of 0.10 (S.D. 0.006) (Figure 4.6).

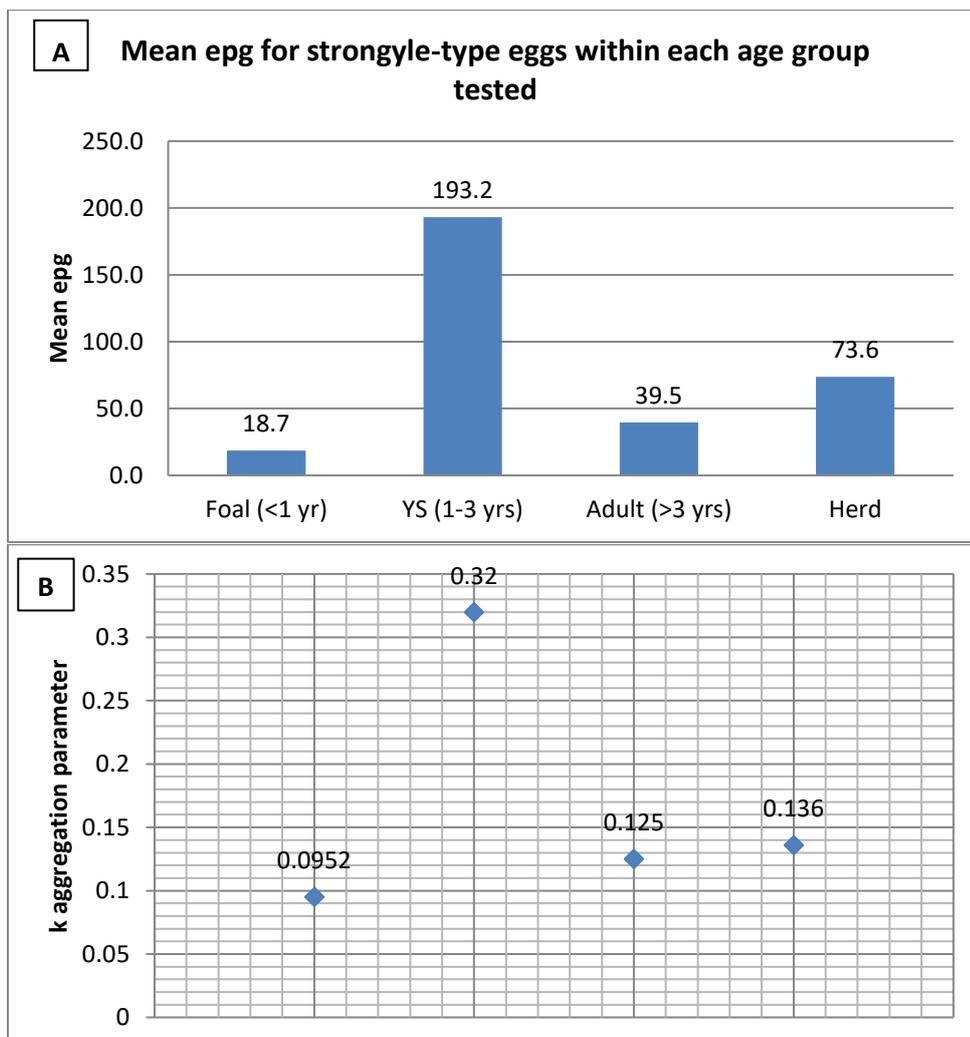
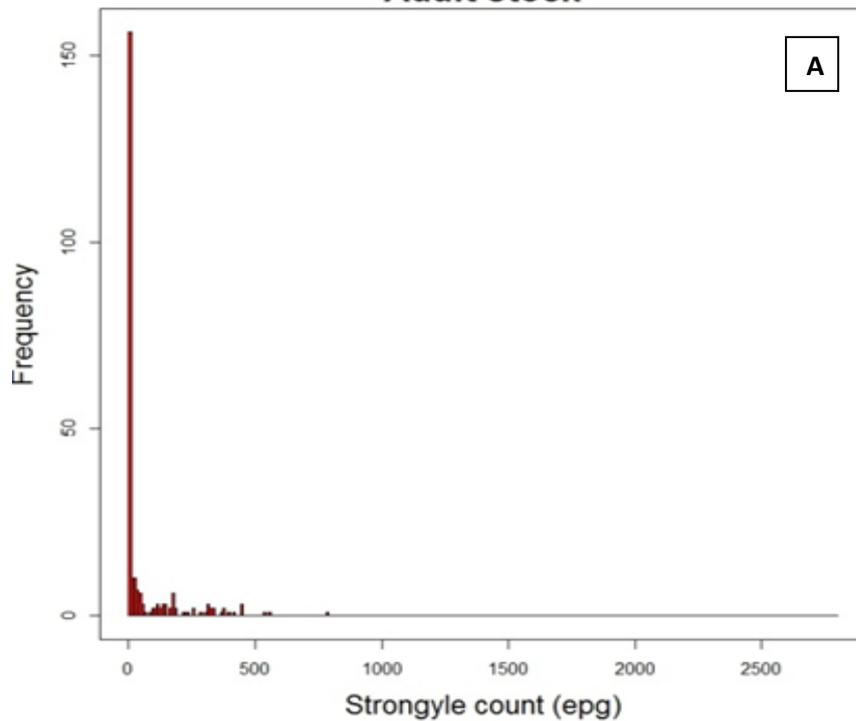


Figure 4.6. Mean strongyle FEC (epg) (A) and distribution of egg counts between age groups (B).  $k$  aggregation parameter of the negative binomial distribution is shown in (B), where a high  $k$  indicates a less-aggregated distribution with higher mean.

### Frequency of baseline Strongyle-type egg counts Adult stock



### Frequency of baseline Strongyle-type egg counts Youngstock

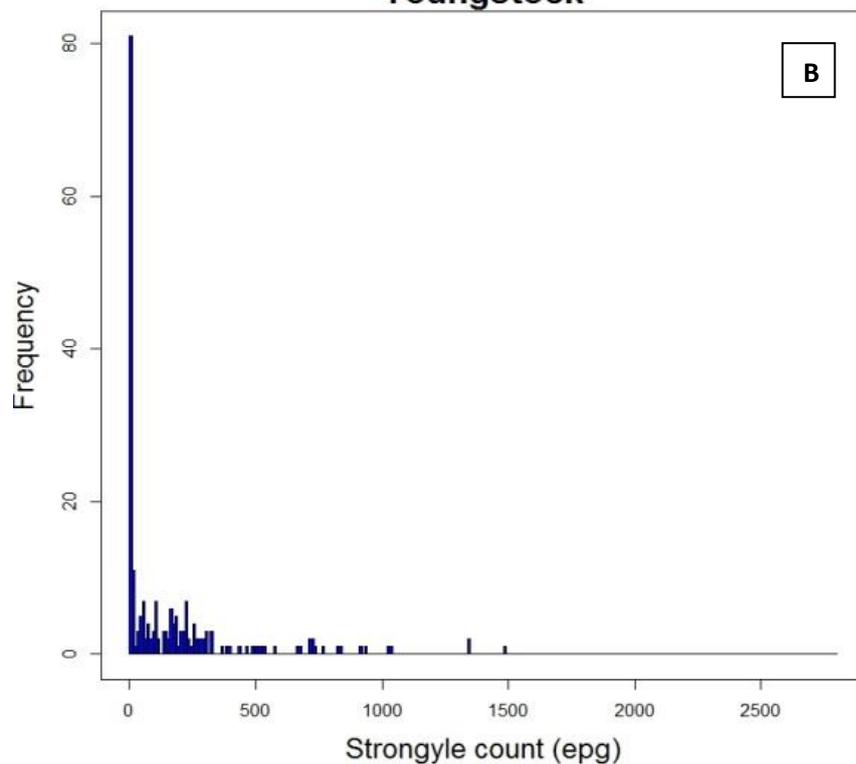


Figure 4.7 The pre-treatment (baseline) results for adult stock (A) and YS (B) on stud A, demonstrating over dispersion/negative binomial distribution within strongyle egg counts only for both age groups, more youngstock than adults demonstrated higher egg counts, a right shift in dispersion. This distribution pattern was seen in cohorts on all other studs except stud E and so is a representative sample of the distributions found.

For *Parascaris equorum*, arithmetic mean of all samples was 6.7epg (range 0-3010.5); adults 0.1epg (range 0-126); YS 2.4epg (range 0-445.5) and 26.1epg (range 0-3010.5) for foals.

Following analysis of data from all studs, each stud was looked at individually.

Unfortunately, despite their initial interest Stud A only provided one set of samples from the YS cohort, due to the low sample number (n=9) and as insufficient time has passed since last anthelmintic treatment, these results were excluded from analysis. Samples were provided from adult horses thus it was possible to determine the proportion (%) of horses positive for strongyle and tapeworm eggs on stud A at different times of the year (Table 4.7). To provide more information for the stud the proportion (%) of horses observed to have epg counts >200epg, 100-199epg, 50-99epg, 0.5-49epg and 0epg was reported (Table 4.8) and the distribution of strongyle FECs for adult horses on stud A (Figure 4.7) and the  $k$  aggregation parameter was reported (Table 4.9). Over-dispersion (a negative binomial distribution) was seen in adult cohorts on all other studs except stud E (Figure 4.7, data for large studs – C, D and F are found in Appendix 4B). The YS cohorts also showed a negative binomial distribution of strongyle FEC values but higher means and  $k$  values were seen in this age group (Table 4.9).

Table 4.7 The proportion (%) of horse's positive for strongyle and tapeworm eggs on stud A at different times of the year

Stud A			Strongyle					Tapeworm					
Time point	Age group	n	Mean (epg)	Median (epg)	Range (epg)	Prevalence % herd positive (95%CI, n)	% herd responsible for 80% egg output (95%CI, n)	<i>k</i> (SD)	Mean (epg)	Median (epg)	Range (epg)	Prevalence % herd positive (95%CI, n)	Prevalence % herd positive (95%CI, n)
March	Adult	159	9.8	0	0-913.5	37.7	3.1	0.09 (0.01)	0	0	0	0	0
						(±7.5%, 60)							
June	Adult	187	9.1	0	0-400.5	38.0	5.9	0.1 (0.01)	0	0	0	0	14.4
						(±7.0%, 71)							(±3.4%, 11)
Sept	Adult	197	18.9	0	0-832.5	42.6	5.0	0.1 (0.01)	0.01	0	0-0.5	1.0	16.8
						(±6.9%, 84)							(±3.1%, 10)

n = number of horses, epg = eggs per gram, *k* (SD) = aggregation factor

Table 4.8 The proportion (%) of horses on stud A observed to have egg per gram (epg) counts >200epg, 100-199epg, 50-99epg, 0.5-49epg and 0epg at different times of year. Data includes proportion of positive (>0epg) results within each egg count band and as a proportion of the total number of horses tested.

Time point	Total tested (n <sup>1</sup> )	Total positive (n <sup>2</sup> )	>200epg		100-199epg		50-99epg		0.5-49epg		0epg
			% of positive results (95%CI, n <sup>2</sup> )	% of total tested (95%CI, n <sup>1</sup> )	% of positive results (95%CI, n <sup>2</sup> )	% of total tested (95%CI, n <sup>1</sup> )	% of positive results (95%CI, n <sup>2</sup> )	% of total tested (95%CI, n <sup>1</sup> )	% of positive results (95%CI, n <sup>2</sup> )	% of total tested (95%CI, n <sup>1</sup> )	% of positive results (95%CI, n <sup>2</sup> )
March	159	60	1.7 (±3.2%, 1)	0.6 (±1.2%, 1)	1.7 (±3.2%, 1)	0.6 (±1.2%, 1)	6.7 (±6.3%, 4)	2.5 (±2.4%, 4)	90.0 (±7.6%, 54)	34.0 (±7.4%, 54)	62.3 (±7.5%, 99)
June	187	71	2.8 (±3.8%, 2)	1.1 (±1.5%, 2)	2.8 (±3.8%, 2)	1.1 (±1.5%, 2)	5.6 (±5.4%, 4)	2.1 (±2.1%, 4)	88.7 (±7.4%, 63)	33.7 (±6.8%, 63)	62.0 (±7.0%, 116)
Sept	197	84	3.6 (±4.0%, 3)	1.5 (±1.7%, 3)	7.1 (±5.5%, 6)	3.1 (±2.4%, 6)	4.8 (±4.6%, 4)	2.0 (±2.0%, 4)	84.5 (7.7%, 71)	36.0 (±6.7%, 71)	57.4 (±6.9%, 113)
Year overall	543	215	2.8 (±2.2%, 6)	1.1 (±0.6%, 6)	4.2 (±2.7%, 9)	1.7 (±1.1%, 9)	5.6 (±3.1%, 12)	2.2 (±1.2%, 12)	87.4 (±4.4%, 188)	34.6 (±4.0%, 188)	60.4 (±4.1%, 328)

n<sup>1</sup> = total number of horses tested; n<sup>2</sup> = number of horses testing positive

Table 4.9 shows *k* aggregation parameters for each age cohort per stud for the time point when the greatest number of faecal samples was submitted for testing. As shown in Table 4.6 highest stock numbers were tested at the following time points for each stud: stud A September; stud C February (adults and YS) and July (foals); stud D January (only adult samples submitted) and August (YS); stud F, March (adults and YS) and stud G May (adults and YS) and September (foals). Studs B, I and H were excluded due to low numbers of horses within each cohort ( $n < 15$  per group). These results show a marked difference between mean values for each age cohort with highest results consistently seen in the YS (Table 4.9). This pattern of results is mirrored for *k* aggregation parameters, with YS having a *k* value typically  $> 1.0$ , followed by adults with *k* values within the range 0.1-0.3 and lowest were foals although sufficient data to produce *k* values was only available on 2 studs. Stud G showed higher *k* aggregation parameters in foals compared to adults but number of results for YS and Foals were low ( $n=10$  and  $12$ , respectively) potentially limiting their accuracy. Stud J showed an aggregation parameter of 0.2, demonstrating good fit with negative binomial distribution, as few YS and foals were tested ( $n=8$ ,  $n=8$  respectively) *k* values were not able to be generated for all age groups.

#### 4.3.4 Large strongyle larval culture results

Given the pathogenic importance of *S. vulgaris* we generated pooled faecal samples from each cohort on studs, at each time-point and analysed them by LC. Of all the cultures performed ( $n=300$ ) only five samples ( $1.7\% \pm 1.4\%$ ,  $n=5/300$ ) were positive for *Strongylus spp* larvae. A maximum of one large strongyle larva was seen per positive culture, where each culture was comprised of ten individuals. All positive cultures were obtained from composite cultures from two studs: stud A,  $n=3$  (1.0% of all samples tested from stud A) and stud C,  $n=2$  (0.7% of all samples tested from stud C, both individuals were imported and in quarantine). These were the only participating studs whose horses travel internationally and have been on diagnostic based treatment the longest.

Table 4.9 The mean egg values and aggregation parameter,  $k$ , for strongyle FECs by age cohorts on each stud and for those with large herd sizes, at the herd level (in bold)

Stud	Time point	Group	N	K	S.D.	mean	S.D.
A	Sept	Adult	197	0.10	0.01	19.0	4.38
C	Feb	Adult	116	0.22	0.03	58.2	11.51
	Feb	YS	53	1.35	0.24	419.7	49.72
	July	Foals	119	0.15	0.03	1.5	0.37
	<b>July</b>	<b>Herd</b>	<b>205</b>	<b>0.10</b>	<b>0.01</b>	<b>42.7</b>	<b>9.45</b>
D	Jan	Adult	25	0.11	0.03	52.0	32.10
	Aug	YS	24	1.08	0.28	781.9	153.60
F	March	Adult	33	0.05	0.02	18.1	13.99
	March	YS	26	1.33	0.34	115.5	19.74
	<b>March</b>	<b>Herd</b>	<b>59</b>	<b>0.16</b>	<b>0.03</b>	<b>61.0</b>	<b>19.62</b>
G	May	Adult	32	0.13	0.04	23.5	11.59
	May	YS	10	0.59	0.23	34.1	14.18
	Sept	Foals	12	0.35	0.13	35.0	17.25
	<b>May</b>	<b>Herd</b>	<b>50</b>	<b>0.17</b>	<b>0.04</b>	<b>22.3</b>	<b>7.74</b>
J	Aug	Adult	31	0.16	0.04	64.2	28.54

A single time point when the highest proportion of each age cohort was tested pre-treatment was chosen for each stud; herd time points reflect where a large proportions of multiple age cohorts were tested.

#### 4.3.5 FECRT and ERP analysis for determining efficacy of anthelmintic drugs

We used the FECRT to assess if the drugs used on stud were effective against strongyle parasites. Rather than specify the use of a given drug we performed FECRT for the drug of choice for that stud, at that particular time-point so that it was reflective of typical stud practice. FECRT were conducted on a total of seven studs. Morphological identification of larvae in pre-and post-treatment cultures for FECRT cohorts was negative for large strongyle larvae, suggesting that only cyathostomin species were present before and after anthelmintic treatment taking limitations of the test into account. Where sufficient numbers of individuals (more than ten horses) were above threshold, FECRT were carried out on adult and YS separately. BZ drugs were tested by FECRT on only one stud, stud G (where MBZ was tested as it was used for the control of strongyle egg output) and showed an arithmetic mean FECRT of -194.5%, identifying that resistance was evident; when calculated using “eggCounts” (Wang & Paul, 2017) results showed a reduction of 0.1% (0.0-0.7%) (Figure 4.8).

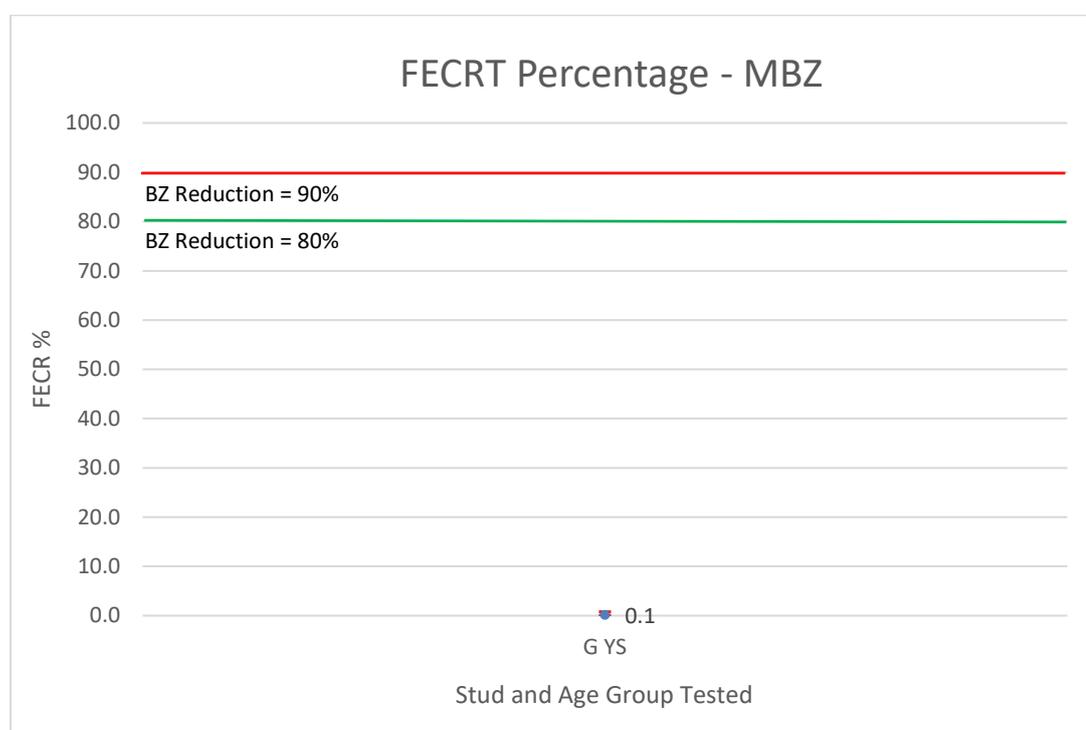


Figure 4.8 The group mean and 95% confidence intervals calculated using eggCounts software (Wang & Paul, 2017) for a mebendazole FECRT on mixed age group horses resident on stud G.

In terms of the second class of anthelmintics, PYR, this was tested on five studs. Stud B recorded arithmetic mean efficacy of 97.7% in YS and 91.8% in adults, above the 90% threshold so identifying the population of strongyles on this stud as drug-susceptible. Modelled values, (eggCounts (Wang & Paul, 2017)) taking variability into account, for stud B YS showed mean and mode FECR values were 98.5% (97.9-99.0%); adult results were 93.2% (92.0-94.4%). The ERP values recorded for Stud B were three weeks for YS and four weeks for adults. Conversely, Stud F arithmetic values showed a FECRT of -8.2% in YS and 77.0% in adults, identifying resistance to PYR on Stud F. Modelled values (eggCounts (Wang & Paul, 2017)) showed FECR mean values for YS and adults as 0.1% (0.0-0.7%) and 69.5% (67.0-71.8%) respectively; mode for adult values was 69.7% (67.2-72.0%). These results confirm arithmetic mean conclusion of resistance.

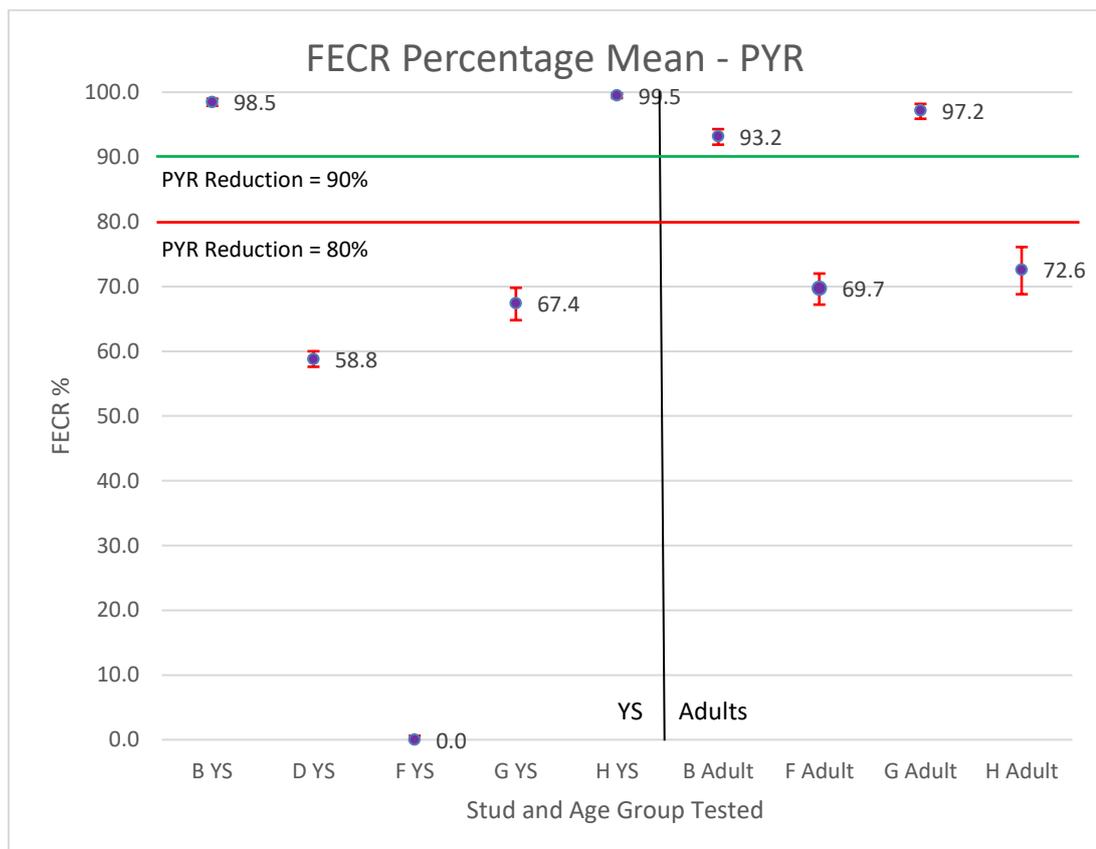


Figure 4.9 The percentage FECR with 95% confidence intervals (eggCounts (Wang & Paul, 2017)) for PYR for studs B, D, F, G, and H. Results are segregated by age group into adults and YS.

Efficacy of PYR on other studs gave a mixed picture: a reduced efficacy on Stud D with mean and mode FECR with 95% CI/HPD of 58.8% (57.6-60.0%) in YS; efficacy of 97.2% (95.9%-98.1%), based on adults data for stud G but not based on YS data, which showed a reduction of 67.4% (65.0-70.0%); and efficacy of 99.5% (99.1-99.7%), in YS on Stud H but not based on data from adult horses, demonstrating a reduction of 72.6% (68.8-76.1%) modelled (Figure 4.9).

The macrocyclic lactone (ML) class of drugs were tested on three studs; independently as IVM on two studs and MOX on three studs (Figures 4.9 and 4.10). Both the adult and YS age cohorts on Stud F showed reductions of 99.3% (99.1-99.5%) for IVM with adult IVM and YS reductions of 100.0% (99.9-100.0%) at two weeks post-treatment (Figure 4.10). The ERPs for Stud F were six weeks for YS and 8 weeks for adults. On Stud D only the YS cohort was tested, which showed a 100.0% (99.9-100.0%) reduction on FECRT, however no ERP was carried out. Only YS cohorts were tested for MOX-sensitivity on studs C and F; YS showed 100.0% (99.9-100.0%) reduction in egg count and stud D a reduction of 94.5% (93.9-95.1%) at two weeks post-treatment (Figure 4.11). ERP was conducted on two of the studs, which showed that by eight weeks post-treatment FEC were greater than pre-treatment values on Stud C and ERP was defined as four weeks on Stud D.

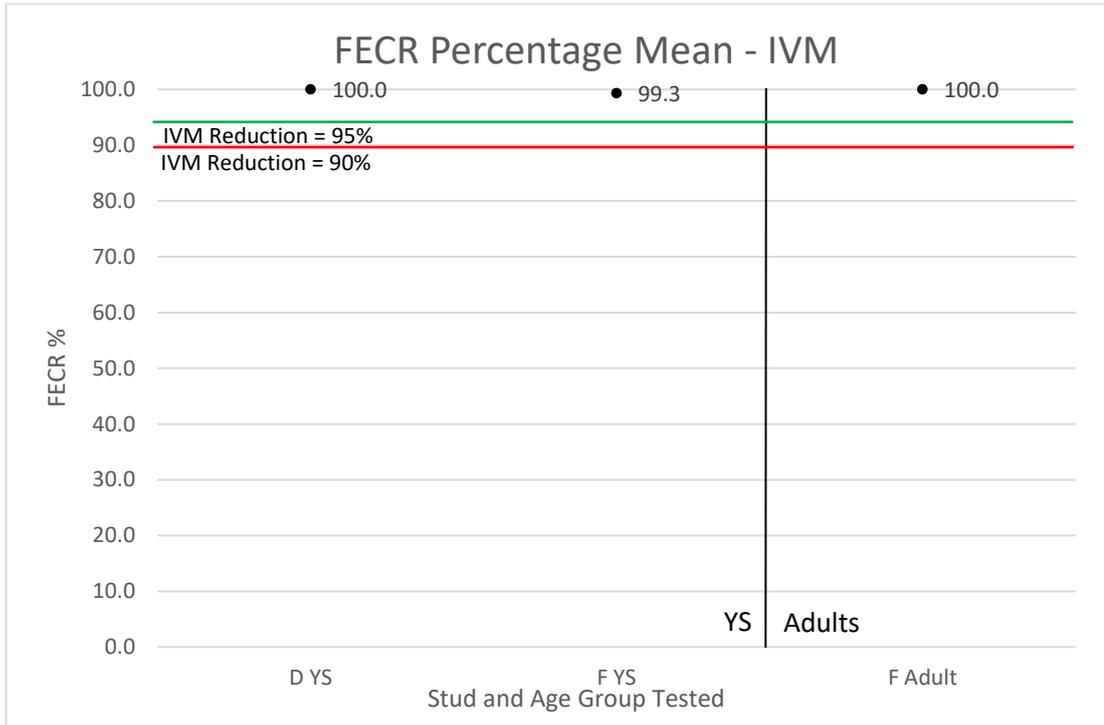


Figure 4.10 Reports the percentage FECR with 95% confidence intervals (eggCounts (Wang & Paul, 2017)) for IVM on studs D and F. Results are segregated by age group into adults and youngstock (YS).

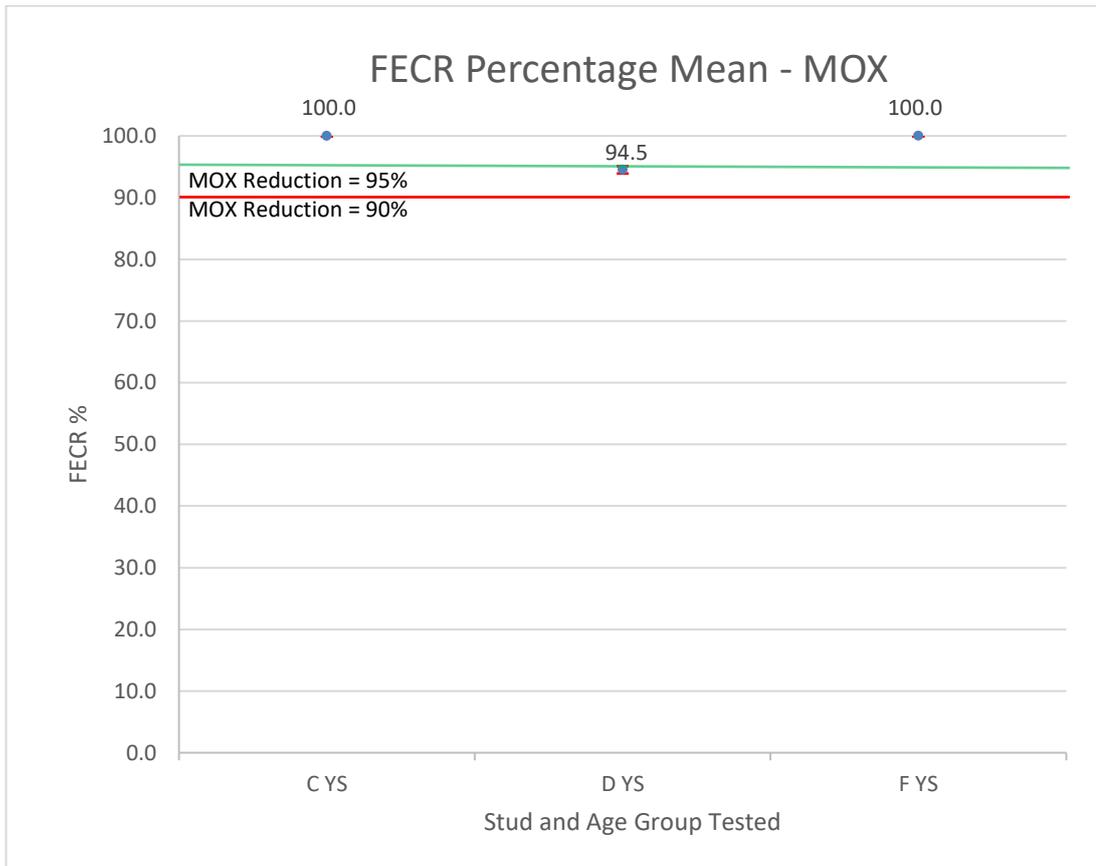


Figure 4.11 Reports the percentage FECR with 95% confidence intervals (eggCounts (Wang & Paul, 2017)) for MOX on studs C, D and F. Results were only generated for youngstock (YS).

## 4.4 Discussion

Understanding the distribution of parasitic infection within the equine population is key to the successful implementation of parasite control strategies that are less dependent on frequent anthelmintic treatment and incorporate targeted drug treatment strategies and management-based controls. This study builds on a body of evidence about the prevalence and distribution of target pathogens in TB (Relf et al., 2013) and non-TB (Tzelos et al., 2017) populations. In terms of prevalence our data is similar to that reported by Relf et al (2013) (Table 4.6). Despite a larger number of samples being examined here the number of samples negative for strongyle eggs was similar in both studies at ~40%. Similarly, results recorded for *Parascaris spp* prevalence (6.5%  $\pm$ 0.9% in 2014/15 and 9%  $\pm$ 1.7 in 2010/11) and tapeworm prevalence (4.0%  $\pm$ 0.7% 2014/15 and 4%  $\pm$ 1.5% in 2010/11) indicate the overall prevalence of infection was the same. When comparing these studies, in terms of the number of studs that were positive for each of the three parasite species, again this was similar between the studies with the exception of an increased number of studs testing positive by FEC for tapeworm eggs. This could be due to the larger number of samples analysed here (which included repeated sampling of the same stud), the smaller number of studs tested compared to Relf et al. (2013) which required further validation. Tapeworm results were based on faecal analysis only and individual prevalence may be much higher due to the limited sensitivity of the FEC for tapeworm detection and the need for better diagnostics for tapeworms is recognised (Nielsen, 2016).

Initially one of our aims was to compare the prevalence of infection on studs following a traditional interval anthelmintic programme versus those studs targeting treatment using FEC, such as that recently reported for non-TB equine populations (Tzelos et al., 2017). Despite our best efforts it was difficult to recruit large numbers of studs but we did capture studs that identified themselves as following both type of regimes: interval (n=5) and targeted selective (n=3). Whilst it is possible we could look at the impact management

factors on strongyle eggs shedding as has been done elsewhere (Tzelos, et al., 2017), the exclusion of some studs due to the small numbers of samples processed was a limiting factor to drawing meaningful comparisons. However, one pertinent consideration that was revealed was although studs stated that they were following a targeted selective approach (studs A, C and D), because they engaged with FEC, they were actually using FECs to drive very frequent use of drug treatment. Of most concern is that when FEC were performed within the ERP for a given drug and tested positive for strongyle eggs, rather than concerns over drug resistance, studs used this information to indicate the need for an anthelmintic treatment, despite the fact that the recommended timeframe had not elapsed since the previous treatment. This is an important consideration going forward with the promotion of targeted selective treatment programmes and needs to form part of the educational framework delivered around the use of FEC to target treatments.

Diagnostic testing is utilised for treatment decision-making in targeted selective protocols during high transmission periods but limited by multiple factors, as discussed in detail within Chapters 1 and 3. One of the most important is what egg threshold studs used for defining the need to treat.

