Prevalence and Control of Strongyle Nematode Infections of Horses in Sweden

Eva Osterman Lind

Faculty of Veterinary Medicine and Animal Science
Department of Biomedical Sciences and Veterinary Public Health
Division of Parasitology and Virology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2005

Acta Universitatis Agriculturae Sueciae
2005:29

ISSN 1652-6880
ISBN 91-576-7028-5

Tryck: SLU Service/Repro, Uppsala 2005

Abstract

Osterman Lind, E. 2005. *Prevalence and control of strongyle nematode infections of horses in Sweden*. Doctoral thesis.
ISSN 1652-6880, ISBN 91-576-7028-5

Strongyle nematodes constitute the most important group of helminths in horses. In this PhD project, based on five separate studies, the overall aim was to investigate the present parasite status of horses in Sweden and to determine the efficacies of the commonly used anthelmintics. It is hoped that the results will serve as a foundation for the development of relevant control strategies in horse establishments.

In a national survey strongyle nematodes were found to be ubiquitous in Sweden, and it was established that young horses on studs had the highest faecal output of strongyle eggs. Furthermore, horses in the south of the country shed more eggs than those in the central and northern parts. Eggs from the pathogenic species *Strongylus vulgaris* were identified in 14% of 110 farms investigated.

In a subsequent study, the prevalence and species composition of small strongyles were investigated in 27 horses on a stud in southern Sweden. Following anthelmintic treatment, worms expelled in faeces were collected and identified to the species level. In addition, the expulsion method was evaluated. Fifteen species of the tribe Cyathostominea were documented for the first time in Sweden. The mean number of species per horse was nine.

The presence of anthelmintic resistance in populations of small strongyles was investigated in two studies using different detection methods, namely the larval development assay (LDA) and the traditional faecal egg count reduction test (FECRT). In the first of these studies, it was concluded that LDA was not a reliable tool for routine monitoring of horse establishments. This was due to difficulties in data interpretation and inexplicable variation in the development of parasite larvae within and between assay plates. The latter study revealed that benzimidazole resistance was widespread despite a low use of these drugs for the past 20 years. Moreover, the first case of pyrantel resistance in Sweden was documented.

Finally, a questionnaire study on parasite control practices on Swedish horse establishments was performed. Most respondents were concerned about anthelmintic resistance, although they considered parasites to be a minor problem in their horse establishments. The survey highlighted the need for changes in routines in order to slow the spread of anthelmintic resistance.

Keywords: Strongyloidea, Cyathostominae, parasitic nematodes, anthelmintic resistance, ivermectin, pyrantel, fenbendazole, control practices

Author's address: Eva Osterman Lind, Department of Biomedical Sciences and Veterinary Public Health, Division of Parasitology and Virology, SLU, P.O. Box 7036, SE-750 07 Uppsala, Sweden

Svensk sammanfattning

Förekomst och kontroll av blodmaskinfektion hos hästar i Sverige

Hästar är värddjur för ett stort antal parasitära maskar (helminter), varav blodmaskar, eller så kallade strongylida maskar, utgör den viktigaste gruppen. I denna doktorsavhandling har förekomst och utbredning av blodmaskinfektion studerats hos svenska hästar. Vidare har artsammansättning och effekt av olika avmaskningsmedel (anthelmintika) undersökts i populationer av små blodmaskar, s.k. cyathostominer. Även en enkätstudie som belyste hur hästägare upplever parasitproblem och vilka åtgärder de vidtar för att förebygga parasitinfektioner hos sina hästar genomfördes.

Syftet med den första delstudien var att undersöka frekvensen av blodmaskägg i träckprov från svenska hästar. Träckprov från 1183 hästar vid 110 stall analyserades varvid antalet blodmaskägg per gram träck (EPG) fastställdes och följande faktorers inflytande på äggutskiljningen undersöktes: a) region i landet, b) typ av besättning, c) hästarnas ålder, d) avmaskningsmedel. Det visade sig att den genomsnittliga äggutskiljningen var högst i södra Sverige. Vidare hade hästar på stuterier ett högre genomsnittligt EPG än hästar på andra typer av gårdar. Unga hästar hade högre EPG än äldre; 2-3 år gamla hästar utskiljde flest ägg. Vi fann inga signifikanta skillnader i EPG beroende på antalet veckor som förflutit sedan senaste avmaskning. Däremot hade hästar som avmaskats med ivermektin i genomsnitt lägre EPG än hästar som avmaskats med pyrantel. Totalt hade 14% av de undersökta besättningarna en eller flera hästar som utskiljde ägg av stora blodmasken, *Strongylus vulgaris*.

I den nästkommande studien undersöktes vilka arter av cyathostominer som förekom hos 27 unga hästar vid ett stuteri i Skåne. Metoden som användes gick ut på att driva ut maskar från tarmen med hjälp av avmaskningsmedel. Hästarna delades in i tre grupper som avmaskades med ivermektin, pyrantel respektive fenbendazol. Under 3 dygn tillvaratogs all avföring som producerades av hästarna. Delprov från varje häst undersöktes under lupp och maskar samlades för att senare artbestämmas. Totalt identifierades 15 arter av cyathostominer. De sex vanligaste arterna (*Cylicostephanus longibursatus, Cylicocyclus nassatus, Cyathostomum catinatum, Cylicocyclus leptostomus, Cylicostephanus minutus* och *C. calicatus*) utgjorde >90% av den tillvaratagna populationen. I genomsnitt var varje häst infekterad med nio arter. Majoriteten av maskarna tillvaratogs under första dygnet efter ivermektin- och pyrantelavmaskning och under det andra dygnet efter avmaskning med fenbendazol.

Den tredje studien syftade dels till att undersöka förekomsten av anthelmintika-resistens hos små blodmaskar och dels till att utvärdera en larvutvecklingstest (LDA) som primärt utvecklats för resistensundersökning av fårparasiter. Fördelen med LDA jämfört med det traditionella testet (faecal egg count reduction test, FECRT) är att träckprov från endast ett tillfälle krävs. Med LDA kan man dessutom undersöka förekomst av resistens mot flera avmaskningsmedel. Antalet EPG bestämdes i träckprov från hästar vid 70 besättningar. Endast besättningar där ägg kunde påvisas undersöktes sedan med larvutvecklingstestet. Åtta gårdar fick uteslutas innan dataanalysen, bland annat på grund av bakterieöverväxt på testplattorna. Resultat erhölls från 54 gårdar. Tyvärr var resultaten svårtolkade och slutsatsen från denna studie var att testet inte var tillförlitligt för rutindiagnostik av resistens hos hästens små blodmaskar.

En FECRT genomfördes sedan i 26 större besättningar. Målet var alltjämt att undersöka förekomsten av anthelmintikaresistens. Utifrån ålder och EPG indelades hästarna på varje gård i tre behandlingsgrupper som avmaskades med ivermektin, pyrantel respektive fenbendazol. Träckprov togs i samband med avmaskning samt efter 7, 14 och 21 dagar. Den genomsnittliga reduktionen av antalet EPG bestämdes därefter för varje behandlingsgrupp. Ivermektin visade sig ha god effekt på samtliga gårdar medan resistens mot fenbendazol förekom hos 73% av de undersökta gårdarna. Pyrantelresistens dokumenterades för första gången i en svensk besättning. I denna studie artbestämdes också små blodmaskar som var misstänkt resistenta mot fenbendazol eller pyrantel.

I projektets sista studie undersöktes avmasknings- och betesrutiner med hjälp av en enkätstudie som innehöll 26 frågor. Enkäten skickade till 627 hästägare/tränare/ridskolechefer som lottats ut bland försäkringstagare i Agria samt från olika medlemsförteckningar. Svarsfrekvensen var överlag hög (71%). Praktiskt taget alla respondenter (99,5%) avmaskade sina hästar minst en gång per år. När det gällde användning av anthelmintika framgick det av studien att styngflugan (*Gasterophilus intestinalis*) har stor betydelse för val av tidpunkt för avmaskning och för preparatval. Således användes makrocykliska laktoner, till exempel ivermektin, i hög utsträckning på hösten. Dessutom ansågs sen höst vara den viktigaste tiden på året för avmaskning. Trots att 72% av respondenterna aldrig hade upplevt några parasitproblem hos sina hästar ansåg de flesta ändå att resistens mot avmaskningsmedel är ett angeläget problem. Studien lyfte fram behovet av att modifiera vissa kontrollrutiner för att förlångsamma spridningen av resistens. Hos alla kategorier av respondenter framkom det att praktiserande veterinärer har en central roll som rådgivare i parasitfrågor. Därför bör man eftersträva ett ökat samarbete mellan parasitologer och kliniskt verksamma veterinärer.

VISHET

Vishet
är att se begränsningen
utgå från den
och överge de falska drömmarna

Mod
Är att ständigt rasera
det ohållbara
och bygga nya drömmar
ur det möjliga

Styrka
är att utgå från sig själv
inse att livet aldrig
blir annorlunda
jag är jag
och marken stannar kvar
under mina fötter

Vishet
återigen
är att sammanfatta allt detta
till ett sätt att leva
begränsad
men öppen mot det obegränsade

Lars Björklund

Contents

Appendix

Papers I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Osterman Lind, E., Höglund, J., Ljungström, B.-L., Nilsson, O. & Uggla, A. 1999. A field study on the distribution of strongyle infections of horses in Sweden and factors affecting faecal egg counts. *Equine Veterinary Journal* 31, 68-72.

II. Osterman Lind, E., Eysker, M., Nilsson, O., Uggla, A. & Höglund, J. 2003. Expulsion of small strongyle nematodes (cyathostomin spp) following deworming of horses on a stud farm in Sweden. *Veterinary Parasitology* 115, 289-299.

III. Osterman Lind, E., Uggla, A., Waller, P. & Höglund, J. 2005. Larval development assay for detection of anthelmintic resistance in cyathostomins of Swedish horses. *Veterinary Parasitology* 128, 261-269.

IV. Osterman Lind, E., Kuzmina, T., Uggla, A., Waller, P. & Höglund, J. A field study on the effect of some anthelmintics on cyathostomins of horses in Sweden. Submitted manuscript.

V. Osterman Lind, E., Rautalinko, E., Uggla, A., Waller, P., Morrison, D.A. & Höglund, J. Parasite control practices on Swedish horse farms. Submitted manuscript.

Some terms and abbreviations

Anthelmintic	drug with efficacy against parasitic worms
AR	anthelmintic resistance
BZ	benzimidazole (an anthelmintic)
EPG	number of eggs per gram of faeces
ERP	egg reappearance period
FBZ	fenbendazole (an anthelmintic)
FEC	faecal egg count
FECR	faecal egg count reduction (percentage)
FECRT	faecal egg count reduction test (*in vivo* test for AR)
Ig	immunoglobulin
IGS	intergenic spacer of ribosomal DNA (rDNA)
ITS-1 and ITS-2	first and second internal transcribed spacer of rDNA
IVM	ivermectin (an anthelmintic)
L1-L5	larval stages in the life cycle of nematodes
Larval inhibition	temporary cessation of larval development in the host
Larval migration	certain species have larval stages that migrate in the body before they settle in their final site
LC	lower 95% confidence limit
LC50 value	the concentration that prevents 50% of the eggs developing to the L3 stage
LDA	larval development assay (*in vitro* test for AR)
LEV	levamisole (an anthelmintic)
PCR	polymerase chain reaction
Prepatent period	time from infection until eggs are detectable in faeces
PYR	pyrantel (an anthelmintic)

Introduction

"Utaf alla de djur, som äro underkastade menniskjans Herrawälde, är Hästen utan twifwel det ädlaste och mäst wärdt wår omsorg; ty hwars tjenst är oss mera nyttig och angenäm än en del andra" (Of all animals submitted to human domination, the horse is doubtless the most noble and deserves the most care; since its service to us is more useful and pleasant than others) wrote Peter Hernquist (1726-1808), the founder of the first veterinary school in Sweden. This statement reflects the prominent position of the horse in society in the 1700s. Horses were the main means of transport, both in the army and for civil transport, until industrialisation and the establishment of railways. During the Second World War the horse still had a central position for smallholders in Sweden, and the estimated number of horses in the 1940s was 600,000, most of which were cold-blooded working horses (Bengtsson, 1993).