As seen within this study, cut-off values for treatment can vary widely, with stud D using 100epg and stud F any positive result (>0epg). The current consensus regarding recommended treatment threshold is what is classified as “high shedding”, defined as 200-500epg within studies such as (Nielsen, et al., 2006) and included in the AAEP guidelines (Nielsen, et al., 2013). However, Nielsen et al (2014b) trace this arbitrary value back to Uhlinger (1993) where the value was found by requesting cut-off values used by commercial laboratories and supported by studies evaluating selective therapy implementing this 100-300epg value (Nielsen, et al., 2014b). Two studies performed in 2010 demonstrated both statistically and by necropsy, that this range of values would leave

50% of the population untreated but provide a 95% reduction in egg count (Kaplan & Neilsen, 2010) and, with a wider cut-off value range of 100-500epg, resulted in reduction of significantly larger strongyle parasite burdens (Nielsen, et al., 2010b). However, the non-linear relationship between FEC and adult strongyle burden has been widely documented and so the results of the latter study require caution when interpreting as the same study also recorded individuals with a FEC of 100epg but luminal adult burden of up to 300,000 strongylid parasites (Nielsen, et al., 2010b). In addition, it is still unknown at what level of infection disease is most likely to occur and the added complication of serious clinical disease resulting from prepatent infection must be considered. To complicate interpretation of FEC further, a multitude of diagnostic tests with varying sensitivities and LDL's are available (Chapter 1, Table 1.1) which affect the interpretation of results as reliability decreases as the LDL is reached (100epg LDL technique McMasters is commonly used in equine practice) (Lester & Matthews, 2014). It is for these reasons that a more bespoke approach is needed.

Given the small number of studs who had expressed a willingness to engage in our FEC and FECRT trial, we chose to best support those studs by devising bespoke test and treatment plans based on current practice. To do this most effectively we gathered knowledge of treatment protocols as well as primary concerns held by the stud veterinary surgeon and owner/manager. One of our indicators of effective parasite control on stud was to look at strongyle FEC distribution. Our data confirmed, on an individual stud basis, that overdispersion of strongyle FEC was present; with a negative binomial distribution of strongyle egg excretion in both adult and YS populations. However, a lower degree of overdispersion was seen in YS (Figure 4.6) and higher mean values were consistently recorded in YS populations (Table 4.9), indicating higher egg shedding of the one to three yo age group as has been shown previously (Relf, et al., 2013). However, this lower level of overdispersion is not considered to preclude implementation of refugia-based targeted

selective treatment regimens for the prevention of large strongyle burdens in younger horses (Relf et al., 2013).

Recent studies have identified that regular removal of faeces from pasture was associated with lower average FEC (Relf et al., 2013; Tzelos et al., 2017). In our study the lowest YS mean epg counts were noted on farms B (144.55epg), H (173.62epg) and C (186.57epg) with lowest adult means seen on studs G (19.68epg), D (51.86epg) and C (49.95epg). No consistent correlation was seen between pasture management practices and these results, for example, Stud C was noted in the lowest three mean epg counts for both YS and adults, despite not practicing faecal removal in adult or YS paddocks and infrequent ruminant grazing. However, both the cited studies used a larger number of studs and so it is challenging to make accurate inferences from our data (Relf, et al., 2013).

Our FECRT data indicated that BZ resistance was well established and that PYR resistance was evident in strongyle populations, whilst IVM and MOX were still efficacious (Figures 4.6 - 4.9). Based on our ERP findings there was a reduction in ERP for ML drugs, which has been observed elsewhere (Geurden et al., 2014; Lyons et al 2011; Relf et al., 2014; Rossano et al., 2010; van Doorn et al., 2014). ERP values on stud F were normal for the adult cohort and reduced by two weeks (ERP = 6 weeks) in YS, showing similar results to initial studies, despite this stud's current and historical intensive usage of this drug. When examining MOX YS cohorts on two of the studs tested showed a marked deviation from the expected 12-13 week ERP for MOX. Stud D showed recovery of egg counts to over ten percent of pre-treatment group mean egg counts by four weeks post treatment and on stud C egg counts exceeded pre-treatment values by eight weeks post-treatment. As discussed in Chapter 1, it is difficult to compare ERP values across studies due to a lack of standardised definitions, protocols and threshold values, but this is consistent with previous reports (Matthews et al., 2014; Tzelos et al., 2017). Difficulties in sampling individuals in accordance with the

fortnightly schedule and compliance were a significant issue when collecting this portion of data. It was not always possible to meet the minimum inclusion criteria as fewer samples were re-sampled post-treatment due to staff time pressures, turn out schedules, stock sales and compliance.

When considering drug resistance it is important that an initial evaluation of a reduced drug efficacy/drug resistance is completed a second time. Our results were generated in duplicate on two separate occasions, giving credibility to the results; in particular those studs where the number of samples submitted were below the eligibility criteria such as stud B. In addition, it must be considered the variety of methods utilised to decide on the dosage of drug given to each individual. Within this study, studs A, B and C evaluated individuals by eye; E, F and G administered one tube/packet of drug per horse and D used the average weight of a horse within the group. To account for this during FECRT we asked managers to obtain as accurate a weight as possible and dose accordingly. Whilst this may limit the confidence in the results, data obtained indicated a trend of reduced efficacy of MOX, the most favoured treatment for YS and quarantine protocols and warrants further investigation and monitoring. In addition to emphasising the need for more intensive and frequent monitoring of drug efficacy, this result highlights the requirement for use of alternative treatments for reduction in egg shedding during high transmission periods, such as PYR, providing PYR efficacy has been demonstrated. In addition, further education on the importance of accurate dosing for both efficacy of individual treatment and to delay the development of resistance is needed.

Another important consideration when interpreting both FECRT and ERP data is the higher strongyle egg shedding demonstrated by the YS population. Immature horses have been reported to have higher parasite burdens and strongyle egg output, which can result in shorter ERP in YS (Rossano et al, 2010). This in turn could lead to incorrect assumptions

about failure of drug efficacy or false interpretation of drug resistance in strongyle populations (Tzelos et al., 2017). This was demonstrated by our data when examining PYR efficacy, which varied both between stud farm and between age cohorts which co-graze on both of these farms. For example, Stud G demonstrated >90% reduction in the adult cohort but <50% mean egg count reduction within the YS; stud H showed a reverse of this situation with adults showing a mean reduction of <60% and YS a 100% result (Figure 4.8). Morphological identification of larvae from both pre-and post-treatment culture of individuals meeting the FECRT inclusion criteria indicate that only cyathostomin spp were present at the time of testing within cohorts under analysis. Both LC and PCR were used by Tzelos et al (2017) in order to ensure results were not compromised by the presence of large strongyle species. As the majority of cultures within our study were negative this indicates only cyathostomin spp were present, PCR is a more reliable method of detection (Tzelos, et al., 2017) but repeated LC of all individuals demonstrated only two positive cultures in any population undergoing FECRT, stud C. Of these positive cultures, both occurred within the adult population from samples that were not included in the FECRT studies, demonstrating that these FECRT results were determining drug efficacy against cyathostomin spp.

As multiple studies have previously highlighted, owner/manager education is a particular focus leading on from our work and that of others (Lester, et al., 2013; Lester & Matthews, 2014; Relf, et al., 2012). This is made more challenging by practical issues such as extensive management of yearling stock during the summer months which limits the number and quality of samples obtained, especially where YS cohorts are kept on sites away from the main site. Another factor creating difficulty in investigation is the high degree of stock movement within the industry as demonstrated for Stud A (Figure 4.2). High workload and demands on staff time limit compliance with diagnostic testing schedules, which in turn limits the value of pre-treatment, efficacy testing and ERP evaluation. The bespoke

approach taken by this study did improve compliance and allowed for development of comprehensive, evidence-based protocols for future parasite control on stud; the advantage of working with a small number of studs is the facilitation of closer liaison regarding the meaning of results and end goal of testing regimes, all of which encouraged compliance.

Questions about knowledge, such as drug activity against specific life cycle stages and optimal usage of drugs such as MOX, highlight the need for improvement with studs F, H and J not utilising it for reduction of encysted cyathostome larval burdens and using IVM or IVM/PRAZ combination products for this purpose. Studs C, D, F, G and J instead believing MOX to be an effective treatment for *Parascaris spp*, correct at time of licensing but not allowing for reports of anthelmintic resistance within this parasitic species. Studs D and G incorrectly identified MOX as effective against *Fasciola hepatica* (Figure 4.3). Aspects such as what defined a targeted selective protocol and the reason for their implementation seemed to create confusion or intermediary “hybrid” regimes. For example, some studs used targeted selective strategies for the strongyle egg count with a treatment threshold of  $\leq 50$ epg. The meaning of egg count values was an area of weakness with anxiety resulting from what would be seen, from a parasitology perspective, as an egg count of no significance. The financial value of TB breeding stock meant that some managers were extremely resistant to reducing the volume of anthelmintic administered due to concerns regarding disease. Similarly, TB studs in the US were only likely to switch to targeted selective regimens if they could be reassured, with evidence, that it slowed the development of anthelmintic resistance without having adverse effects on horse health (Robert et al., 2014). If providing this evidence is widely considered vital to the future implementation of surveillance-based drug treatment programmes, it poses a significant challenge.

# Chapter 5 – Evaluation of parasitic infection on a stud with a colic outbreak

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## 5.1 Introduction

*Anoplocephala perfoliata* is the most common tapeworm species present within UK horses and has been shown to be associated with colic in horses (Beroza, et al., 1986b; Owen, et al., 1989; Pearson, et al., 1993; Proudman & Trees, 1999). Recent prevalence studies, conducted using post-mortem examination and enumeration, within the UK have shown a prevalence of approximately 50-55% (Morgan, et al., 2005; Pittaway, et al., 2014), with global prevalence ranging from 13-82% depending on geographical location and diagnostic method utilised (Gasser, et al., 2005; Slocombe, et al., 2007) (See Appendix 1A). Currently, only two drugs are licensed for the treatment of tapeworms in horses; PRAZ, at 2.5mg/kg, and PYR, at 38mg/kg and neither drug has persistent action.

Tapeworm infection has been identified as a significant risk factor for spasmodic and ileal impaction colic (Proudman, et al., 1998) and the risk of these two forms of colic was shown to increase with increasing tapeworm infection intensity, as determined by the anti-12/13 kDA IgG(T) ELISA (Proudman, et al., 1998; Proudman & Holdstock, 2000). Tapeworm burdens numbering higher than 20 parasites are considered by some to indicate high risk of colic (Pearson, et al., 1993; Proudman & Trees, 1996a). Proudman and Holdstock (2000) performed a case-control study that suggested a strong association between tapeworm infection and colic. This study showed that a horse that was positive by coprological (FEC) analysis had an increased odds ratio of 23.9 (P value 0.002) of being at risk from colic, whilst the anti-12/13 kDA IgG(T) ELISA OD values in the study revealed a dose-dependent effect; with an ELISA OD of >0.800 indicating a significantly increased risk of colic (odds ratio of 21.1, p value 0.009). Similarly, Back et al (2013) identified that a horse with one or

more *A. perfoliata* eggs in its faeces had a 16 times higher risk of colic, however in this study they did not find a significant association between colic and the median ELISA OD value, suggesting the presence of tapeworm eggs faeces may be a more useful indicator of colic risk. A lack of association between spasmodic colic and strongyle FEC has been reported when the mean strongyle egg count was 131epg (Proudman, et al., 1998), but has been implicated when a higher mean FEC of 900-2000 epg were detected (Uhlinger, 1990).

An extensive review of the detection of tapeworm infection in horses is provided in Chapter 3, where we report the comparison of two faecal detection methods; DCF and CF (validated to simultaneously detect both strongyle and ascarid eggs at 1epg LDL), identifying that the CF method can detect eggs in faeces with similar accuracy to the DCF method. FEC techniques are hampered by the intermittent and unpredictable nature of segments being shed, meaning that faecal diagnostic tests have low sensitivity with a range in sensitivity from 8-61% when compared to the gold standard of post mortem examination (Kjaer, et al., 2007; Meana, et al., 2005; Proudman & Edwards, 1992; Slocombe, 2004; Tomczuk, et al., 2014). Sensitivity can be improved statistically to 92% when removing horses with low burdens from analysis (Proudman & Edwards, 1992). A significant advantage of either of these methods of faecal analysis is that the presence of eggs in faeces indicates current infection. The other major diagnostic means of detecting tapeworm infection is detection of anti-12/13kDa IgG(T) antibodies in serum (ELISA test) (Proudman & Trees, 1996a) or saliva (Equisal™) (Lightbody, et al., 2016), both commercially available as diagnostic tests. The ELISA has a maximum sensitivity of 68% and specificity of 71% (Kjaer, et al., 2007) whilst Equisal™ demonstrated 83% and 78% sensitivity and specificity respectively (Lightbody, et al., 2016) where the detection limit was set at >20 tapeworms. The threshold used for “high” burdens of >20 is used by multiple studies and evidenced by the positive relationship between degree of mucosal damage and parasite number with <20 considered non-clinically relevant (Edwards, 1999; Gasser, et al., 2005;

Lightbody, et al., 2016), although Kjaer et al (2007) considered this figure a conservative estimate of a non-pathogenic infection intensity. However, both these tests have the limitation that they are detecting exposure rather than current infection, which also compromises their ability to determine the impact of treatment interventions. The anti-12/13kDa IgG(T) ELISA has been indicated as a favourable option for monitoring herd level infection intensity (Proudman and Holdstock, 2000), whilst the FEC has been considered more useful at the individual horse level (Kjaer et al., 2007).

## 5.2 Methods

### 5.2.1 Stud details

The stud (referred to as Stud E in Chapter 4) was a small, closed herd private stud farm stud based in South East England that was referred to this study at the UoFL in autumn 2014, by a veterinarian who was concerned about the high incidence of colic on farm. Specific details of the stud are provided in Table 4.2 but are provided here for context. The stud had ~55 horses, 25 mares, ten YS (YS), ~18 foals and three companions/teasers on a 500 acre site. Mares visit other stud farms for the duration of the stud season; returning horses are quarantined for over seven days on return to home premises, managed indoors overnight and turned out during the day depending on climatic conditions. During spring, summer and autumn horses are provided with 16-24hrs/d at grass, access is restricted during the winter months with horses having 6-10hrs/d. Grazing land is rested and rotated between groups on an annual basis, grazed by cattle/sheep monthly with mechanical faecal removal on a weekly basis. The same pastures are used for grazing in subsequent years for mares and YS respectively.

Historically the stud did engage with ELISA testing for tapeworm exposure with all horses tested recording a moderate and high infection intensity result. In Sept 2014 an initial ELISA test was performed through the commercial outlet Diagnosteq, UoFL. FEC for nematode eggs were not routinely performed and were only carried out on suspicion of disease, with

treatment threshold at >0epg. The clinical history of the stud was provided and at the time of referral 70% of adult stock (mares) were reported as having had colic, requiring veterinary intervention during the 2014 season. The referring veterinarian reported chronically high levels of tapeworm-related colic within the broodmare cohort from May-October. Tapeworm parasites were seen grossly during colic surgery. No cases of colic were reported in the YS cohort.

Advice on timing and choice of anthelmintic product was based on veterinary opinion, although products were entirely purchased online. In the 12 months preceding the start of the project IVM, MOX, PYR and PRAZ were administered by various members of stud staff on a two to three monthly basis for adult stock as well as at signs of disease. When dosing, prior to the start of the project, one tube/packet of anthelmintic was administered per horse. During the course of our treatment schedule the stud was advised to base treatments on individual weights in order to reduce the risk of under-dosing and sub-optimal efficacy. Spring, autumn and at suspicion of clinical disease were considered particularly important occasions for the administration of anthelmintics.

### **5.2.2 Sample collection**

Sampling was initiated pre-treatment in February 2015 following referral, the stud expressed concerns and wished to conduct both serological and coprological analysis. At each time-point samples were collected as paired faecal and serum samples. FEC was performed by the DCF and CF method described in sections 2.8 and 2.2 respectively, with results recorded qualitatively for tapeworm eggs. Strongyle and ascarid eggs were detected by the CF method. Results for strongyle eggs were quantitative and recorded in epg with *A. perfoliata* results recorded as positive or negative. Faecal sampling protocols, as detailed in Chapter 2, were issued to the farm manager and veterinary surgeon and all submitted faecal samples were tested in duplicate as described previously. Sampling was performed

at regular intervals: February 2015, April 2015, June 2015, September 2015, April 2016 and July 2016 (Figure 5.1).

### 5.2.3 Sampling and Treatment timetable

Due to the concern regarding potentially high levels of tapeworm related clinical disease an intensive treatment protocol was initiated. Treatment involved administration of PRAZ, at 2.5mg/kg (Equitape™) +/- MOX (Equest Pramox™), approximately every three months.

Drug treatment was performed on the day of sampling or immediately on receipt of test results and recommended dose provided by means of a weigh tape. The sampling and treatment protocol is shown in Figure 5.1.

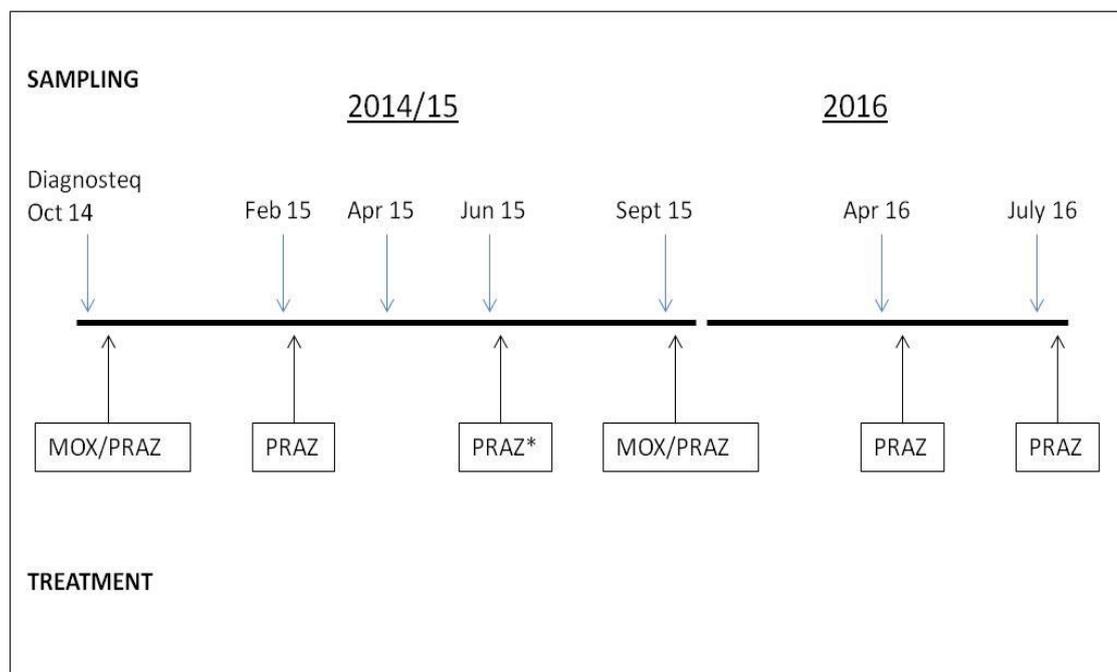


Figure 5.1 The sampling and treatment time frame on stud for the period October 2014 to July 2016. Diagnosteq staff conducted testing in October 2014, all other samples were processed as part of this project. Treatment followed immediately after sampling for all except Apr 15. Anti-cestode products used were praziquantel alone (PRAZ) or in combination with moxidectin (MOX/PRAZ). \*One horse at Jun 15 sampling had strongyle egg count >200epg and was administered an ivermectin/praziquantel combination product.

## 5.3 Results

### 5.3.1 Strongyle and *Parascaris spp* FEC

All 139 horses for which faecal samples were submitted (n=78 adult, n=61 YS) were

analysed for the presence of strongyle and ascarid eggs using the CF method. In total 59

(42.4%  $\pm$ 8.2%) of the whole herd were positive (egg of one or above) for strongyle eggs with 24 strongyle positive (30.8%  $\pm$ 10.2%) in the adult cohort and 34 (55.7%  $\pm$ 12.5%) in the YS cohort. Highest values, at individual testing points, were recorded in April 2015 (41.67%  $\pm$ 27.9%; n=5/12) and July 2016 (42.86%  $\pm$ 25.9%; n=6/14) in adults and June 2015 within the YS group (76.92%  $\pm$ 22.9%; n= 10/13). A mean value of 14.7 strongyle egg was recorded (range 0 -342 epg) in the adult cohort and a mean of 1.98epg (range 0-19.5 epg) in YS. Of positive results recorded, 100.0% of YS had a FEC of <50epg, whilst in adults 72 (92.3%  $\pm$ 5.9%) of adults recorded <50epg with 3.9% ( $\pm$ 4.3%, n=3/78) and 2.6% ( $\pm$ 3.5%, n=2/78) recording strongyle FEC of 50-200 epg and >200epg, respectively. All LCs were negative for large strongyle infective stage larvae; cyathostomin L3 were present for those cultures that were FEC positive.

With respect to ascarid eggs, none of the 78 adult horse samples showed presence of ascarid eggs, whilst nine (14.8%  $\pm$ 8.9%) of YS samples were positive for ascarid eggs (range 0.5-112.5 epg). Ascarid eggs were detected most frequently in April 2015 with seven of the total nine positive results seen at this time point. A total of 17 foals from the 2015 foal cohort were tested between February and June 2015 by CF and DCF with all individuals negative for ascarids and tapeworm. Four individuals showed positive strongyle counts, one during April and three during June (range 0.5-7.5epg; mean 2.25epg).

### **5.3.2 Tapeworm Diagnostic Results**

Two coprological and one serological test were used to detect tapeworm infection on the stud.

#### **5.3.2.1 Coprological testing**

Using the CF method of FEC for samples from adult horses on stud, a total of 58 (74.4%  $\pm$ 9.7%) recorded negative egg counts, three horses had FEC less than one epg, fourteen below two epg and three horses had FEC above two epg. Similar values were seen with the alternative DCF method with 79.5% ( $\pm$ 9.0%, n=62/78) recording a negative egg count. The

mean epg count for the CF method was 0.75 epg (range 0-7) and 0.47 epg (range 0-4) for the DCF. Fewer samples were submitted for YS (n=46) however the trend was the same with 75.4% ( $\pm 10.8\%$ , n=46/61) of all YS samples recording a negative egg count by both the CF and DCF method, respectively. The mean epg count for the CF method in YS was 0.8 epg (range 0-4) and 0.58 epg (range 0-3.27) for the DCF method. The number of samples submitted per time point is shown in Table 5.1.

Table 5.1 The number of samples submitted from the stud for each of the time points for adult and young stock

Adult stock						
	Feb 15	Apr 15	Jun 15	Sept 15	Apr 16	July 16
ELISA	9	11	16	23	11	16
CF/DCF	12	12	15	15	9	15
Young stock						
	Feb 15	Apr 15	Jun 15	Sept 15	Apr 16	July 16
ELISA	18	18	15	8	2	2
CF/DCF	17	17	12	-	-	-

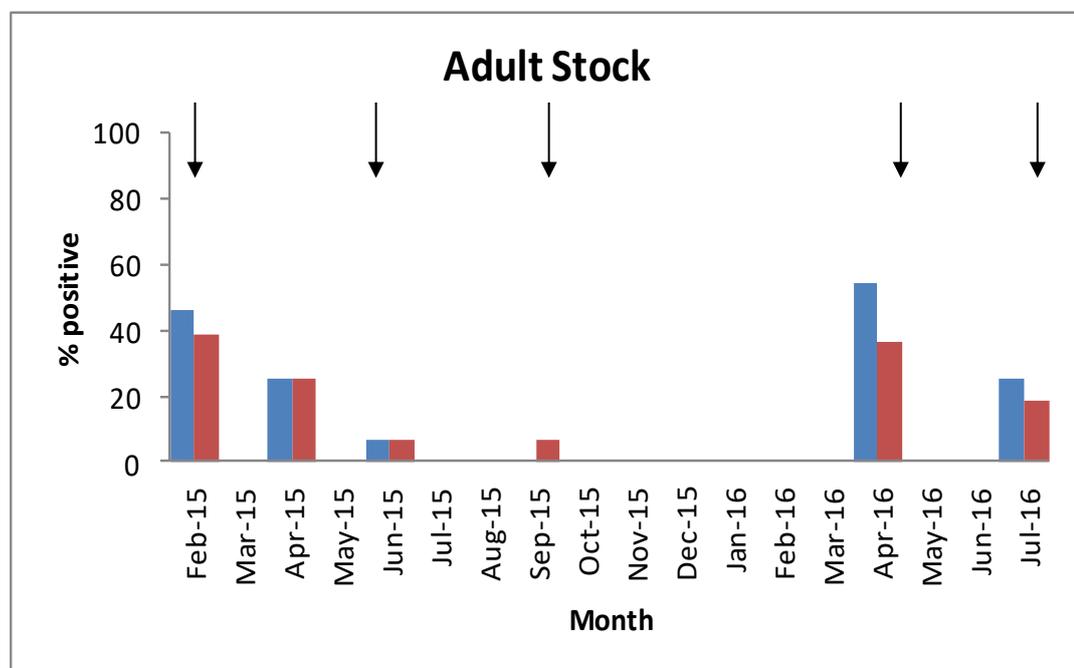


Figure 5.2. The percentage of samples submitted from adult stock that tested positive on FEC for tapeworm eggs by the CF method (blue bars) and DCF (red bars) between February 2015 and July 2016. Each treatment with praziquantel (+/- moxidectin) is shown by the black arrow.

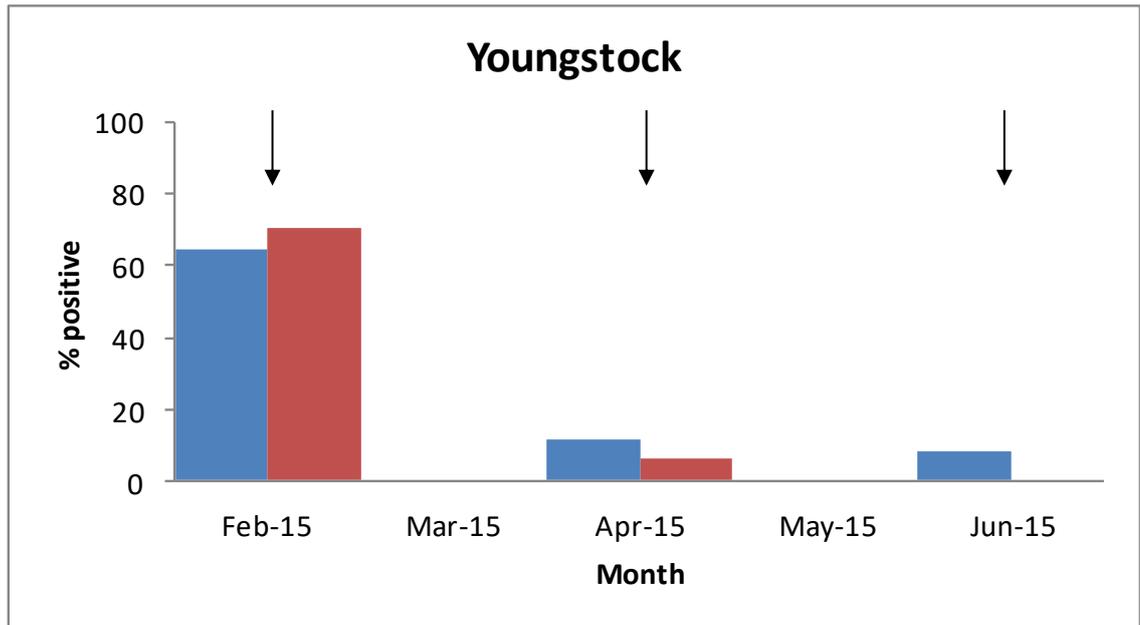


Figure 5.3. The percentage of samples submitted from YS that tested positive on FEC for tapeworm eggs by the CF method (blue bars) and DCF (red bars) between February 2015 and June 2015. Each treatment with praziquantel (+/- moxidectin) is shown by the black arrow.

The greatest proportion of animals positive by FEC was in February 2015 and April 2016 in adult stock (Figure 5.2) and February 2015 in YS (Figure 5.3). A decline in number of horses positive was seen as the year progressed (Figures 5.2 and 5.3) after treatment was instigated at approximately three month intervals. In adult stock a rise in FEC was seen in the following April (2016), when a six month timeframe elapsed since the previous treatment.

### 5.3.2.2 Serological testing (ELISA)

Of the total number of samples subjected to testing by the ELISA from adult stock (n=86), 6 (7.0% ±5.4%) recorded an OD <0.200, 32 (37.2% ±10.2%) had an OD value 0.201-0.700 and 48 (55.8% ±10.5%) recorded a value >0.700. For YS, of the 64 samples tested, two (3.1% ±4.3%) recorded an OD <0.200, 14 (21.9% ±10.1%) had an OD value 0.201-0.700, whilst 48 (75.0% ±10.6%) had an OD of >0.700. These data show that in both cohorts more than half the horses recorded an OD value indicating high tapeworm infection intensity. Throughout

the period of study 94.7% ( $\pm 3.6\%$ ,  $n=142/150$ ) of all samples were reported to have a moderate to high tapeworm infection intensity above the threshold for treatment.

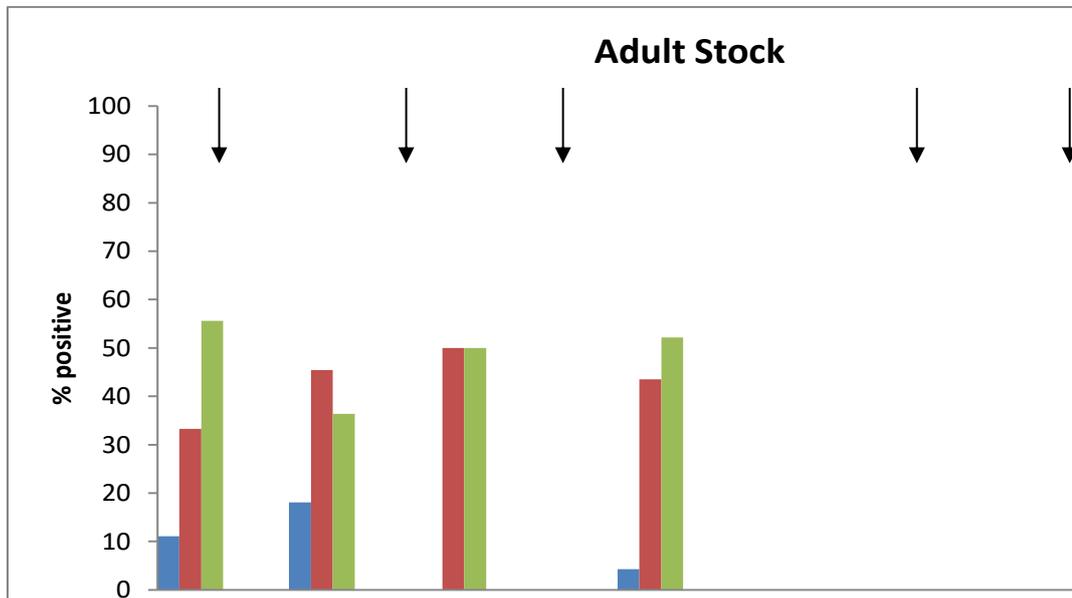


Figure 5.4. The percentage of samples submitted from adult stock that gave OD values in the low (<0.200) for tapeworm infection intensity (blue bars), moderate (OD= 0.201-0.700) tapeworm infection intensity (red bars) and high (OD >0.700) tapeworm infection intensity (green bars) between February 2015 and July 2016. Each treatment with praziquantel (+/- moxidectin) is shown by the black arrow.

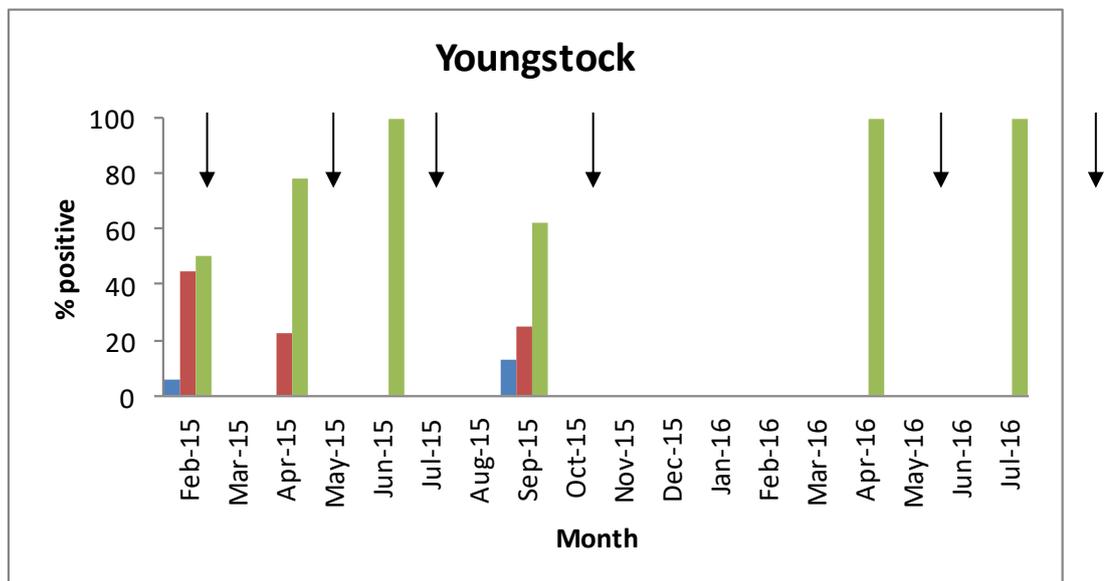


Figure 5.5. The percentage of samples submitted from young stock that gave OD values in the low (<0.200) for tapeworm infection intensity (blue bars), moderate (OD= 0.201-0.700) tapeworm infection intensity (red bars) and high (OD >0.700) tapeworm infection intensity (green bars) between February 2015 and June 2015. Each treatment with praziquantel (+/- moxidectin) is shown by the black arrow.