There is a current increase in the number of horses in Sweden. Today, there are 250-300,000 horses, of which the great majority are leisure and sports horses. The Swedish horse sector has an annual turnover of approximately 20 billion SEK, of which 50% is attributed to gambling (Anonymous, 2004). It is primarily within the trotting discipline that gambling is of significance. Furthermore, horse riding is the greatest sport practised by girls and women, and there are approximately 900 riding clubs in Sweden. Since horses are so popular in Sweden, they are of major veterinary importance. Indeed, in some regions they are the major source of calls to veterinarians. Therefore, an understanding of the parasitological problems and appropriate prevention methods is of significance. This thesis addresses the role of strongyle nematodes in the Swedish horse industry.

Background

Strongyle nematodes of the horse

The horse is host to a great number of gastrointestinal parasite species, of which nematodes of the family Strongylidae, commonly called strongyle nematodes or strongyles, are the most important. These parasites are ubiquitous and live as adults in the large intestine of equids. Strongyle nematodes also occur in other domestic livestock, for example *Chabertia ovina* in sheep and *Oesophagostomum* spp. in ruminants and pigs. The main characteristic feature of strongyle nematodes is a well-developed buccal capsule, the shape and size of which are important for species identification. Strongyle nematodes of equids (horse, donkey, zebra) are classified into the subfamilies Strongylinae and Cyathostominae, sometimes categorized as large and small strongyles, respectively. Within the subfamily Cyathostominae, virtually all species parasitizing horse belong to the tribe Cyathostominea. In 2001, the recommendation to use the term *cyathostomins* as a common name for members of the tribe Cyathostominea was adopted by the World Association for the Advancement of Veterinary Parasitology (Lichtenfels, Gibbons & Krecek, 2002).

In 1780, the first strongyle nematode, *Strongylus equinus*, was described by Müller (Lichtenfels, 1975), and during the following century descriptions and naming of several strongyle species were published. In the definitive monograph by Ralph Lichtenfels (1975), 56 species of strongyle nematodes of domestic equids were described in detail. Since then the classification of these parasites has undergone some revision, partly due to the development of modern molecular-based techniques. These techniques have opened the door for studies on the molecular relationships between species. Research groups, mainly in Australia (Hung *et al.*, 2000) and the UK (McDonnell *et al.*, 2000), are currently working on establishing the phylogenetic relationships of the two subfamilies. By comparing DNA sequences it has been shown that the genera with small cylindrical buccal capsules are likely to have evolved from those with large buccal capsules (Hung *et al.*, 2000).

Morphology

Nematodes have a cylindrical form, and the body is covered by a colourless layer, the cuticle. The digestive system includes the mouth, the oesophagus and the intestinal tube, which terminates in an anus in females and a cloaca in males. The female reproductive organs comprise ovary, oviduct and uterus, whereas in the male this comprises the testis and a vas deferens. The males also posses accessory organs – the most significant being the spicules and the gubernaculum. These organs are essential for species identification of ruminant trichostrongyloids, but are of less importance for the identification of horse strongyles.

The morphological differentiation of the strongyle species of horses is primarily based on characteristics of the cephalic end (head) of the worm (Lichtenfels, 1975) (Fig 1). The buccal capsule is well-developed. Many strongyles have a distinct thickening that extends dorsally in the buccal capsule, the so-called dorsal gutter. A mouth collar, which is part of the cuticle, surrounds the mouth opening; sometimes it contains sub median and lateral cephalic papillae. On the anterior edge of the mouth collar leaf-like structures, so-called leaf crowns, are formed. Usually there are two such crowns, the external and the internal, with the internal being located distally to the external. Some genera have a unique structure called the extra-chitinous support for the external leaf crown, which is a sclerotized ring anterior to the buccal capsule.

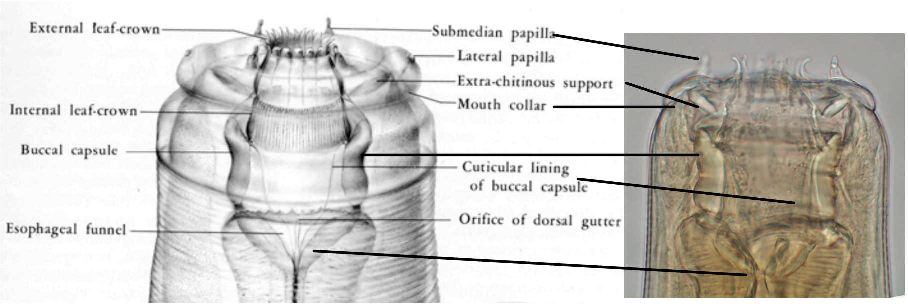


Fig. 1. Head of *Coronocyclus coronatus* (schematic drawing to the left; photo x 200 to the right). The schematic figure shows some of the characteristics used for species identification. The drawing is used by courtesy of Dr. R. Lichtenfels. (Photo: E. Osterman Lind)

Species

Strongylinae

The species of Strongylinae have large spherical or funnel-shaped buccal capsules, often containing some kind of teeth at the base. The genera included in the subfamily Strongylinae are: *Strongylus* (Müller, 1780), *Oesophagodontus* (Railliet & Henry, 1902), *Triodontophorus* (Looss, 1902), *Craterostomum* (Boulenger, 1920) and *Bidentostomum* (Tshoijo, 1957). In the future there may be a revision of the current systematics of the subfamily, as phylogenetic studies have indicated that *Oesophagodontus robustus, Triodontophorus* spp. and *Craterostomum acuticaudatum* are more closely related to the subfamily Cyathostominae (Hung *et al.*, 2000).

Strongylus, the most studied genus, encompasses four species: *S. edentatus, S. equinus, S. vulgaris* and *S. asini*. The latter has been isolated only from the zebra and the donkey. The length of these nematodes varies from 20-45 mm, and they are fairly stout. The buccal capsule is globular, and for *S. equinus, S. vulgaris* and *S. asini* it contains prominent teeth that are characteristic of the different species. *Oesophagodontus* consists of the single species *O. robostus,* which is considered to be rare (Lichtenfels, 1975). Seven species belong to the genus *Triodontophorus*, all equipped with three pairs of large teeth extending into the buccal capsule. *Craterostomum*, the smallest of the large strongyles, consists of only two rare species, of which *C. acuticaudatum* occurs worldwide (Lichtenfels, 1975). The genus *Bidentostomum* with one species, *B. ivaschkini*, is the latest addition to the subfamily (Lichtenfels, 1975; Lichtenfels *et al.*, 1998).

Cyathostominae

The buccal capsule of cyathostomins is short, cylindrical or annular and the worms are generally smaller than the large strongyles, although some species within the genus *Cylicocyclus* may reach 25 mm in length. A number of studies on the prevalence of the cyathostomins throughout the world are based on the morphological keys by Lichtenfels (1975). Here he described 41 species, assigned to the following eight genera: *Caballonema* (Abuladze, 1937), *Cyathostomum* (Molin, 1861), *Cylicocyclus* (Ihle, 1922), *Cylicodontophorus* (Ihle, 1922), *Cylicostephanus* (Ihle, 1922), *Cylindropharynx* (Leiper, 1911), *Gyalocephalus* (Looss, 1900) and *Poteriostomum* (Quiel, 1919). However, Hartwich (1986) later revised the systematics of the cyathostomins and reorganised some of the species into two new genera: *Coronocyclus* (Hartwich, 1986) and *Parapoterostomum* (Hartwich, 1986). Moreover, the genus *Petrovinema* (Ershov, 1943) was resurrected, whereas *Gyalocephalus* was placed separately from the tribe Cyathostominea. In the checklist published in 1998 (Lichtenfels *et al.*, 1998), three additional genera that had been acknowledged by Dvojnos and Kharchenko (1994) were included: *Hsiungia* (K'ung & Yang, 1964), *Skrjabinodentus* (Tshoijo, in Popova, 1958) and *Tridentoinfundibulum* (Tshoijo, in Popova, 1958). Thus, within the tribe Cyathostominea there are currently 13 genera recognised, comprising 52 species and one subspecies in equids. *Cylicocyclus asinus* in the donkey is the most recently described species (Lichtenfels, Gibbons & Krecek, 2002; Matthee, Krecek & Gibbons, 2002). To date, 11 of the 52 species have been recorded exclusively in donkeys and zebras.

Life cycles

Strongylus spp.

The life cycles of the *Strongylus* species are direct, but are somewhat complicated as they involve somatic migrations of larval stages. The prepatent periods (the time from infection until eggs are detectable in faeces) for members of this genus vary from 6 months (*S. vulgaris*) to 10-12 months (*S. edentatus*) (Urquhart *et al.*, 1996).

So-called verminous aneurysms in the arteries caused by *S. vulgaris* larvae have been recognised for a long time. In 1949 it was reported that 90% of the horses autopsied in Sweden had such aneurysms (Brinck, 1949). Although different theories had been proposed for how the larvae migrate to the mesenteric arteries (Popova, 1955), details of the life cycle remained vague until experimental infections of foals were carried out in the 1960s and 1970s. The following developmental cycle for *S. vulgaris* was suggested by Duncan and Pririe (1972): The third stage larvae (L3) on pasture, still enclosed in the cuticle of the second stage (L2), are ingested by grazing horses. The larvae exsheath and penetrate the mucosa of the small and large intestine within a few days. During the following week the larvae moult to the fourth stage (L4) and enter the lumen of small arterioles of the intestine. The L4s migrate up the arterial tree against the blood flow, and by 14-21 days they reach the cranial mesenteric artery, where they grow considerably in size for a period of 3-4 months before they moult to the fifth stage (L5). The L5s exsheath, and with the blood flow the young adults return down the mesenteric arteries to the wall of the caecum and colon, where they encapsulate in the subserosa, forming nodules of 5-8 mm in diameter. Eventually these nodules rupture and the young adults are released into the lumen of the large bowel. After another 6-8 weeks in the gut lumen the young adults have developed into sexually mature worms. Occasionally lesions elsewhere in the arterial system occur owing to aberrant migration of larvae.

Cyathostominae

The life cycles of the individual cyathostomin species are still not known in detail, and hence the route presented here is currently assumed to be applicable for all members of the subfamily. The ingested L3s exsheath in the small intestine before they enter the glands of Lieberkühn in the caecum and colon. In the base of these glands the larvae penetrate the mucosal layer, where they subsequently become enclosed by fibroblasts that form capsules. Some species, for example the large species *C. insigne* prefer to burrow deeper into the submucosa, whereas smaller species such as *C. longibursatus* stay in the mucosa (Ogbourne, 1978). In the wall of the large intestine the L3s moult to L4s. At some point the L4s break out of the capsules and enter the gut lumen (Fig 2), where the fourth moult takes place. The adult worms appear to have different site preferences in the large intestine depending on species (Ogbourne, 1978). Interestingly, the adults are usually found in an anatomic location distal to the preferred site of larval development. Thus, it appears that cyathostomins migrate posteriorly during maturation.