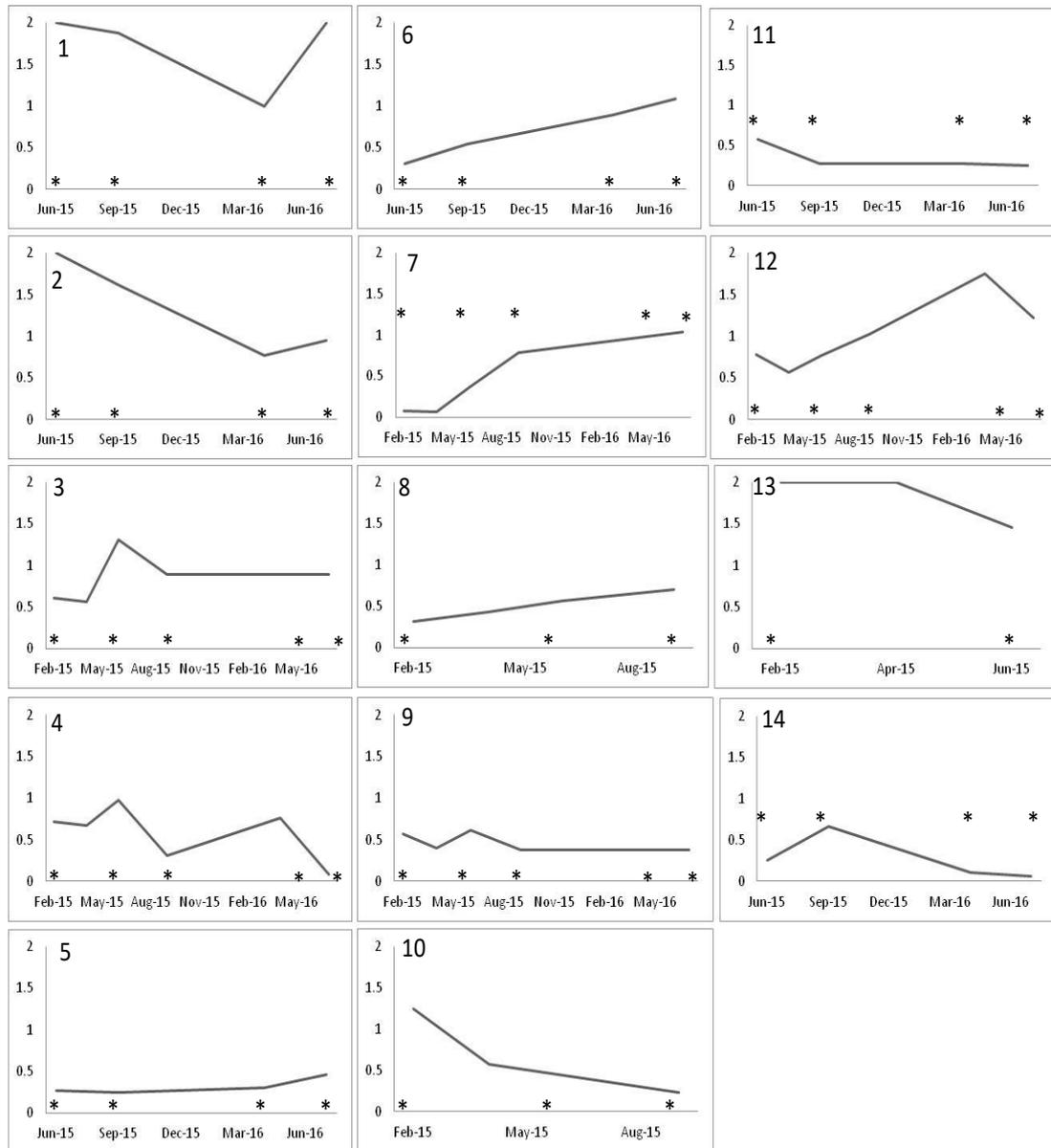


Figure 5.6. Antibody detection ELISA OD values from 14 adult horses repeatedly sampled between February 2015 and July 2016. Values on the Y axis are OD values between 0 and 2. Each horse is plotted separately over the timeframe. Each treatment with praziquantel (+/- moxidectin) is shown by an asterisk.

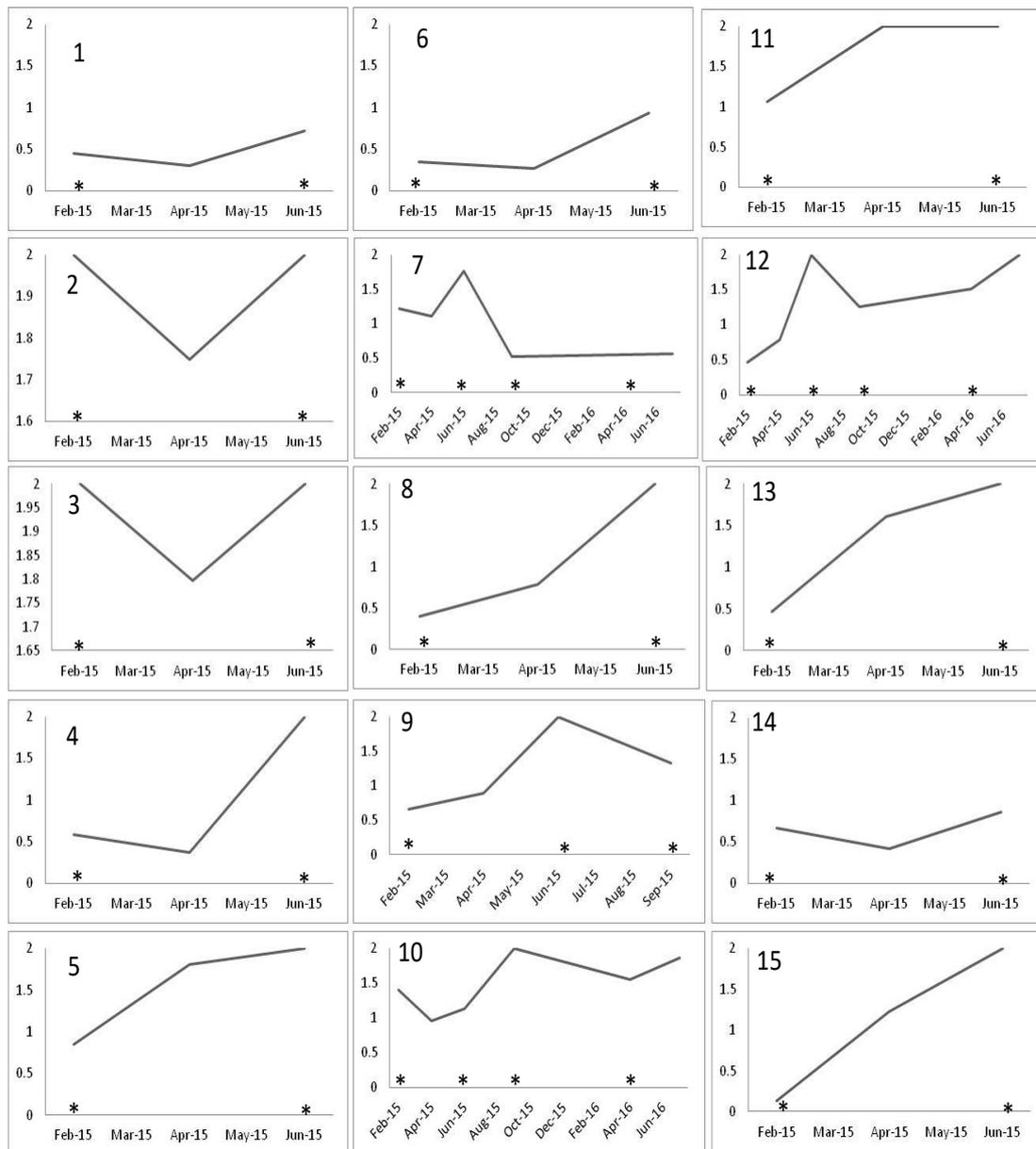


Figure 5.7. Antibody detection ELISA OD values from 15 YS repeatedly sampled between February 2015 and June 2015. Values on the Y axis are OD values between 0 and 2. Each horse is plotted separately over the timeframe. For the majority of horses sampling took place in February 2015, June 2015 and September 2015. A subset of horses were sampled over a longer time frame (nos. 7, 9, 10 and 12). Each treatment with praziquantel (+/- moxidectin) is shown by an asterisk.

When considering individual time points there is no apparent decline in OD values for either adult or YS cohort, despite frequent treatment (Figure 5.4 and 5.5). In fact in both cohorts the proportion of horses recording an OD value >0.201, the threshold for treatment (shown as the red and green bars in Figures 5.4 and 5.5) appears to increase over time. The time point when the greatest proportion of horses recorded an OD value >0.201 was June 2015, when 100% of adult and YS recorded >0.201 OD values.

In terms of individual animals tested, the greatest number of adults tested was 23, whilst for YS it was 17 (see Table 5.1). The majority of animals were repeatedly tested over an 18 month period for adults or a nine month period for YS. For animals where more than three sampling time points were tested, the ELISA OD value was plotted against time of year to observe if there was a change in titre over time and to determine if OD values declined post treatment. The values for adult horses and YS are shown in Figures 5.6 and 5.7, respectively.

## 5.4 Discussion

Infection with *Anoplocephala spp*, *A. perfoliata* being the most common species in temperate climates, is thought to predispose and potentially result in a range of colic syndromes both medical and surgical (Beroza, et al., 1986b; Bigletti & Garbagnati, 2002; Proudman & Trees, 1999; Veronesi, et al., 2009). Horses which graze are at risk due to incidental ingestion of the intermediate forage mite host which contains the infective stage of the parasite. Once ingested, tapeworm feed, grow and mature at the ileocaecocolic junction of the gastrointestinal tract resulting in ulceration from plug feeding and increased peristaltic rate due to irritation of the mucosa (Pavone, et al., 2010; Pavone, et al., 2011). The parasite has a PPP of six months, putting yearlings and older at risk of patent infection; peak infection intensity has been recorded in YS (defined as six months to two years old) followed by a decrease and plateau in three to 15 year old adults with subsequent increase in horses >15 years (Proudman, et al., 1997). This pattern was seen within the current study

when examining the proportions of adult and YS cohorts recording high infection intensity values in April 2015 and 2016, June 2015 and July 2016 (Figures 5.4 and 5.5).

Detection of tapeworm infection is challenging due to irregular shedding of mature proglottids in faeces and a limited range of validated diagnostic tests available whilst detection of current infection by ELISA is limited by the persistence of antibodies with antibody titres reported to remain high for up to six months in some individuals (Proudman & Trees, 1996a; Proudman & Trees, 1996b) following clearance of infection. This was observed here with some horses showing a rising titre over time, despite intensive treatment (Figure 5.6 and Figure 5.7). However it could be seen that when an ELISA OD was provided at ~six weeks post treatment (April 2015 following the February treatment) there was a general trend towards a drop in titre (see Figure 5.6 nos. 3, 4, 9, 10, 12 and Figure 5.7 nos. 1, 2, 3, 4, 6, 7, 10, 14), before it rose again at the next sampling time point (June 2015). Tomczuk et al. (2015) demonstrated a degree of seasonality within the proportion of mature stages present in horses undergoing post-mortem in Poland; January – March results showed a 12-fold higher mature to immature burden with highest mean parasite burdens found from October – December, potentially having a significant effect on coprological results relying on patent infection. This seasonality has been debated based on the degree of climatic variability between seasons, with Agneessens et al (1998) finding no significant difference at different points of the year, a finding refuted by studies performed in Central and Eastern Europe by Tomczuk et al (2015). Tomczuk et al (2015) found prevalence of 11.1% in November compared to 3.8% in June within a slaughter horse population (n=724) with highest number of proglottids between January and April, with Rehbein et al (2013) reporting similar results in Germany (n=2,013). As *A. perfoliata* transmission is dependent on an intermediate host, climatic factors affecting the oribatid mite will inevitably affect the potential infectivity of grazing land with Gergocs et al (2011) demonstrating increased environmental intensity with high humidity and moderate

temperature. Drugs which are currently available for use show no persistence of action, PRAZ and PYR at double nematocidal dose, result in potential immediate reinfection post-treatment due to a reservoir of orbatid mite vectors on pasture especially when considering the permanent pasture grazing practiced on this stud, this is possibly what is observed here. Alternatively, it could indicate a lack of efficacy of PRAZ against immature stages and re-infection and establishment of infection within the interim period. The horse is exposed to excretory/secretory (ES) antigen throughout infection, with highest levels seen as proglottids are released. This, combined with lack of efficacy of PRAZ against prepatent infection, may result in high ELISA antibody titres from infection comprised of large numbers of larvae and immature stages, undetectable by coprological examination and potentially post mortem (Lightbody, et al., 2016). This can result in a failure of antibody levels to reduce following treatment, especially if the immune system is primed by previous exposure. These immature stages subsequently mature after the anthelmintic has been eliminated from the body, leading to faster recovery of patency (Lightbody, et al., 2016). These factors were also highlighted and specifically considered within validation of Equisal™ by Lightbody et al (2016) in order to assess the range of use of the test.

Another important consideration is that YS were 2014 born foals so at the time of first sampling in February 2015, they had been grazing for approximately one year, yet this was sufficient for 75.0% of them to record an OD value of >0.700, indicative of high infection intensity. The fact that 75.0% fall within this category suggests a significant reservoir of infection in the orbatid mite population, resulting in heavily contaminated pasture. In the YS cohort reported here the high number of horses demonstrating an OD >0.700, indicating high infection intensity, correlates with previous findings by Proudman et al (1997) where higher parasite burdens were found in horses less than two years old. However, this study included foals, which was not possible within our study as this age cohort was not serum tested by the stud due to concerns regarding handling and development of

thrombophlebitis as a result of venepuncture (Proudman, et al., 1997). In addition, the oldest broodmare retained by the stud was 15 years of age, meaning that significant numbers of stock within each age category were not present for comparison.

Relf et al (2013) recorded a prevalence of 4% for all samples tested (n=672) across 22 UK TB studs in 2011, when using the same test stud E showed a prevalence of 27.3%, much higher than this previous study. When using ELISA results, prevalence on stud E was below that found in post-mortem studies (50-55%, see Chapter 1 and Appendix 1A), this is most likely explained by limitations of diagnostic testing including faecal egg shedding variation and individual differences in immune response and detection limit (Morgan, et al., 2005; Pittaway, et al., 2014). Nevertheless, the high prevalence reported on this stud, together with the number of animals shedding eggs in faeces and recording high antibody titres suggests a very high level of tapeworm transmission on stud. Work performed by Hoglund et al (1995) showed that the highest parasitic burdens were reported in horses that spent most time on pasture. On stud E, stock are allowed 16-24 hours of grazing per day Spring, Summer and Autumn and six to ten hours during winter. An additional factor may be the oribatid mites distinct preference for specific soil types, potentially contributing to differences in geographical distribution (van Nieuwenhuizen, et al., 1994). Survival of oribatid mites within dry forage, which stud E cohorts are provided high amounts of, and various types of bedding have not been extensively investigated but if survival is possible this could potentially allow for year-round exposure. Although pasture management, in the form of sweeping, was said by the veterinary surgeon to be performed regularly, the regime was stated to be more intermittent by the stud manager and pastures were reutilised year on year.

This study found consistently high levels of infection in both age cohorts with an unexplained predisposition to clinical disease within the adult population despite higher

levels of infection intensity, as determined by ELISA, within the YS cohort. The stud veterinary surgeon has noted colic cases are always mares with foals at foot at pasture and cases occur primarily April to August. Evidence from Veronesi et al (2009) reinforced evidence for a positive correlation between egg shedding and non-surgical cases of colic. Due to the extensive management of YS and potentially self-resolving nature of spasmodic colic it is possible that some clinical cases are self-limiting and either not seen or not recognised as being associated with tapeworm infection (Beroza, et al., 1986b; Proudman & Trees, 1999; Bigletti & Garbagnati, 2002; Veronesi, et al., 2009). The stud vet reported a decrease in the number of mares experiencing colic during the 2015, 2016 seasons, following our intervention. Cases within 2015 comprised 20 colics, primarily large colon displacement and tympany with two requiring surgical intervention; in 2016 11 colic episodes were recorded but none required surgery and in 2017 six colic cases occurred with one mare euthanased intraoperatively due to a colon torsion. One potentially important observation is that of the five horses which underwent surgery, no tapeworm were found on gross examination intraoperatively. The total number of colic cases may not truly reflect actual case number as mild to moderate episodes of spasmodic colic may go unreported. However, to establish if tapeworm infection is associated with colic on stud E it would be best to conduct a case-control study as previously described (Proudman & Holdstock, 2000), with controls from within the same cohort as cases (e.g. adult horses that did not have a history of colic vs adult horses with a history of colic).

In this study PRAZ, which should be used under license biannually was used more frequently due to the clinical concerns and following discussions with veterinarians at the Philip Leverhulme Equine Hospital, UoFL. It was agreed that an intensive treatment protocol of quarterly administration was necessary. As a result pre-treatment results can also be considered as 12 week post-treatment measurements. Despite the intensive regimen of drug administration no significant decrease in antibody titre was observed. A previous

study by Traversa et al (2008) demonstrated positive antibody titres of treated horses at 12 weeks post treatment. When it came to FEC, our data showed a decrease in the number of horses that were shedding eggs in faeces over time and following anthelmintic treatment intervention for both the adult and YS cohort (Figures 5.2 and 5.3). The FEC methods used are not quantitative and therefore no conclusion can be drawn regarding efficacy or changes in tapeworm burden based on FEC (Traversa, et al., 2008) but the downwards trend is encouraging in terms of reduced egg shedding.

Despite the initiation of a high-intensity, quarterly, PRAZ treatment regime antibody titres did not decrease within the second year, although the stud veterinary surgeon reported a decrease in clinical cases. The most likely explanation for this is a combination of residual environmental burden and the difficulties of compliance. The timing of sample collection and treatment was challenging due to extensive management during the summer months and raising YS from weaning to rising two year olds, plus decreased staff engagement during sale and sale preparation periods. As YS were on permanent turn-out, and managed extensively, following the completion of weaning obtaining individual faecal samples was not possible as this required the entire cohort to be brought in and housed which was not viable due to labour and time. It is for this reason that adult cohort tapeworm samples were primarily focused on. Following testing and treatment with a combination product in September 2015 (MOX/PRAZ) the follow up sampling and treatment three months later (Dec/Jan 2016) did not take place and this may have allowed an increased level of contamination to occur on pasture. Further follow-up of this stud after 12-24 months of intensive testing and treatment would yield more conclusive data on the impact of our intervention.

An interesting observation from faecal diagnostic results are atypically low prevalence and intensity of strongyle and ascarid egg shedding in all age groups when compared to other

studs tested concurrently (see Chapter 4). Ascarid prevalence in foals and the numbers of eggs shed by the YS cohort were markedly lower than noted in previous studies (Relf, et al., 2013). The previous study performed by Relf et al stated mean prevalence on stud of strongyle eggs as 56% compared to the 42% reported here for stud E. Of FEC recorded in this study 12.5% of individuals aged two to 14 years recorded values of >200epg, compared to 3.5% on stud E. Reduced prevalence of ascarids were also noted on stud E recording whole herd prevalence of 6.7% in adults, 15.8% in YS and 0.0% in foals; compared to Relf's findings of 9% of all faecal samples tested, 38% of foals, 4% of yearlings and 3% of two to four year olds testing positive with 18% of foals recording values of >200epg. For strongyle species, when compared to all studs participating in the current drug efficacy study (Chapter 4) and data presented in published studies, a much higher degree of overdispersion of egg count was noted on stud E with significantly reduced overall farm prevalence and individual levels of egg shedding (Relf, et al., 2013). This may indicate that pasture management for strongyle eggs was effective; that the role of the intermediate host when feeding dry forage has been underestimated or that colonisation by tapeworms impacts on establishment of infection. There is currently no published data or studies regarding the effect of co-infection with cestodes and nematodes and this area would be a potential avenue for future research.

# Chapter 6 – Large Scale Questionnaire Study

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## 6.1 Introduction

Intensive anthelmintic administration, termed interval or intensive dosing, where drugs are given to stock at set intervals to control intestinal parasites by suppressing egg shedding has been recommended for several decades (Drudge & Lyons, 1966). As discussed in Chapter 1, this regime was initiated in horses due to a high prevalence of *Strongylus spp* and associated clinical disease, of which some forms were associated with high mortality such as intestinal infarction causing colic due to formation of mesenteric emboli (McCraw & Slocombe, 1976; Reinemyer, 2012; Slocombe & McCraw, 1973; Tolliver, et al., 1987). However, due to the increased prevalence of anthelmintic resistance in the cyathostomin and *Parascaris spp*, intensive regimes are no longer sustainable. Targeted selective treatment protocols, where only “high shedders” identified by diagnostic testing are administered anthelmintics during high transmission periods have been developed. This enables identification of horses that contribute most to transmission of parasites and was investigated in a cohort of studs as discussed in Chapter 4 (Kaplan & Neilsen, 2010; Nielsen, et al., 2013; Reinemyer, 2012).

With the change in “best practice” between these two parasite control regimes, education of horse owners and farm/stud managers regarding optimal usage of anthelmintics is important. By identifying areas of lack of knowledge and sub-optimal parasite control practices, provision of evidence-based advice enables effective and sustainable parasite control can be achieved optimising the health of stock on stud farms. Previous studies have shown that most (>85%, range 88-100%) of participants from all types of equine establishments use interval programmes for control of intestinal parasites (Lind, et al., 2007; Lloyd, et al., 2000; O'Meara & Mulcahy, 2002; von Samson-Himmelstjerna, et al.,

2009). Relf et al. (2012) found that 88% of 61 UK TB studs used anthelmintics intensively and this was similar in TB breeding establishments in New Zealand (Bolwell, et al., 2015) and Italy (Papini, et al., 2015). In the latter studies, interval dosing was used on 80% of mares on TB and Standardbred (SB) farms (Bolwell, et al., 2015). A more recent survey of UK leisure and competition horse owners in 2013 revealed that 15% 193 of respondents stated that they used intensive programmes (Stratford, et al., 2013). In the latter study 44% of respondents stated that they used “strategic” programmes and 40% used “targeted selective” anthelmintic strategies (Stratford, et al., 2013). This might suggest a change in parasite control strategies being used in the leisure/competition horse population in the UK. In the USA, 67.9% of 112 TB studs surveyed used a rotational intensive anthelmintic-based control programme (Robert, et al., 2015). However, it is unknown what current practices are being used on UK TB stud farms and if there has been a shift away from intensive programmes for intestinal parasite control.

Within targeted selective protocols, control is focussed on diagnostic testing and pasture management (Reinemyer, 2012), as detailed in Chapter 4. The use of coprological diagnostics varies between both geographical area and study populations. Studies have reported that 81% of 110 South African (Matthee, et al., 2002) and 59% of 55 Irish TB stud farms used FEC (O'Meara & Mulcahy, 2002). Surveys undertaken between 2012 and 2015 on TB studs showed over 50% of 61 UK studs (Relf, et al., 2012), 30% of studs in Kentucky (Robert, et al., 2015) and 22% of 136 New Zealand studs used FEC (Bolwell, et al., 2015), but of these 0%, ~30% and 64% respectively conducted testing routinely (Relf, et al., 2012; Bolwell, et al., 2015; Robert, et al., 2015). A large proportion of studs performed diagnostics at the time when parasite-associated disease was a suspected “signs of disease” in the UK and New Zealand surveys (Relf, et al., 2012; Bolwell, et al., 2015).

The aims of this study were to determine current parasite control practices on UK TB studs with a particular focus on use of pasture management practices, administration of anthelmintics and use of targeted selective strategies. This would enable comparisons to be made with the findings of Relf et al. (2012) to determine if and how equine parasite control programmes have changed on UK TB stud farms since that time.

## **6.2 Materials and Methods**

### **6.2.1 Questionnaire**

A questionnaire was designed (Appendix 6A) and was divided into 11 sections, composed of 82 questions (detailed in the following sub-sections). The questionnaire was trialled, by distribution to studs participating in the FEC project (Chapter 4) and collecting feedback, prior to the final version being distributed to a wide selection of stud farms.

#### **6.2.1.1 *Demographic information and perception of parasite control / sources of information.***

Each participant was asked to provide numbers of permanent stock, delineated by defined age and sex groups, on the stud at the time of the questionnaire being completed.

Participants were asked how important they considered parasite control to be on a stud farm and if they associated high and low burdens of the following helminth parasites with disease: cyathostomins; *Strongylus spp*; *Parascaris spp*; *Anoplocephala perfoliata* and *Fasciola hepatica*. Participants were also asked to provide information about sources of information about parasite control and use of specific anthelmintic products.

#### **6.2.1.2 *Grazing and Pasture management***

The acreage owned, rented or used for grazing was requested in order to gauge the size of the farm and the workload required for grazing management including the number of hours of grazing per day available to stock, on average, within each season. Questions also included the type and frequency of pasture management strategies.

#### **6.2.1.3** *Wormer advice and usage in adult stock*

Administration protocols within adult stock was investigated, including details about the anthelmintic regime used, additional or specific occasions considered to pose a high risk of gastrointestinal parasitism, specific drugs utilised and frequencies of drug class rotation and administration. In addition details about anthelmintic administration, asking for details regarding the individual administering anthelmintics and how the dosage was calculated.

#### **6.2.1.4** *Foal and yearling worming practices*

This section of the questionnaire was designed to assess participants' understanding of age-specific risks (such as *Parascaris spp* in horses under 18 months old) and to evaluate the appropriateness of the chosen regime for these age groups and how this differed from parasite control in adult horses.

#### **6.2.1.5** *Horse movement and quarantine*

Participants were asked to provide information about the duration of stay for visiting stock, how this group were grazed and any relevant pre-arrival checks that were usually undertaken. The same information was requested for each stud's own stock that travelled and/or were housed elsewhere including descriptions about differences in quarantine protocols for both groups.

#### **6.2.1.6** *Anthelmintic Resistance*

These questions were designed to assess the level of concern participants had regarding anthelmintic resistance. In addition, participants were asked if they were aware of anthelmintic resistance on their stud, including how and by whom this was confirmed.

#### **6.2.1.7** *Parasite-related disease*

Participants were asked within this section to define the number and, if known or suspected, the aetiology of clinical disease cases on stud in order to investigate the prevalence of confirmed and suspected parasite-related disease. Respondents' views on changes in prevalence and parasite/s posing greatest risk were also collected.

#### **6.2.1.8 Diagnostic testing and use of results**

This section assessed the use and perceived value of diagnostics for stock health and pasture management. FECRT conduction prevalence was investigated, including asking for details of results where available.

#### **6.2.1.9 Targeted selective worming**

Knowledge about the principle and prevalence of usage was investigated within this section. Participants, if using targeted selective worming, were asked to provide opinions on their personal experiences with the change in regime related to the previous sections (disease prevalence, FEC results, and subjective view of benefits). Participants were provided with a large comments section for any feedback on the survey or additional information about their experiences with parasite control.

#### **6.2.2 Study population**

Stud farms were initially identified by retrieval of the first 200 listings from DoT. This information was cross-referenced with the Newmarket Farm Owners Association list in order to account for outdated listings resulting from stud closure, sale and renaming. In order to be eligible to participate in the study, stud farms were required to be breeding TB stock for racing purposes and to be located within England, Scotland or Wales (due to management differences in Ireland). Following initial telephone contact with the first 50 studs, many studs had either changed purpose (e.g. to riding schools, sports horse breeding, holiday cottages), and so additional criteria were required to be met including having an:

- Active, updated website
- Active Facebook/Twitter page
- References within racing literature (Racing Post, sales records of Tattersalls/Weatherbys/Doncaster (AKA Goffs)) – recorded as having bought or sold stock in 2015)

- Public Access - company finance, business tax and revenue records showing “active” status

Through this process, additional stud farms were identified which met all of the above criteria and were subsequently included. Additional databases including “Yorkshire Racing” and county-level historical data were also used in order to maximise participant numbers and eliminate bias from invitation of only studs from a single database (DoT). Bloodstock agents were also included, as discussed in Chapter 1 within TB industry structure, three distinct groups exist: studs which also perform sales preparation and consignment; consignment and pinhooking only; or sales preparation, consignment and pinhooking syndication. Only those meeting the description of the first category, where breeding was conducted on-site, were eligible and were invited to participate.

### **6.2.3 Distribution**

Studs were grouped into batches of approximately 50, and following initial contact, it was determined whether participants wished to complete the questionnaire by telephone, post or online. The questionnaire was identical for each.

#### **6.2.3.1 Telephone Distribution**

Initially, an introductory letter, consent form and information sheet were posted to the first 50 stud farms requesting a telephone interview within a two week interval and working day timeframe, instructions on how to pre-book a time and date by email, text or phone were included (Monday-Sunday, 9am – 9pm) to fit into the managers schedule. This was followed up with a phone call within the date and time range specified.

#### **6.2.3.2 Postal Distribution**

Following stud feedback regarding concerns about discussing business over the phone and lack of telephone availability, studs were sent a paper copy of the questionnaire and associated paperwork. The first mailing was conducted during November/December 2015. Telephone contact was made with studs that had not returned a completed questionnaire

in January 2016. Those which had not responded by February 2016 were sent a second questionnaire pack in March/April, followed by an email reminder two weeks later.

#### **6.2.3.3 Online Distribution**

The online survey was advertised through Facebook, Twitter feeds and/or websites of the following organisations: Racing Post; Doncaster (GOFFS) Sales House; Tattersalls Sales House; Weatherbys; RosSDales Veterinary Surgeons; Thoroughbred Breeders Association (TBA); Horserace Betting Levy Board (HBLB); Liphook Equine Hospital; UofL Diagnosteq Laboratory and Veterinary School Alumni.

#### **6.2.4 Data analysis**

Prior to analysis, all matrix and multiple-answer responses were converted into categorical independent variables and were assigned numerical values, where true missing data were present and follow-up was unsuccessful a value of zero was entered and only valid responses counted. Yes/no questions were converted into binary independent variables and continuous variables such as stock and acreage were left in raw format for descriptive analysis. For multiple sections a filter question was included and if the section was not applicable the respondent was instructed to skip to the next section, this resulted in variation in denominators which was accounted for during descriptive statistical analysis and displayed within results. All statistical analysis and graphical representations were performed using Microsoft Excel (2016). Descriptive analysis of the data included frequencies of each response as percentage of respondents for each question in order to account for true missing data and variable denominators to express the responses obtained as a proportion of data obtained, 95% confidence intervals were calculated for all proportions. The mean, median and mode were calculated for each section of matrix questions. For continuous variables, the mean, median and range were calculated.

### **6.2.5 Ethical Approval**

Ethical approval for the study was obtained from the UofL Veterinary Ethics Committee (VREC 243a).

## **6.3 Results**

### **6.3.1 Stud farm recruitment**

At the time of recruitment, DoT had a total of 482 UK stud farm listings - 405 in England, 19 in Wales and 14 in Scotland. As many DoT listings as possible were manually verified as detailed below, those showing current activity were shortlisted for invitation and a representative sample, including all geographical regions, were invited to participate in the study. Suffolk recorded the highest number of studs per county (n=67), with these studs comprising 16.5% of total DoT listings, likely due to traditional centring of the racing industry in Newmarket. Graphical representations (Appendix 6B) show the proportion of studs from each geographical region registered on DoT compared to geographical range of those studs investigated for participation, demonstrating representative sampling.

In total, 243 studs were shortlisted for invitation to participate in the study (60.5% of all DoT listings); 19.2% (n=47) were no longer TB studs or stud farms of any kind, 1.2% (n=3) were satellite studs for larger corporations and nine were not able to be verified and were assumed to no longer be active. Of those invited, 49 were not present on the DoT listing (20.0%).

### **6.3.2 Response Rates and Stud Demographics**

The overall response rate was 21.2% (n=39/243). A total of 30 (76.9%, n=30/39) of questionnaires were returned by post, eight (20.5%, n=8/39) were completed online and one (2.6%, n=1/39) was completed by telephone.

Studs who provided a location (n=37/39) were spread around England (Figure 6.1) with most located in the Southern half of England, with a focal point in the South-East around the Newmarket area (Figure 6.1). Of the 37 studs who provided a geographical location,

27.0% (n=10/37) were located in and around Newmarket, Cambridgeshire; 10.8% (n=4/37) were located in Wiltshire; 8.1% of respondents were located in Somerset (n=3/37); Lincolnshire and Oxfordshire both had two responding studs (5.4%, n=2/37) and, where other counties were represented, only a single stud responded for each (2.7%, n=2/37).

The majority of stock on stud were broodmares (mean 29.2 per stud) with a wide range of grazing acreages (mean 320 acres, range 14-4500 acres) as shown in Table 6.1. Calculation of an accurate stocking density was not possible due to highly variable numbers of yearlings and foals and the information provided gave the total acreage owned, rented or used for grazing as a maximum potential value (not taking into account the acreage rested or left unused at any one time).

Table 6.1 Mean, median and range provided by participating studs for number of permanent stock held on site and grazing acreage owned, rented or used for grazing purposes.

<b>Parameter</b>	<b>Mean</b>	<b>Median</b>	<b>Range</b>
Stallions (n)	1.2	0	0-11
Geldings/Teasers (n)	1.8	0	0-25
Broodmares (n)	29.2	15.5	1-280
Yearlings (n)	17.7	8.5	0-250
Foals (n)	16.5	10	0-175
Donkeys (n)	0	0	0
Grazing acreage (acre)	320	127.5	14-4500



Figure 6.1 Geographical map of questionnaire respondents showing the majority within the South of England with a cluster located in the Newmarket area and outliers in Scotland, Manchester and Derbyshire.

### 6.3.3 Grazing and pasture management

During spring stock were provided with 6-24hr/d turn-out; during summer, 100% of studs (n=39/39) gave stock 16-24 hr/d on pasture; in autumn the majority of studs, 82.1% ( $\pm 12.0\%$ , n=32/39) of studs provided 16-24 hours grazing and 6-10hrs/d was the most grazing time allowed in the winter months (Table 6.2). All studs responded to this question.