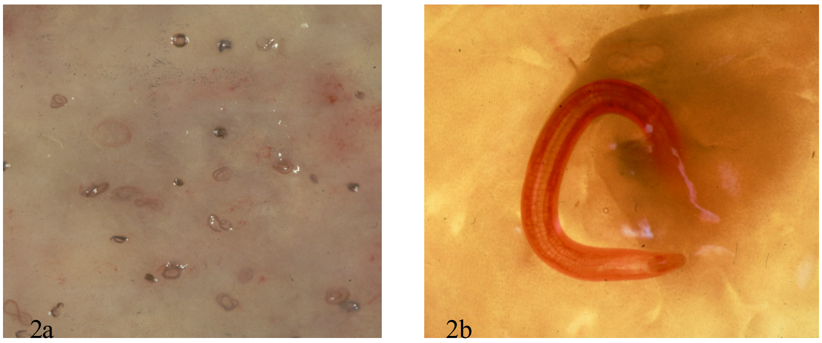


Fig. 2a) Cyathostomin fourth stage larvae (L4) encysted in the intestinal mucosa; *b)* L4 larva entering the intestinal lumen from the mucosa. (Photo: B. Ekberg, SVA and J. Höglund, SLU)

A proportion of the early stage larvae (EL3) will temporarily cease their development in the mucosa/submucosa (Eysker, Jansen & Mirck, 1984). This inhibited condition is considered to be a normal part of the life cycle (Coles *et al.*, 2003), and it may last for a prolonged period of time. Gibson (1953) demonstrated that inhibited larvae still could resume development in stabled horses after three years. Larval inhibition is a common phenomenon in nematode parasites of domestic livestock. Nevertheless, it is not known which factors initiate the process. For nematodes of ruminants, experimental infections have indicated that host immunity, population size and seasonal conditioning of larvae are all important (Armour & Duncan, 1987; Eysker, 1997a). These factors have also been discussed as potential triggers for cyathostomin larvae. Both in cattle and horses in the Northern Hemisphere it has been shown that the proportions of inhibited larvae are greater in the cold season, when the conditions on pasture are harsh for the infective larvae (Armour & Duncan, 1987; Eysker, Boersema & Kooyman, 1990; Collobert-Laugier *et al.*, 2002).

The prepatent period for cyathostomins is generally 2-3 months, although it varies depending on larval inhibition (Urquhart *et al.*, 1996). Also, the age of the horse seems to have an impact on the prepatent period, as it has been shown that it is longer in old horses than in helminth-naïve foals (Smith, 1978). The average prepatent period observed in a Scottish study of naturally infected horses was 54-62 days (Love & Duncan, 1992b).

Prevalence

The three *Strongylus* species that infect horses are common throughout the world, particularly *S. vulgaris*, which is also recognised as the most pathogenic species of the large strongyles. However, thanks to the widespread use of modern anthelmintics (deworming drugs), the occurrence of *S. vulgaris* has greatly decreased in recent decades (Herd, 1990). In herds where anthelmintics have not been used to control parasites, the prevalence has remained high (Gawor, 1995).

Virtually all grazing horses are infected with cyathostomins. A great proportion of the total burden consists of larval stages in the intestinal mucosa and submucosa. The number of cyathostomins in the gut lumen may vary from a few thousand to more than 1,200,000 (Ogbourne, 1975a). Commonly, 90% of the adult worms are distributed throughout the dorsal and ventral colon, and the remaining 10% are found in the caecum (Reinemeyer, 1986; Gawor, 1995; Collobert-Laugier *et al.*, 2002).

Although more than 40 cyathostomin species may infect the horse, prevalence studies worldwide have shown that the great majority of the cyathostomin populations within horse herds are composed of fewer than 10 species (Ogbourne, 1976; Reinemeyer *et al.*, 1984; Torbert *et al.*, 1986; Krecek, Reinecke & Horak, 1989; Mfitilodze & Hutchinson, 1990; Gawor, 1995; Silva *et al.*, 1999; Lichtenfels *et al.*, 2001). Typically, one horse is infected with 5-10 of the common species (Lichtenfels, Gibbons & Krecek, 2002). *Cylicostephanus longibursatus, Cylicocyclus nassatus* and *Cyathostomum catinatum* have been reported to be the three most common species (Anderson & Hasslinger, 1982; Reinemeyer *et al.*, 1984; Mfitilodze & Hutchinson, 1990; Bucknell, Gasser & Beveridge, 1995; Silva *et al.*, 1999). However, these studies were carried out prior to the re-descriptions of *Cylicocyclus nassatus* and *Cylicocyclus ashworthi* (Lichtenfels *et al.*, 1997), and due to the morphological similarities between the two species, it is most likely that the prevalence of *Cylicocyclus ashworthi* had been under-estimated in favour of *Cylicocyclus nassatus.* In a study performed more recently in Scotland *Cylicocyclus ashworthi* was reported to be the most abundant species (Lichtenfels *et al.*, 2001).

Epidemiology

Strongyle eggs are passed with the faeces, and under good conditions they will hatch into first stage larvae (L1) within 2-4 days. The embryonated eggs tolerate low temperatures (even freezing), which will only delay further development (Uhlinger, 1991). The rate of hatching is directly proportional to the temperature of the environment (Ogbourne, 1972). Laboratory studies have shown that at temperatures between 25-33°C hatching is completed within 24 hours, whereas below 5°C the eggs remain viable but do not no hatch (Mfitilodze & Hutchinson, 1987). The first stage larvae are susceptible to freezing temperatures as well as to desiccation. When faecal material dries quickly the eggs hatch but the L1s do not develop further (Ogbourne, 1972). The L1s feed on bacteria before they develop to the second stage. The L2s are quite resistant to desiccation, but like the L1s they die following prolonged periods of cold.

For the development of the pre-infective stages into infective L3s, and further survival of L3s in the faeces, temperature and moisture interact. However, unless it is very dry, temperature is the limiting factor for development into L3s. Under laboratory conditions, L3s can only be recovered from faeces that has been incubated between 10-35°C. This takes 15-24 days at the lowest temperature and 3 days at the highest (Mfitilodze & Hutchinson, 1987). In Scotland, L3s were recovered from herbage samples from plots 3-4 weeks after the faeces had been deposited during May-October (Ramsey *et al.*, 2004).

In common with many gastro-intestinal nematodes of livestock, the infective L3s retain their L2 cuticles, which is beneficial for prolonged survival on herbage (Urquhart *et al.*, 1996). Thus, the L3s do not feed but live on stored nutrients. They survive longer in the desiccated state than under moist conditions because in the former state the larvae are not active and hence conserve energy, whereas in the latter situation higher temperatures stimulate migration from faeces to the surrounding herbage and cause a depletion of the stored energy (Mfitilodze & Hutchinson, 1987). It has been demonstrated that L3s do not all migrate from faeces simultaneously; the faecal deposits act as reservoirs from which the larvae are intermittently released (Ogbourne, 1972). The plot study in Scotland showed that under warm conditions very few infective larvae remained in the faeces after 4 weeks (Ramsey *et al.*, 2004).

It is well known that the L3s are capable of surviving severe cold, especially under a protective snow cover which somewhat stabilises the climatic variations (Urquhart *et al.*, 1996). Thus, the Swedish winter is not an obstacle for the L3s on pasture; a considerable number of infective larvae deposited in August-September may survive over the winter (Lindberg, 1976). Furthermore, it has been observed that the vegetation on old pasture is more favourable for overwintering larvae than is new pasture (Nilsson & Andersson, 1979). Nevertheless the overwintered larvae do not usually constitute a major problem the following summer. Studies in Scotland, Canada and Sweden have indicated that by late May and June, the numbers of overwintered infective larvae on the pasture are reduced to very low levels due to depletion of food store and dilution with fresh grass (Duncan, 1974;

Lindberg, 1976; Slocombe, Valenzuela & Lake, 1987). More important are the L3s originating from eggs passed in the current grazing season.

Figure 3 illustrates schematically the pattern of strongyle infections in mares and foals over the grazing season in temperate climates. A spring rise in the faecal output of eggs is seen (Poynter, 1954; Mirck, 1981; Herd, 1986; Klei, 1992). These eggs are derived from adult parasites that developed from larvae, which were ingested the previous year and subsequently inhibited over the winter. Often the output of eggs culminates with a peak in July-September, when the conditions become optimal for development of free-living stages on pasture (Herd, Willardson & A., 1985). In the northern USA, peak numbers of adult cyathostomins present in the large intestine have been observed in March and September (Reinemeyer *et al.*, 1986).

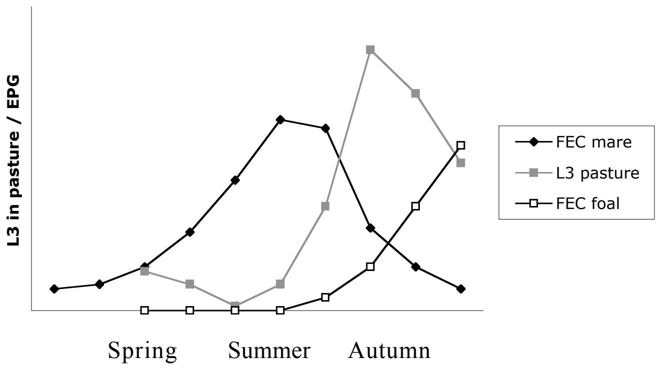


Fig. 3. Schematic illustration of the epidemiology of strongyle infections in mares and foals over the grazing period in temperate climates. The x-axis shows the season and the y-axis the levels of infective larvae (L3s) on pasture and strongyle eggs per gram of faeces (EPG). The figure is based on Urquhart *et al.* (1996) and unpublished data from Sweden.

The eggs develop to infective L3s that accumulate in the faeces on pasture. Coinciding with rainfall in late summer-early autumn, massive increases in the numbers of L3s in herbage are typically seen (Duncan, 1974; Nilsson & Andersson, 1979; Mirck, 1981; Craig, Bowen & Ludwig, 1983; Herd & Willardson, 1985; Polley, 1986; Slocombe, Valenzuela & Lake, 1987). Depending on the geographic location, climatic differences cause different transmission patterns. Thus, in central Texas the increase in larval recoveries does not occur until October and the mares do not exhibit a spring rise in the faecal egg output (Craig, Bowen & Ludwig, 1983). In southern Louisiana transmission occurs during all seasons, with the greatest numbers of L3s being present on the pasture in the winter and the lowest numbers in the hot summer months (Baudena *et al.*, 2000a; Chapman, French & Klei, 2001).

It is difficult to predict pasture infectivity since many factors, for example rainfall, temperature, moisture and degradation of faeces, influence the development and survival of eggs and larvae. This was obvious in a series of long-term studies performed on nematodes of cattle in Sweden (Dimander, 2003). The proportion of overwintering larvae differed tenfold both over the season and from one year to the next.

Pathogenicity

Strongylus vulgaris

The pathogenic effects of adult *S. vulgaris* are related to the parasite's ingestion of mucosa plugs of the large intestine. The feeding habits of the adults result in damage to the mucosal layer and occasionally rupture of blood vessels. Although the effects of adults in the gut are of less significance than those caused by the larvae, there is no doubt that heavy infections may cause unthriftiness and anaemia in the horse (Urquhart *et al.*, 1996).

The pathogenic effects of *S. vulgaris* infection are primarily caused by the migrating larval stages. The larvae induce endoarteritis in the mesenteric artery and its branches. This provokes thickening of the arterial wall, thrombus formation, and potential infarction and necrosis in areas of the intestine. The term "verminous aneurysm" refers to true aneurysms with dilation and thinning of the arterial wall that may occur as a result of migrating larvae. The clinical significance of lesions caused by *S. vulgaris* larvae depends on their magnitude and location. Abdominal distress and colic are regarded as the most common clinical symptom. In foals, clinical symptoms such as a rise in temperature, anorexia, depression and abdominal pain have been observed within a few weeks following experimental infection (Duncan & Pirie, 1973). Experimentally, it has also been found that larval migration may induce extra-vascular lesions such as eosinophilic pneumonia and eosinophilic granulomas in the epicardium and liver (Turk & Klei, 1984). Under natural conditions, severe symptoms are rarely seen because foals may tolerate large numbers of larvae ingested in small doses over a long period (Urquhart, 1996). A study of natural infection of foals on highly contaminated pasture showed that exposure for one month was associated with debility and weight loss (Thamsborg *et al.*, 1998). Even though old horses are susceptible to infection with *S. vulgaris*, the complications of infarction and colic are most prevalent in young horses. Generally, colic initiated by *S. vulgaris* is more frequently seen during the winter than at other times of the year. This is most likely due to the number of larvae in the arterial system, with the highest numbers being present during the winter months (Ogbourne, 1975b).

Cyathostominae

Historically, cyathostomins have been considered as essentially non-pathogenic, even though parasites in the large intestinal mucosa were observed to be associated with diarrhoea over 150 years ago (Love, Murphy & Mellor, 1999) and detailed descriptions of the clinical manifestations of cyathostominosis were made over 50 years ago (Velichkin, 1952). The pathogenic effects of cyathostomins were probably overlooked by veterinary practitioners owing to the dramatic symptoms that were seen in horses suffering from *S. vulgaris* infection. However, the decline of *S. vulgaris* and the rise of anthelmintic-resistant cyathostomins over the past decades has changed the view of the relative importance of these parasites. Today, cyathostomins are considered to be the principal nematode pathogens of the horse (Love, Murphy & Mellor, 1999).

Adult cyathostomins are regarded to be of minor clinical importance, although infections with adult worms may be associated with mild diarrhoea. As with *S. vulgaris*, the larval stages are responsible for most of the damage caused. In the mucosa and submucosa the larvae are surrounded by a fibrous capsule, and there may be an inflammatory reaction with eosinophils, which is usually more intense in the submucosa (Jubb, Kennedy & Palmer, 1985). Goblet hypertrophy and hyperplasia around the encapsulated larvae are frequently seen. Emergence of the larvae from the gut wall into the lumen causes rupture of the muscularis mucosae and intense eosinophilia and oedema, followed by infiltration of neutrophils and macrophages. Heavily infected horses may carry as many as 60 larvae per square centimetre of the large intestine mucosa (Jubb, Kennedy & Palmer, 1985).

A clinical syndrome termed larval cyathostominosis (Lichtenfels, Gibbons & Krecek, 2002) may occur following synchronised reactivation of inhibited larvae and subsequent mass emergence of L4s into the intestinal lumen. Risk factors associated with the development of clinical disease include horse age, season and recent anthelmintic treatment (Reid *et al.*, 1995). Larval cyathostominosis is an acute disease that primarily affects horses younger than six years, although it may be diagnosed in horses of all ages. The condition is seasonal, and in temperate climates it often occurs in January-May, when the mucosal larvae resume development after inhibition (Giles, Urquhart & Longstaffe, 1985). Thus, to some extent it can be compared to type II ostertagiosis in cattle (Soulsby, 1986). Sometimes larval cyathostominosis is seen in single individuals (Reilly, Cassidy & Taylor, 1993), or sometimes as outbreaks in groups of horses (Kelly & Fogarty, 1993). The main clinical effect attributable to cyathostominosis is weight loss (Love, Murphy & Mellor, 1999). Other typical clinical features are: profuse, sudden-onset diarrhoea, which can become chronic; loss of body condition; weakness but normal appetite; and subcutaneous oedema of the limbs and ventral abdomen (Paul, 1998). Death is relatively common; a mortality rate of >50% has been reported (Love, 1995). In the UK, it has been shown that larval cyathostominosis is the commonest cause of chronic diarrhoea in horses older than 1 year (Love, Mair & Hillyer, 1992). The diagnosis is based on the history of the affected horse and the presence of cyathostomin larvae in faeces. In addition, more unspecific findings, such as negative or low faecal egg counts, neutrophilia, hypoalbuminemia and hyperglobulinemia with an increase in □□globulins, support the diagnosis.

Besides the classical seasonal syndrome, a variety of other clinical manifestations have been associated with cyathostomin infection. These involve non-specific colic (Uhlinger, 1990), recurrent diarrhoea in aged ponies (Mair, 1993), and multifocal non-strangulating infarction of the large intestine (Mair & Pearson, 1995). Horses may also demonstrate mild or subclinical symptoms, such as loss of condition, weight loss and disturbed intestinal motility, possibly as a result of a protein losing enteropathy induced by the cyathostomins (Proudman & Matthews, 2000). In a Swedish study of naturally infected yearlings, a significantly lower average weight gain was observed in a group of untreated horses compared to an ivermectin treated group. It was interpreted that these differences were caused by developing cyathostomins (Nilsson, Hellander & Jerneld, 1990).

Immunity

It has long been accepted that young horses are more susceptible to strongyle infections than are older animals, but the mechanisms involved in the immune responses are complex and not much studied. A number of observations indicate that immunity is acquired with increasing age. High faecal egg counts and clinical cyathostominosis are more frequently seen in young horses, i.e. those under six years. There are also age differences in the prepatent period for cyathostomins and the time required for reappearance of strongyle eggs in faeces after deworming. These features are shorter in young animals compared to older ones (Smith, 1978; Love & Duncan, 1992a; Klei & Chapman, 1999). Moreover, in studies of cyathostomin burdens of naturally infected horses, increased species diversity has been observed in foals and yearlings compared to horses older than 8 years (Klei & Chapman, 1999).

Evidence of acquired immunity against strongyle infections has also been obtained from experimental infections. Studies on *S. vulgaris* showed that horses previously exposed to infection developed a significant resistance to re-infection and less clinical signs than animals reared worm-free (Duncan & Pirie, 1973). A more recent study of foals showed that previous exposure to heavily contaminated pasture induced immunity against challenge infection with mixed strongyle L3s. Furthermore, a second challenge of exposed individuals resulted in expulsion of existing nematodes (Monahan *et al.*, 1997). This expulsion was similar to self-cure, a well known Th2-mediated immune reaction that is often described from nematode infections of sheep (Tizard, 2000).

The immune response of the host is directed against different stages of the life cycle. Higher numbers of early L3s and other encysted stages have been demonstrated in the mucosa of previously exposed ponies compared to those unexposed (Chapman *et al.*, 2002). It was suggested that some host factors induce larval inhibition in previously exposed ponies. In another study, comparison of cyathostomin burdens of previously exposed foals and helminth-naïve foals also revealed higher proportions of mucosal stages in the previously exposed animals, but they had lower total burdens (Love & Duncan, 1992b). However, compared to adults (mean age 8.6 years), grazed foals and yearlings had higher numbers of inhibited larvae.

Generally, gastrointestinal nematodes are coupled to the Th2-type lymphocyte response, which includes secretion of the following cytokines: interleukin-4 (IL-4), IL-5, IL-6, IL-9, IL-10 and IL-13. The Th2 response involves mobilisation of eosinophils, intestinal mast cell accumulation and eventually IgE production. Thus, parasitized animals may have greatly elevated numbers of eosinophils and IgE levels. These effectors may result in type I hypersensitivity reactions such as urticaria, which occasionally occur in helminth infections (Tizard, 2000). The most common immunological feature of helminth infections is recruitment of eosinophils. As mentioned previously, large numbers of eosinophils may often be observed around the cyathostomin larvae in the gut wall. Peripheral eosinophilia is a more variable finding in horses with strongylosis. Following experimental infection with cyathostomin L3s of previously exposed adult ponies, eosinophil counts rose rapidly within 10 days and remained high for two years (Smith, 1978). It was suggested that the prolonged eosinophilia was related to the presence of inhibited larvae. In a more recent study, ponies immunised with radiation-attenuated *S. vulgaris* L3s prior to challenge infection showed marked eosinophilia after challenge compared to ponies immunised with crude soluble somatic extracts of parasites and to control ponies (Monahan *et al.*, 1994). Also a study of foals exposed to mixed strongyle infections for one month revealed a substantial increase in eosinophils in the blood after two weeks of grazing (Thamsborg *et al.*, 1998). However, in clinically affected horses, eosinophilia is not a consistent finding, and the absence of eosinophilia does not exclude the diagnosis of strongyle infection (Uhlinger, 1991; Love, 1995). In a report of 15 clinical cases of larval cyathostominosis, leucocytosis was frequently present but was associated with an increase in neutrophils rather than eosinophils (Giles, Urquhart & Longstaffe, 1985).

In conclusion, although many of the observations indicate development of immunity with age, it is clear that acquired resistance to strongyle infections is incomplete. Thus, heavy worm burdens may be found in individuals of all ages, and horses may contribute to infected pastures throughout their lives.

Diagnosis

Determining the number of strongyle eggs (Fig 4b) per gram of faeces (EPG) is the most widely used method for diagnosing infection with adult strongyle parasites. The main disadvantage of faecal egg counts is that they do not reflect the burden of sexually immature stages – L5s and L4s in the lumen, developing L4s and L3s in the mucosa, and inhibited L3s in the mucosa. Therefore, faecal egg counts are considered to be of little merit when larval cyathostominosis is suspected. Moreover, factors such as worm-density effects on egg production, uneven distribution of eggs in the faeces and faeces consistency, must also be recognised. However, faecal egg counts are useful for comparing the efficacy of anthelmintic compounds, detecting drug resistance, and determining the correct interval between anthelmintic treatments (Herd, 1992; Warnick, 1992).

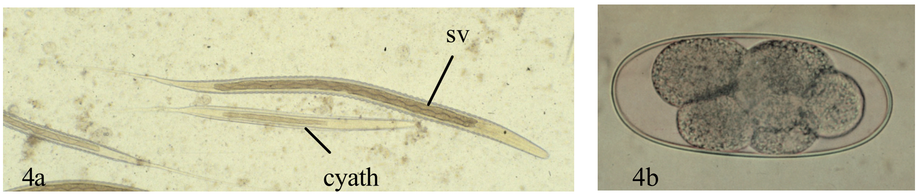


Fig. 4a) Third stage larvae (L3) from *S. vulgaris* (sv) and Cyathostominae (cyath); *b)* strongyle nematode egg. (Photo: SWEPAR)

Since it is not possible to distinguish strongyle eggs of different species morphologically, faecal samples are cultured for 10-14 days at 20-25°C to allow the development to L3s, which may be collected by means of the Baermann procedure (Anonymous, 1986). Based primarily on the number and shape of the intestinal cells of the L3, the majority of the large strongyles can be morphologically differentiated from one another and from cyathostomins, but different species of cyathostomins are still not distinguishable (Fig 4a). It has recently been reported that the Baermann technique is also a successful method for the recovery of immature cyathostomin larvae in the faeces of clinically diseased horses (Nautrup Olsen *et al.*, 2003).

Within the past decade, molecular approaches have been developed that enable species identification of pre-adult stages of strongyle nematodes (reviewed by Gasser *et al.*, 2004; Matthews *et al.*, 2004). Recent advances include the characterization of strongyle nematode ribosomal DNA sequences. The first and second internal transcribed spacers (ITS-1 and 2) (Gasser *et al.*, 1996) and the intergenic spacer (IGS) (Hodgkinson *et al.*, 2001) have been used as genetic markers for species identification. From the ITS sequences, species-specific oligonucleotide primers for some of the most common species (*S. vulgaris, C. catinatum, C. nassatus, C. longibursatus* and *C. goldi*) have been designed and used in a PCR system, thus allowing species-specific amplification of parasite DNA in eggs and larvae (Hung *et al.*, 1999). Recently, it was reported that IGS oligoprobes from six species (*C. ashworthi, C. nassatus, C. insigne, C. longibursatus, C. goldi*, and *C. catinatum*) have been used in a PCR-ELISA (hybridisation assay) for the detection of PCR products from L4s collected from diarrhoeic horses (Hodgkinson *et al.*, 2003). It was found that the samples from the diseased horses contained at least two species, suggesting that larval cyathostominosis is caused by mixed species infections. There is an on-going research project where IGS oligoporbes are used to study the effect of anthelmintic treatment at the species level (Matthews *et al.*, 2004). Also a microchip-based capillary electrophoresis technology has been employed successfully for species differentiation of closely related cyathostomins (Posedi *et al.*, 2004).