Where pastures were rotationally grazed by sheep/cattle (82.1%  $\pm 12.0\%$ , n=32/39) this occurred most commonly either annually (35.9%  $\pm 15.1\%$ , n=14/39) or occasionally (25.6%  $\pm 13.7\%$ , n=10/39) with monthly and weekly ruminant grazing being reported to be

performed by 10.3% ( $\pm 13.7\%$ ,  $n=10/39$ ) each. Harrowing was undertaken on an annual basis by 59.0% ( $\pm 15.4\%$ ,  $n=23/39$ ) of all studs, occasionally by 18.0% ( $\pm 12.0\%$ ,  $n=7/39$ ), monthly by 15.4% ( $\pm 11.3\%$ ,  $n=6/39$ ), weekly by one stud (2.6%  $\pm 5.0\%$ ,  $n=1/39$ ). Only two studs stated that harrowing was never performed (5.1%  $\pm 6.9\%$ ,  $n=2/39$ ).

Table 6.2 The average hours per day (hrs/d) access to grazing provided by season

<b>Q8. On average, how much access to grazing (hours per day - hrs/d) do horses have during...</b>					
	<b>0 hrs/d % studs (n)</b>	<b>1-5 hrs/d % studs (n)</b>	<b>6-10 hrs/d % studs (n)</b>	<b>11-15 hrs/d % studs (n)</b>	<b>16 - 24 hrs/d % studs (n)</b>
Spring (March - May)	0	7.7 (3)	35.9 (14)	23.1 (9)	33.3 (13)
Summer (June - August)	0	0	0	0	100 (39)
Autumn (September - November)	0	0	2.6 (1)	15.4 (6)	82.1 (32)
Winter (December - February)	2.3 (1)	23.1 (9)	43.6 (17)	10.3 (4)	20.5 (8)

Faeces were removed from a large proportion or all of grazing land on approximately half of responding studs (53.9%  $\pm 15.9\%$ ,  $n=20/38$ ); four studs provided multiple answers where nursery/foal/small paddocks were cleaned but larger paddocks were not. This resulted in 64.0% ( $\pm 18.9\%$ ,  $n=16/24$ ) stating they used machines, most commonly the Nicholson Paddock Sweeper, 28.0% ( $\pm 18.2\%$ ,  $n=7/24$ ) performed faecal removal manually and 8% ( $\pm 11.1\%$ ,  $n=2/24$ ) performed a mixture of techniques with manual removal being most commonly performed for nursery paddocks. Of those removing faeces from the majority of paddocks, when asked about frequency, 30.0% ( $\pm 20.1\%$ ,  $n=6/20$ ) performed pasture cleaning twice weekly, commonly nursery paddocks were cleaned with greater frequency, 35.0% ( $\pm 20.9\%$ ,  $n=7/20$ ) performed faecal removal occasionally/sporadically with 10.0% ( $\pm 13.1\%$ ,  $n=2/20$ ) removing faeces monthly, 15.0% ( $\pm 15.6\%$ ,  $n=3/20$ ) fortnightly and 10.0% ( $\pm 13.1\%$ ,  $n=2/20$ ) weekly.

Paddock resting was most commonly performed monthly (35.9% ±15.1%, n=14/39) or occasionally (35.9% ±15.1%, n=14/39) with yearly resting performed by 23.1% (±13.2%, n=9/39). Two studs stated that resting was never performed. Rotation between groups of horses was highly variable between studs with one stud rotating on a weekly basis, 43.6% (±15.6%, n=17/39) monthly, 12.8% (±10.5%, n=5/39) annually, 25.6% (±13.7%, n=10/39) occasionally and 15.4% (±11.3%, n=6/39) never rotating groups.

### 6.3.4 Perception of parasites and equine disease

Figure 6.2 shows that the majority of stud owners were “very concerned” about high burdens of any of the parasites specified (63.9% [±15.7%, n=23/36] - 88.9% [±10.3%, n=32/36]) with low burdens of *Fasciola hepatica* stated as causing high concern by 63.9% (±15.7%, n=23/36) of respondents.

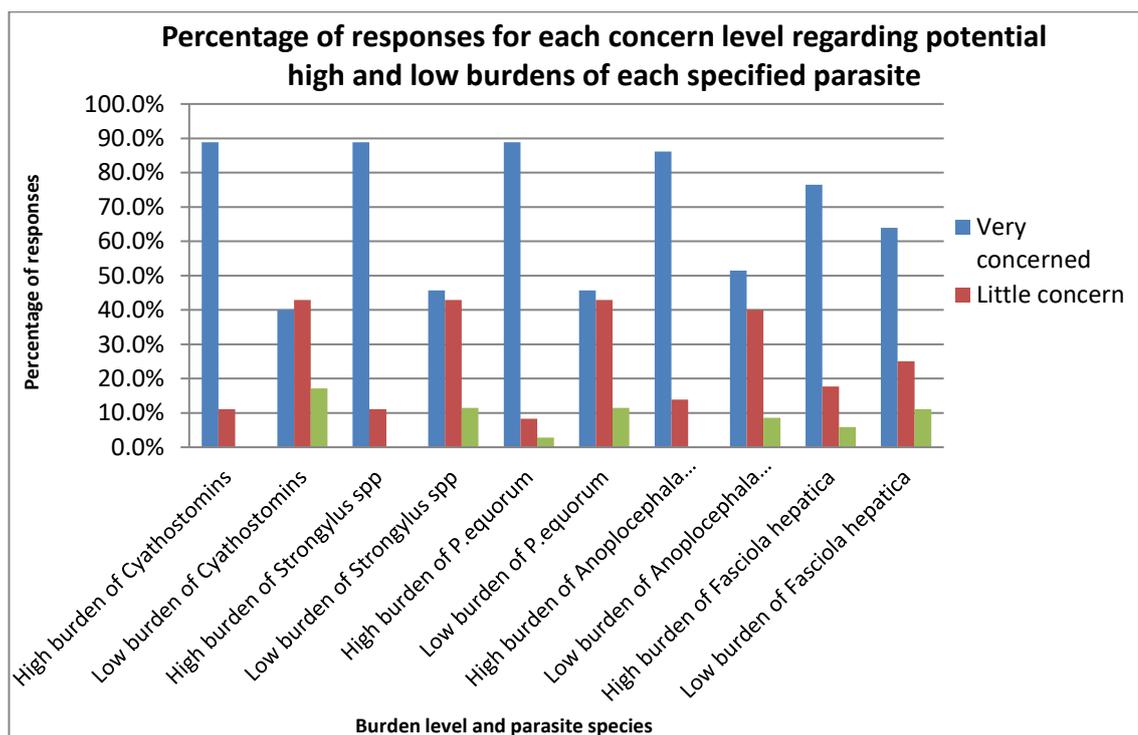


Figure 6.2 Responding studs level of concern at the potential presence of each parasite species in either a high or low burden on the participants’ stud.

This question had a variable response rate, one stud only indicated an answer where there was concern and responses were only shown to high burdens of all parasites with the

exception of *F. hepatica* where low burden was indicated to be of concern. For “high burden” categories n=36/39 studs responded; “low burden” categories were answered by n=35/39 respondents; for *F. hepatica* “high” and “low” were answered by n=34/39 and n=36/39 respectively.

### 6.3.5 Movements and quarantine

Over half of responding studs (62.9%  $\pm$ 16.0%, n=22/35) accepted visiting stock with 80.6% ( $\pm$ 12.9%, n=29/36) stating that their horses also visited other premises. Those studs responding “no” to accepting visiting stock and/or sending stock away did not complete the remainder of the related section. The mean number of visiting horses per stud was 25.8 (median 22.5, range 2-70). Visiting stock boarding times were stated by 86.36% ( $\pm$ 14.3%, n=19/22) to fall primarily into a single category, with 13.64% ( $\pm$ 14.3%, n=3/22) stating two distinct groups, this resulted in n=25 responses for most common average boarding duration. Of these responses, boarding time was most often, 56.0% ( $\pm$ 19.5%, n=14/25) of cases, dependant on the individual horse or owner. For stock being sent away for boarding, three studs declined to give further information, 76.92% ( $\pm$ 16.2%, n=20/26) felt one category was most applicable to their practices; 19.23% ( $\pm$ 15.1%, n=5/26) felt two categories were most accurate and 3.85% ( $\pm$ 7.4%, n=1/26) indicated three categories. Of these responses it was most common (54.6%  $\pm$ 17.0%, n=18/33) for stock to remain at the host stud for one to four months. For those visiting, classification of duration of stay as “other” most often specified six weeks boarding, correlating with stock sent to other studs. For home stock sent to external stud farms, many comments referred to the duration of stay being dependant on whether horses stayed within mainland UK or travelled to Ireland for covering and/or boarding. Within the latter scenario horses stayed longer (Figure 6.3).

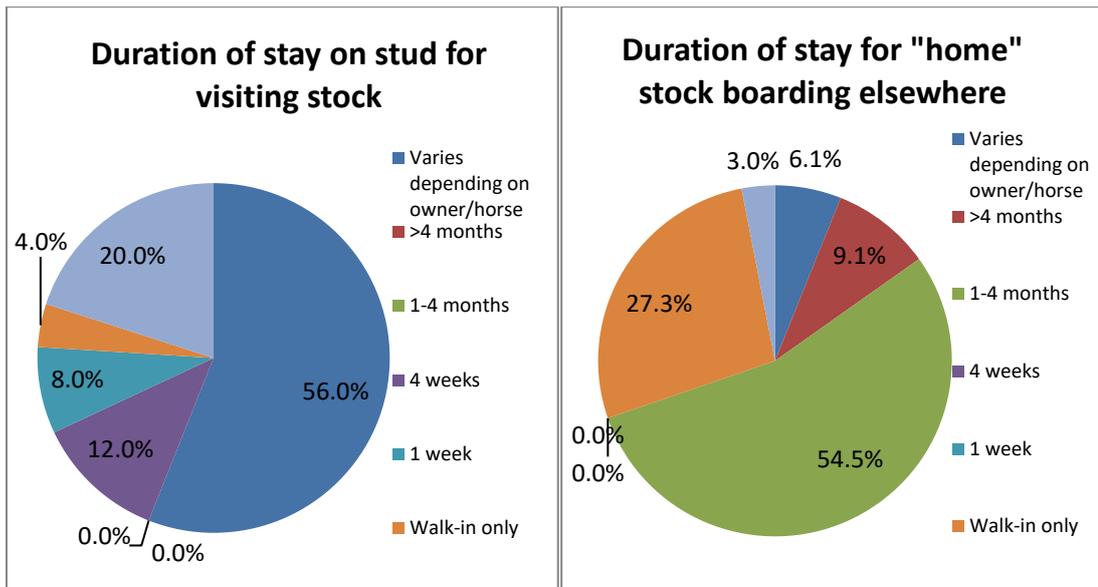


Figure 6.3 Comparative length of stay between those horses visiting participating studs and mares visiting other studs.

When asked about how concerned respondents were about returning mares introducing pathogens onto home premises (Figure 6.4), only one stud of the 29 to whom this section was applicable declined to comment, the responding studs (n=28) were most concerned about infectious non-reproductive disease such as “strangles” (“very concerned” = 57.1% ±18.3%, n=16/28), parasites resistant to anthelmintics and increased pasture contamination by parasites (46.4% ±18.5%, n=13/28 for both). Infectious reproductive disease was rated as “very”, “slightly” and “not” concerning approximately equally (39.9% ±18.1%, n=11/28; 32.1% ±17.3%, n=9/28; 28.6% ±16.7%, n=8/28 respectively). A few respondents (7.1% ±9.5%, n=2/28) elaborated within the comments section stating that industry biosecurity, testing and certification prior to mating were good and was why infectious reproductive disease was of little concern to them, as effective preventive and stringent protocols already exist and are a legal requirement.

When asked about quarantine, 77.3% (±17.5%, n=17/22) had one protocol with five studs stating two durations used resulting in 27 responses in total. Of these, 74.1% (±16.5%, n=20/27) of responses stated that visiting horses were quarantined on arrival with fewer

stating that returning home stock are also isolated upon return (63.3%  $\pm$ 17.2%, n=19/30 responses, two studs stating two differing protocols depending on where horses return from and one declined to give further details). For both groups duration of quarantine was most often more than seven days regardless of where they had visited (40.7%  $\pm$ 18.5%, n=11/27 and 41.7%  $\pm$ 19.7%, n=10/24 for visiting and returning stock). For returning stock 12.5% ( $\pm$ 13.2%, n=3/24) of respondents quarantined horses for one to two days if returning from a mainland UK farm or two to seven days regardless of where they had visited. For the former group it was specified by two studs that returning specifically from Irish sites was an influential factor and resulted in prolonged isolation for a fixed three week period. Other respondents answered that the protocol for returning stock heavily depended on the point of origin but conditions for isolation differed, with segregation for two to seven days only if showing signs of respiratory disease (yearlings) and only if returning from outside the UK/Ireland or dependant on the specific stud visited (4.2%  $\pm$ 8.0%, n=1/24 per answer). One stud reported that quarantine was not necessary for returning from walk-in visits or trips to a stud visited previously (one stud per answer, 4.2%  $\pm$ 8.0%, n=1/24 respectively). A single stud did not quarantine either visiting or returning stock due to lack of facilities and in comparison with quarantine protocols for visiting stock, 8.33% ( $\pm$ 11.1%, n=2/24) did not feel that any form of quarantine was necessary regardless of available facilities.

Prior to visiting stock arrival, 86.4% ( $\pm$ 14.3%, n=19/22) of studs never requested or checked pre-arrival FEC; 63.6% ( $\pm$ 20.1%, n=14/22) requested a three month worming history pre-arrival with 13.6% ( $\pm$ 14.3%, n=3/22) stating this was preferred but optional; two studs (9.1%  $\pm$ 12.0%, n=2/22) requested this from mares only, potentially due to only receiving mares; 9.1% ( $\pm$ 12.0%, n=2/22) never requested or checked and a single stud (4.6%  $\pm$ 8.7%, n=1/22) investigated worming history only on an occasional/random check basis.

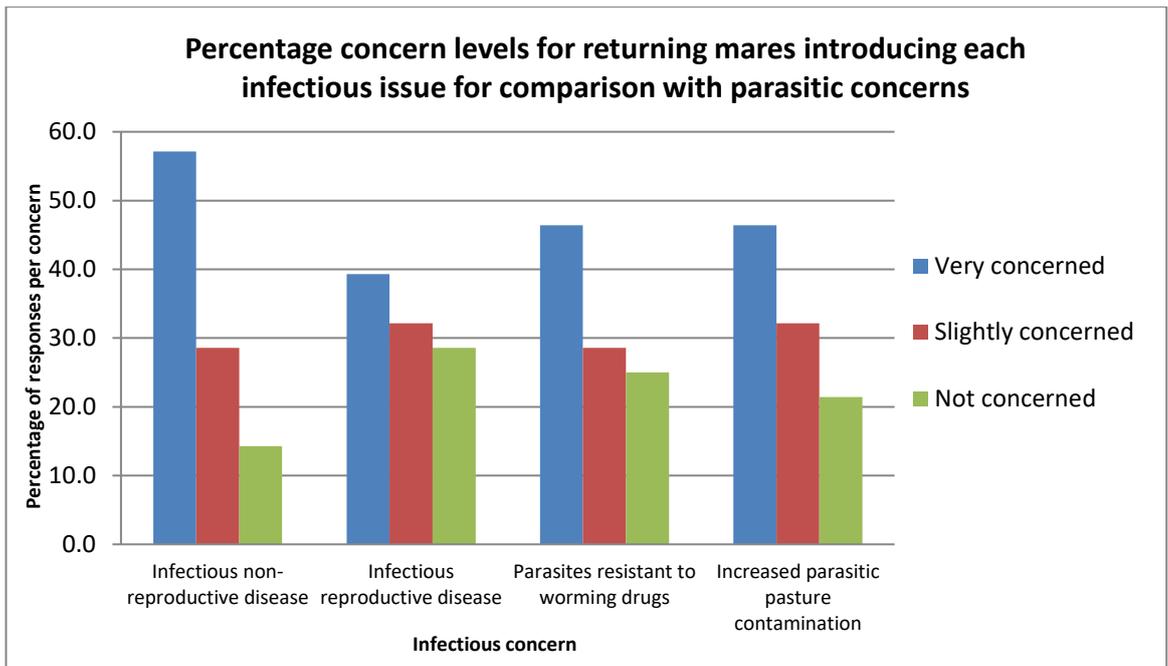


Figure 6.4 Percentage of respondents classifying level of concern as “very”, “slightly” or “not” with respect to introduction of - infectious reproductive or infectious non-reproductive disease for comparison with concern levels presented by increased parasitic pasture contamination and drug resistance originating from returning mares.

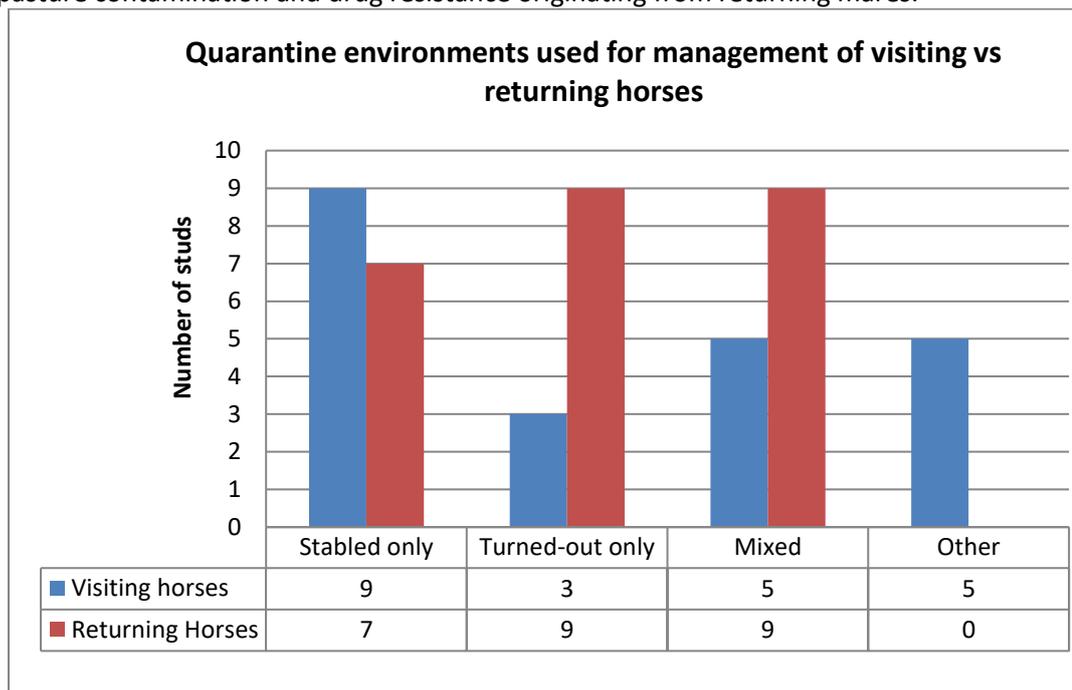


Figure 6.5 Comparative environmental management strategy for quarantine for both visiting and returning (or “home”) horses.

Studs classing management of quarantined visiting horses as “other” primarily stated that it depended on the point of origin (22.7%  $\pm$ 17.5%, n=5/22), whether there were foals at foot and the temperament of the mare or YS. One stud stated that only YS showing active signs of respiratory disease were quarantined. Of the 22.7% ( $\pm$ 17.5%, n=5/22) conducting mixed location management of visiting horses, 60.0% ( $\pm$ 42.9%, n=3/5) were turned out during the day and stabled at night with 40.0% ( $\pm$ 42.9%, n=2/5) stating that it depended on the time of year and weather as to how quarantine was conducted. In contrast to visiting horses, more returning mare quarantine periods were conducted completely at pasture. A combination of turnout and stabling was most common for both groups’ quarantine, and fewer returning stock were kept purely stabled (Figure 6.5).

FECs were equally classed as always or never being performed for visiting and returning stock as seen in Table 6.3. Those studs who stated they only used FEC for disease diagnosis (16.7%  $\pm$ 12.2%, n=6/36, three studs declined to respond) conducted faecal examination primarily on visiting stock. Respondents who utilised FEC occasionally and those who used the results for treatment decision making, did so most commonly on returning horses. Serum tapeworm ELISA testing was reported to be used infrequently within either of the latter groups but, when used, it was exclusively utilised for returning home stock. Testing was used more frequently in horses with suspicion of disease or if the site was known to have historical issues with parasites. The salivary ELISA (Equisal™, ADB) was used occasionally by two studs.

Table 6.3 Comparative quarantine practices for visiting versus returning stock.

<i>Origin</i>	<b>FEC performed % (95%CI, n/total)</b>		<b>Blood Tapeworm ELISA % (95%CI, n/total)</b>		<b>Saliva Tapeworm ELISA % (95%CI, n/total)</b>		<b>Wormed on FEC results % (95%CI, n/total)</b>		<b>All wormed pre- turnout % (95%CI, n/total)</b>	
	<i>Visit</i>	<i>Home</i>	<i>Visit</i>	<i>Home</i>	<i>Visit</i>	<i>Home</i>	<i>Visit</i>	<i>Home</i>	<i>Visit</i>	<i>Home</i>
<b>Always/ Every visiting horse</b>	27.8 (±20.7, 5/18)	25.0 (±16.0, 7/28)	0	0	0	0	23.1 (±22.9, 3/13)	31.8 (±19.5, 7/22)	52.6 (±22.5, 10/19)	45.5 (±20.8, 10/22)
<b>If point of origin has history of parasitic disease</b>	0	14.3 (±13.0, 4/28)	0	4.0 (±7.7, 1/25)	0	0	0	0	0	0
<b>If mare has high egg count on return</b>	0	0	0	0	0	0	7.7 (±14.5, 1/13)	18.2 (±16.1, 4/22)	10.5 (±13.8, 2/19)	0
<b>If suspicious of disease</b>	22.2 (±19.2, 4/18)	3.6 (±6.9, 1/28)	0	8.0 (±10.6, 2/25)	0	0	7.7 (±14.5, 1/13)	0	15.8 (±16.4, 3/19)	0
<b>Occasionally</b>	5.6 (±10.6, 1/18)	17.9 (±14.2, 5/28)	0	8.0 (±10.6, 2/25)	0	10.0 (±13.1, 2/20)	7.7 (±14.5, 1/13)	4.6 (±8.7, 1/22)	0	13.6 (±14.3, 3/22)
<b>Never/Not necessary</b>	44.4 (23.0, 8/18)	39.3 (±18.1, 11/28)	100.0 (14/14)	80.0 (±15.7, 20/25)	100.0 (14/14)	90.0 (±13.1, 18/20)	53.9 (±27.1, 7/13)	45.5 (±20.8, 10/22)	21.1 (±18.3, 4/19)	40.9 (±20.5, 9/22)

In visiting horses, 35.7% ( $\pm 25.1\%$ , n=5/14 who responded) of studs administered anthelmintics to all horses, despite having no access to grazing during their stay. One respondent stated that treatment without grazing access only occurred when there was suspicion of parasite related disease. All studs responding allowed visiting mare's access to grazing. In returning mares, a similar pattern of results was found for studs administering an anthelmintic to all horses regardless of the quarantine protocol. The majority of respondents (52.6%  $\pm 22.5\%$ , n=10/19 conducting quarantine) did not feel that blanket worming of returning horses was necessary prior to turn-out, one stud wormed occasionally, with the remainder complying with a blanket worming protocol (42.1%  $\pm 22.2\%$ , n=8/19).

While visiting, 36.7% ( $\pm 17.7\%$ , n=11/29) of respondents stated that visiting horses were grazed only with other visiting mares following quarantine (including anthelmintic administration if required), 20.0% ( $\pm 14.7\%$ , n=6/29) without quarantine, however although the majority of respondents (71.4%  $\pm 19.3\%$ , n=15/21) felt a single category surmised their practices, three studs gave two and three responses each. Other responses included 26.7% ( $\pm 16.3\%$ , n=8/29) being grazed with resident stock after quarantine (including anthelmintic administration if required) and 16.7% ( $\pm 13.7\%$ , n=5/29) grazing visiting mares alone in a small paddock. No respondents declared that grazing visiting stock with permanent residents was routine without quarantine protocols being followed. The way in which frequently travelled home mares were grazed when residing on their home premises was more variable between studs with 30% ( $\pm 20.1\%$ , n=6/20, two studs declined to comment) of respondents stating that they frequently grazed travelled mares of the same age or mixed age group 25.0% ( $\pm 19.0\%$ , n=5/20), and 25.0% ( $\pm 19.0\%$ , n=5/20) grazed frequently travelling mares with non-mobile resident stock of the same age or a mixed age group (20.0%  $\pm 17.5\%$ , n=4/20).

### 6.3.6 Parasite related disease

Only 25.7% ( $\pm 14.5\%$ ,  $n=9/35$ ;  $n=4$  studs declined to comment) respondents declared incidences of parasite-related disease on their stud farms, all of which had one to five confirmed cases within the previous two years. Of these, an equal number ( $46.7\% \pm 25.2\%$ ,  $n=7/15$  total responses regarding diagnosis) were diagnosed by personal/staff opinion and veterinary diagnosis with a single reported incident being confirmed by veterinary opinion without diagnostic testing. Surgical and medical colic were the only categories determined to be parasite related by veterinary diagnosis; worms in faeces and diarrhoea lasting less than one week recorded the same number of responses for staff opinion and veterinary diagnosis. Those diagnosed by personal/staff opinion without any declared veterinary input were non-specific signs of disease including poor performance, loss in/failure to gain condition, and weight loss.

The most common presentations of parasitic disease were visible worms in faeces ( $n=5/15$  reported signs,  $33.3\% \pm 23.9\%$  of all responses) and colic  $26.7\% (\pm 22.4\%, n=4/15)$ . The most common parasites confirmed as causing disease were strongyles ( $33.3\% \pm 23.9\%$ ,  $n=5/15$  for both cyathostomins and *Strongylus spp*). *Parascaris spp* was considered to be responsible for parasite associated disease on three studs ( $25.0\% \pm 26.7\%$ ,  $n=4/12$  responses) and *A. perfoliata* on one ( $8.3\% \pm 15.6\%$ ,  $n=1/12$ ). Three respondents stated that three parasites were responsible for disease cases ( $37.5\% \pm 33.5\%$ ,  $n=3/8$ , one stud declined to comment),  $12.5\% (\pm 22.9\%, n=1/8)$  said two and  $50.0\% (\pm 34.6\%, n=4/8)$  gave one parasite sole responsibility for disease cases. When asked for personal opinion on parasite-related disease case prevalence,  $33.3\% (\pm 30.8\%, n=3/9)$  of respondents stated that there had been an increase in this; one stated an increase on their stud, and two had noted an increase in cases within the local area.

### 6.3.7 Anthelmintic advice, purchase and use

All studs administered anthelmintics to horses on their premises. Only one source of advice was used in 51.4% of respondents ( $\pm 16.6\%$ ,  $n=18/35$ ) with 40.0% ( $\pm 16.2\%$ ,  $n=14/35$ ) using two or three sources of advice. As shown in Figure 6.6, the most common reference source was their veterinary surgeon (71.4%  $\pm 15.0\%$ ,  $n=28/35$  studs; 37.9%  $\pm 11.7\%$ ,  $n=25/66$  total responses) followed by articles in equine magazines (25.7%  $\pm 14.5\%$ ,  $n=9/35$  studs; 13.6%  $\pm 8.3\%$ ,  $n=9/66$ ) and feed shops/worming suppliers (20.0%  $\pm 13.3\%$ ,  $n=7/35$  studs; 10.6%  $\pm 7.4\%$ ,  $n=7/66$ ). Twelve respondents (34.3%  $\pm 15.7\%$ ,  $n=12/35$  studs; 18.2%  $\pm 9.3\%$ ,  $n=12/66$ ) stated that no advice was required as their existing strategy and personal experience was sufficient.

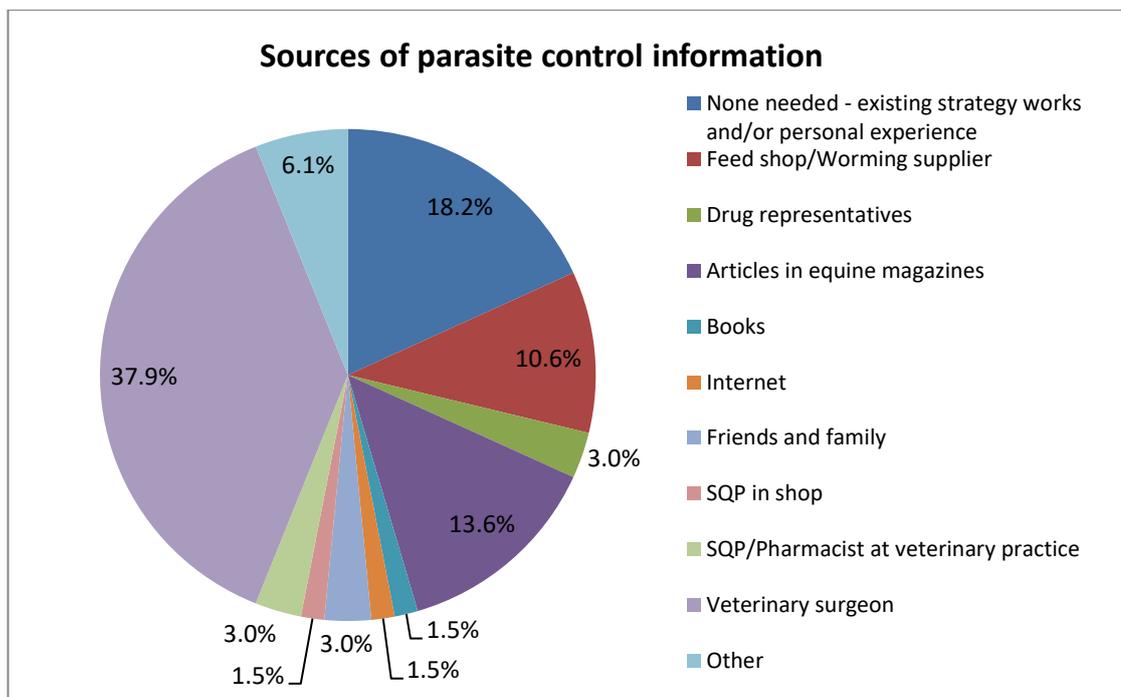


Figure 6.6 The percentage of respondents using each of the specified sources of information for worming protocols and advice.

Anthelmintic products were most often obtained from online shops (31.6%  $\pm 14.8\%$ ,  $n=12/38$ ) or other sources such as general equine suppliers (23.7%  $\pm 13.5\%$ ,  $n=9/38$ ).

Although the majority of advice was obtained from the stud veterinary surgeon, veterinary

practices were the least common source of anthelmintic product purchase (5.3%  $\pm$ 7.1%, n=2/38) as seen in Figure 6.7.

Rotation between drug classes was most commonly performed at set intervals (65.71%  $\pm$ 15.7%, n=23/35) whereas 25.7% ( $\pm$ 14.5%, n=9/35) of respondents chose the drug used based on FEC or ELISA results. All respondents stated they were aware of what was meant by the term 'drug class'.

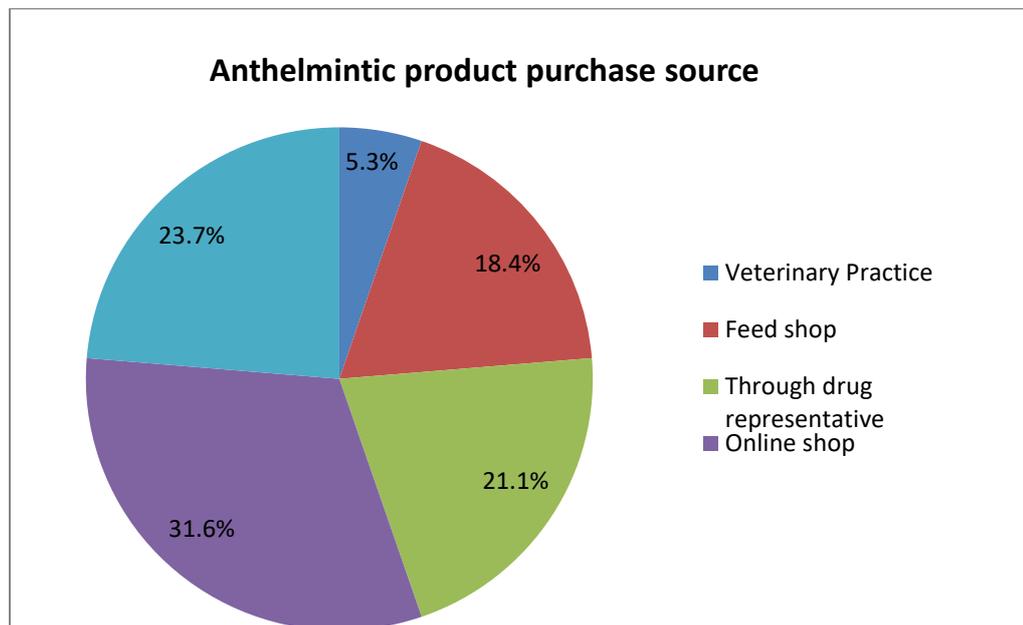


Figure 6.7 Most frequent source of worming product purchase for survey participants.

In order to calculate dosage, studs most often had one blanket protocol (85.7%  $\pm$ 11.6%, n=30/35) but some respondents stated that they altered the strategy according to age group (14.3%  $\pm$ 11.6%, n=5/35). Assessment of weight "by eye" was most common (43.2%  $\pm$ 14.6%, n=19/44 total responses), followed by administration of one tube or packet of drug per horse (23.7%  $\pm$ 12.4%, n=10/44). The remaining respondents used weight of the heaviest animal within the grazing group (11.4%  $\pm$ 9.4%, n=5/44), individual weigh-taping (9.1%  $\pm$ 8.5%, n=4/44) or use of a weigh-bridge (6.8%  $\pm$ 7.4%, n=3/44). When deciding what dosage to administer, 85.71% ( $\pm$ 11.6%, n=30/35) of responding studs stated only one was used, five studs stated more than one weight measuring technique was used on their

individual stud. Where different strategies were used based on age group being treated it was most common for adults to be given an approximate dosage, estimated by eye or one tube/packet each. For foals and YS these were dosed according to the heaviest weight within the age group, by weighbridge or weigh-tape. How the heaviest or average weight for age or grazing groups were determined was not declared.