A method for detecting mucosal larval stages would be valuable in the diagnosis of larval cyathostominosis. Recently, promising results were obtained in this field, whereby studies on immune responses to larval somatic antigen revealed increases in serum IgG(T) in response to mucosal larvae within the prepatent period (Dowdall *et al.*, 2002). Two putative diagnostic antigen complexes were identified, and subsequently their specificity was evaluated using sera from experimentally and naturally infected horses and helminth-naïve ponies (Dowdall *et al.*, 2003; Dowdall *et al.*, 2004). The results from these studies indicated the possibility of developing an immunoassay to be used in clinical cases of diarrhoea, or weight loss in horses.

Control

The goal for control of horse strongyle infections is to minimise the number of eggs and resultant infective L3s on the grazing areas, and thereby prevent clinical and subclinical disease. There are various approaches on how to achieve this goal, but traditionally control has relied on regular treatment with anthelmintic drugs. Of course, the most reliable control method would be to prevent horses from grazing, but for animal welfare reasons this is not applicable in Sweden.

Initially, the anthelmintic control programmes were primarily aimed at the *Strongylus* species, especially the pathogenic *S. vulgaris*. The prevalence of *S. vulgaris* and colic related to this parasite has indeed decreased, but unfortunately a widespread and intensive use of anthelmintics has led to the selection of drug-resistant cyathostomin populations. Based on the mode of action, there are currently three main classes of anthelmintic drugs used against strongyle nematodes: 1) the benzimidazoles (e.g. thiabendazole, oxibendazole and fenbendazole); 2) the tetrahydropyrimidines (pyrantel); and 3) macrocyclic lactones (ivermectin and moxidectin). When first introduced, all of these drugs exerted high efficacies (>90%) against adult stages of strongyle nematodes. On pre-adult stages, however, the efficacy varies. The macrocyclic lactones are very potent against *S. vulgaris* larvae (Wescott, 1986; Monahan *et al.*, 1996), whereas unsatisfactory efficacies have been reported for benzimidazoles and pyrantel (Duncan *et al.*, 1977; Eysker, Boersema & Kooyman, 1991). As regards cyathostomin pre-adult stages, a high efficacy of ivermectin has been shown against luminal L4s, but not against inhibited early L3s, late L3s and mucosal L4s, not even at elevated drug doses (Eysker, Boersema & Kooyman, 1992; Klei *et al.*, 1993). Moxidectin is as efficacious as ivermectin against adults and luminal L4s, but in addition mucosal L4s are successfully reduced by this drug (Eysker, 1997b). The inhibited early L3s are considered to be the least anthelmintic-susceptible stages, making the strategy of "treat and move to clean pasture" less successful for horses than for cattle and sheep. Nevertheless, a controlled dose-and-slaughter study demonstrated that treatment with fenbendazole for five days was highly effective against adult worms as well as inhibited L3s (Duncan, Bairden & Abbott, 1998). However, in a field study on the efficacy of a five-day course of fenbendazole against benzimidazole-resistant populations, this regime failed to reduce the faecal egg counts to acceptable levels (Chandler, Collins & Love, 2000). It was concluded that five-day treatment with fenbendazole may be of therapeutic benefit but should be avoided in prevention programmes on premises with confirmed benzimidazole resistance.

There are different routines for anthelmintic treatment, which include: a) interval dose systems with treatments of all horses every 6-8 weeks; b) strategic treatments of all horses at critical points of time, based on the seasonality of development and survival of L3 on the pasture; and c) targeted treatments of individual horses with faecal egg counts above a certain number of eggs per gram (EPG). In the USA, daily and monthly administration of anthelmintic drugs has also been practiced (Herd & Majewski, 1994). With increasing anthelmintic resistance problems, it is vital to conserve the efficacy of existing compounds. There seems to be a trend among horse parasitologists and veterinary practitioners to abandon the older interval dosing systems in favour of targeted treatment programmes that are designed individually for different herds (Coles *et al.*, 2003; Sangster, 2003). The concept is to reduce the number of treatments and thereby the selection pressure for anthelmintic resistance. The EPG distribution within a herd is most often skewed, with a majority of the individuals excreting few or no eggs. Due to this, a change from interval treatment to targeted ivermectin treatment has been shown to markedly reduce the total number of anthelmintic treatments (Duncan & Love, 1991; Krecek *et al.*, 1994; Little *et al.*, 2003). It has been proposed that some horses are pre-disposed to parasite infection, and that these so-called "wormy" horses serve as a reservoir of infection for the herd. Although not yet confirmed by long-term field studies, the finding of significant correlations between paired faecal egg counts of the same individuals supports this theory (Gomez & Georgi, 1991; Döpfer *et al.*, 2004).

Egg reappearance period (ERP) is the term for the time interval from anthelmintic treatment until strongyle eggs are again detected in the faeces. The ERP thus constitutes a guide for the establishment of treatment intervals. Generally, ERP varies from 4-12 weeks. Moxidectin has the longest suppressive effect on strongyle egg output (Boersema, Eysker & Canderaar, 1998; Martin-Downum *et al.*, 2001). However, not only the choice of anthelmintic drug affects the variability of ERP, but also factors such as herd size, age of horses, pasture and management factors, and climatic/seasonal variations (Uhlinger, 1992). Therefore, proper timing of treatment intervals can be difficult to determine; the best estimate would be obtained when faecal egg counts are performed for the grazing group.

Anthelmintic treatment represents one possibility for breaking the life cycle of the strongyle nematodes and thus reducing the number of eggs deposited on pasture. There are also other methods which should be integrated with chemotherapy, especially in young horses where reduced efficacy of anthelmintic treatments and shorter ERPs have been reported (Herd & Gabel, 1990). The value of pasture hygiene is usually highlighted in parasite control recommendations, although there are associated practical obstacles. It has been shown that removal of faeces from the pasture twice weekly provides a highly effective control, even superior to the use of anthelmintics (Herd, 1986). In addition, the grazing areas increase substantially when the defecated areas are cleaned. Twice weekly removal of faeces ensures that strongyle L3s do not develop and migrate to the grass before the manure is removed. Since it has been shown in Scotland that very few infective larvae develop within the first week after deposition (Ramsey *et al.*, 2004), it is reasonable to believe that removal of manure once per week would be sufficient in northern Europe. Pasture clipping and chain harrowing are procedures frequently practiced in Sweden. However, instead of killing the larvae by desiccation, which is the idea, the result of incorrect timing could be the opposite – an effective spreading of viable larvae over the pasture. Other grazing management strategies that diminish the accumulation of larvae on pasture include: less intensive grazing, mixed or alternate grazing with sheep/cattle, grazing on aftermath, re-seeding, and pasture burning.

Biological control with nematode-trapping fungi, which are natural predators of nematodes, is a non-chemical approach for control of strongyle nematodes. Trials employing the fungus *Duddingtonia flagrans* on cattle, sheep and pig farms have been successful in reducing the numbers of infective larvae (Larsen, 1999). Chlamydospores, a form of resting spores of *D. flagrans*, are fed to the animals, and on pasture the infective larvae are trapped and destroyed by an adhesive three-dimensional network of hyphae. Studies with horses in Denmark demonstrated that *D. flagrans* significantly reduced the number of L3s on pasture as well as the acquired worm burden in tracer foals (Larsen *et al.*, 1996; Fernández, 1997). Also, evidence from field studies performed in a subtropical climate support the use of *D. flagrans* as a potential biological control agent in equine parasite control programmes (Baudena *et al.*, 2000b).

Finally, attempts have been made to vaccinate horses orally with irradiation-attenuated *S. vulgaris* L3s for protection against this parasite. Encouraging results were obtained in experimental studies on non-immune foals that were vaccinated prior to challenge infections (Klei *et al.*, 1982). In subsequent long-term studies of vaccinated foals, duration of protection against acute verminous arteritis was seven months (Klei *et al.*, 1989). However, there were also indications that vaccination would be most efficacious in combination with anthelmintic control programmes. Although the results from these studies performed in the 1980s were promising, a commercial *S. vulgaris* vaccine has not eventuated. This is probably due to the diminished occurrence of the parasite in areas where macrocyclic lactones have been used. In addition, there are practical and ethical problems involved in producing large numbers of strongyle larvae in donor horses.

Anthelmintic resistance

Intensive and widespread use of anthelmintic compounds has resulted in the development of drug resistance among the parasites, which constitutes a serious threat to effective control of parasite infections. The problem is most acute in small ruminants, where anthelmintic resistance has developed against all drug classes. In some Southern Hemisphere countries, resistance to multiple drugs has reached levels that make chemotherapy-dependent sheep farming impossible (Chandrawathani *et al.*, 2004). The first finding of resistance against modern broad-spectrum anthelmintics in strongyle nematodes of horses was in 1965 – only four years after thiabendazole had been introduced onto the market (Drudge & Lyons, 1965). Although anthelmintic resistance has occasionally been suggested to occur among *Strongylus* species, the problems in cyathostomin populations have been recognised worldwide (reviewed by French & Klei, 1983; Kaplan, 2002).

Definitions

Anthelmintic resistance is defined as a genetically transmitted loss of sensitivity in worm populations that were previously sensitive to the same drug (Köhler, 2001).

Side resistance exists where the resistance to a compound is the result of selection by another compound with similar mode of action.

Reversion is a decrease in the frequency of resistant individuals in a population following removal of the selecting agent (Prichard *et al.*, 1980).

Mechanisms of resistance

The mode of action of benzimidazoles, the first class of broad spectrum anthelmintics, is related to their ability to bind with high affinity to helminth β-tubulin, which, along with α-tubulin, polymerises to form microtubule structures in the parasite cells (Martin, Robertson & Bjørn, 1997). Disruption of microtubule formation inhibits vital functions such as mitosis and transport within the cell. Ultimately, the benzimidazoles cause starvation of the parasite, and they are also ovicidal. The effect of these drugs is slower than those that bind directly to ion-channels, for example the macrocyclic lactones. Two isotypes of β-tubulin have been identified in nematodes: isotype-I and isotype-II, which have separate genes and several alleles. Benzimidazole resistance is associated with a progressive loss of alleles of isotype-I and a total loss of isotype-II for β-tubulin in the nematode population (Martin, 1997). This reduction in the number of isotype alleles seems to produce a resistant phenotype, whose tubulin binds with a lower affinity to the drug. In the sheep nematodes *Haemonchus contortus* and *Teladorsagia circumcincta*, benzimidazole resistance has been linked primarily to a phenylalanine-tyrosine polymorphism at codon 200 of the isotype-I gene (Kwa, Veenstra & Roos, 1994; Elard & Humbert, 1999). However, more recently the finding of mutations at other locations suggests that the genetics for benzimidazole resistance is more complex, and may vary between different species (Wolstenholme *et al.*, 2004). In cyathostomins of the horse the mechanism for benzimidazole resistance appears to involve more than one mutation. Codons 167 and 200 of isotype-I are considered to be of importance (Kaplan, 2002; Samson-Himmelstjerna *et al.*, 2002; Drögemüller *et al.*, 2004).

The second class of drugs, the tetrahydropyrimidines, act selectively as agonists at synaptic and extra-synaptic nicotinic acetylcholine receptors on the surface of nematode muscle cells, thus causing contraction and spastic paralysis (Martin, 1997). Resistance against tetrahydropyrimidines is considered to be related to alteration in the nicotinic acetylcholine receptors, but the molecular basis for differences between sensitive and resistant worms is still unclear (Wolstenholme *et al.*, 2004).

Macrocyclic lactones, the third class, act as agonists of glutamate at the glutamate-gated chloride channels (Köhler, 2001). This leads to an increased Cl^- permeability followed by hyperpolarisation of the muscle cell membrane and paralysis. It has been suggested that the nematode pharynx is an important target for these drugs, and that starvation is the real nematocidal effect (Sangster, 1999; Köhler, 2001). The mechanism of resistance against macrocyclic lactones is also uncertain, although some hypotheses have been proposed based on observations in ruminant trichostrongylid nematodes and the free-living nematode *Caenorhabditis elegans* (Wolstenholme *et al.*, 2004).

In conclusion, the mechanisms of anthelmintic resistance are more intricate than was previously believed. More knowledge is required in this area if endoparasites are to be successfully controlled by chemotherapeutics.

Selection for resistance

At maximum (100%) efficacy of a drug, no parasites survive and resistance would not develop. However, it is believed that genes conferring resistance occur naturally in all worm populations. Chemotherapy removes susceptible worms, whereas some individuals, possessing genes that code for resistance, survive and reproduce. Short treatment intervals provide greater drug exposure and so increase the selection rate for resistance. Over time, the frequency of heterozygous resistant individuals decreases until homozygous resistant worms dominate, so that the drug is tolerated by the majority of the worms. Thus, in phenotypically highly resistant populations there are in theory close to 100% homozygous individuals. This process is likely to be more rapid if the resistance is linked to a dominant gene and/or if the resistance is linked to enhanced fitness. Once resistance has been established, the population does not appear to revert to susceptibility in the absence of the selecting drug (Sangster, 1999).

There are also factors other than parasite genetics that are of importance for the appearance of resistance. Short generation times in parasites results in faster accumulation of resistant alleles in the population. The generation time in large strongyles is considerably longer than in cyathostomins; this could be one contributing factor for the absence of resistance in the large strongyles. Parasites in so-called *refugia* represent the fraction that is not selected by drug treatment. They are very important because the higher the proportion in refugia then the slower will be the selection for resistance (Martin, Le Jambre & Claxton, 1981; van Wyk, 2001). In cyathostomins, treatment with, for example, pyrantel has no effect on mucosal larvae; hence these stages together with free-living stages on pasture are not selected for resistance. Conversely, treatment with larvicidal drugs would result in smaller proportions of the population in refugia, and thus the selection pressure would increase, especially if anthelmintic treatment is performed at a point of time when few free-living stages are present on pasture. Furthermore, computer models have suggested that long-acting drugs, like moxidectin, select more strongly for resistance than do short-acting compounds because of their exposure to worms at subtherapeutic doses in the drug elimination phase (Dobson, Le Jambre & Gill, 1996).

Detection of resistance

A variety of *in vivo* and *in vitro* tests are available for the detection of anthelmintic resistance. All of these tests are associated with certain drawbacks in terms of sensitivity, interpretation, reliability and cost.

The *controlled test* is the most reliable method for assessing anthelmintic efficacy, but it is rarely used since it is related to high costs. Parasitized horses are randomly allocated to treated and untreated groups. The drug is administered, and after 1-2 weeks the animals are euthanised and necropsied. The efficacy of the drug is determined by comparing the number of parasites in the treated animals with the number in the untreated animals.

In a *critical test* the individual infected animals serve as their own controls. Following drug administration, faeces is collected daily until necropsy. The number of expelled parasites is counted and compared with the total number of parasites, i.e. expelled worms + those remaining at necropsy.

The *faecal egg count reduction test (FECRT)* is the most frequently used method for detecting and monitoring the presence of anthelmintic resistance in horse herds. The reduction in faecal egg counts before and 10-14 days after anthelmintic treatment is calculated, and then assessed according to certain resistance criteria. The World Association for the Advancement of Veterinary Parasitology (WAAVP) has published recommendations for the detection of anthelmintic resistance in equine nematodes (Coles *et al.*, 1992). However, with reference to reported studies these methods have not been adopted; numerous ways of performing, calculating and interpreting the status of resistance have been suggested. The FECRT is easy to conduct but it requires many animals with positive egg counts – something that can be hard to obtain. Moreover, the sensitivity is low; at least 25% of the worms within a population have to be resistant before it is evident by FECRT (Martin & Jarrett, 1989).

Anthelmintic resistance can also be detected by *in vitro* methods, of which the *egg hatch assay (EHA)* is the most widely used. The EHA was developed for detection of benzimidazole resistance, as this class of anthelmintics prevents embryonation and hatching of strongyle eggs. Eggs are incubated in increasing serial concentrations of anthelmintic, and by calculating the percentage of hatching at each concentration a dose-response curve and an EC50 value (concentration required to kill 50% of the eggs) can be obtained. This method requires undeveloped eggs, which limits its use in routine diagnostics. As is the case with the FECRT, the EHA only detects resistance levels above 25% (Martin & Jarrett, 1989).

The *larval development assay (LDA)* is an *in vitro* method where the developing larva is the site of action of the drug that is investigated. Eggs are cultured for seven days in a microtitre plate containing serial concentrations of different anthelmintics, usually from all three drug classes. For each anthelmintic, the percentage development of L3 larvae is calculated for each concentration, and subsequently the dose-response and the LC50 values (concentration where development to L3 is inhibited in 50% of larvae) are determined. The advantage of LDA is that anthelmintics with different modes of action can be tested simultaneously. The test has been successfully used in field surveys of sheep flocks (Lacey *et al.*, 1990). However, for equine strongyles LDA appears to be associated with interpretation, reliability and repeatability problems (Tandon & Kaplan, 2004).

Genetic tests detect the presence of resistance alleles in a nematode population. The design of these tests requires knowledge on the molecular and biochemical mechanisms of anthelmintic resistance, but to date these are not well understood. There are also other tests such as the larval paralysis test, motility tests, migration tests and biochemical tests, but these have not been frequently used in horse nematodes.

The resistance situation in horse strongyles

Benzimidazole resistance in cyathostomin populations, as determined by FECRT, is widespread throughout the world, and is likely to be present in all areas where these drugs have been used. In a Swedish study in 1986, benzimidazole resistance was found in all but one of 23 herds examined (Nilsson, Lindholm & Christensson, 1989). These figures were in accordance with results from other studies performed in Europe and the USA. Ten of the most common species of cyathostomins have been shown to be resistant (Slocombe, 1992), and it has been suggested that the majority, maybe all, of the cyathostomin species include resistant genotypes that accumulate in situations where increased selection pressure is applied (Kaplan, 2002).

Pyrantel compounds have been used extensively against horse strongyles since the 1970s. Still, resistance against pyrantel was not documented until 1996 in the USA (Chapman *et al.*, 1996). This report was followed by others from Norway, Denmark and the southern USA (Ihler, 1995b; Craven *et al.*, 1998; Kaplan, 2004). The fact that resistance has been slow to develop against pyrantel could be partly related to the proportion (0-10%) of susceptible genotypes of worms that remain in the intestine following treatment, thus diluting resistant genotypes, if present.

The only class of anthelmintics that remains efficacious on equine establishments where benzimidazole and pyrantel resistance has been recognised are the macrocyclic lactones. Despite being excessively used for 20 years, there are still no reports of resistance to ivermectin in horse strongyles. Nevertheless, it is still believed that resistance against the macrocyclic lactones will inevitably occur in horse cyathostomins (Sangster, 1999). Whether a frequent use of moxidectin, the most recently marketed macrocyclic lactone, could accelerate the selection process has been a topic of considerable debate and discussion (Coles *et al.*, 2003; Abbott *et al.*, 2004).

Aims of the study

With increasing numbers of performance horses in Sweden and a widespread benzimidazole resistance in equine strongyles, there is an obvious requirement for knowledge of the present parasite situation in Swedish horse herds. This information is vital for the development of sustainable endoparasite control of equines in Sweden.

The objectives of this project are:

- To investigate the occurrence and distribution of strongyle nematode infected horses in relation to geographic location, type of establishment, horse age, and herd size. A specific objective was to estimate the prevalence of *S. vulgaris*.

- To identify the cyathostomin species and estimate their prevalence and abundance in Swedish horses.

- To determine the efficacies of three classes of anthelmintic compounds used in horses and to identify anthelmintic-resistant cyathostomin species.

- To investigate current practices used for parasite control in different types of horse establishments in Sweden.

Methodological considerations

Selection of farms and horses

For preference, establishments and horses should be selected randomly. However, with the exception of study V, this was not feasible as a certain number of faecal samples was required from each farm, and in study III and IV the samples also had to be positive for strongyle eggs. In study IV, horses on 40 farms were sampled in order to find 26 farms with a sufficient number of egg-positive horses. The farms included in study III and IV were to a great extent located via local veterinarians. The selection of the farm in study II was based on the history of restricted use of anthelmintics and the presence of sufficient numbers of young horses. In study V, the respondents were selected randomly from different sources representing different types of horse managements.

Faecal examinations

In studies I, III and IV, faecal samples were collected by the horse owners or managers and sent in for analysis. Faecal strongyle egg counts were carried out using a modified McMaster technique based on 3 g of faeces (Anonymous, 1986), with a minimum detection level of 50 eggs per gram of faeces (EPG). When eggs were observed only outside the grid of the chamber, the EPG was set to 10. To evaluate the reliability of the faecal egg counts, 10 samples in study I were analysed twice. The correlation between these analyses was 0.95. To estimate the proportions of cyathostomin eggs, and to identify farms infected with *S. vulgaris*, larval cultures were performed. Faecal samples were placed in plastic beakers with perforated lids and then incubated at 24-25°C for 10-14 days. Third stage larvae were then collected by means of the Baermann procedure, killed in Lugol's solution, and differentiated either as *S. vulgaris* or cyathostomin species (Thienpont, Rochette & Vanparijs, 1986). At least 100 larvae per sample were examined.

Worm expulsion for species identification

Studies on the prevalence and abundance of different cyathotomin species are usually performed post mortem, as this gives a complete picture of the parasitological status, including mucosal and submucosal larval stages. However, experimental studies are costly, and horses examined at abattoirs are often old and have diverse grazing and deworming histories. Therefore, another method was used for the investigation of cyathostomin species (II, IV). This method was based on the recovery and identification of worms expelled in faeces during three days following anthelmintic treatment. The horses selected for study II were 1-5 years of age and were shedding ≥200 strongyle EPG prior to deworming. The faeces from each horse were collected and weighed. Following the calculation of the number of cyathotomins per 100 g faeces in subsamples, the total number of cyathostomins expelled was estimated by multiplying by the total amount of faeces produced. The main expulsion of worms occurred during the first day following deworming with ivermectin and pyrantel, and during the second day with fenbendazole. The highest proportions of larvae were expelled from the ivermectin treated individuals. It was concluded that the expulsion method was practicable and that this method could represent an alternative, or complement, to post mortem examinations. In view of anthelmintic resistance, which has been demonstrated for fenbendazole and pyrantel, ivermectin would be the most appropriate drug to use for this purpose.