#### **6.3.7.1 Adults**

When treating adult stock it was most common for anthelmintics to be administered on a set treatment schedule (46.2%  $\pm$ 13.5%, n=24/52 total responses from 35 studs) with 34.6% ( $\pm$ 12.9%, n=18/52) of remaining studs utilising diagnostic testing in treatment decision-making. Anthelmintics were most frequently administered by a specific member of staff (65.7%  $\pm$ 15.7%, n=23/35) and for the majority, this responsibility was that of the stud owner or manager (56.5%  $\pm$ 20.3%, n=13/23 with a specific nominated member of staff). Fewer respondents stated that this was performed by a stud groom/head girl or assistant stud grooms (43.5%  $\pm$ 20.3%, n=10/23).

Following administration of anthelmintics, 60.0% ( $\pm$ 16.2%, n=21/35) of studs did not keep horses off the pastures. Of those stabled following treatment (40.0%  $\pm$ 16.2%, n=14/35), 71.4% ( $\pm$ 23.7%, n=10/14) were removed from pasture for one to two days whereas the remainder (14.3%  $\pm$ 18.3%, n=2/14 each) kept horses off pasture for less than one day and less than one week respectively. No studs prevented access to pasture for seven days or more. When resuming grazing post-treatment, 62.9% ( $\pm$ 16.0%, n=22/35) of studs did not move horses to clean pasture and 37.1% ( $\pm$ 16.0%, n=13/35) did. For those studs that declared the duration of time elapsed between treatment and turnout the mean, median and range were 4.1, 2 and 0-21 days respectively.

When asked which anthelmintics had been used on their studs over the past 12 months (Table 6.4), a maximum of seven active ingredient/s (8.6%  $\pm$ 9.3%, n=3/35 responses) and a

modal value of three drugs (31.4% ±15.4%, n=11/35) were selected as having been used by participants in adult cohorts. The second most common number of active ingredient/s were five (25.7% ±14.5, n=9/35) and four (17.1% ±12.5%, n=6/35). Of the drugs listed, the most commonly used was IVM (21.9% ±6.6%, n=33/151 total responses for all drugs used) followed by MOX (16.6% ±5.9%, n=25/151) with third most common tied between BZ class drugs and MOX/PRAZ combination products (14.6% ±5.6%, n=22/151).

Table 6.4. Usage of each drug on participating studs within the 12 months prior to questionnaire completion (section 3 – Wormer advice and use question 21 – Appendix 6A)

**21. Which of the following wormers have been used at your stud in the past 12 months?**

<b>Drug (example trade names provided)</b>	<b>% responses (95%CI, n/n total)</b>
Ivermectin (e.g. Eqvalan; Eraquell; Bimectin; Vectin; Noromectin)	21.9 (±6.6, 33/151)
Moxidectin (e.g. Equest)	16.6 (±5.9, 25/151)
Benzimidazoles (e.g. Panacur; Panacur five day guard; Telmin)	14.6 (±5.6, 22/151)
Pyrantel (e.g. Strongid-P; Pyratape)	11.3 (±5.0, 17/151)
Praziquantel (e.g. Equitape)	10.6 (±4.9, 16/151)
Ivermectin/Praziquantel (e.g. Eqvalan Duo; Equimax)	9.3 (±4.6, 14/151)
Moxidectin/Praziquantel (e.g. Equest Pramox)	14.6 (±5.6, 22/151)
Other, please specify	1.3 (±1.8, 2/151)

As shown in Table 6.5, macrocyclic lactone (ML) class of drugs were most frequently used as single-drug products (51.0% ±14.1%, n=25/48 responses when answers were grouped according to drug class), followed by combination preparations (macrocyclic lactone/PRAZ, 20.4% ±11.5 n=10/48). The most frequently used product (by trade name) was Equest (26.5% ±12.4%, n=13/49 written responses).

Table 6.5 The breakdown of original written responses provided by respondents showing most popular brand/trade names incorporating relisting by active ingredient and drug class.

<b>As written by respondents</b>	<b>% (95%CI, n/n total)</b>	<b>By active drug/s</b>	<b>% (95%CI, n/n total)</b>	<b>By Drug Class</b>	<b>% (95%CI, n/n total)</b>
Equest	26.5 (±12.4, 13/49)	Moxidectin	30.6 (±12.9, 15/49)	Macrocyclic Lactone	ML only 51.0 (±14.0, 25/49)
Strongid-P	10.2 (±8.5, 5/49)	Ivermectin	16.3 (±10.3, 8/49)	ML-Praz combo	20.4 (±11.3, 10/49)
Ivermectin	10.2 (±8.5, 5/49)	Pyrantel	12.2 (±9.2, 6/49)	Tetrahydropyrimidine	12.2 (±9.2, 6/49)
Equimax	8.2 (±7.7, 4/49)	Ivermectin-Praziquantel	12.2 (±9.2, 6/49)	Benzimidazole	8.2 (±7.7, 4/49)
Panacur	6.1 (±6.7, 3/49)	Fenbendazole	8.2 (±7.7, 4/49)	Other	Vary, etc. 6.1 (±6.7, 3/49)
Equest pramox	6.1 (±6.7, 3/49)	Moxidectin-Praziquantel	6.1 (±6.7, 3/49)	Praz only	2.0 (±4.0, 1/49)
Varies/Varies as much as possible/Drugs alternated	6.1 (±6.7, 3/49)	Varies/Varies as much as possible/Drugs alternated	6.1 (±6.7, 3/49)		
Eraquell	4.1 (±5.5, 2/49)	Noromectin	4.1 (±5.5, 2/49)		
Noromectin	4.1 (±5.5, 2/49)	Noromectin-Praziquantel	2.0 (±4.0, 1/49)		
Moxidectin	4.1 (±5.5, 2/49)	Praziquantel-only	2.0 (±4.0, 1/49)		
Ivermectin-praziquantel	4.1 (±5.5, 2/49)				
Eqvalan	2.0 (±4.0, 1/49)				
Equitape	2.0 (±4.0, 1/49)				
Pyrantel	2.0 (±4.0, 1/49)				
Benzimidazole	2.0 (±4.0, 1/49)				
Noropraz	2.0 (±4.0, 1/49)				

Respondents considered spring (23.8%  $\pm$ 8.1%, n=25/105 total responses from 35 studs) and autumn (21.9%  $\pm$ 7.9%, n=23/105) the most important time periods for administering anthelmintics. Prior to introduction of new equines (12.4%  $\pm$ 6.3%, n=13/105), foaling (10.5%  $\pm$ 5.9%, n=11/105) and summer were also considered important to a lesser degree. Few studs reported pre-turnout (9.5%  $\pm$ 5.6%, n=10/105) and winter (6.7%  $\pm$ 4.8%, n=7/105) as important times for administering anthelmintics. The majority of studs felt that there were multiple occasions when administration of anthelmintics was important (28.6%  $\pm$ 15.0%, n=10/35 responding studs; 25.7%  $\pm$ 14.5%, n=9/35, two or three occasions respectively).

#### 6.3.7.2 Foals & Youngstock

The majority of respondents reported that cyathostomins were of either 'major' or 'minor' significance in foals (45.2%  $\pm$ 17.5%, n=14/31 responding studs; 38.7%  $\pm$  17.1%, n=12/31 respectively) whereas for YS these were considered a 'major concern' by most (65.5%  $\pm$ 17.3%, n=19/29 responding studs). The majority of respondents reported *Strongylus spp*, *Parascaris spp* and *A. perfoliata* to be of major concern in foals (63.3%  $\pm$ 17.2%, n=19/30; 73.3%  $\pm$ 15.8%, n=22/30; 57.1%  $\pm$ 17.9%, n=16/30 respectively) and YS (75.0%  $\pm$ 16.0%, n=21/28; 55.2%  $\pm$ 18.1%, n=16/29; 67.9%  $\pm$ 17.3%, n=19/28).

YS and adult worming protocols differed on 48.5% ( $\pm$ 17.1%, n=16/33) of studs with 47.6% ( $\pm$ 21.4%, n=10/21 total responses from 16 studs) of respondents stating that anthelmintics were more frequently administered to the YS cohort; 23.8% ( $\pm$ 18.2%, n=5/21) used different anthelmintics; 14.3% ( $\pm$ 15.0%, n=3/21) used more frequent FECs and 4.8% ( $\pm$ 9.1%, n=1/21) answered that a smaller dose, less frequent treatment or increased monitoring (general parameters and condition) of stock was used. A wide range of anthelmintics and number of drugs per annum were used in this group (Table 6.6).

### 6.3.7.3 Foals

The majority of respondents (88.2%  $\pm$ 10.8%, n=30/34) stated that foals had different anthelmintic administration routines compared to adult stock, with 59.5% ( $\pm$ 15.8%, n=22/37 responses from 30 studs) stating that these were administered more frequently to foals; 27.0% ( $\pm$ 14.3%, n=10/37) used different anthelmintics; 5.4% ( $\pm$ 7.3%, n=2/37) specified a different dosage was used; 2.7% ( $\pm$ 5.2%, n=1/37) for each of the following was also recorded – first anthelmintic treatment delivered by stomach tube, increased frequency of FEC and routine FECRT after each treatment. The first treatment was most often given at three to four weeks of age (47.1%  $\pm$ 16.8%, n=16/34 studs) or five to six weeks (35.3%  $\pm$ 16.1%, n=12/34). Of these 5.9% ( $\pm$ 7.9%, n=2/34) gave the first treatment at birth to two weeks and seven to eight weeks respectively with the remainder (5.9%  $\pm$ 7.9%, n=2/34) treating at over eight weeks or “other”. As for YS, anthelmintic drugs and frequency of administration per year varied widely (Table 6.7).

### 6.3.8 Anthelmintic resistance

All but one respondent was concerned about resistance to anthelmintics (97.1%  $\pm$ 5.7%, n=33/34 studs) with 67.7% ( $\pm$ 15.7%, n=23/34) choosing the highest category (“very concerned”). A total of 37.5% ( $\pm$ 16.8%, n=12/32) felt they had experienced a reduction in efficacy of anthelmintics and that problems had been noticed within all drug classes. When asked to provide more detail, seven studs (43.8%  $\pm$ 24.3%, n=7/16) considered that IVM efficacy was compromised with one specifying the issue was specifically in YS. Benzimidazoles were selected by 25.0% ( $\pm$ 21.2%, n=4/16) with one stud stating that issues existed with cyathostomins and 12.5% ( $\pm$ 16.2%, n=2/16) reported that PYR and MOX had reduced efficacy. One stud stated that the latter anthelmintic seemed less effective against pinworm; a single stud felt that the effectiveness of anthelmintics was reduced “across the board”.

Table 6.6 Frequency of use per annum for each active ingredient/combination within youngstock groups

<b>What drugs would you use for youngstock (1 - 3 years old)?</b>						
	<b>Once per year</b> % (95%CI, n/n total)	<b>Twice per year</b> % (95%CI, n/n total)	<b>Three times per year</b> % (95%CI, n/n total)	<b>&gt; Three times but others also used</b> % (95%CI, n/n total)	<b>Only drug used (entire year)</b> % (95%CI, n/n total)	<b>Not used at all</b> % (95%CI, n/n total)
Ivermectin (e.g. Eqvalan, Eraquell, Bimectin)	15.6 (±12.6, 5/32)	15.6 (±12.6, 5/32)	18.8 (±13.5, 6/32)	18.8 (±13.5, 6/32)	0	31.3 (±16.1, 10/32)
Moxidectin (e.g. Equest)	25.8 (±15.4, 8/31)	19.4 (±13.9, 6/31)	3.2 (±6.2, 1/31)	9.7 (±10.4, 3/31)	0	41.9 (±17.4, 13/31)
Pyrantel (e.g. Strongid-P, Pyratape-P)	16.1 (±12.9, 5/31)	6.5 (±8.6, 2/31)	3.2 (±6.2, 1/31)	3.2 (±6.2, 1/31)	3.2 (±6.2, 1/31)	67.7 (±16.5, 21/31)
Benzimidazoles (e.g. Panacur, Telmin)	19.4 (±13.9, 6/31)	3.2 (±6.2, 1/31)	6.5 (±8.6, 2/31)	9.7 (±10.4, 3/31)	3.2 (±6.2, 1/31)	58.1 (±17.4, 18/31)
Ivermectin/Praziquantel (e.g. Eqvalan Duo, Equimax)	9.7 (±10.4, 3/31)	25.8 (±15.4, 8/31)	3.2 (±6.2, 1/31)	0	0	61.3 (±17.1, 19/31)
Moxidectin/Praziquantel (e.g. Equest Pramox)	19.4 (±13.9, 6/31)	16.1 (±12.9, 5/31)	0	9.7 (±10.4, 3/31)	0	54.8 (±17.5, 17/31)
Praziquantel only (e.g. Equitape)	12.9 (±11.8, 4/31)	9.7 (±10.4, 3/31)	0	3.2 (±6.2, 1/31)	0	74.2 (±15.4, 23/31)

Table 6.7 Active ingredients used within a foals first year of life and the frequency per annum

	<b>Once per year</b> % (95%CI, n/n total)	<b>Twice per year</b> % (95%CI, n/n total)	<b>3 times per year</b> % (95%CI, n/n total)	<b>&gt; 3 times but others also used</b> % (95%CI, n/n total)	<b>Only drug used (entire year)</b> % (95%CI, n/n total)	<b>Not used at all</b> % (95%CI, n/n total)
Ivermectin (e.g. Eqvalan, Eraquell, Bimectin)	5.7 (±7.7, 2/35)	17.1 (±12.5, 6/35)	22.9 (±13.9, 8/35)	31.4 (±15.4, 11/35)	5.7 (±7.7, 2/35)	17.1 (±12.5, 6/35)
Moxidectin (e.g. Equest)	20.6 (±13.6, 7/34)	5.9 (±7.9, 2/34)	0	5.9 (±7.9, 2/34)	2.9 (±5.7, 1/34)	64.7 (±16.1, 22/34)
Pyrantel (e.g. Strongid-P, Pyratape-P)	8.8 (±9.5, 3/34)	8.8 (±9.5, 3/34)	8.8 (±9.5, 3/34)	14.7 (±11.9, 5/34)	2.9 (±5.7, 1/34)	55.9 (±16.7, 19/34)
Benzimidazoles (e.g. Panacur, Telmin)	17.7 (±12.8, 6/34)	8.8 (±9.5, 3/34)	14.7 (±11.9, 5/34)	11.8 (±10.8, 4/34)	2.9 (±5.7, 1/34)	44.1 (±16.7, 15/34)
Ivermectin/Praziquantel (e.g. Eqvalan Duo, Equimax)	5.9 (±7.9, 2/34)	11.8 (±10.8, 4/34)	5.9 (±7.9, 2/34)	2.9 (±5.7, 1/34)	0	73.5 (±14.8, 25/34)
Moxidectin/Praziquantel (e.g. Equest Pramox)	20.6 (±13.6, 7/34)	0	0	2.9 (±5.7, 1/34)	2.9 (±5.7, 1/34)	73.5 (±14.8, 25/34)
Praziquantel (e.g. Equitape)	20.6 (±13.6, 7/34)	0	0	2.9 (±5.7, 1/34)	0	76.5 (±14.3, 26/34)

Only 15.2% ( $\pm 12.2\%$ ,  $n=5/33$ ) of studs had directly seen the effects of, or had been told, that there was drug resistance present on their stud. The evidence, where detail was disclosed, for this was both objective and subjective, stated as high FEC post-treatment ( $60.0\% \pm 42.9\%$ ,  $n=3/5$ ), drug failure ( $20.0\% \pm 35.1\%$ ,  $n=1/5$ ) and *A. perfoliata* results always coming back high/medium ( $20.0\% \pm 35.1\%$ ,  $n=1/5$ ). The majority of studs ( $87.9\% \pm 11.1\%$ ,  $n=29/33$ ) did not have confirmed resistance (as supported by FECRT) to any drugs; of the four that did (one incomplete response), results were evenly split between IVM and BZs. The status on these four studs were all determined differently with one result recorded for each of the following: FECRT; veterinary opinion; disease following anthelmintic administration giving personal confirmation, no veterinary input and the last was unsure of how resistance was determined.

### 6.3.9 Diagnostic testing and use of results

FEC had been performed by  $80.6\% (\pm 12.9\%, n=28/36)$  of studs and  $13/36 (36.1\% \pm 15.7\%)$  performed tapeworm serum ELISA tests. Of those that undertook FEC,  $75.9\% (\pm 15.6\%, n=22/29)$  had FECs performed by their veterinary practice,  $14.8\% (\pm 12.6\%, n=4/29)$  by an external laboratory and  $3.7\% (\pm 6.6\%, n=1/29)$  by an on-site laboratory.

FECs were most commonly performed every two to three months ( $31.4\% \pm 15.0\%$ ,  $n=11/36$  responses). On  $17.1\% (\pm 12.2\%, n=6/36)$  of studs FECs were only conducted when there was suspicion of parasite-related disease and the same number tested horses during quarantine. One stud ( $2.8\% \pm 5.4\%$ ,  $n=1/36$ ) used FEC only for returning horses and the remaining five ( $13.9\% \pm 11.3\%$ ,  $n=5/36$ ) for both visiting and returning horses. Following receipt of FEC results the most common threshold for treatment was any positive result (any result  $>0\text{epg}$ ) ( $33.3\% \pm 17.3\%$ ,  $n=9/28$ ). The remainder of responses were relatively evenly spread between the remaining results with two, three or four responses per answer including  $14.8\% (\pm 13.0\%, n=4/28)$  of respondents who treated all horses regardless of FEC and  $14.8\% (\pm 13.0\%, n=4/28)$  that treated only those with FEC  $>200\text{epg}$ .

Where tapeworm ELISAs were performed (40.7%  $\pm$ 18.5%, n=11/27), their primary function was as a screening tool on an annual basis (36.4%  $\pm$ 28.4%, n=4/11) or horses at suspicion of having parasite-associated disease (36.4%  $\pm$ 28.4%, n=4/11) with a wide spread of responses for all possible answers and treatment thresholds.

Post or email was used for delivery of diagnostic test results for 70.0% ( $\pm$ 16.4%, n=21/30) of studs undertaking testing. The majority (85.7%  $\pm$ 17.5%, n=18/30) of reports included advice on individuals requiring treatment; 26.7% ( $\pm$ 15.8%, n=8/30) were provided in person, all with advice regarding which horses required treatment with a single stud being provided with results in person after which anthelmintics were purchased from the same person/company.

When asked how valuable participants perceived FEC and ELISAs to have been in a variety of transmission and disease reduction parameters, FEC testing was classed as important or very important for all listed descriptors with its use in evaluating pasture management strategies achieving the highest percentage of "important" classifications and determination of which horses to treat highest in the "very important" categories (Figure 6.8). In contrast, ELISA testing was classed as neutral for transmission reduction and herd health parameters and equally neutral or important/very important for screening herd levels or determining which horses to treat (Figure 6.9).

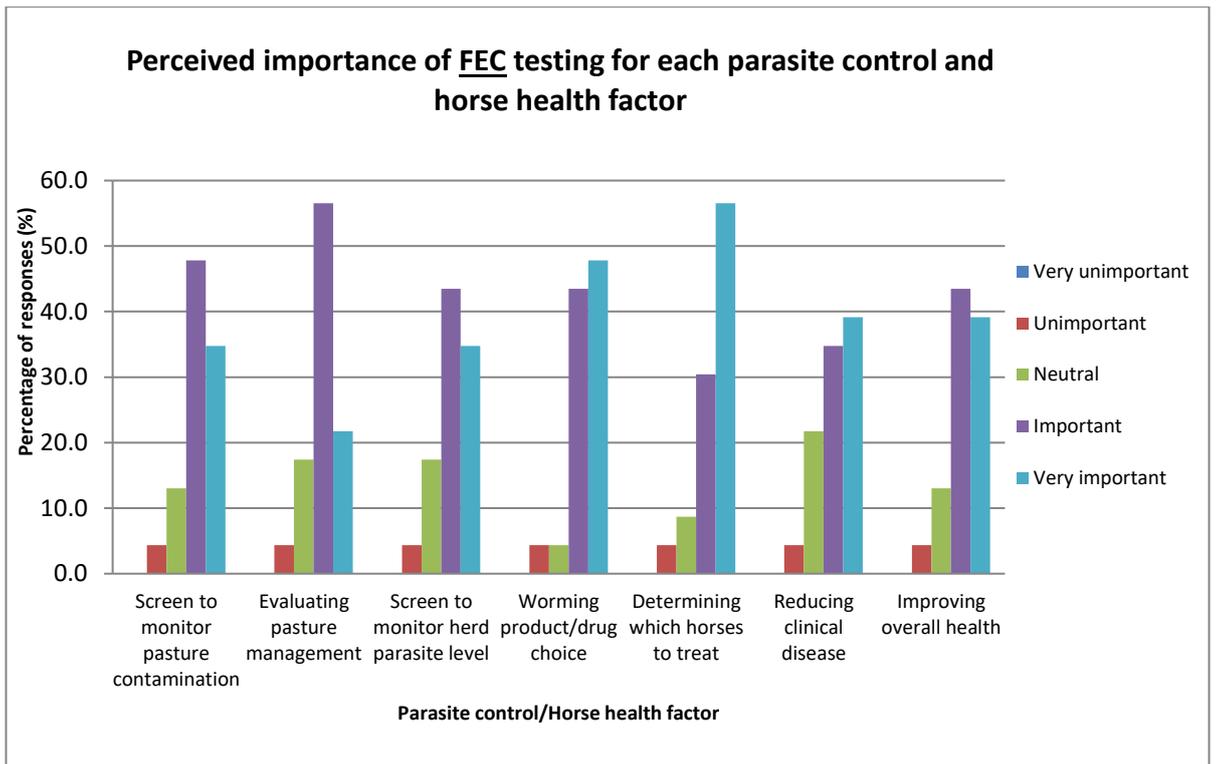


Figure 6.8 Perceived importance of FEC testing for a variety of factors important for controlling parasite transmission and herd health.

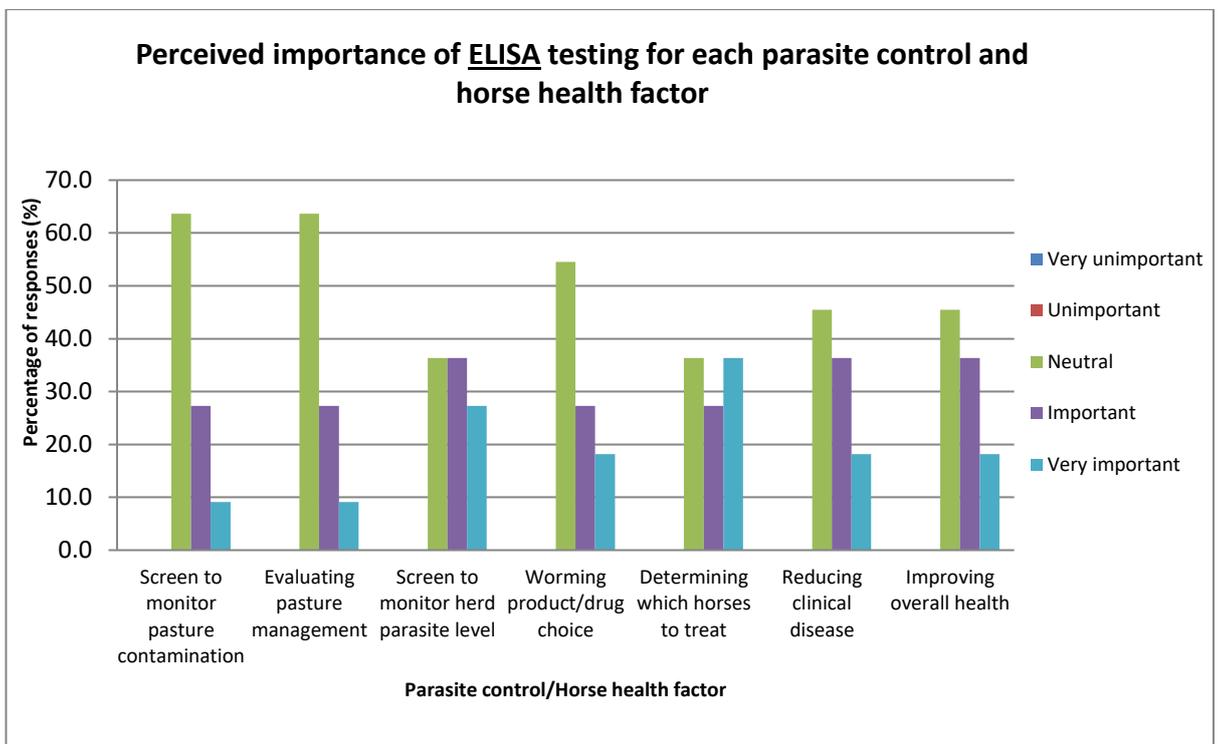


Figure 6.9 Participants perception of the importance of ELISA testing for a range of parameters involved in reduction of parasite transmission and herd health.

### 6.3.10 FECRT

Half (50%  $\pm$ 16.3%, n=18/36) of responding studs were aware of what a FECRT was with 27.3% ( $\pm$ 14.6%, n=10/36) having previously had one performed on their stud.

Approximately equal proportions of FECRT had been performed in the YS/foal combined age group compared to adults (Figure 6.10).

Of those performing FECRT, 30% of respondents ( $\pm$ 28.4%, n=3/10) tested one, two or six age categories with the remaining 10% ( $\pm$ 18.6%, n=1/10) testing three age groups. In 42.9% ( $\pm$ 36.7%, n=3/7) of tests, eggs were observed in post-treatment samples and resistance was reported to have been observed in all ages with the exception of four to six year olds and 11-14 year olds. When age classification is simplified as either foal, YS or adult, resistance was confirmed in all age classes with two instances in foal groups, two in YS and three in adult cohorts.

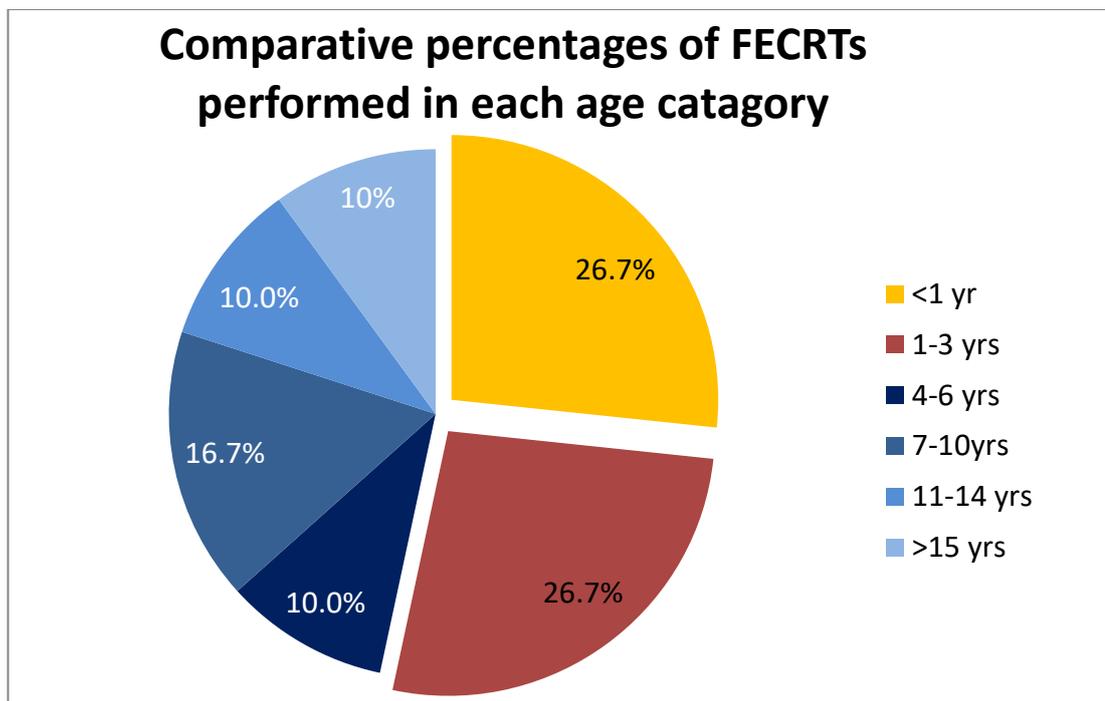


Figure 6.10 Comparative percentage of FECRTs performed for each age category with foals in yellow, YS in red and adults shown in blue sub-categorised by specific age.

### 6.3.11 Targeted selective worming – defining the protocol and personal experiences with targeted selective treatments

Of 35 participants who stated that they understood what was meant by targeted selective control strategies, 60.0% ( $\pm 16.2\%$ ,  $n=21/35$ ) answered correctly. A correct answer was defined as inclusion of the option: “basing all worming treatments on faecal egg counts performed every three to four months” or the same principle paraphrased under “other”. The most common combination was “basing all worming treatments on faecal egg counts performed every three to four months” with “using specific drugs each season to target specific parasites”.

Over half of the respondents (60.0%  $\pm 16.2\%$ ,  $n=21/35$ ) stated they currently used targeted selective treatments. However, 47.6% ( $\pm 21.4\%$ ,  $n=10/21$ ) of this sub-group of participants incorrectly defined the principle of this protocol; of the 40.0% ( $\pm 16.2\%$ ,  $n=14/35$ ) not using a targeted selective regime, 71.4% ( $\pm 23.7\%$ ,  $n=10/14$ ) selected the correct definition.

Of the 21 studs that considered themselves to be following targeted selective protocols, 70.0% ( $\pm 20.1\%$ ,  $n=14/20$ ) changed over more than two years ago, 20.0% ( $\pm 17.5\%$ ,  $n=4/20$ ) one to two years ago and 10.0% ( $\pm 13.1\%$ ,  $n=2/20$ ) less than one year ago. An equal number felt that either there had been no change or a decrease in worm egg counts overall since changing their strategy. When discussing specific species, the majority reported no change in strongyles (68.8%  $\pm 22.7\%$ ,  $n=11/16$ ), *Anoplocephala spp* (69.2%  $\pm 25.1\%$ ,  $n=9/13$ ) or *Oxyuris equi* (75.0%  $\pm 24.5\%$ ,  $n=9/12$ ) egg counts and 50.0% ( $\pm 24.5\%$ ,  $n=8/16$ ) felt there had been either no change or a decrease in *Parascaris equorum* FEC. The majority of respondents (57.9%  $\pm 22.2\%$ ,  $n=11/19$ ) performed fewer anthelmintic treatments with 26.3% ( $\pm 19.8\%$ ,  $n=5/19$ ) using the same number and the remainder (15.8%  $\pm 16.4\%$ ,  $n=3/19$ ) using more.

For all specified clinical syndromes, the majority of studs felt that there had been no change in incidence, a few respondents felt there was a decrease primarily of “worms in

faeces" (35.3% ±22.7%, n=6/17) but no studs recorded an increase of incidence of any clinical signs they considered to be related to gastrointestinal worm burdens.

All respondents were confident that their definition of targeted selective treatment controlled parasite transmission. All respondents stated that this regime had important beneficial impacts with 31.58% (±20.9%, n=6/19) stating one primary benefit, 26.3% (±19.8%, n=5/19) two or three benefits and 15.8% (±16.4%, n=3/19) specifying all four benefits as shown in Figure 6.11.

With the exception of a single stud, all respondents (94.7% ±10.0%, n=18/19) stated that they would recommend changing over to targeted selective treatment to others; the remaining stud stated that use of targeted selective treatment should be determined on a farm-by-farm basis, depending on individual circumstances and an individual risk assessment.

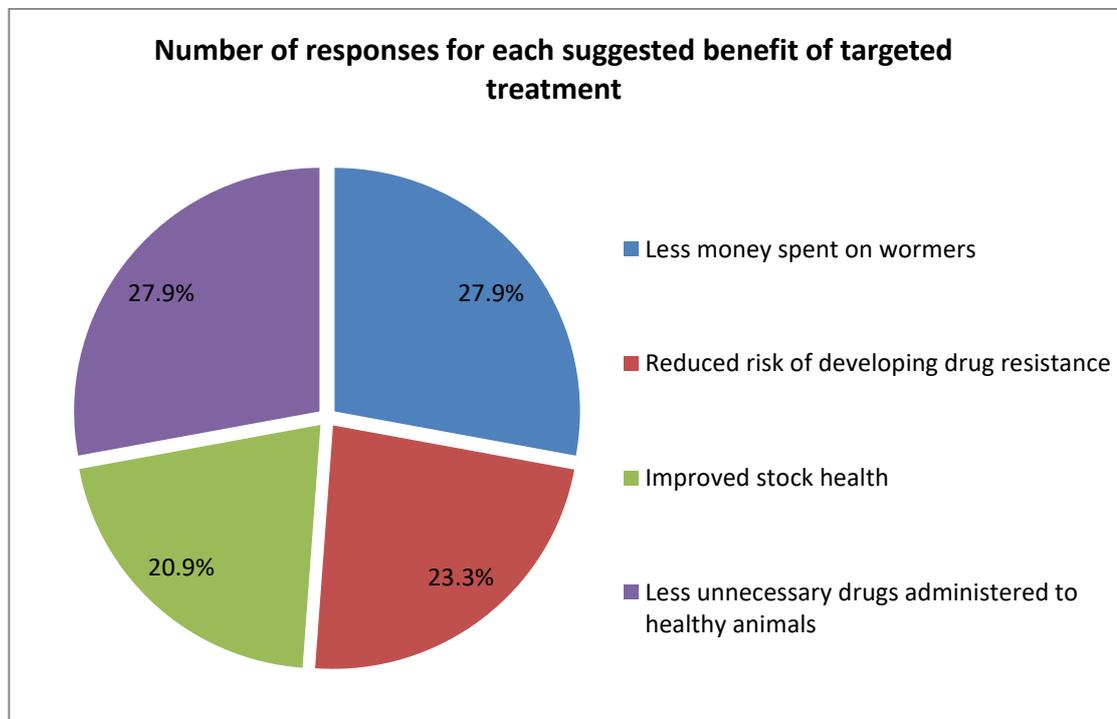


Figure 6.11 The overall number of responses for each suggested benefit of a targeted selective treatment regime.

### 6.3.12 Comments

A wide range of comments were added by nine participants; others respondents did not comment and so detailed analyses of these data were not performed. Two participants stated that they believed that changes in practice are not reaching, or being implemented, by larger equine establishments with two studs adding that they felt this was due to the relative ease of routine anthelmintic administration and low cost of anthelmintics. One stud reported that both veterinary and SQP training needed significant improvement and in many cases was “inadequate”, and that there should be increased availability of parasitology CPD.

## 6.4 Discussion

The aim of this study was to investigate if and how parasite control practices have changed on TB stud farms in the UK and to provide a comparison to previous data obtained in 2012 (Relf, et al., 2012). Such information enables researchers and veterinary surgeons to determine whether continued education of stud farms is required to try to maximise the use of best practice, particularly in reducing unnecessary overuse of anthelmintics and promotion of anthelmintic drug resistance. This study found that a greater proportion of UK TB stud farms utilised (or considered that they utilised) targeted selective use of anthelmintics. However, use of macrocyclic lactone drugs was high within all age classes, control strategies (i.e. interval or targeted selective) and pasture management regimes and FECRT testing remained uncommon.

There appeared to be a large increase in the proportion of UK TB studs stating that they used targeted selective treatment (60.0%  $\pm$ 16.2%, n=21/35) compared to 22% of study respondents (n=61) in the study by Relf et al (2012). However, when investigating what respondents understood by the term ‘targeted selective treatment’ it is more likely that around 30% of the studs surveyed were using truly targeted selective treatment strategies. This is an interesting finding and would appear to suggest that UK TB stud farm managers

are aware of the use of targeted selective treatments and are implementing altered strategies, compared to the traditional interval programmes. However, the findings of the study would also appear to show that information about how to implement truly targeted selective worming programmes is a key area of education for these establishments.

Within targeted selective protocols, pasture management is a key factor in reducing both pasture infection and intestinal parasite burdens. In the current study the majority of studs did not undertake regular (46.2%  $\pm$ 15.6%, n=18/39 “Never”; 18.0%  $\pm$ 12.0%, n=7 “occasional/sporadic” removal) or effective removal of faeces from pastures, with a large number still relying on sporadic harrowing in larger paddocks housing both adult and YS groups (excluding foals kept on “nursery” grazing). Harrowing has been demonstrated to distribute larvae across pasture and to increase the infective potential of paddocks in temperate climates such as the UK (Corbett, et al., 2014; Reinemyer, 2012) and so this is a major area for improvement. Barriers stated by studs in the current study comments, and reported by farms participating in the drug efficacy study (Chapter 4), regarding implementation of more appropriate methods such as faeces removal include having large paddocks incorporating a range of terrain and large overall acreage. The utilisation of both large (such as Nicholson paddock cleaner™) and smaller, more robust machinery (such as the Muck Truck™) could combat terrain issues and/or a return to historical systems of employment of “pasture grooms”, whose sole responsibility was pasture care and hygiene, may aid in relieving time constraints although the short term financial impact and viability of these measures would remain a concern for many studs. This combination of factors was stated by some respondents as being a reason why removal of faeces was unrealistic due to lack of staff-time and/or machinery suitable for the terrain. In addition, high costs on large establishments both in terms of staff-time and cost of mechanical equipment (Reinemyer, 2012) are likely to play a role. Pasture management was more frequently performed in nursery paddocks. The importance of regular, effective faecal removal as part

of pasture management strategies is therefore an area for further education and improvements. The cost-benefits of spending more money on pasture management should also be highlighted in educational material and should be weighed up against the economic savings of using targeted selective rather than interval strategies thereby reducing the costs of purchasing anthelmintics.

Another primary component of targeted selective management is the use of diagnostics for treatment decision-making during periods of potentially high transmission (April – Sept) and to test the efficacy of anthelmintics used (Kaplan & Neilsen, 2010). In the current study there was an apparent increase in the proportion of studs using FEC (80.6%  $\pm$ 12.9%, n=29/36) where 30.6% ( $\pm$ 15.0%, n=11/36) detailed undertaking FEC testing every two to three months in comparison to the study performed by Relf et al. (2012) in which no studs utilised FEC on a routine basis. Reporting of FEC was most usually performed by each stud's usual veterinary practice rather than sending them to independent laboratories (75.9%  $\pm$ 15.6%, n=22/29 veterinary practice; 13.8%  $\pm$ 12.6%, n=4/29 independent laboratories). This highlights the importance of appropriate veterinary advice based on correct interpretation of results and best practice regarding ongoing parasite testing and the importance of concurrent, suitable pasture management. Targeted selective treatment strategies should include FECs performed in adult stock at approximately six to eight week intervals. In the current study this interval (two to three months) was reported to be utilised most frequently. Although participants reported FEC to be 'very important' in treatment decision-making, it was evident that UK TB studs continue to administer anthelmintics regardless of the results or at extremely low thresholds (i.e. any positive result). This is a concern and suggests that utilisation of FEC more frequently has not necessarily correlated with a reduction in true anthelmintic usage. In contrast to FEC, very few studs in the current study conducted tapeworm ELISA testing and only a minority considered them to be important in relation to any parasite control and strategy

assessment factors. This is an area for future investigations to determine barriers to undertaking testing such as economic and time-related factors (e.g. serum ELISA and the need for a blood sample to be performed or costs of this being undertaken by a veterinary surgeon) or ease of use (e.g. saliva based test which requires feed and water to be withheld for 30 minutes beforehand).

FECRT are recommended for studs using anthelmintics within any parasite control regime in order to reduce the risk of disease and high burdens due to treatment failure. Within the current study almost all studs were concerned about anthelmintic resistance (97.1%  $\pm$ 5.7%, n=33/34), this is a higher percentage than seen in any previous studies on either UK TB or leisure horse population, which may indicate an increased awareness of this issue in UK TB studs (Relf, et al., 2012; Stratford, et al., 2013). A larger proportion of respondents had undertaken a FECRT in the current study (27.8%  $\pm$ 14.6%, n=10/36) compared to 12% (n=7/61) in a previous UK TB stud survey (Relf, et al., 2012) which may represent increased practical implementation of methods to prevent anthelmintic resistance.

Veterinary surgeons were stated by the majority of respondents in the current study to be the most utilised source of information about controlling intestinal parasites. However, the results of the current study suggest that best-practice methods, whilst being understood in principle, are not being undertaken in practical terms. Therefore, there appears to be potential knowledge gap or failure of knowledge transfer from research to veterinary surgeons or from veterinary surgeons to clients regarding the underlying theory and principles of targeted selective management. Specifically, there appear to be confusion regarding the use and interpretation of diagnostics and in veterinary surgeons providing correct, evidence-based advice to UK TB stud farms. As FECs and FECRT, are commonly reported to be performed by veterinary practices, and serum ELISAs require a veterinary surgeon for sample collection, there are many opportunities for discussion of thresholds,

potential and correct uses of diagnostics with owners, especially in light of stud owners reported preference for face-to-face interaction when seeking advice. These results emphasise a need for improvement in both veterinary and client education within the TB breeding industry regarding the practical usage of targeted selective strategies if such a strategy is to achieve its intended aims.

Similar studies conducted previously have considered the issue of quarantine but most have not requested specific information about the practical methods utilised to reduce potential parasite infective burdens on pasture. The results from the current study indicated that more studs travel their mares than accept visiting stock (80.6% [ $\pm 12.9\%$ ,  $n=29/36$ ] and 62.9% [ $\pm 16.0\%$ ,  $n=22/35$ ] respectively), and that studs accepting visiting stock had an average intake of 26 individuals per year with a wide range (2-70 individuals/year) from various stud farms. Duration of stay, and so potential impact on pasture contamination, varied between respondents. Where mares were sent away, they were usually away for an average of six to eight weeks, approximately equal to the PPP of cyathostomins. Therefore returning mares could represent a potential source of parasites making suitable management of these mares important in reducing the parasite burden. A smaller proportion of studs conducted quarantine for the over seven day period for returning mares compared to those visiting (63.3% [ $\pm 17.2\%$ ,  $n=19/30$ ] and 74.1% [ $\pm 16.5\%$ ,  $n=20/27$ ] respectively). Finances were reported to be utilised for FEC testing in returning mares whereas this was mostly focussed on anthelmintic purchase and blanket administration of these to visiting stock. A small proportion of studs (21.4%  $\pm 15.2\%$ ,  $n=6/28$ ) reported that they did not consider returning mares to require either treatment or testing, despite concerns regarding introduction of anthelmintic resistance and increased pasture contamination, which participants rated approximately equal in terms of importance to infectious non-reproductive and reproductive pathogens. This is another area for improved education.

Sources of advice on intestinal parasite control and purchase of anthelmintics have previously been investigated in mixed establishment studies, although in limited detail (Bolwell, et al., 2015; Hinney, et al., 2011; Lendal, et al., 1998; Lind, et al., 2007; Lloyd, et al., 2000; Papini, et al., 2015; Relf, et al., 2012; Robert, et al., 2015; Stratford, et al., 2013). Of those who sought advice in the current study, 71.4% ( $\pm 15.0\%$ ,  $n=25/35$  studs) used their veterinary surgeon as the primary source of guidance. This is similar to previous studies conducted on TB studs in which vets were used on 70-75% of studs (Relf, et al., 2012; Robert, et al., 2015). The next most frequent sources of information accessed by respondents in the current study were articles in equine publications and feed shops/wormer suppliers. It is not possible to compare previous TB studies, as data on these topics were not collected (Relf, et al., 2012; Robert, et al., 2015). Almost a fifth of respondents thought that no advice or information on anthelmintic administration was needed regarding changes in practice, as current methods are “tried and tested”. In contrast to the popularity of web-based systems for advertising and information transfer, internet sites and forums only constituted 1% of responses. Stud owners/managers appeared in the current study to favour a face-to-face, interactive, approach to obtaining advice on intestinal parasite control (51.4%  $\pm 16.6\%$ ,  $n=18/35$ ) compared to independent or indirect information gathering, through books, articles, internet, and commercial, feed shop/worming supplier or drug representatives. This potentially presents an optimal method for future knowledge transfer and the requirement for more hands-on bespoke parasite control strategy formulations involving face-to-face discussion.

In contrast to veterinary surgeons being the primary source of information about anthelmintics used, only 5.3% ( $\pm 7.1\%$ ,  $n=2/38$ ) of studs in the current study purchased these from their veterinary practice with online shops being most popular. This finding is likely to be due to the lower costs that online-only pharmacies are able to offer compared to the high mark-up (an average of 90-150%), on pharmaceutical products from veterinary

surgeries (Materni & Tumblin, 2013). Managers of TB studs may therefore have less opportunities to obtain advice on best practice by choosing to purchase anthelmintics from sources that are cheaper but do not include information or advice from veterinary professionals.

Specific sections that were not completed in part or full included the disease prevalence and aetiology section. The low response rate for this section may be due to a genuinely low disease prevalence or a reluctance of stud managers to disclose clinical issues. The latter may be perceived as resulting from poor management and therefore damaging to the stud's reputation, despite the confidential nature of the study. Studies have shown that answers perceived to be socially undesirable are under-reported, with questions that are deemed intrusive to be systematically refused or evaded by respondents (Foddy, 1993; Sacktt, 1979).

The current study is based on the responses from 39 UK TB studs. This was a relatively low response rate despite repeated attempts to increase uptake through re-distribution, multiple completion options and reminders. However, this was similar to the response rate in the study by Relf et al (2012). It was not possible to survey exactly the same respondents who participated in the Relf et al (2012) study which would have provided the most accurate information about how practices have changed between the two time-frames. However, the methods by which TB studs were identified and contacted was similar and the current study had a similar geographic distribution of participants compared to the Relf et al (2012) study. This was a relatively long questionnaire that requested detailed information which may have reduced the response rate due to time constraints. Timing of studies such as this, within a time-pressured context such as stud farms, is challenging. The study was designed to be distributed "out of season" when no foaling or covering was taking place. The initial deployment period (October-December) however coincided with

foal and yearling sales, resulting in many managers and owners being absent during the primary contact periods (telephone interviews and contact confirmation). Similar studies, such as the study conducted by Relf et al (2012) were also conducted relatively recently, resulting in some participants feeling that, as they had already spent time completing a similar survey/s, further participation would be of no benefit to them. During discussion, managers also felt that their time was too limited and valuable for repeated questioning on the same or a perceived similar topic. It is also important to consider that those who chose to complete the questionnaire considered this topic to be of greater importance and so respondents may be biased towards TB studs that have more current, best practice policies on controlling intestinal parasites.

Questionnaires, although useful in order to assess population level practices and beliefs, have many sources of bias that need to be taken into account when interpreting the results (Choi & Pak, 2005). A separate study found that telephone questionnaires, the first strategy in the current study, were more likely to be met by increased suspicion and encourage participants to present a more socially desirable image than when conducted in person. The former observation was noted during the course of the current study and the consequent low uptake, combined with the difficulties of arranging a suitable time to speak with the manager were the driving forces for the change in format from telephone to multi-format completion choice (Holbrook, et al., 2003). Multiple formats for questionnaire completion make surveys as accessible and inclusive as possible (Choi & Pak, 1998).

Overall, this study found that concerns about anthelmintic resistance appear to have increased compared to the previous study of UK TB stud farms (Relf, et al., 2012).

Veterinary surgeons remain the primary source of information, although purchasing habits appear to be changing in order to reduce stud overhead operational costs (Relf, et al., 2012). The uptake of targeted selective programmes appears to be increasing on UK TB

stud farms but more education directed towards to stud managers and veterinary surgeons with particular emphasis on implementation of removal of faeces as part of pasture hygiene rather than harrowing, the least effective method in reducing pasture infective potential (Corbett, et al., 2014), and the correct use of diagnostic testing is needed. The use of FEC appears to be more frequently utilised but there appears to be a lack of knowledge about interpretation of FEC results and un-necessary use of anthelmintics appears to be common. FECRT was more frequently performed on the studs surveyed in the current study compared to previous studies, indicating a potential trend towards improved awareness of sustainable parasite control on UK TB stud farms. The reliance on the macrocyclic lactones also continues which is a concern regarding development of anthelmintic resistance. Quarantine is an area that respondents considered important in reducing infectious disease on UK TB studs but development of appropriate protocols that can be implemented on UK TB studs appears to be another potential area for improved education within the industry.

# Chapter 7 – General Discussion

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This chapter will summarise the research objectives, significant findings and conclusions of the study. Within this section recommendations related to the current study and future research requirements will also be addressed.

Helminth infection has a significant impact on the health and welfare of managed horses. Infection is ubiquitous in grazing horses and, without effective preventative treatment, can result in serious disease. Most commonly, disease is characterised by a multitude of non-specific signs such as weight loss, diarrhoea, colic and ill-thrift (Feary & Hassel, 2006).

Control of infection and disease has historically relied on anthelmintic drugs, administered at regular intervals based on the ERP for a given drug at the time of licensing. This strategy, known as interval dosing, became best practice in the 1960s due to a high prevalence of *Strongylus vulgaris* and related disease, thromboembolic colic (Drudge, et al., 1966; Slocombe, et al., 1973; Slocombe, et al., 1976; Tolliver, et al., 1987). The introduction of the macrocyclic lactone class of anthelmintics, which showed persistence of action and a high degree of efficacy, in combination with regular dosing, reduced the prevalence and incidence of *S. vulgaris* – associated disease, through suppression of egg output and reduced transmission (DeLay, et al., 2001; Drudge, et al., 1966; Duncan, et al., 1991).

However, interval dosing has been a factor in promoting anthelmintic resistance in parasite populations; a particular challenge is that resistance threatens the control of other nematode parasites of importance such as the cyathostomes and *Parascaris equorum*.

Within the cyathostomin species, resistance to the BZ class is now considered to be widespread, with this class of anthelmintics no longer recommended for use. FECRT studies have shown 40-50% of farms across Europe and the USA harbour PYR resistant cyathostomin populations, in addition to multi-drug resistant strains (Table 1.4, Chapter 1).

The most concerning development is documentation of reduced ERP values for the

macrocyclic lactone class, specifically IVM and MOX (Table 1.4, Chapter 1). In contrast to cyathostomin species, it is the macrocyclic lactone class which requires performance of a FECRT if it is to be used for control of *Parascaris spp* due to widespread resistance reports from post mortem studies, disease case reports and FECRT; approximately 50% of *Parascaris spp* populations in Europe and the USA have shown resistance and reports of reduced ERP in multiple studies and there are also some concerns about concurrent resistance to BZ class drugs for this parasite, although this remains to be widely confirmed (Table 1.4, Chapter 1). Horses remain susceptible to patent infection by these helminths throughout their lives exposure resulting in the need for life-long parasite control, with the exception of *Parascaris spp* where immunity usually develops by 18-24 months of age. In addition, a potential role for *Fasciola hepatica* in what is currently classified as idiopathic hepatic disease is emerging, posing a new element for consideration in preventative parasite control programmes.

With no new drugs currently in development for use in horses, conservation of currently available drugs is of primary importance. Best practice reflects this through increased reliance on evidence-based administration, through targeted selective treatment and limitation of exposure where possible through effective pasture management. Many challenges exist when considering widespread uptake of targeted selective strategies: tailor-made farm parasite control programmes, including FECRT to determine drug efficacy on stud; development of financially viable, sensitive and specific diagnostic tests, especially for detection of prepatent infection; the uptake of effectively implemented targeted selective regimes and dissemination of accurate information for effective implementation on TB stud farms.

Most currently available diagnostic tests rely on faecal analysis which have inherent limitations which require improvement before targeted selective regimes can be pursued.

Faecal diagnostics rely on presence and detection of patent infection, removing their utilisation for detection of potentially pathogenic prepatent burdens in the case of cyathostomes, *S. vulgaris* and *F. hepatica*. Individual daily variability in the number of eggs shed, lack of even distribution around the faecal pile and hatching of larvae prior to testing can introduce significant errors in results, meaning that FEC can only be seen as a single time point value with reliability dependant on optimal sampling and handling (Lester, et al., 2012; Nielsen, et al., 2010). In addition, techniques vary in their LDL, sensitivity, specificity and species of parasite detected (Table 1.1, Chapter 1). FECRT is currently the pre-mortem gold standard for evaluation of drug efficacy (Nielsen et al., 2013), limited by the lack of equine specific cut off values as well as the potential sources of error previously discussed (Chapter 1, section 1.5.3.1).

An ideal faecal diagnostic test would display high sensitivity and specificity, low LDL and detection of a wide range of parasite species. Within the current study we demonstrated that the CF technique may present a potential solution. The CF technique is already validated for detection of strongyle and ascarid eggs, with a detection limit of one egg (Bartley, et al., 2011), and can detect *Strongyloides westerii* eggs (Relf, et al., 2013). The current study demonstrated its potential for the detection of *A. perfoliata* eggs in a similar way to the current faecal diagnostic of choice, DCF (Chapter 3). By detecting of a range of parasitic species this test shows potential as an efficient multi-species detection screen. The limitation of this technique is expense of equipment necessary, potentially reducing the use in general equine practice and so availability to TB studs.

The McMaster technique is a widely available faecal screen, requiring little specialist equipment and time to perform, detecting strongyle and ascarid eggs with LDL's of 15-100epg (Lester & Matthews, 2014). The extension of this test to provide a screen for *F. hepatica* eggs is a potentially important addition to diagnostic screening. Co-grazing of

pasture with ruminant stock is commonly practiced, shown in the current study (82.1%  $\pm$ 12.0%, n=32/39), presenting a potential risk for liver fluke in horses. It is thought that the prevalence of infection in horses may be higher than previously thought and so an accessible and inexpensive surveillance method could encourage uptake amongst stud owners.

Serological testing for *A. perfoliata* has been available for several decades, utilised at a herd level to assess exposure. The variability of antibody response and subsequent half-life is a limitation of this test, appearing in the current study to result in under-utilisation (36.1%  $\pm$ 15.7%, n=13/36). A new commercially available diagnostic test is the Equisal™ salivary antibody ELISA, which shows good sensitivity and specificity compared to the currently available ELISA; one major advantage for owners is that they are able to sample and test independently of their vet, increasing accessibility by reducing cost. This test demonstrates a positive correlation with post mortem and paired pre-validated serum ELISA results. Interpretation is issued with results, giving owners guidance on treatment requirements. However, the current study showed that TB stud owners/managers appear to prefer face-to-face interaction, with the most common source of information being their veterinary surgeon (37.9%, n=25/66 responses). The “direct to owner” approach for interpretation and advice potentially reduces contact with vets, therefore limiting their impact on the development of bespoke best practice targeted selective parasite control programmes, incorporating site-specific drug efficacy and conservation of anthelmintics through more targeted selective approaches to treatment through inclusion and correct use of new diagnostic tests.

An encouraging result regarding sustainability of parasite control is the apparent increase in implementation of targeted selective regimes compared with Relf et al’s (2012) findings: 60.0% ( $\pm$ 16.2%, n=21/35) in the current study compared to 88% using interval treatment

with 0% routinely utilising FEC (Relf, et al., 2012). Stud owners/managers in the current study were unanimously “very concerned” regarding anthelmintic resistance. However, despite this concern the level of anthelmintic use, specifically reliance on macrocyclic lactones (ML), does not seem to be reducing, the current study showed on 71.4% ( $\pm 12.6\%$ ,  $n=35/49$  responses) of studs ML or ML-PRAZ combination products were most commonly used (Chapter 6). Many studs considered themselves to be using targeted selective regimes but when asked could not identify the key principle of this control format (60.0%  $\pm 16.2\%$ ,  $n=21/35$  of those stating they used targeted selective control). Those studs which engaged with FEC for routine surveillance saw reduced ERP values, interpreting this as a requirement for more frequent anthelmintic administration rather than investigating the cause, demonstrating a key area for education and improvement in the use of diagnostics. In addition, within both the anthelmintic efficacy and questionnaire studies those studs using targeted selective regimes used a wide range of treatment cut-off values from  $>0$  to 200epg. Cut-off values noted are below currently accepted treatment thresholds defining “high shedders”, noted as 200-500epg (Nielsen, et al., 2013). The current study demonstrated significant areas for improvement in implementation of best practice parasite control, particularly in regard to pasture hygiene, with 46.1% ( $\pm 15.6\%$ ,  $n=18/39$ ) never removing faeces from pasture, and correct use of FEC including treatment thresholds. The use of FEC for diagnosis, or on suspicion of, disease was reported on 17.1% ( $\pm 12.5\%$ ,  $n=6/35$ ) studs surveyed (Chapter 6), highlighting the importance of education regarding the use and interpretation of FEC results.

Many of the studs included in the anthelmintic efficacy section of the current study presented with clinical concerns, with further testing demonstrating differences in drug efficacy between cohorts on the same stud. This emphasises the need for a bespoke approach to formulation of parasite control regimens. This type of approach screening for presence of parasite species with subsequent age-specific drug efficacy evaluation can

allow for the conservation of macrocyclic lactones through use of alternative adulticidal products during high transmission periods, once efficacy has been proven, in addition to more effective YS preventative treatment through targeting treatments to parasite species present.

Bespoke approaches also allow for in-depth assessment and discussion of non-therapeutic based control strategies such as pasture management. Through evaluation of overdispersion of cyathostome FEC in adult populations, quoted as 20-30% of individuals carrying 80% of the total worm burden (Crofton, 1971; Anderson, et al., 1978; Shaw, et al., 1995; Shaw, 1998), effectiveness of pasture management strategies can be assessed. Relf et al (2013) and Lester et al (2013) demonstrated that on well managed farms, 11-15% of adult stock excrete 80% of total egg output. Bespoke approaches may facilitate uptake of these more holistic approaches to parasite control through provision of evidence to stud managers to encourage implementation of improved pasture management and responsible anthelmintic use through highlighting stud specific resistance issues. This degree of input creates an increased opportunity for discussion and monitoring to allow vets greater input into preventative healthcare programmes, as is currently the case for cattle and sheep “herd health” programmes. These programmes have shown success in cattle and sheep with standardisation and publication of guidelines such as Sustainable Control of Parasites in Sheep (Sustainable Control of Parasites in Sheep (SCOPS) Group, 2012) and Control of Worms Sustainably (COWS, 2012); there is a significant gap in standardised guidelines for horses in the UK, with the only current guidelines published by the American Association of Equine Practitioners (AAEP). The challenge in production and implementation of guidelines is the unique nature of varying horse industries with one blanket policy not necessarily appropriate for all establishments, especially as anthelmintic sensitivity within cyathostome and *Parascaris spp* populations may vary from site to site, the impact of horse age demographics present on site and a wide range of other site specific factors.

Standardisation of elements such as FEC thresholds indicating a need for treatment and efficacy cut-off values specific to individual parasite species would be advantageous to promote consistency in practice and allow for confident implementation of targeted selective regimes but further research is required to provide a firm evidence base for these values.

An important potential barrier to implementation of bespoke herd health type parasite control programmes is the level of knowledge and confidence of vets regarding best practice parasite control and interpretation of diagnostic test results especially when requiring a case by case individualised approach. Another important source of information for many owners which must be considered are SQPs. As contact is often face-to-face during anthelmintic purchase, which the current study has shown does not often occur at veterinary practices, SQPs are advantageously positioned to provide advice on targeted selective protocols. As for vets this relies on accessible, consistent and accurate CPD to allow for optimal dissemination of reliable and consistent advice. The provision of combined CPD for vets and SQPs could potentially facilitate consistency in the advice given and accessibility of information, with free of charge webinars and seminars being used as a short term incentive to increase uptake. This approach may also help to bridge any potential gaps between research and prescribers for increased confidence in dissemination of information to owners. Within large animal practice there is a large knowledge and practical base for herd health planning which has, so far, been underutilised within the equine field, a more multidisciplinary approach with adaptation of software and knowledge transfer between large animal practitioners and other professionals may aid in time-efficient bespoke whole herd planning.

While these approaches could help in moving forward our approach to equine parasite preventative strategies, yard owners/managers and individual owners must also be actively

involved and considered in order to increase compliance, ensuring that the knowledge is not only being effectively transferred but used. This, in the authors opinion, relies heavily on active face-to-face interactions of vets and other professionals with owners, allowing a forum for open questioning and discussion as an increase in passive advice (i.e. magazines, internet sites, owner-focused CPD webinars) can only go so far, although remains a useful secondary approach. A direct, active approach is especially challenging within the leisure horse industry, where all horses on a single yard may have different owners. In this situation, arranging regular yard meetings to monitor implementation, at least in the initial phase, may be of great benefit. However, these active approaches require time and potentially incur a cost for yards/owners which may not be feasible. The involvement of pharmaceutical companies, which have knowledgeable representatives who are often ambulatory and have good communication skills, could potentially bridge this gap but would still need to liaise with the farms veterinary surgeon in order to maintain consistency for monitoring. The provision of clear guidelines and rationale in an owner-friendly format could provide a linkage for follow-up discussions and planning where regular meetings are not possible. A hybrid approach of active and passive knowledge transfer and dissemination of educational materials to owners could also involve the next generation of yard staff and horse owners through The Pony Club; seminars within owner-focused conference events such as “Your Horse Live” or large competitive events. For each of these solutions there are practical aspects to consider, however, until interventions are attempted an optimised strategy cannot be formulated.

With an optimisation in uptake of implementation of new practices we must also consider future research which is required to ensure that best practice continues to improve, and does not neglect parasites of importance. With the increased awareness of the potential impact to equine health of *Fasciola hepatica*, especially with the recommendation of ruminant co-grazing, the continuation in development of diagnostic tests which are

financially viable is essential. For cost-effective implementation, the development of combined assays may be an avenue for investigation, for example a single salivary or serum sample which can be used for multiple parasite tests, may be positive in terms of implementation and compliance. More reliable tests for parasites currently of secondary concern in the UK, such as *Strongylus spp*, potentially may prevent a resurgence in clinical impact in combination with a worldwide consensus on optimal preventative protocols while still using anthelmintics as conservatively as possible. All of these potentially important advances focus on a practical, accessible, cost-effective and holistic approach to parasites of concern on UK equine establishments.

In advancing our approach to parasite detection, surveillance and preventative strategies the next point to consider is how we can assess the impact of these interventions. Currently, the Department for Environment, Food and Rural Affairs (DEFRA) and the National Animal Disease Information Service (NADIS) compile and publish disease reports. DEFRA's quarterly equine disease surveillance report contains figures from large commercial laboratories on the number of samples tested and positive results, the expansion of this to include as many laboratory facilities as possible would aid in monitoring of uptake in diagnostic testing. Data from this source does not however provide information on how diagnostics are being used and disease surveillance data from NADIS relies on reporting by practitioners; increased information gathering from both of these sources requires an easy user-friendly interface in addition to emphasis on laboratories and clinicians regarding compliance with communication. Regular surveying of horse/yard owners with year on year compilation of data may aid in demonstrating any shifts in practices through retrospective studies after a given period. Short surveys linked to both veterinary and owner websites as well as major social media platforms could aid in ongoing data capture, and is used by many organisations in the form of single question interactive polls. The collection of clinical data from first opinion and referral practices would add an

important element to diagnostic testing figures and survey results to form snapshots at regular intervals, although clinical data must be interpreted cautiously until more reliable diagnostic tests are developed due to often non-specific clinical syndromes. Collection of abattoir findings, as for ruminants, would add to this although currently only one UK abattoir processes horses resulting in potential bias and a limited sample size.

Interpretation of clinical (including abattoir) data should be done with care in order to account for potential lag-phase in findings as practices change. A fully rounded approach would require central co-ordination to allow for development of comparable datasets and organised data capture, a national working group could aid in ensuring collection and analysis of such information.

In conclusion, there are many areas for improvement and barriers to implementation of targeted selective and sustainable parasite control regimes which have been highlighted by the current and previous studies (Relf, et al., 2012; Relf, et al., 2013). The improvement of diagnostics to provide tests which are sensitive and specific; allow for multi-species detection of both patent and prepatent infection; are widely accessible and financially viable are key factors in the reduction of anthelmintic use. However, with advancement in diagnostics to provide targeted selective treatment plans, the knowledge gap in interpretation of results and the relationship between parasitic burden and disease must be addressed. Due to the multitude of factors involved in control of parasite burdens with reduced use of drugs, presence of multiple species, increasing anthelmintic resistance concerns and complex nature of TB studs, a bespoke approach to implementation of targeted selective treatment is required. The need for the reduction in the use of anthelmintics and change in preventative strategies is evident from widespread resistance in multiple parasitic species. Although awareness of anthelmintic resistance and the need for change in use of currently available drugs appears to be increasing, implementation and understanding of targeted selective protocols is an area where further dissemination of

information from the research community to vets and stud farms, followed up with careful monitoring of parasite species prevalence and clinical impact as uptake improves, is needed to ensure maintenance of stock health and welfare.

# Appendix 1A

Country	Prevalence ( <i>A.perfoliata</i> )	Study Population Size	Author
United Kingdom Studies			
UK	58%	103	Owen et al., 1988
UK	80%	20	Pearson et al., 1993
Ireland	51%	363	Fogarty et al., 1994
UK	52%	81	Morgan et al., 2005
UK	55%	75	Pittaway et al., 2014
European (non-UK) Studies			
Albania	10%	240	Velaj et al., 2014
Bavaria	25%	127	Beelitz & Gothe, 1997
Bavaria	38%	100	Beelitz & Gothe, 2001
Belgium	28.9%	270	Agneessens et al., 1998
Central Europe	7.2%	724	Tomczuk et al., 2015
Germany	28.5%	2,013	Rehbein et al., 2013
Netherlands	21%	70	Borgsteede & van Beek, 1996
Norway	20%	201	Ihler et al., 1995
Poland	4%	50	Gawor, 1995
Spain	22%	107	Meana et al., 1998
Spain	24%	372	Meana et al., 2005
Spain	23%	121	Rodriguez-Bertos et al., 1999
Sweden	65%	470	Nilsson et al., 1995
The Americas and Australia			
Australia	32%	57	Mfitalodze & Hutchinson, 1989
Australia	62%	138	English, 1979
Australia	4.9%	140	Dunsmore & Jue Sue, 1985
Australia	29%	150	Bucknell et al., 1995
Australia	38.5%	130	Williamson et al., 1997
Brazil	21.2%	310	Sangioni et al., 2000
Canada	0-13%	434	Skotarek, 2008
New Zealand	82%	65	Bain & Kelly, 1977
USA, Kentucky	54%	363	Lyons et al., 1983
USA, Kentucky	53%	396	Lyons et al., 1984
USA, Kentucky	Foals = 30% Yearlings and above = 60%	Foals = 87 Yearlings and older = 186	Lyons et al., 1987
USA, Kentucky	64% for TBs 54% for non- TBs	118	Benton & Lyons, 1994
USA, Kentucky	100%	16	Lyons et al., 2006
USA, Louisiana	46.7% (1986) 52.1% (2000)	117	Chapman et al., 2002
USA, Massachusetts	32%	28	Beroza at al., 1986
USA, Ohio	18%	55	Reinemyer et al., 1984

# Appendix 2A

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## Faecal Sampling

### Sample collection

1. Sub-sample from 3-5 separate areas (or three whole "pellets") from a fresh, <4 hours old, faecal pile (figure 1)
2. Expel all air from the bag and store at 4°C prior to shipping
  - Squeeze faeces into the bottom of the bag and roll bottom to top while applying pressure to remove air, then seal.
    - This creates an airless (anaerobic) environment to delay development of eggs and provide a more accurate result.
3. Send to the laboratory as soon as possible.

**Figure 1 – Example sampling sites**



Cara Hallowell-Evans

 Demarcates example sampling sites within a faecal pile.

# Appendix 4A

1/12/2016

Study participation

## Stud Information

Hello and thank you for participating in our study, your contribution is extremely valuable to our work. This study is being conducted by the University of Liverpool, funded by the HBLB. This section concerns basic details of your stud farm. All results are kept securely and confidentially. Data will be analysed and anonymised prior to inclusion in the PhD thesis and all scientific publications. Thank you for your time.

1. Stud name
2. Stud postcode
3. Email
4. Veterinary Practice
5. How many of the following **permanently reside** at your stud?
 

Stallions	<input type="text"/>
Geldings	<input type="text"/>
Companion horses/teasers	<input type="text"/>
Broodmares	<input type="text"/>
Youngstock (1-3 years old)	<input type="text"/>
Foals	<input type="text"/>
Donkeys	<input type="text"/>
6. Does your stud accept **visiting** equines?

## Equine movements & quarantine procedures for visiting equines

This section looks at your quarantine policy when your stud farm receives visiting ADULT equines.

7. On average, how long do visiting equines stay?
  - Varies depending on individual horses/owners
  - 1 year
  - 1 month
  - 1 week
  - Other (please specify) / Additional comments
8. Which of the following checks are performed **pre-arrival for visiting stock**?
 