Larval development assay (LDA)

In study III, the prevalence of anthelmintic resistance was investigated by the use of DrenchRite^{TM} (Horizon Technology, Roseville, Australia), an LDA that has been developed and commercialised for monitoring of resistance in sheep nematodes. The original DrenchRite^{TM} plate contains thiabendazole, levamisole, thiabendazole-levamisole combination, and two ivermectin analogues. Since pyrantel is one of the most frequently used drugs in horses, we used a modified assay plate where the thiabendazole-levamisole combination wells were substituted by pyrantel. Unfortunately, owing to technical problems with the pyrantel formulation, the delivery of the modified plates was delayed, and the first 19 farms were tested with the original DrenchRite™ plate. One plate was analysed for each farm, and the test was performed essentially as described in the DrenchRite™ user manual (Anonymous, 1996).

Faecal egg count reduction test (FECRT)

In study IV, the prevalence of anthelmintic resistance in horse establishments was investigated by the FECRT, which estimates the percentage reduction of faecal egg counts following anthelmintic treatment. Treatment groups of 3-8 individuals were used, and FECs were performed 0, 7, 14 and 21 days after deworming. Unlike the recommendation to use arithmetic means of egg output (Coles *et al.*, 1992), the FECRs were calculated using geometric means according to Bjørn *et al.* (1991). Compared to arithmetic means, the impact of extreme values is reduced in geometric means, which was considered advantageous in the assessment of resistance at the farm level. The horses served as their own controls, as there were too few egg-positive horses on some farms for the inclusion of untreated control groups. In compliance with a suggestion that resistance criteria should be based on original drug efficacy data (Pook *et al.*, 2002), FECR cut-off values were set to 95% for fenbendazole and ivermectin and to 90% for pyrantel. For ivermectin and fenbendazole, resistance was reported when the FECR was <95% and the lower confidence limit (LC) of the reduction was <90% (Anonymous, 1989). Pyrantel resistance was reported when the FECR was <90% and the LC <80% (Pook *et al.*, 2002). If only one of the criteria was met, resistance was reported as suspected.

Questionnaire

In study V, parasite control practices used by Swedish horse owners were investigated. A questionnaire about anthelmintic usage and pasture management was developed and tested on 60 horse owners in a pilot study before a final, revised version, including an explanatory letter, was posted to 627 randomly selected establishments. The questionnaire comprised 26 questions, of which three were open-ended and the rest closed (for details, see paper V). To increase the number of respondents, a reward in the form of an informative book was sent to respondents who had completed the questionnaire. Moreover, reminder letters were sent to those who had not returned the questionnaire after two and four weeks, respectively. Respondents who had completed less than half of the questions were excluded from the data analysis (four respondents).

Statistical analyses

Data summaries and descriptive analyses were calculated in Excel (Microsoft Corporation). Statistical analyses were variously performed using Super ANOVA (Abacus Concepts), StatView™ (SAS Institute Inc.) and Stata® (Stata Corporation) for Macintosh (Apple Computer Inc.) and SYSTAT®, (SPSS Inc.) for Windows. The DrenchRite™ data were analysed in the LOGIT program to obtain LC50 values (Dobson *et al.*, 1987).

The Chi-square test was used to compare prevalence of strongyle infections in study I and for comparison of results obtained for different types of establishments in study V. Analysis of variance (ANOVA) was used to compare EPG values in study I, to calculate the influence of anthelmintic and number of days after treatment on the parasite expulsion in study II, and to compare LC50 values in study III. In studies I and III, the ANOVA calculations were made on transformed EPG and LC50 values, respectively. The Kruskal-Wallis test was applied to compare FECR data obtained on different sampling days in study IV. The Spearman rank correlation test was used for measuring a rank-order relationship between sets of data in studies I and III. The significance level for all statistical tests was set to $p \leq 0.05$.

Results and discussion

The occurrence of strongyle infections of horses in Sweden

Initially in this project, the occurrence and distribution of strongyle infections in horses were investigated in a field study based on faecal egg counts (FECs). Such an investigation had not previously been carried out in Sweden. This nationwide study showed that strongyle infections were highly prevalent in Swedish horse herds: 78% of 1183 examined horses on 110 farms were found to shed nematode eggs. Furthermore, some factors that influenced the faecal egg output were identified. Knowledge of these factors could be of value for identifying types of establishments that would potentially run a greater risk of having problems with strongyle nematodes.

Horse age and faecal egg output

Although there was considerable individual variation in the FECs, the average number of eggs per gram of faeces (EPG) was found to be significantly higher in young horses (<5 years) than in adult horses (Fig 5). A peak in the faecal egg output was seen at the age of 2-3 years. The majority of horses older than 4 years had low EPG values – 59% shed ≤100 EPG (Fig 6). However, it is important to note that a few (7%) of adult individuals still had >1000 EPG and, consequently, would constitute a great contamination source on pasture.

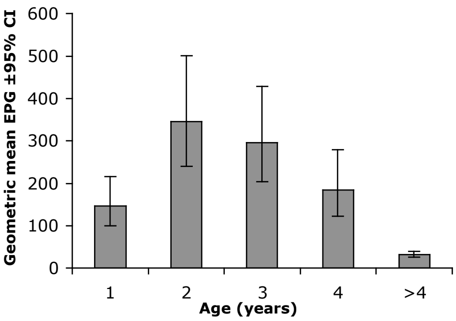


Fig 5. Faecal output of strongyle eggs in relation to age. A peak output of EPG was evident at the age of 2-3 years (I).

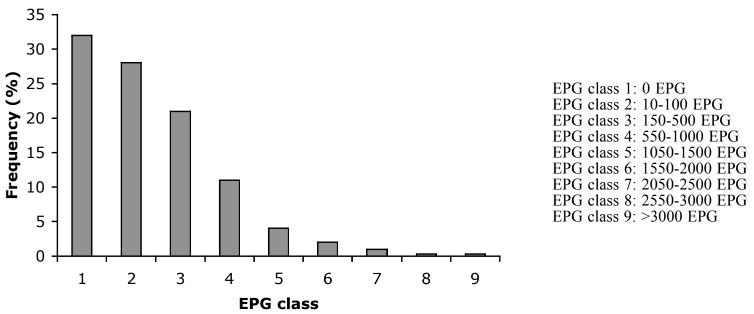
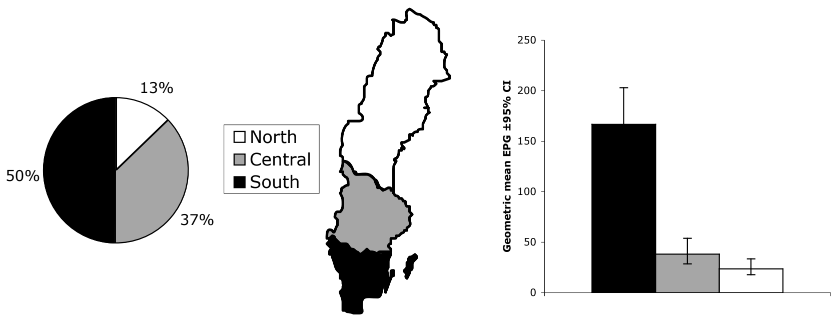


Fig. 6. Frequency distribution of faecal output of strongyle eggs in 679 horses older than 4 years (I).

Study I was carried out in January-March. Since the majority (89%) of the horses had been treated with non-benzimidazole anthelmintics in the previous autumn, after they had left the grazing areas, it was assumed that the strongyle eggs were primarily produced by adult worms, which developed from inhibited larvae known to be refractory to anthelmintics. One possible explanation for higher EPG values in young horses could be that young horses generally harbour greater numbers of mucosal larval stages than do older individuals (Love & Duncan, 1992b; Höglund *et al.*, 1997). The development of an age-related immunity, which results in lower worm burdens in old individuals compared to young, has been demonstrated in experimental studies using ponies of different ages (Monahan *et al.*, 1998). Decreased worm fecundity is another manifestation of immunity that could explain the age differences observed in mean EPG values. For example, it has been shown that immunity to *Ostertagia ostertagi* in calves results in a reduction of worm fecundity (Claerebout & Vercruysse, 2000). An additional reason for higher EPG values in young horses could be that the anthelmintic treatments performed in the previous autumn were less effective in young horses compared to adult animals. This suggestion is based on the interactions that were found between anthelmintic treatment and horse age.

Geographic location and faecal egg output

The field study also revealed that the mean faecal egg output differed significantly between the south, central and north of Sweden: the highest mean EPG was observed in the south and the lowest in the north. Climate factors and grazing intensity were suggested to be of importance for these regional differences. A warmer climate in the south of Sweden results in a longer grazing season, which is often extended until late October-November. In addition, the time when temperature favours development of infective larvae in the grass is obviously longer in the south. Ingestion of high numbers of infective larvae in the autumn, in combination with an increased degree of larval inhibition at this time of the year (Eysker, Boersema & Kooyman, 1990), probably contributed to higher mean faecal egg counts in horses from the south. A higher grazing intensity in the south than in the north is indicated by recent data on the officially estimated number of horses in the country, published by the Swedish Board of Agriculture (http://www.sjv.se/startsida/amnesomraden/statistikfakta; 28-Feb-2005). Based on these data, the estimated total number of horses in Sweden is 271,000, and it can be concluded that 50% are found on establishments in the south of the country (Fig 7). However, these data do not confirm that the horse density is significantly different on the actual grazing areas in the different regions. A region-related difference was also observed in the level of anthelmintic resistance in study II (page 39).



a b c

Fig 7a, b) Proportional distribution of horses in three different regions of Sweden according to data published by the Swedish Board of Agriculture (2005); *c)* Geometric means of strongyle EPG in the same regions (I).

Farm type and faecal egg output

With reference to the results of the faecal egg counts in study I, stud farms represent the environment where problems with strongyle infections are most likely to occur. The mean EPG of horses on stud farms was significantly higher than the EPG of horses on other types of establishments. The difference in horse age distribution between stud farms and other types of establishments is probably of significance for the observed differences in the mean faecal egg output. The proportion of horses younger than five years was higher on stud farms (55%) than on other types of establishments (23%). A large proportion (50%) of young horses on stud farms was also shown in study V. Typically, on stud farms high numbers of horses graze per unit of land. Large stud farms may accommodate up to 200 mares and foals each for a few months during one summer season. This, in combination with a relatively low treatment frequency (42%) of new arrivals (V), contributes to a high infective pressure on the pastures of these establishments.

Species involved in strongyle infections

In previous studies on strongyle infections of horses in Sweden, much attention has been paid to *S. vulgaris* (Tolling, 1976; Nilsson & Andersson, 1979), whereas data about cyathostomin species has been lacking.

Strongylus vulgaris

In study I, *S. vulgaris* larvae were identified in larval cultures from 15 (14%) of 110 establishments. The overall proportion of individuals infected with *S. vulgaris* was 4.8%, which was in accordance with a previous Swedish abattoir study, where 3.6% of the horses were found to shed *S. vulgaris* eggs (Höglund *et al.*, 1997). However, in study I *S. vulgaris* first had to be identified in a pooled sample from a farm before individual examinations took place, which meant that individuals shedding low numbers of these eggs might have been overlooked. In study IV, where larval cultures were performed on pooled samples from 26 farms, as many as eight samples (31%) were positive for *S. vulgaris*. One must take into account, though, that farms included in the latter study had been selected on the basis of faecal egg counts; at least nine horses on each farm had ≥200 EPG.

In Sweden (Nilsson, Lindholm & Christensson, 1989), as well as in other parts of the world (Reinemeyer *et al.*, 1984; Herd, 1990; Bucknell, Gasser & Beveridge, 1995), where modern broad-spectrum anthelmintics have been frequently used, the prevalence of *S. vulgaris* has decreased markedly since the mid 1980s. However, although the majority of Swedish horse owners treat their horses in late autumn with either ivermectin or pyrantel (I,V), it can be concluded that low levels of *S. vulgaris* infections are still prevalent, and we need to consider the potential presence of this species when endoparasites control recommendations are formulated.