	Every visiting horse	Stallions only	Mares only	Overseas mares only	Overseas stallions only	Preferred but optional	Occasionally/Random checking	Never requested/checked
Pre-arrival faecal egg count	<input type="checkbox"/>	<input type="checkbox"/>						
Recent (3 month) worming history	<input type="checkbox"/>	<input type="checkbox"/>						
Parasitic disease history of home premises	<input type="checkbox"/>	<input type="checkbox"/>						
9. Are visiting equines wormed **on arrival**?

<https://survey.liv.ac.uk/Print.aspx?SurveyID=88K384L&Title=N&Breaks=N&AllPages=Y&Pages=>

1/13

- Yes
  - No
  - Other, please specify
- 

10. Are visiting mares...

- Grazed alone in a small paddock
  - Grazed with other visiting mares only - AFTER quarantine
  - Grazed with other visiting mares only - NO quarantine
  - Grazed with resident stock - AFTER quarantine
  - Grazed with resident stock - NO quarantine
  - Visiting mares are only stabled/have no access to grazing
  - Other, please specify
- 

11. Are **visiting** equines quarantined and for how long?

- Yes - >7 days
- Yes - 7 days
- Yes - <7 days
- Yes - 1-2 days
- Yes - <1 day
- No

**Quarantine Protocol for Visiting Stock**

This question is to look at the level of quarantine you feel is necessary for your stud farm when receiving visiting ADULT equines.

12. Are VISITING horses in quarantine kept...

- Turned out only
  - Stabled only
  - Mixed, please specify
- 

13. Which of the following are part of your quarantine protocol?

	Always/Every visiting horse	Only if home site has history of parasitic disease	Only if pre-arrival faecal egg count is positive	Occasionally	Rarely	Never
Faecal egg count performed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wormed based on FEC results	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wormer administered for all visiting equines prior to turn-out	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wormer administered for all visiting equines but NO turn out	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**Resident ("home") equines travel**

This section considers your ADULT MARES which travel to other studs for breeding or boarding purposes.

14. Do **your stock** visit other premises?

**Resident ("home") equines travel**

This section considers your ADULT MARES which travel to other studs for breeding or boarding purposes and quarantine (if any).

15. On average, how long do **home** equines stay on **other premises**?

- Less than a week
- 1 week
- 1 month
- 1 year
- Stud season

- Depends on individual horse
  - Other (please specify) / Additional comments
- 

16. Are your stock quarantined upon **return to home premises**?

- Yes - > 7 days
  - Yes - 7 days
  - Yes - <7 days
  - Yes - 1-2 days
  - Yes - <1 day
  - No/Not necessary
  - Other (please specify)
- 

17. Upon return (whether in quarantine or not) is a wormer administered...

- Yes - always
- No
- If faecal egg count is high

18. Do you perform faecal egg counts on returning stock prior to turn-out?

- Yes
- No
- Only if suspicious of disease/high burden

19. How concerned are you about returning mares introducing the following onto your stud...

	Very concerned	Slightly concerned	Not concerned
Infectious non-reproductive disease	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Infectious reproductive disease	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Parasites resistant to worming drugs	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Increased parasitic pasture contamination	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

20. Considering mares which travel frequently to other studs, when at "home" are they...

- Grazed with other frequently travelled mares - same age group
  - Grazed with other frequently travelled mares - mixed age group
  - Grazed with non-travelling resident stock - same age group
  - Grazed with non-travelling resident stock - mixed age group
  - Other, please specify
- 

**Quarantine and Re-integration protocol for returning stock**

These questions relate to quarantine and re-integration protocols for returning ADULT stock.

21. Are RETURNING horses in quarantine kept...

- Stabled only
  - Turned out only
  - Mixed, please specify
- 

22. Which of the following are part of your quarantine and re-integration protocol?

	Always/Every returning horse	If site of visit has history of parasitic disease	If mare has high egg count on return	If suspicious of disease	Occasionally	Not necessary
Faecal egg count performed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wormed based on FEC results	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wormer administered to all in quarantine pre-turn out	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wormer administered to all prior to turn-out	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

### Grazing & Pasture management

These questions look at your current grazing and pasture management strategies.

23. Approximately how many acres of land do you own, rent or use for equine grazing purposes?

24. Do you co-graze visiting and resident equines?

- Yes  
 No - separate grazing  
 No - no visiting equines  
 Other, please specify

25. How frequently are grazing areas on your establishment...

	Daily	Weekly	Monthly	Yearly	Occasionally	Never
Grazed by cattle or sheep	<input type="radio"/>					
Harrowed and/or clipped	<input type="radio"/>					
Rested from grazing	<input type="radio"/>					
Rotated between groups of equines	<input type="radio"/>					

26. On average, how much access to grazing (hours per day - hrs/d) do horses have during...

	0 hrs/d	1-5 hrs/d	6-10 hrs/d	11-15 hrs/d	16 - 24 hrs/d
Winter (December - February)	<input type="radio"/>				
Autumn (September - November)	<input type="radio"/>				
Summer (June - August)	<input type="radio"/>				
Spring (March - May)	<input type="radio"/>				

27. How often do you remove faeces from pasture?

- Occasionally  
 Monthly  
 Fortnightly  
 Weekly  
 Twice weekly  
 Daily  
 Never  
 Differs seasonally - please specify

28. How are faeces removed from pasture?

- Machine  
 Manually  
 Mixture of both

### Parasites of concern and related disease

This section asks about your perception of the risk posed by each parasite, how concerned you are about their presence within your stock and related disease.

29. How concerned are you would be about the presence of each of the parasites below, in high or low numbers, on your stud farm?

	Study participation		
	Very concerned	Little concern	No concern
High burden of Small strongyles (Cyathostomins)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Low burden of Small strongyles (Cyathostomins)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
High burden of Large strongyles (e.g. Strongylus vulgaris)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Low burden of Large strongyles (e.g. Strongylus vulgaris)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
High burden of Roundworm (Parascaris equorum)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Low burden of Roundworm (Parascaris equorum)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
High burden of Tapeworm (such as Anoplocephala perfoliata)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Low burden of Tapeworm (such as Anoplocephala perfoliata)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
High burden of Liver fluke (Fasciola hepatica)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Low burden of Liver fluke (Fasciola hepatica)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

30. Have there been any instances of worm-related illness at your stud?

### Parasite-related disease

These questions focus on incidents of parasite-related disease, whether diagnosed or suspected, on your stud.

31. Have there been instance of any of these worm-related illnesses at your stud?

Please tick all that apply

	Personal/Staff opinion	Veterinary opinion	Veterinary diagnosis
Poor performance	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Worms in faeces	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diarrhoea - lasting 1-4 weeks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diarrhoea - lasting <1 week	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Loss in/failure to gain condition	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lower weight gain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Weight loss	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Larval cyathostomosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Colic	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

32. On average, how many cases of parasite-related disease have you had in the past 2 years?

33. Have you noticed, on your stud or from speaking to others, an change in the number of parasite-related disease cases?

Please tick all that apply

- Yes - increase on my stud  
 Yes - more reports in the local area  
 Yes - decrease on my stud  
 Yes - decrease in reports in the local area  
 No change

34. Of the parasites listed below which do you consider to be/have been diagnosed as being responsible for periods of illness within your equines?

Please tick all that apply

- Small redworm (Cyathostomins)  
 Large strongyles (e.g. Strongylus vulgaris)  
 Roundworm (Parascaris equorum)  
 Tapeworm  
 Liver fluke (Fasciola hepatica)  
 Other, please specify

**Wormer administration and advice**

35. Are wormers administered to horses at your stud?

**Worming advice and drug purchase**

This section aims to determine your sources for worming advice and drug purchase, along with what is most important to you when considering which product to buy.

36. What is the MOST IMPORTANT factor to you when choosing which drug/product to use?

- Price
- Veterinary opinion
- Advice from SQP/Pharmacist within veterinary practice
- Advice from SQP/Pharmacist within feed shop
- Online reviews and forums
- Advice from experienced friends/family
- Positive personal experience with product/brand

37. Do you receive ALL advice and purchase ALL worming products from your veterinary surgery/team?

- Yes - Advice and worming products ALL from vets (no other sources for either)
- Some advice and wormers - other sources used in addition
- Advice only - All wormers purchased elsewhere
- All worming products only - Advice from elsewhere
- No veterinary advice and no wormers purchased from vets

**Information on worming**

This question is aimed towards defining where you gather information about worming practice and drug choices.

38. Where do you obtain information on worming?

Please tick all that apply

- None needed - existing strategy works well
- Feed shop/Worming supplier
- Drug representatives
- Articles in equine magazines
- Friends and family
- SQP in shop
- SQP/Pharmacist at veterinary practice
- Veterinary surgeon
- Other, please specify

39. Where do you BUY your worming products?

- Veterinary Practice
- Feed shop
- Through drug representative
- Online shop

**Administration and use of wormers**

This section asks for information on your administration and use of worming drugs in ADULT EQUINES on your stud.

40. Who administers the wormers?

Please state individual/s role

41. On what basis do you decide to worm horses?

FEC = faecal (worm) egg count

- Set treatment/dosing programme
- At signs of disease (e.g. poor weight gain, condition, etc)
- Following FEC by independent laboratory/person (no vet. advice)
- Following FEC by internal laboratory (no vet advice)
- Following FEC by vet with their recommendation to treat
- Veterinary recommendation (no FEC)

42. How frequently do you administer wormers to ADULT horses?

- 4 weeks or less
- 5-6 weeks
- 7-8 weeks
- 2-3 months
- 4-6 months
- 7-12 months
- > 12 months
- Other, please specify

43. Are equines kept off of pasture after worming?

- Yes for <1 day
- Yes for 1-2 days
- Yes for <7 days
- Yes for 7 days
- Yes for >7 days
- No

44. Are equines moved to "clean" pasture after worming?

- Yes
- No

45. Which of the following wormers have been used at your stud in the past 12 months?

Please tick all that apply

- Ivermectin (e.g. Equivalan; Eraquell; Bimectin; Vectin; Noromectin)
- Moxidectin (e.g. Equest)
- Benzimidazoles (e.g. Panacur; Panacur 5 day guard; Telmin)
- Pyrantel (e.g. Strongid-P; Pyratape)
- Praziquantel (e.g. Equitape)
- Ivermectin/Praziquantel (e.g. Equivalan Duo; Equimax)
- Moxidectin/Praziquantel (e.g. Equest Pramox)
- Other, please specify

46. For each you have used, which parasites are you aiming to eliminate?

	Moxidectin/Praziquantel (e.g. Equest Pramox)	Ivermectin/Praziquantel (e.g. Equivalan Duo; Equimax)	Praziquantel (e.g. Equitape)	Pyrantel (e.g. Strongid- P; Pyratape- P)	Benzimidazoles (e.g. Panacur; Panacur 5 day guard; Telmin)	Moxidectin (e.g. Equest)	Ivermectin (e.g. Equivalan; Eraquell; Bimectin)
Small strongyle (cyathostomin) ADULTS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Small strongyle (cyathostomin) ENCYSTED LARVAE	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Large strongyles (e.g. S.vulgaris) ADULTS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Large strongyles (e.g. S.vulgaris) LARVAE	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Roundworm (Parascaris equorum) ADULTS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Roundworm (Parascaris equorum) LARVAE	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tapeworm (e.g. Anoplocephala perfoliata)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Liver fluke	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

(Fasciola hepatica)

47. Do you consider any of the following occasions particularly important for administering wormers?

Tick all that apply

- Spring (March; April; May)
- Summer (June; July; August)
- Autumn (September; October; November)
- Winter (December; January; February)
- Prior to grazing turnout
- Prior to introduction of new equines
- Foaling
- Suspicion of parasite-related disease
- Other, please specify

48. Do you know what is meant by the term wormer/drug class?

- Yes
- No

**Drug wormer/class rotation practices**

49. How often do you rotate between wormer/drug classes?

- Every 2-3 months
- After each treatment
- Every 6 months
- Every year
- Infrequently
- Never

**Administration and use of wormers**

These questions ask further questions about administration and dosage decision making with worming drugs in ADULT STOCK.

50. What wormer do you use most frequently?

51. What was the last wormer you used and the month it was used in?

Wormer

Month of use

52. How are individual horses dosages calculated?

- Weighbridge individually
- Weighttape individually
- Each horses weight is estimated by eye
- One tube/packet of drug per horse is administered
- All are dosed according to the weight of the heaviest animal in the group
- All are dosed based on the average weight of a horse in that age group
- Other, please specify

**Resistance to worming drugs**

Resistance to worming drugs is an increasing concern within the equine buisness and pleasure-horse communities, these questions address your personal experience and opinions of current resistance levels and concerns.

53. Are you concerned about resistance to wormers?

54. In your experience, do any wormers seem to be less effective than they used to be?

Please name product/drug

Product/Drug

Product/Drug

Product/Drug

Product/Drug 

55. For each parasite, do you have a suspicion of, or have had diagnosed, resistance to wormers on your stud?  
 \*Resistance confirmed by performing faecal egg count reduction test ^Resistance suspected - wormed prior to parasite-related disease but NO faecal egg count reduction test performed on affected horse, or others on premises.

	Confirmed resistance after signs of disease*	Confirmed resistance with NO signs of disease*	Suspected resistance after signs of disease ^	Suspected resistance with NO signs of disease	No suspected or confirmed resistance
Small redworm (cyathostomins)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Large strongyles (e.g. strongylus vulgaris)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Roundworm (Parascaris equorum)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pinworm (Oxyuris equi)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tapeworm (e.g. Anoplocephala perfoliata)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

56. Have you seen or been told that there is resistance to a worming drug on your stud?

- Yes  
 No

### Resistance to worming drugs on your stud

57. To which drug/s has resistance been documented in at your stud?

- Ivermectin (e.g. Equivalan; Eraquell; Bimectin; Vectin; Noromectin)  
 Moxidectin (e.g. Equest)  
 Benzimidazoles (e.g. Panacur; Panacur 5 day guard; Telmin)  
 Pyrantel (e.g. Strongid-P; Pyratape)  
 Other, please specify

58. How was resistant status to this drug/s determined?

- Faecal egg count reduction test  
 Veterinary opinion drug has resistance on your stud  
 Veterinary advice drug does not work generally  
 Disease from specific parasite following wormer administration - Veterinary opinion  
 Personal observation from previous experience with drug  
 Disease from specific parasite following wormer administration - personal experience (no veterinary input)  
 Not sure

### Foal worming practices

This section looks at your worming protocols for foals.

59. Which parasite/s are of concern to you when considering the health of your **foals** and **youngstock** (1-3 years of age)?

	Small redworm (Cyathostomins)	Large strongyle (e.g. Strongylus vulgaris)	Roundworm (Parascaris equorum)	Tapeworm (Anoplocephala spp)
Major concern - Foals	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Minor concern - Foals	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
No concern - Foals	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Major concern - Youngstock	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Minor concern - Youngstock	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
No concern - Youngstock	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

60. What age (in weeks) are foals when they receive their **first** worming treatment?

- 0-2  
 3-4

- 5-6  
 7-8  
 >8  
 Other, please specify

61. Are foals wormed differently to adult equines?

- Yes  
 No

62. What drugs would you use in the **first year** of a foals life?

Please tick all that apply

	Once per year	Twice per year	Three times per year	More than three times but other products also used	Only drug used for the entire year	Not used at all
Ivermectin (e.g. Equivalan, Eraquell, Bimectin)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Moxidectin (e.g. Equest)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pyrantel (e.g. Strongid-P, Pyratape-P)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Benzimidazoles (e.g. Panacur, Telmin)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Ivermectin/Moxidectin (e.g. Equivalan Duo, Equimax)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Moxidectin/Praziquantel (e.g. Equest Pramox)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

63. Are youngstock (1-3 years old) wormed differently to adult stock?

- Yes  
 No

64. What drugs would you use for **youngstock** (1 - 3 years old)?

Please tick all that apply

	Once per year	Twice per year	Three times per year	More than three times but other products also used	Only drug used for the entire year	Not used at all
Ivermectin (e.g. Equivalan, Eraquell, Bimectin)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Moxidectin (e.g. Equest)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pyrantel (e.g. Strongid-P, Pyratape-P)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Benzimidazoles (e.g. Panacur, Telmin)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Ivermectin/Moxidectin (e.g. Equivalan Duo, Equimax)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Moxidectin/Praziquantel (e.g. Equest Pramox)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

### Faecal Egg Counts

65. Do you have faecal egg counts performed on samples from your horses?

- Yes  
 No

### Faecal Egg Counts and Use of Results

This section aims to discover how many are using faecal egg counting for both screening and decision making purposes and how treatment thresholds (if any) are determined.

66. Who performs these?

Tick all that apply

- Veterinary Practice  
 On-site stud laboratory  
 External laboratory  
 Mobile faecal egg counting laboratory  
 Other, please specify

67. How often are faecal egg counts performed?

Please tick all that apply

- Monthly  
 Every 2-3 months  
 Every 6 months  
 Annually  
 Infrequently/Randomly  
 Under suspicion of a parasite-related illness  
 During quarantine (please specify visiting equines, home equines or both)

68. In what form are FEC results provided? Is advice on whether to treat and wormer treatments all provided by the same individual/company?

- By post/email - report on which need treatment  
 By post/email - results only, no advice given  
 In person - horses which require treatment identified  
 In person - results only, no advice given  
 By post/email - Results given and wormer purchased from same person/company  
 In person - Results given and wormer purchased from same person/company  
 Other, please specify

69. On receiving faecal egg count results do you treat...

(egg = eggs per gram)

- Only horses with positive egg counts (anything above 0egg)  
 All horses regardless of egg count  
 Only horses with egg counts above 50 egg  
 Only horses with egg counts above 100egg  
 Only horses with egg counts above 200egg  
 FEC report includes a list of horses which need treatment and these are treated  
 Other threshold, please specify

70. How valuable do you feel faecal egg counts are/have been for each of the following...

	Very Unimportant	Unimportant	Neutral	Important	Very Important
Screen to monitor pasture contamination	<input type="radio"/>				
Evaluating pasture management strategies	<input type="radio"/>				
Screen to monitor parasite level within the herd	<input type="radio"/>				
Deciding which drug to use based on types of parasites detected	<input type="radio"/>				
Determining which equines to treat	<input type="radio"/>				

71. Within which age group are your FEC results generally highest? (1 = Generally HIGHEST; 5 = Generally LOWEST; 0 = Don't know)

Rank the items below, using numeric values starting with 1.

1-3 years (youngstock) 4-6 years 7-10 years 11-14 years 15 years and above

**Faecal egg count reduction testing**

72. Do you know what a faecal egg count reduction test (FECRT) is?

-- None -- ▾

73. Has a FECRT been performed at your stud?

-- None -- ▾

**FECRT Results**

74. For which age groups was a FECRT performed?

Please tick all which apply

- <1 year old  
 1-3 years (Youngstock)  
 4-6 years  
 7-10 years  
 11-14 years  
 15 years and above

75. For your FECRT please provide

Date test performed

Drug/product tested

76. Were eggs observed in **post-treatment** samples?

- Yes  
 No

77. If resistance (eggs post-treatment) was observed, within which age group/s was this?

Please tick all that apply

- <1 year old  
 1-3 years (Youngstock)  
 4-6 years  
 7-10 years  
 11-14 years  
 15 years and above  
 No resistance detected

**Targeted Strategic Worming**

These questions look at your knowledge of targeted strategic worming protocols and, if you have used them, how effective you believe them to be.

78. What does "Targeted strategic worming" mean to you?

- Basing all worming treatments on faecal egg counts performed every 3-4 months  
 Treating all horses once per year with a wormer but no diagnostics between treatments  
 Treating all horses every three months with a wormer  
 Treating only horses which seem to have parasite-related disease  
 Using specific drugs each season to target specific parasites  
 Other, please specify

79. Does your stud use Targeted Strategic Treatment?

- Yes  
 No

**Targeted Strategic Worming - your experiences**

80. For approximately how many months have you been using targeted strategic worming?

81. When looking at your egg count data have you seen an...

- Overall increase in worm egg counts  
 Overall decrease in worm egg counts

No change in egg counts

82. Do you use...

- More worming treatments  
 Less worming treatments  
 Approximately the same number

83. Comparing first FEC results to most recent have you seen a change in prevalence of these parasitic species?

	Increase in egg count	Decrease in egg count	No change in egg count
Small strongyles (Cyathostomins)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Ascarids (Parascaris equorum)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tapeworm (Anoplocephala spp)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pinworm (Oxyuris equi)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

84. Since starting targeted treatment have you seen a change in instances of worm-related illness at **your stud**?

	Increase	Decrease	No change in number
Worms in faeces	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diarrhoea - lasting 1-4 weeks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diarrhoea - lasting <1 week	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Loss in/failure to gain condition	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lower weight gain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Weight loss	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Colic	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Larval cyathostomosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Poor performance	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

85. Do you feel confident that targeted treatment controls parasite transmission on your stud?

-- None -- ▾

86. What are the benefits to **you and your business** of targeted treatment?

Please tick all that apply

- Reduced overhead costs  
 Reduced risk of developing drug resistance  
 Improved health of stock  
 Confidence that less unnecessary drugs are administered to healthy animals  
 No benefits  
 Other, please specify

87. Would you recommend changing over to targeted treatment to others?

- Yes  
 No  
 Not sure  
 Other, please specify

### Study participation

If you would like to participate further please tick "yes" below and leave a contact email or telephone number.

88. Would you be interested in participating in a wormer resistance study?

- Yes  
 No

# Appendix 4B

## Stud C

Time point	Group	n	Strongyle-type					Ascarid					Tapeworm	
			Mean (epg)	Median (epg)	Range (epg)	Prevalence (% herd/n)	80% egg output (% herd/n)	k (SD)	Mean (epg)	Median (epg)	Range (epg)	Prevalence (% herd/n)	80% egg output (% herd/n)	Prevalence (% herd/n)
Feb	Adults	116	58.0	4.5	0-778	75/87	7.2/20	0.2 (0.03)	0.3	0	0-30	0.9/1	NA	1.7/2
	YS	53	419.5	303	0-2569.5	100/53	47.2/25	1.4 (0.2)	1.8	0	0-58.5	5.7/3	NA	5.7/3
April	YS	54	120.7	93	0-531	100/54	46.3/25		1.7	0	0-42	5.6/3	NA	0
May	Adults	48	76.8	0	0-1800	47.9/23	6/3		0.02	0	0-1	2.1/1	NA	0
	Foals	30	50.6	0	0-1498.5	36.7/11	<1		102.2	0	0-3010.5	10/3	NA	0
	Herd	78	57.2	0	0-1800	43.6/34	3.9/3		33.7	0	0-3010.5	5.1/4	NA	0
June	Adults	26	67.4	0	0-1314	38.5/10	3.9/1		0	0	0	0	0	0
	Foals	84	21.5	0	0-1476	36.9/31	1.2/1		33.4	0	0-846	20.2/17	8.3/7	0
	Herd	110	32.4	0	0-1476	37.3/41	2.7/3		25.5	0	0-846	15.5/17	6.4/7	0
July	Adults	37	15.32	1	0-117	56.8/21	6.2/6		0	0	0	0	0	0
	YS	49	163.2	87	0-742.5	77.6/38	34.7/17		6.7	0	0-328.5	6.1/3	NA	0
	Foals	119	1.41	0	0-52.5	31.1/52	9.2/11	0.2 (0.03)	53.6	0	0-1246.5	34.5/41	9.2/11	0

	<b>Herd</b>	205	42.6	0	0-742.5	46.8/96	9.8/20	0.1 (0.01)	32.7	0	0- 1246.5	21.5/44	5.9/12	0
<b>Aug</b>	<b>Adult</b>	32	9.3	0	0-94.5	40.6/13	5.6/5		0	0	0	0	0	0
	<b>YS</b>	30	234.2	186.8	0-693	100/30	46.7/14		2.3	0	0-31.5	13.3/4	NA	0
	<b>Foals</b>	101	0.7	0	0-24.5	16.8/17	5.9/6		6.7	0	0-288	7.9/8	4.0/4	0
	<b>Herd</b>	163	45.4	0	0-693	36.8/60	9.2/15		4.6	0	0-288	7.4/12	3.1/5	0
<b>Sept</b>	<b>Foals</b>	119	6.1	0	0-127.5	37.8/45	8.4/10		4.6	0	0-126	13.5/16	5.9/7	0
<b>Oct</b>	<b>Foals</b>	105	43.9	0	0-657	48.6/51	11.4/12		3.3	0	0-121.5	10.5/11	3.8/4	0
	<b>YS</b>	35	6.4	0	0-85.5	34.3/12	14.3/5		1.8	0	0-29	8.6/3	NA	0
<b>Year overall</b>	<b>Adult</b>	261	50.0	0.5	0-1800	59.2/154	11.2/29		0.1	0	0-31	0.8/2	NA	0.8/2
<b>Year overall</b>	<b>Foals</b>	559	15.9	0	0- 1498.5	31.6/192	3.0/18		24.7	0	3010.5	17.2/96	3.6/20	0
<b>Year overall</b>	<b>YS</b>	223	186.6	91.5	0- 2569.5	84.8/200	33.6/75		2.8	0	0-328.5	7.6/17	2.7/6	1.4/3
<b>Year overall</b>	<b>Herd</b>	104 3	62.5	0.5	0- 2569.5	51.3/535	11.2/117		13.7	0	0- 3010.5	11.5/120	2.3/24	0.5/5

Descriptive statistics for all testing time points and age groups including year overall for both individual age groups and the herd overall for stud C.

Group/ Age	Month	# tested	% +ve Str	>200	100-199	50-99	0.5-49	0
				(% +ve/% total)	(% +ve/% total)	(% +ve/% total)	(% +ve/% total)	(% total)
Adult	Feb	116	75	11.5/8.6	12.6/9.5	10.3/7.8	65.5/49.1	25
	April	1	NA	NA	NA	NA	NA	NA
	May	48	47.9	13.0/6.3	13.0/6.3	4.4/2.1	69.6/33.3	52.1
	June	26	38.5	12.5/3.9	25.0/7.7	12.5/3.9	75.0/23.1	61.5
	July	37	56.8	0/0	4.8/2.7	23.8/13.5	71.4/40.5	43.2
	Aug	32	40.6	0/0	0/0	7.7/3.1	92.3/37.5	59.4
	Sept	NA	NA	NA	NA	NA	NA	NA
	Oct	NA	NA	NA	NA	NA	NA	NA
	<b>OVERALL</b>	<b>260</b>	<b>59.2</b>	<b>9.1/5.4</b>	<b>11.0/6.5</b>	<b>11.0/6.5</b>	<b>68.8/40.8</b>	<b>40.8</b>
YS	Feb	53	100	66	15	17.0	1.9	0

	April	54	100	20	24	22.2	33	0
	May	NA	NA	NA	NA	NA	NA	NA
	June	NA	NA	NA	NA	NA	NA	NA
	July	49	77.6	50/39	8/6	18.4/14.3	23.7/18.4	22.5
	Aug	30	100	43	23	13.3	20	0
	Sept	2	NA	NA	NA	NA	NA	NA
	Oct	35	34.3	0/0	0/0	8.3/2.9	91.7/31.4	65.7
	<b>OVERALL</b>	<b>223</b>	<b>84.8</b>	<b>41/35</b>	<b>16/14</b>	<b>17.5/14.8</b>	<b>24.9/21.1</b>	<b>15.3</b>
Foals	Feb	NA	NA	NA	NA	NA	NA	NA
	April	1	NA	NA	NA	NA	NA	NA
	May	30	36.7	9.1/3.3	0/0	0/0	90.9/33.3	63.3
	June	84	36.9	3.2/1.2	0/0	3.2/1.2	93.6/34.5	63.1
	July	119	31.1	0/0	0/0	2.7/0.8	97.3/30.3	68.9
	August	101	16.8	0/0	0/0	0/0	100/16.8	83.2
	Sept	119	37.8	0/0	4.4/1.7	6.7/2.5	88.9/33.6	62.2
	Oct	105	48.6	16/8	16/8	5.9/2.9	62.8/30.5	51.4
	<b>OVERALL</b>	<b>608</b>	<b>31.6</b>	<b>5.2/1.6</b>	<b>5.2/1.6</b>	<b>3.7/1.2</b>	<b>85.9/27.1</b>	<b>68.4</b>
	Herd	Feb	169	82.8	32.1/26.6	12.9/10.7	13.6/11.2	41.4/34.3
April		56	96.4	20.4/19.6	24.1/23.2	22.2/21.4	33.3/32.1	3.6
May		78	43.6	11.8/5.1	8.8/3.9	2.9/1.3	76.5/33.3	56.4
June		110	37.3	4.9/1.8	4.9/1.8	4.9/1.8	85.4/31.8	84.6
July		205	46.8	19.8/9.3	4.2/2.0	13.5/6.3	62.5/29.3	53.2
August		163	36.8	21.7/8.0	11.7/4.3	8.3/3.1	58.3/21.5	63.2
Sept		121	38.8	0/0	4.3/1.7	4.3/1.7	91.5/35.5	61.2
Oct		141	44.7	12.7/5.7	12.7/5.7	6.4/2.8	68.3/30.5	55.3
<b>OVERALL</b>		<b>1043</b>	<b>51.3</b>	<b>19.1/9.8</b>	<b>10.8/5.6</b>	<b>10.7/5.5</b>	<b>59.4/30.5</b>	<b>48.7</b>

Egg count bands for each parasitic species at each time point within both age strata and the herd overall on stud C.

## Stud D

Time point	Group	n	Strongyle-type					Ascarid					Tapeworm	
			Mean (epg)	Median (epg)	Range (epg)	Prevalence (% herd/n)	80% egg output (% herd/n)	k (SD)	Mean (epg)	Median (epg)	Range (epg)	Prevalence (% herd/n)	80% egg output (% herd/n)	Prevalence (% herd/n)
Jan	Adult	25	51.9	0	0-382.5	48/12	12/3	0.1 (0.03)	0	0	0	0	0	4/1
Feb	YS	16	101.1	88.8	0-355.5	100/16	43.75/7		0	0	0	0	0	0
March	YS	8	53.3	36	0-144	87.5/7	37.5/3		0	0	0	0	0	25/2
April	YS	7	42.9	1	0-198	57.1/4	28.6/2		0	0	0	0	0	28.6/2
May	YS	28	207.1	26.8	0-1161	92.9/26	25/7		0.04	0	0-1	3.6/1	NA	60.7/17
June	YS	37	93.0	1.5	0-1899	59.5/22	10.8/4		0	0	0	0	0	0
Aug	YS	50	524.7	369	?-2736	88/44	36/18	1.1 (0.3)	0	0	0	0	0	0
Year overall	YS	146	259.0	58.5	0-2736	81.5/119	21.2/31		0.007	0	0-1	0.7/1	NA	14.4/21

Descriptive statistics for each testing time point of cohorts on stud D.

Group/ Age	Month	# tested	% +ve Str	>200 (% +ve/% total)	100-199 (% +ve/% total)	50-99 (% +ve/% total)	0.5-49 (% +ve/% total)	0 (% total)
Adult	Jan	25	48	25/12	0/0	16.7/8	58.3/28	NA/52
YS	Feb	16	100	12.5	25	25	37.5	0
	March	8	87.5	0/0	28.6/25	28.6/25	42.9/37.5	NA/12.5
	April	7	57.1	0/0	50/28.6	0/0	50/28.6	NA/42.9

	May	28	92.9	34.6/32.14	15.4/14.3	0/0	50/46.4	NA/7.1
	June	37	59.5	18.2/10.81	13.6/8.1	9.1/5.4	59.1/35.1	NA/40.5
	August	50	88	63.6/56	9.1/8	11.4/10	15.9/14	NA/12
YS	OVERALL	146	81.5	36.1/29.5	16.0/13.0	10.9/8.9	37.0/30.1	NA/18.5

Percentages of positive animals within each egg count band at each time point on stud D

## Stud F

Time point	Group	n	Strongyle						Ascarid				Tapeworm	
			Mean (epg)	Median (epg)	Range (epg)	Prevalence (% herd/n)	80% egg output (% herd/n)	<i>k</i> (SD)	Mean (epg)	Median (epg)	Range (epg)	Prevalence (% herd/n)	80% egg output (% herd/n)	Prevalence (% herd/n)
March	Adult	33	18	0	0-553.5	27.3/9	<1	0.05 (0.02)	0	0	0	0	0	0
	YS	26	115.2	90.5	0-284	100/26	46.2/12	1.3 (0.3)	4.6	0	0-102	7.7/2	NA	0
	Herd	59	60.9	3.5	0-553.5	59.3/35	22.0/13	0.2 (0.03)	2	0	0-102	3.4/2	NA	0
April	Adult	31	32.6	0	0-235.5	48.4/15	12.9/4	NA	0	0	0	0	0	6.5/2
	YS	26	193.8	152.3	0-724.5	96.2/25	42.3/11	NA	0.9	0	0-24	3.9/1	NA	0
	Herd	57	106.1	13.5	0-724.5	70.2/40	24.6/14	NA	0.4	0	0-24	1.8/1	NA	3.5/2
June	Adult	32	113.1	25.3	0-789	78.1/25	25-Aug	NA	0	0	0	0	0	0
	YS	19	213.7	160.5	0-720	89.5/8	31.6/6	NA	0	0	0	0	0	0
	Herd	51	98.7	25.3	0-789	82.4/42	25.5/13	NA	0	0	0	0	0	0
July	Adult	25	223.8	183	0-450	88/22	40/10	NA	0	0	0	0	0	0
	YS	51	429	283.5	0-1489.5	100/45	49.0/25	NA	0	0	0	0	0	0
	Herd	76	377.8	270	0-1489.5	96.1/73	44.7/34	NA	0	0	0	0	0	0
Aug	Adult	33	116.4	8.5	0-531	69.7/23	24.2/8	NA	0	0	0	0	0	0

	YS	20	99.2	0.25	0-913.5	50/10	7/1.7	NA	0	0	0	0	0	0
	Herd	53	109.9	2.5	0-913.5	62.3/33	17.0/9	NA	0	0	0	0	0	0
Sept	Adult	64	22.2	3	0-163.5	57.8/37	15.6/10	NA	0	0	0	0	0	0
	YS	33	119.4	47.5	0-1039.5	84.9/28	27.3/9	NA	0	0	0	0	0	0
	Herd	97	55.3	4.5	0-1039.5	67.0/65	18.6/18	NA	0	0	0	0	0	0
Oct	Adult	13	15.7	5	0-63	76.9/10	30.8/4	NA	0	0	0	0	0	0
Year overall	Adult	231	61.1	2	0-789	61.0/134	16.5/38	0.2 (0.02)	0	0	0	0	0	0.9/2
Year overall	YS	162	217.9	135	0-1489.5	88.9/144	35.2/57	0.4 (0.04)	0.9	0	0-102	1.9/3	NA	0
Year overall	Herd	393	127.8	19	0-1489.5	72.52/285	23.66/93	0.2 (0.02)	0.4	0	0-102	0.8/3	NA	0.5/2

Group /Age	Month	# tested	% +ve Str	>200 (% +ve/% total)	100-199 (% +ve/% total)	50-99 (% +ve/% total)	0.5-49 (% +ve/% total)	0 (% total)
Adult	March	33	27.3	11.1/3.0	0/0	0/0	88.9/24.2	72.7
	April	31	48.4	20/9.7	6.7/3.2	6.7/3.2	66.7/32.3	51.6
	June	32	78.1	24/18.8	20/15.6	4/3.1	52/40.6	21.9
	July	25	88	31.8/28	22.7/20	9.1/8	36.4/32	12
	August	33	69.7	34.8/24.2	17.4/12.1	0/0	47.8/33.3	30.3
	Sept	64	57.8	0/0	21.6/12.5	2.7/1.6	75.7/43.8	42.2
	October	13	76.9	0/0	0/0	20/15.4	80/61.5	23.1
	OVERALL	231	61.0	17.7/10.8	16.3/10.0	5.0/3.0	61.0/37.2	39.0
YS	March	26	100	23.1	19.2	30.8	26.9	0

	April	26	96.2	48/46.2	16/7.7	4/3.9	32/30.8	3.9
	June	19	89.5	23.5/21.1	17.7/15.8	17.7/15.8	41.2/36.8	10.5
	July	51	100	64.7	25.5	5.9	3.9	0
	August	20	50	20.0/10.0	10.0/5.0	0/0	70.0/35.0	50
	Sept	33	84.9	21.4/18.2	25/21.2	7.1/6.1	46.4/39.4	15.2
	OVERALL	162	88.9	41.7/37.0	22.2/19.8	11.8/10.5	24.3/21.6	11.1
Herd	March	59	59.3	20/11.9	14.3/8.5	22.9/13.6	42.9/25.4	40.7
	April	57	70.2	37.5/26.3	12.5/8.8	5.0/3.5	45.0/31.6	29.8
	June	51	82.4	23.8/19.6	19.0/15.7	9.5/7.8	47.6/39.2	17.7
	July	76	96.1	54.8/52.6	24.7/23.7	6.9/6.6	13.7/13.2	4.0
	August	53	62.3	30.3/18.9	15.2/9.4	0/0	54.6/34.0	37.7
	Sept	97	67.0	9.2/6.2	23.1/15.5	4.6/3.1	63.1/42.3	33.0
	OVERALL	393	72.5	29.8/21.6	19.3/14.0	8.4/6.1	42.5/30.8	27.5

Percentages of positive animals within each egg count band at each time point on stud F

# Appendix 6A

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## Parasite Control on Thoroughbred Studs

*University of Liverpool*

*Funded by HBLB*

We would be very grateful if you would be willing to complete this questionnaire.

This study is being conducted by the University of Liverpool, funded by the HBLB.

All results are kept *securely and confidentially*. Data will be analysed and *anonymised* prior to inclusion in the PhD thesis and all scientific publications.

Thank you for your time.

Should you have any comments, please contact –

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Liverpool .  
L3 5RF

## Section 1 Stud Information

In the first section we would like to collect some basic information about your stud farm.

1. Response ID	
----------------	--

3. Email – Please only include if you would like a copy of our findings	
---	--

5. How many of the following permanently reside at your stud?	
Stallions	
Geldings	
Companion horses/teasers	
Broodmares	
Youngstock (1-3 years old)	
Foals (0-12 months)	
Donkeys	

6. How important do you think gastrointestinal/gut worms and worm treatment are in relation to a stud farm?	Tick one box
Extremely important	
Moderately important	
Fairly important	
Not important	

## Section 2 Grazing & Pasture management

These questions look at your current grazing and pasture management strategies.

<b>7.</b>	<b>Approximately how many acres of land do you own, rent or use for equine grazing purposes?</b>

<b>8. On average, how much access to grazing (hours per day - hrs/d) do horses have during...</b>					
	0 hrs/d	1-5 hrs/d	6-10 hrs/d	11-15 hrs/d	16 - 24 hrs/d
Spring (March - May)					
Summer (June - August)					
Autumn (September - November)					
Winter (December - February)					

<b>9. Are grazing areas on your establishment...</b>					
	Weekly	Monthly	Yearly	Occasionally	Never
Grazed by cattle or sheep					
Harrowed					
Rested from grazing					
Rotated between groups of horses					

<b>10.</b>	<b>How often do you remove faeces from pasture?</b>	<b>Tick one box</b>
	Twice weekly	<input type="checkbox"/>
	Weekly	<input type="checkbox"/>
	Fortnightly	<input type="checkbox"/>
	Monthly	<input type="checkbox"/>
	Occasionally/Sporadically	<input type="checkbox"/>
	Never	<input type="checkbox"/>
	Differs seasonally - please specify	

<b>11.</b>	<b>How are faeces removed from pasture?</b>	<b>Tick one box</b>
	Machine*	<input type="checkbox"/>
	Manually	<input type="checkbox"/>
	Mixture of both *	<input type="checkbox"/>
<ul style="list-style-type: none"> <li>• Please state type of machine use</li> </ul>		

12. How concerned are you would you be about the presence of each of the parasites below, in high or low numbers, on your stud farm?

	Very concerned	Little concern	No concern
<b>High burden</b> of Small strongyles ( <i>Cyathostomins</i> )			
<b>Low burden</b> of Small strongyles ( <i>Cyathostomins</i> )			
<b>High burden</b> of Large strongyles (e.g. <i>Strongylus vulgaris</i> )			
<b>Low burden</b> of Large strongyles (e.g. <i>Strongylus vulgaris</i> )			
<b>High burden</b> of Roundworm ( <i>Parascaris equorum</i> )			
<b>Low burden</b> of Roundworm ( <i>Parascaris equorum</i> )			
<b>High burden</b> of Tapeworm (such as <i>Anoplocephala perfoliata</i> )			
<b>Low burden</b> of Tapeworm (such as <i>Anoplocephala perfoliata</i> )			
<b>High burden</b> of Liver fluke ( <i>Fasciola hepatica</i> )			
<b>Low burden</b> of Liver fluke ( <i>Fasciola hepatica</i> )			

**Section 3**  
**Wormer advice and use**

<b>13.</b>	<b>Are wormers (anthelmintics) administered to horses of any age at your stud?</b>	<b>Tick one box</b>
	Yes	
	No	Skip to Section 5

**Worming advice and drug purchase**

This section aims to determine your sources for worming advice and drug purchase, along with what is most important to you when considering which product to buy.

<b>14.</b>	<b>Where do you obtain information on worming?</b>	<b>Please tick all that apply</b>
	None needed - existing strategy works and/or personal experience	
	Feed shop/Worming supplier	
	Drug representatives	
	Articles in equine magazines	
	Books	
	Internet	
	Preferred website	
	Preferred forum	
	Friends and family	
	SQP in shop	
	SQP/Pharmacist at veterinary practice	
	Veterinary surgeon	
	Other, please specify	

<b>15.</b>	<b>Where do you MOST FREQUENTLY BUY your worming products?</b>	<b>Tick one box</b>
	Veterinary Practice	
	Feed shop	
	Through drug representative	
	Online shop	
	Other, please specify	

**Administration of wormers to ADULT stock**

This section asks for information on your administration and use of worming drugs in ADULT HORSES on your stud.

<b>16.</b>	<b>Who administers the wormers?</b>	<b>Tick one box</b>
	Set Person *	
	* Who? (Job role)	
	Member of stud staff (varies)	

17. On what basis do you decide to worm horses? <i>FEC = faecal (worm) egg count</i>		Tick one box
Set treatment/dosing programme		
At signs of disease (e.g. poor weight gain, condition, etc)		
Following FEC by...	Independent laboratory/person <b>NO</b> veterinary advice on results	
	On-site/Internal laboratory <b>NO</b> veterinary advice on results	
	Internal or independent laboratory <b>WITH</b> veterinary advice on results	
	Veterinary practice laboratory <b>WITH</b> veterinary advice on results	
Following tapeworm ELISA	Blood test	
	Saliva test	
Veterinary recommendation (no FEC)		

18. How frequently do you administer wormers to ADULT horses?		
Every 4 weeks or less		
Every 5-6 weeks		
Every 7-8 weeks		
Every 2-3 months		
Every 6 months		
Once per year		
Less than once per YEAR		
Based on Diagnostics ONLY	FEC is high (>200 eggs per gram)	
	FEC is moderate (100 – 200 eggs per gram)	
	FEC is positive (1-99 eggs per gram)	
	<i>Treated ONLY if (tick all that apply) –</i>	
	Tapeworm ELISA identifies HIGH burden	
	Tapeworm ELISA identifies MODERATE burden	
	Tapeworm ELISA identifies LOW burden	
Other, please specify		

19. Are horses kept off of pasture after worming?		Tick one box
YES for...	<1 day	
	1-2 days	
	<7 days	
	7 days	
	>7 days	
NO		

20.	Are horses moved to "clean" pasture after worming?	
	Yes *	
	* After how many days following treatment are equines moved to clean pasture?	
	No	

21.	Which of the following wormers have been used at your stud in the past 12 months?	Tick all that apply
	Ivermectin (e.g. Equivalan; Eraquell; Bimectin; Vectin; Noromectin)	
	Moxidectin (e.g. Equest)	
	Benzimidazoles (e.g. Panacur; Panacur 5 day guard; Telmin)	
	Pyrantel (e.g. Strongid-P; Pyratape)	
	Praziquantel (e.g. Equitape)	
	Ivermectin/Praziquantel (e.g. Equivalan Duo; Equimax)	
	Moxidectin/Praziquantel (e.g. Equest Pramox)	
	Other, please specify	

22.	Do you consider any of the following occasions particularly important for administering wormers?	Tick all that apply
YES	Spring (March; April; May)	
	Summer (June; July; August)	
	Autumn (September; October; November)	
	Winter (December; January; February)	
	Prior to grazing turnout	
	Prior to introduction of new equines	
	Foaling (Pre and/or immediately post)	
	Other, please specify	
	NO	

23.	What wormer do you use most frequently?

24.	How are individual horses dosages calculated?	Tick one box
	Weighbridge individually	
	Weightape individually	
	Each horses weight is estimated by eye	
	One tube/packet of drug per horse is administered	
	All are dosed according to the weight of the heaviest animal in the <i>grazing</i> group	
	All are dosed based on the average weight of a horse in that <i>age</i> group	
	Other, please specify	

### Drug/wormer class rotation practices

25.	How often do you rotate between drug/wormer classes?	Tick one box
	YES – at set intervals	
	NO	
	Wormer chosen at random	
	Drug chosen based on FEC/ELISA results	
	Other (please specify)	
	Unsure what is meant by wormer /drug CLASS	

## Section 4

### Foil and youngster worming practices

This section looks at worming protocols for foals and youngster (1-3 years of age) on your stud farm. Some questions refer specifically to EITHER foals or youngster.

26. Which parasite/s are of concern to you when considering the health of your foals and youngster (1-3 years of age)?

	Small redworm ( <i>Cyathostomins</i> )	Large strongyle (e.g. <i>Strongylus vulgaris</i> )	Roundworm ( <i>Parascaris equorum</i> )	Tapeworm ( <i>Anoplocephala spp</i> )
Major concern - Foals				
Minor concern - Foals				
No concern - Foals				
Major concern - Youngstock				
Minor concern - Youngstock				
No concern - Youngstock				

27. What age are foals when they receive their first worming treatment?	Tick one box
0-2 weeks	
3-4 weeks	
5-6 weeks	
7-8 weeks	
>8 weeks	
Other, please specify	

28. Are foals wormed differently to adult equines?	Tick one box
Yes*	
<ul style="list-style-type: none"> <li>How is worming different? (e.g. different drugs, more frequent treatment, etc)</li> </ul>	
No	

29. What drugs would you use in the first year of a foals life? Please tick all that apply						
	Once per year	Twice per year	3 times per year	> 3 times but other products also used	Only drug used for the entire year	Not used at all
Ivermectin (e.g. Equivalan, Eraquell, Bimectin)						
Moxidectin (e.g. Equest)						
Pyrantel (e.g. Strongid-P, Pyratape-P)						
Benzimidazoles (e.g. Panacur, Telmin)						
Ivermectin/Moxidectin (e.g. Equivalan Duo, Equimax)						
Moxidectin/Praziquantel (e.g. Equest Pramox)						
Praziquantel (e.g. Equitape)						

30.	Are youngstock (1-3 years old) wormed differently to adult stock?	Tick one box
	Yes	
	<ul style="list-style-type: none"> <li>How is worming different? (e.g. different drugs, more frequently, etc)</li> </ul>	
	No	

31. What drugs would you use for youngstock (1 - 3 years old)? Please tick all that apply						
	Once per year	Twice per year	Three times per year	> Three times but other products also used	Only drug used for the entire year	Not used at all
Ivermectin (e.g. Equivalan, Eraquell, Bimectin)						
Moxidectin (e.g. Equest)						
Pyrantel (e.g. Strongid-P, Pyratape-P)						
Benzimidazoles (e.g. Panacur, Telmin)						
Ivermectin/Moxidectin (e.g. Equivalan Duo, Equimax)						
Moxidectin/Praziquantel (e.g. Equest Pramox)						
Praziquantel only (e.g. Equitape)						

## Section 5 Horse movements & quarantine procedures for visiting stock

This section looks at your quarantine policy when your stud farm receives visiting ADULT horses.

32. Does your stud accept visiting horses?	Tick one box
Yes *	
* Approximately how many per year?	
No Proceed to Section 6	

33. On average, how long do visiting horses stay?	Tick one box
Varies depending on individual horses/owners	
1 year	
1 month	
1 week	
Walk-in only	
Other (please specify) / Additional comments	

34. Are any of the following checks performed PRE-arrival for visiting stock?								
	Every visiting horse	Stallions only	Mares only	Overseas mares only	Overseas stallions only	Preferred but optional	Occasional/ Random checking	Never requested/ checked
Pre-arrival faecal egg count								
Recent (3 month) worming history								

35. Are visiting mares...	Please state Yes or No
Grazed alone in a small paddock	
Grazed with other visiting mares only - AFTER quarantine (including worming if required)	
Grazed with other visiting mares only - NO quarantine	
Grazed with resident stock - AFTER quarantine (including worming if required)	
Grazed with resident stock - NO quarantine	
Visiting mares are only stabled/have no access to grazing	
Other, please specify	

36. Are visiting horses quarantined and for how long?	Tick one box
Yes - >7 days	
Yes - 7 days	
Yes - <7 days	
Yes - 1-2 days	
Yes - <1 day	
No Skip to Section 6	

## Quarantine Protocol for Visiting Stock

These questions are to look at the level of quarantine you feel is necessary for your stud farm when receiving visiting ADULT horses.

37.	Are VISITING horses in quarantine kept...	Tick one box				
	Turned out only					
	Stabled only					
	Mixed, please specify					
38.	Are any of the following part of your quarantine protocol (on ARRIVAL)?					
<i>Please tick all which apply</i>	YES - Always/Every visiting horse	YES – Only if home site has history of parasitic disease	YES – Only if pre-arrival faecal egg count is positive	YES - Occasionally	YES - Rarely	NO
Faecal egg count (FEC) performed						
Tapeworm ELISA performed	Blood					
	Saliva					
Wormed based on...	FEC result					
	ELISA result					
Wormer administered for all visiting horses prior to turn-out	<b>If yes</b>					
	Specific drug/product (please specify)					
	Non-specific drug/product (please specify how selected)					
	Product recommended based on faecal egg count results					
Wormer administered for all visiting equines but NO turn out	<b>If yes</b>					
	Specific drug/product (please specify)					
	Non-specific drug/product (please specify how selected)					
	Product recommended based on faecal egg count results					

## Section 6 Resident ("home") equine travel

This section considers your ADULT MARES which travel to other studs for breeding or boarding purposes.

<b>39. Do your stock visit other premises?</b>	<b>Tick one box</b>
Yes	
No <span style="margin-left: 100px;">Skip to Section 7</span>	
<hr/>	
<b>40. On average, how long do "home" horses stay on other premises?</b>	<b>Tick one box</b>
Walk-in only	
1 week	
2-4 weeks	
1-4 months	
> 4 months	
Depends on individual horse	
Other (please specify) / Additional comments	
<hr/>	
<b>41. Do you quarantine horses upon return to home premises?</b>	
Yes*	*How long for?
	1-2 days
	2-7 days
	> 7 days
No/Not necessary	
Other (please specify)	
<hr/>	
<b>42. Upon return (whether in quarantine or not) would you administer a wormer?</b>	<b>Tick one box</b>
Yes – Always	Product of choice
Yes – Sometimes	Product of choice
Yes – Only if FEC is considered to be high	Product of choice
Yes – If tapeworm ELISA is considered to be high	Product of choice
No	
<hr/>	
<b>43. Do you perform faecal egg counts and/or tapeworm ELISA testing on returning stock prior to turn-out?</b>	<b>Tick one box</b>
Yes – FEC only	
Yes – FEC <i>and</i> ELISA	
Yes – ELISA only	
Yes – Only if suspicious of disease or high burden ( <b>NO</b> testing performed)	
No	

44. Are you concerned about returning mares introducing any of the following onto your stud...			
	<u>Very</u> concerned	<u>Slightly</u> concerned	<u>Not</u> concerned
Infectious non-reproductive disease (e.g. respiratory diseases such as Strangles)			
Infectious reproductive disease			
Parasites resistant to worming drugs			
Increased parasitic pasture contamination			

45. For mares that travel <i>frequently</i> to other studs, when at "home" are they...	Tick one box
Grazed with other frequently travelled mares - same age group	
Grazed with other frequently travelled mares - mixed age group	
Grazed with non-travelling resident stock - same age group	
Grazed with non-travelling resident stock - mixed age group	
Other, please specify	

## Quarantine and Re-integration protocol for returning stock

This section looks at what quarantine (if any) you feel is necessary for ADULT MARES when returning to home premises after a period away for breeding or boarding.

46. Are horses RETURNING to your stud (having visited another stud) in quarantine kept...	Tick one box
Stabled only	
Turned out only	
Mixed, please specify	
No quarantine necessary	

47. Are any of the following part of your quarantine and re-integration protocol for RETURNING stock?						
<i>Tick all that apply</i>	YES - Always/Every returning horse	YES - If site of visit has history of parasitic disease	YES - If mare has high egg count on return	YES - If suspicious of disease	YES – Occasionally	NO / Not necessary
Faecal egg count performed						
Tapeworm ELISA performed	Blood					
	Saliva					
Wormed based on FEC results	If YES					
	Specific drug/product (please specify)					
	Non-Specific drug/product					
Wormer administered to all in quarantine pre-turn out	Product based on FEC/ELISA recommendation					
	If YES					
	Specific drug/product (please specify)					
Wormer administered to all prior to turn-out	Non-Specific drug/product					
	Product based on FEC/ELISA recommendation					
	If YES					
Wormer administered to all prior to turn-out	Specific drug/product (please specify)					
	Non-Specific drug/product					
	Product based on FEC/ELISA recommendation					

## Section 7

### Resistance to worming drugs

Resistance to worming drugs is an increasing concern within the equine business and pleasure-horse communities, these questions address your personal experience and opinions of current resistance levels and concerns.

<b>48. Are you concerned about resistance to wormers?</b>		<b>Tick one box</b>
Yes	Very concerned	
	Slightly concerned	
No		
Unsure		
<b>49. In your experience, do any wormers seem to be less effective than they used to be?</b>		
	<b>Tick one box</b>	<i>Please name product/drug(/s)</i>
YES (please specify product/drug)		1.
		2.
		3.
NO		
<b>50. Have you seen evidence of or been told that there is resistance to a worming drug on your stud?</b>		<b>Tick one box</b>
Yes *		
• If confirmed then how and by whom (job role)/what evidence have you seen?		
No		
<b>51. Has resistance been CONFIRMED at your stud?</b>		<b>Tick all which apply</b>
YES	Ivermectin (e.g. Equivalan; Eraquell; Bimectin; Vectin; Noromectin)	
	Moxidectin (e.g. Equest)	
	Benzimidazoles (e.g. Panacur; Panacur 5 day guard; Telmin)	
	Pyrantel (e.g. Strongid-P; Pyratape)	
Other, please specify		
NO		
<b>52. How was resistant status to worming drug/s determined?</b>		<b>Tick one box</b>
Faecal egg count reduction test		
Veterinary opinion drug has resistance on your stud		
Veterinary advice drug does not work generally		
Disease from specific parasite following wormer administration - Veterinary opinion		
Personal observation from previous experience with drug		
Disease from specific parasite following wormer administration - personal experience (no veterinary input)		
Not sure		

## Section 8 Parasite-related disease

These questions focus on incidents of parasite-related disease, whether diagnosed or suspected, on your stud.

53.	Have there been any instances of worm-related illness at your stud?	Tick one box	
	Yes		
	No	Skip to Section 9	
54. If yes, what type of parasite related disease was seen and how was this diagnosed? Please tick all that apply			
	Personal/Staff opinion	Veterinary opinion (no tests performed)	Veterinary diagnosis
	Poor performance		
	Worms in faeces		
	Diarrhoea - lasting 1-4 weeks		
	Diarrhoea - lasting <1 week		
	Loss in/failure to gain condition		
	Lower weight gain		
	Weight loss		
	Larval cyathostomiasis		
Colic	Treated MEDICALLY		
	Treated SURGICALLY		
55.	On average, how many cases of CONFIRMED parasite-related disease have you had in the past 2 years on your stud?	Tick one box	
	0		
	1-5		
	5-10		
	>10		
	Prefer not to say		
56.	Do you think, based on your stud or from speaking to others, there has been a change in the number of parasite-related disease cases?	Please tick all that apply	
YES	INCREASE on <u>my stud</u>		
	INCREASE in reports from the <u>local area</u>		
	DECREASE on <u>my stud</u>		
	DECREASE in reports from the <u>local area</u>		
	NO change		
57.	Of the parasites listed below which have been CONFIRMED as being responsible for periods of illness?	Please tick all that apply	
	Small redworm ( <i>Cyathostomins</i> )		
	Large strongyles (e.g. <i>Strongylus vulgaris</i> )		
	Roundworm ( <i>Parascaris equorum</i> )		
	Tapeworm (such as <i>Anoplocephala perfoliata</i> )		
	Liver fluke ( <i>Fasciola hepatica</i> )		
	Other, please specify		

## Section 9 Diagnostic testing for worms and use of results

This section aims to discover how many studs are using faecal egg counts and tapeworm ELISA testing; whether they are used for screening and/or decision making purposes and how treatment thresholds are determined.

58. Do you have faecal egg counts performed on your horses?	Tick one box
Yes	
No*	

59. Do you have tapeworm ELISA tests performed on your horses?	Tick one box
Yes	Blood test
	Saliva test
No*	

- - If you answered **NO** to **Q58 AND Q59** please **skip to section 10**
- - If you answered **YES** to **EITHER** please **continue to question 60**

60. Who performs faecal egg counts?	Tick all that apply
Veterinary Practice	
On-site stud laboratory	
External laboratory	
Mobile faecal egg counting laboratory	
Other, please specify	
FEC not performed	

61. How often are faecal egg counts performed?	Please tick all that apply
Monthly	
Every 2-3 months	
Every 6 months	
Annually	
Infrequently/Randomly	
Only when there is suspicion of a parasite-related illness	
During quarantine	Visiting equines
	Home (returning) equines
	Both home and visiting
FEC not performed	

62.	How often are tapeworm ELISA tests performed?	Please tick all that apply	
	Monthly		
	Every 2-3 months		
	Every 6 months		
	Annually		
	Infrequently/Randomly		
	Under suspicion of a parasite-related illness		
	During quarantine	Visiting equines	
		Home (returning) equines	
		Both	
	Not performed		

63.	In what form are FEC and/or ELISA results provided? Is advice on whether to treat and wormer treatments all provided by the same individual/company?	Please tick all which apply	
		FEC	ELISA
	By post/email - report on which need treatment		
	By post/email - results only, no advice given		
	In person - horses which require treatment identified		
	In person - results only, no advice given		
	By post/email - Results given and wormer purchased from same person/company		
	In person - Results given and wormer purchased from same person/company		
	Other, please specify		

64.	On receiving faecal egg count results do you treat... (epg = eggs per gram)	Tick one box
	Only horses with positive egg counts (anything above 0epg)	
	All horses regardless of egg count	
	Only horses with egg counts above 50 epg	
	Only horses with egg counts above 100epg	
	Only horses with egg counts above 200epg	
	FEC report includes a list of horses which need treatment and these are treated (threshold not specified)	
	Other threshold, please specify	
	Not performed	

65. On receiving tapeworm ELISA results do you treat...	Tick one box
All horses regardless of result	
Horses with "high" results ONLY	
Horses with "moderate" and "high" results ONLY	
All horses with a positive result ("low", "moderate" and "high")	
ELISA report includes a list of horses which need treatment and these are treated (results not specified)	
Other, please specify	
Not performed	

66. How valuable do you feel faecal egg count and/or ELISA results (depending on whether you use both or just one of these diagnostic tests on your stud) have been for each of the following...		Very Unimportant	Unimportant	Neutral	Important	Very Important
Screen to monitor pasture contamination	FEC					
	ELISA					
Evaluating pasture management strategies	FEC					
	ELISA					
Screen to monitor parasite level within the herd	FEC					
	ELISA					
Worming product/drug choice	FEC					
	ELISA					
Determining which equines to treat	FEC					
	ELISA					
Reducing clinical disease	FEC					
	ELISA					
Improving overall health	FEC					
	ELISA					

## Section 10

### Faecal egg count reduction testing (FECRT)

This section asks about faecal egg count reduction testing, if this has been performed on your stud and if so what the results were.

67. Do you know what a faecal egg count reduction test (FECRT) is?	Tick one box
Yes	
No	

68. Has a FECRT been performed at your stud?	Tick one box
Yes	
No	Skip to Section 11

69. For which age group/s was a FECRT performed?	Please tick all which apply
<1 year old	
1-3 years (Youngstock)	
4-6 years	
7-10 years	
11-14 years	
15 years and above	

70. For your FECRT please provide	
Date test performed	
Drug/product tested	

71. Were eggs observed in post-treatment samples?	Tick one box
Yes	
No	

72. If resistance (eggs post-treatment) was observed, within which age group/s was this?	Please tick all that apply
<1 year old	
1-3 years (Youngstock)	
4-6 years	
7-10 years	
11-14 years	
15 years and above	
No resistance detected	

## Section 11 Targeted Strategic Worming

These questions look at your knowledge of targeted strategic worming protocols and, if you have used them, how effective you believe them to be.

<b>73. What does "Targeted strategic worming" mean to you?</b>	
Basing all worming treatments on faecal egg counts performed every 3-4 months	
Treating all horses once per year with a wormer but no diagnostics between treatments	
Treating all horses every three months with a wormer	
Treating only horses which seem to have parasite-related disease	
Using specific drugs each season to target specific parasites	
Other, please specify	

<b>74. Does your stud use Targeted Strategic Treatment?</b>	<b>Tick one box</b>
Yes	
No	Please skip to last page

### Targeted Strategic Worming *Your experiences*

<b>75. When did you change to targeted strategic worming?</b>	<b>Tick one box</b>
>2 years ago	
1-2 years ago	
<1 year ago	

<b>76. When looking at your egg count results (SINCE changing to a targeted program) have you seen an...</b>	<b>Tick one box</b>
Overall increase in worm egg counts	
Overall decrease in worm egg counts	
No change in egg counts	

<b>77. Do you now use...</b>	<b>Tick one box</b>
More worming treatments	
Less worming treatments	
Approximately the same number	

**78. Comparing first FEC results to most recent have you seen a change in prevalence of these parasitic species?**

<i>Tick one per parasite</i>	Increase in egg count	Decrease in egg count	No change in egg count
Strongyles ( <i>Small – Cyathostomin spp/ Large – Strongylus spp</i> )			
Ascarids ( <i>Parascaris equorum</i> )			
Tapeworm ( <i>Anoplocephala spp</i> )			
Pinworm ( <i>Oxyuris equi</i> )			

**79. Since starting targeted treatment have you seen a change in instances of worm-related illness at your stud?**

	Increase	Decrease	No change in number
Worms in faeces			
Diarrhoea - lasting 1-4 weeks			
Diarrhoea - lasting <1 week			
Loss in/failure to gain condition			
Lower weight gain			
Weight loss			
Colic	Req. MEDICAL treatment		
	Req. SURGICAL treatment		
Larval cyathostomiasis			
Poor performance			

**80. Do you feel confident that targeted treatment controls parasite transmission on your stud?** Tick one box

Yes	
No	

**81. Do you feel targeted treatment has benefited your business?** Please tick all that apply

YES	Less money spent on wormers	
	Reduced risk of developing drug resistance	
	Improved health of stock	
	Confidence that less unnecessary drugs are administered to healthy animals	
Other, please specify		
NO benefits		

**82. Would you recommend changing over to targeted treatment to others?** Tick one box

Yes	
No	
Unsure	
Other comments about targeted treatment	

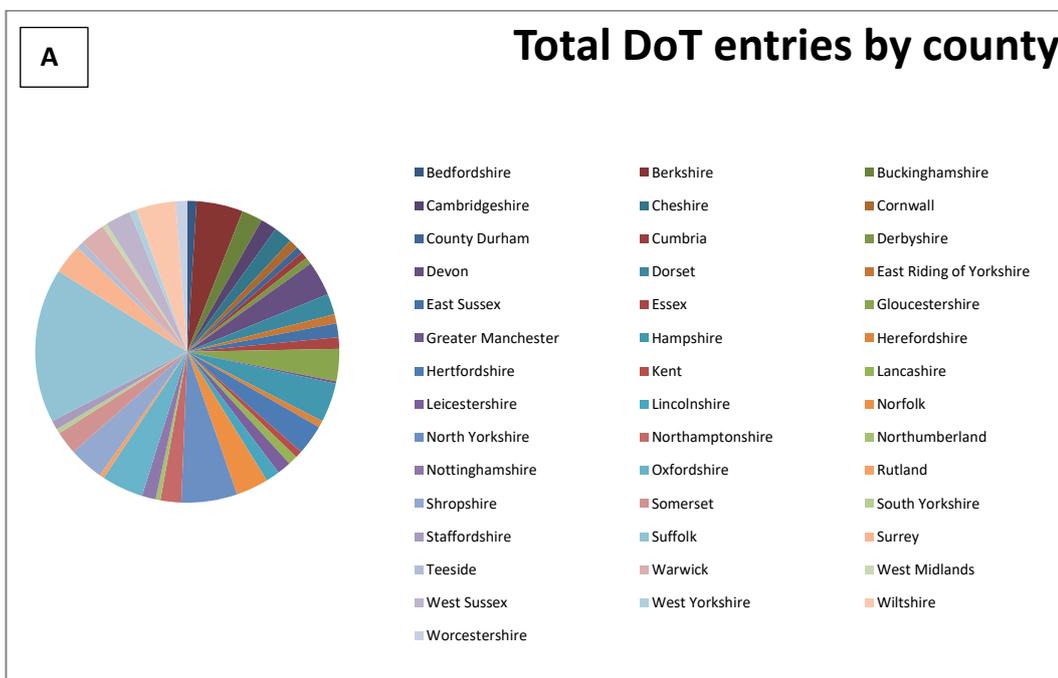
Do you have any comments you would like to share with us?

Once the questionnaire study is complete a results report will be sent to all participating studs.

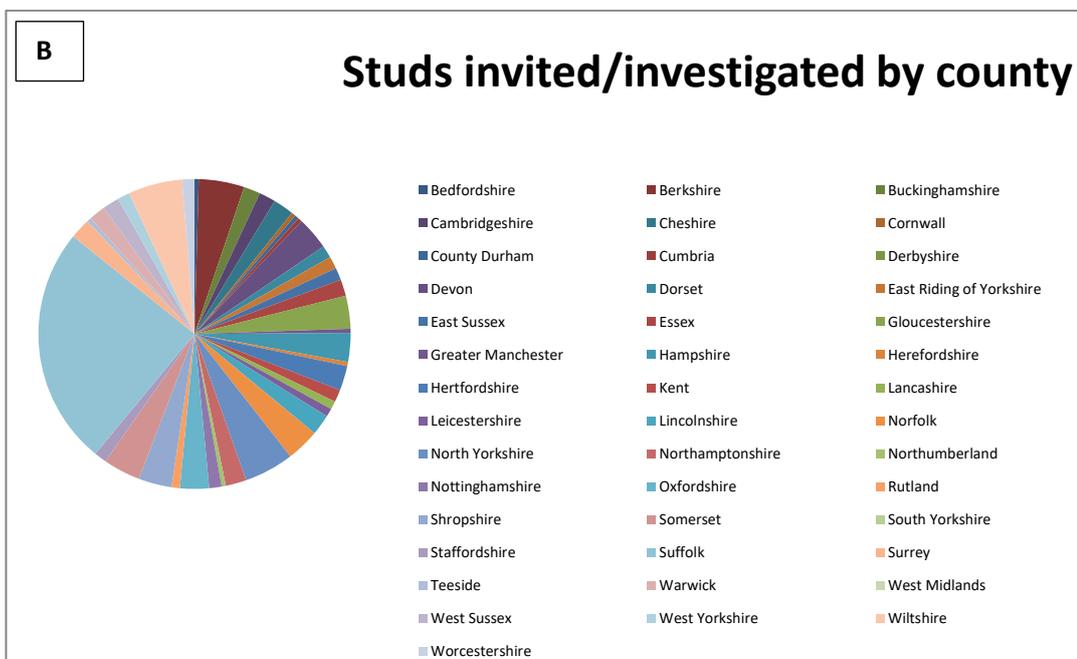
**Thank you** for your time to complete this questionnaire, your participation is invaluable to the continuation of our work.

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# Appendix 6B



A: The demographic spread of all DoT entries, included those unverified by this study in order to show the geographic spread of all stud farms listed.



B: The studs which were invited or investigated from each county for comparison with A in order to show that a geographically representative sample of entries was investigated for inclusion in the study using a random sample of DoT listings.

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