Cyathostominae

The data on cyathostomin species of Swedish horses is based on the identification of worms that were expelled following anthelmintic treatment. There is one report from Germany where a similar expulsion technique was used to estimate the species composition (Anderson & Hasslinger, 1982). Generally, studies on the prevalence and abundance of cyathostomins have been carried out by necropsy examinations. Compared to most such studies, not only the recovery technique was different in our study but also the selection of horses. The horses that participated in study II were all from the same farm, and presumably they were not representative of the whole Swedish horse population.

The presence of several cyathostomin species in the same individual host was confirmed in study II as well as in study IV. The mean number of species per horse was nine (II), which is in accordance with other studies (Reinemeyer *et al.*, 1984; Mfitilodze & Hutchinson, 1990; Gawor, 1995). Of 41 cyathostomin species described in horses, it is well documented that less than 10 species dominate. We found that six species, *Cylicostephanus longibursatus, Cylicocyclus nassatus, Cyathostomum catinatum, Cylicocyclus leptostomum, Cylicostephanus minutus* and *Cylicostephanus calicatus,* comprised 91% of the total cyathostomin burden (II). The same species have also been reported among the 10 most prevalent species in other studies, performed in geographically distinct regions (Ogbourne, 1976; Reinemeyer *et al.*, 1984; Eysker, Jansen & Mirck, 1986; Torbert *et al.*, 1986; Krecek, Reinecke & Horak, 1989; Bucknell, Gasser & Beveridge, 1995; Gawor, 1995; Silva *et al.*, 1999).

From six genera of the tribe Cyathostominea, the following 15 species were identified from 27 horses (II): *Cyathostomum catinatum, C. pateratum, Coronocyclus coronatus, C. labiatus, C. labratus, Cylicocyclus ashworthi, C. insigne, C. leptostomus, C. nassatus, Cylicostephanus calicatus, C. minutus, C. longibursatus, C. goldi, Petrovinema poculatum,* and *Parapoteriostomum mettami*. A few specimens of *Gyalocephalus capitatus* were also recorded. Thirteen of these cyathostomin species were also identified in study IV. In order to retrieve large numbers of worms, 1-5 year old horses with ≥300 EPG were used in study II, which was performed on a stud farm in the south of Sweden. Still, 5-6 species that had been recorded in some other European studies (Çirak, Hermosilla & Bauer, 1996; Lichtenfels *et al.*, 2001; Collobert-Laugier *et al.*, 2002) were not identified. The absence of these species is likely to be related to the number of worms that were identified per horse. An average of 132 worms per horse were identified, and this has been found to be an insufficient sample size to accurately estimate the species present in a population (Chapman, Kearney & Klei, 2003). Chapman and co-workers (2003) showed that the mean number of species per individual increased from 11 to 25 when 5% aliquots of the large intestine contents were completely examined, instead of 200 worms from each animal.

Anthelmintic efficacy on strongyle nematodes

Studies I and II revealed that strongyle nematodes are common in Swedish horses, particularly in animals younger than five years, distributed on stud farms in the south of the country. The fact that 99.5% of Swedish horse owners regularly deworm their horses (V) reflects the reliance on anthelmintic drugs for controlling helminth infections. The objective of studies III and IV was to investigate the efficacy of anthelmintics commonly used to prevent strongyle infections. In a field study on the efficacy of various benzimidazole and non-benzimidazole compounds in 1986, a widespread prevalence of benzimidazole resistance was demonstrated (Nilsson, Lindholm & Christensson, 1989). As a consequence of that study the use of benzimidazole compounds decreased dramatically in Sweden, and today these compounds represent a very small percentage (< 0.5%) of anthelmintics used in Swedish horses (Figs 8 and 10). Since we were interested to investigate the possibility of whether reversion to susceptibility would have taken place, benzimidazole drugs were included in studies III and IV.

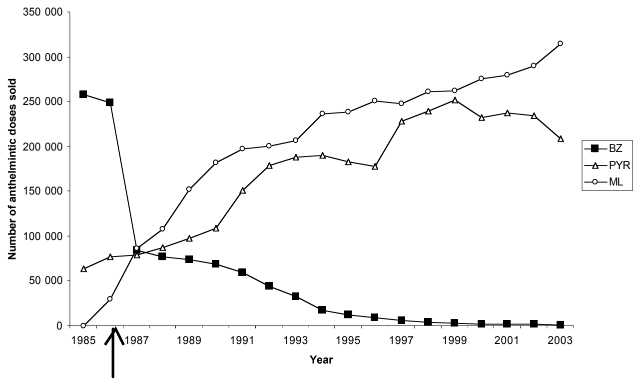


Fig 8. Number of anthelmintic doses sold per 500 kg bodyweight of horse in Sweden during 1985-2003, according to Apoteksbolaget (Swedish Pharmacy Chain), the sole retailer of medical products in Sweden. Benzimidazole resistance was evident in a FECRT study performed in 1986 (arrow). BZ – benzimidazoles; PYR – pyrantel; ML – macrocyclic lactones.

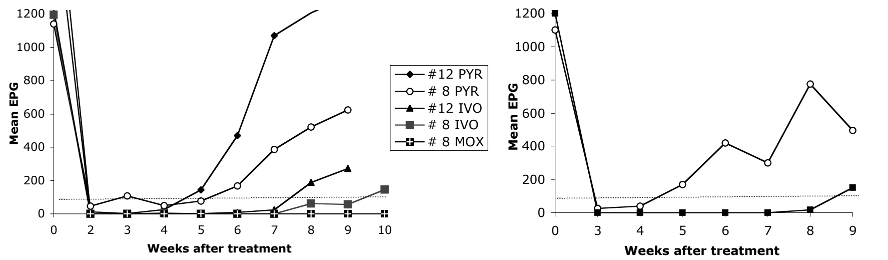
In study III, 70 horse establishments were sampled, and examined for the presence of anthelmintic resistance by the LDA DrenchRite^{TM}. On the whole, interpretation of the LDA data was problematic, owing to lack of established cut-off values and variations in the larval development within and between assay plates. From eight farms LC50 values could not be obtained because of poor development of larvae and bacterial overgrowth. Further evaluation of LDA should optimally include reference strains with known *in vivo* susceptibility and/or resistance to anthelmintics. However, this is complicated owing to the multi-species nature of strongyle infections in equines. In agreement with Tandon & Kaplan (2004), it was concluded that this LDA is not yet a sufficiently reliable tool for the assessment of the resistance status in populations of cyathostomins.

Nevertheless, some of the results may be discussed. The LC50 values (concentration that prevents 50% of the eggs developing to the L3 stage) for thiabendazole and levamisole were generally lower than what has been reported from other horse studies (Ihler & Bjørn, 1996; Craven *et al.*, 1999; Pook *et al.*, 2002; Tandon & Kaplan, 2004), but were still slightly higher than those obtained in a herd of feral horses that had not been treated with anthelmintics for at least 25 years (Young *et al.*, 1999). These findings were interesting, since levamisole and benzimidazole compounds had never been used (levamisole), or used infrequently (benzimidazoles), in Swedish horses for more than a decade.

With the exception of pyrantel, the mean LC50 values obtained for anthelmintics tested by DrenchRite^{TM} were significantly higher in the south of

Sweden than in the north. This implies that cyathostomin populations in the south are less susceptible to anthelmintics. Unfortunately, this hypothesis was not tested in study IV as too few of the farms selected for study IV were in the northern part of the country. However, the presence of lower susceptibility in the south is not surprising since the horse density and the anthelmintic treatment intensity are higher in this region (III).

To investigate further the anthelmintic resistance situation in Sweden, a FECRT was subsequently carried out (IV). In addition, the egg reappearance periods (ERP) for the most frequently used drugs were determined on two farms, one located in the south and one in the north. It is believed that a reduction in the ERP is one of the first signs of anthelmintic resistance (Sangster, 1999). In all 26 establishments included in study IV, the FECRT showed that ivermectin reduced the mean EPG by >99%, irrespective of sampling day (7, 14 or 21 days after treatment). The ERP for ivermectin was 8-10 weeks, which is comparable with results obtained on one of these farms eight years earlier (Osterman *et al.*, 1996; Fig 9). Thus, based on these results, it can be concluded that the efficacy of ivermectin against strongyle nematodes is still excellent in Sweden. The ERP for moxidectin was also tested on one farm; however data were not presented in the paper (Fig 9). It was found that the ERP for moxidectin was more than 14 weeks, which is comparable to previous studies conducted in the Netherlands (Boersema, Eysker & Canderaar, 1998) and the USA (Martin-Downum *et al.*, 2001).



2003 1995

Fig 9. Egg reappearance patterns following treatment with three anthelmintic drugs commonly used in Sweden. Treatment groups of 8-10 horses were tested on two farms in 2003 (IV) and one farm in 1995. Farm #8 was located in the south and farm #12 in the north. PYR – pyrantel; IVO – ivermectin and MOX – moxidectin.

With pyrantel, the resistance situation was slightly more worrying. In 26 establishments tested for this drug, the reduction of eggs varied from 95-100%; but based on the criteria we used for resistance, there were nine establishments that were assessed as suspected resistant (page 33) on at least one of the three sampling days. Using large treatment groups, subsequent examinations of two farms clearly showed that one farm met the criteria for pyrantel resistance for three consecutive years. This was the first documented case of pyrantel resistance in cyathostomins of horses in Sweden. Even if the ERP for pyrantel did not appear to be reduced since 1995, henceforth we certainly have to be attentive to the emergence of pyrantel resistance in other farms. Pyrantel-resistant cyathostomin populations have been previously reported in the USA (Chapman *et al.*, 1996; Woods *et al.*, 1998; Tarigo-Martinie, Wyatt & Kaplan, 2001; Kaplan *et al.*, 2004), Denmark (Craven *et al.*, 1998) and Norway (Ihler, 1995a). In agreement with other studies (Chapman *et al.*, 1996; Kaplan, 2002 and references therein), it appeared that several of the most prevalent cyathostomin species were capable of developing resistance against pyrantel and benzimidazole. In study IV, 10 and 13 species suspected to be resistant against pyrantel and benzimidazole, respectively, were identified.

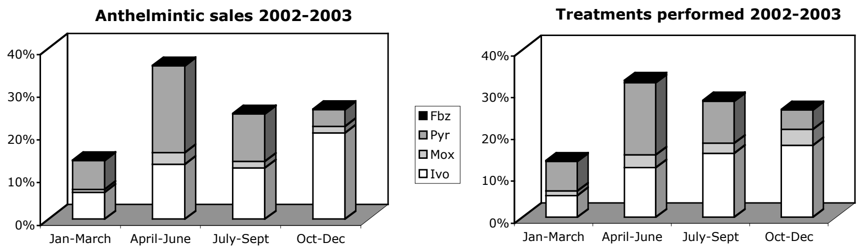
The FECRT also showed that benzimidazole resistance is still widespread in cyathostomin populations of Swedish horses. Reversion to susceptibility had not occurred despite a very low use of these drugs for the previous 15 years. More than 70% of the establishments met the criteria for fenbendazole resistance (IV). The mean FECR was 86%, ranging from 56-100%. Prevention of cyathostomin-related disease by a five-day course of fenbendazole has never been adopted in Sweden, and with resistance still being prevalent it is not likely that the use of benzimidazoles against strongyles will increase. However, since problems with *Parascaris equorum* refractory to the macrocyclic lactones (Boersema, Eysker & Nas, 2002; Hearn & Peregrine, 2003) appear to have increased in Sweden, it is possible that the benzimidazole compounds will remain on the horse anthelmintic market.

Control practices

With increasing numbers of horses in Sweden and a heavy reliance on anthelmintics, to which resistance has been documented in two out of the three substance classes, it was important to scrutinise the control practices used on horse establishments, and to recognise information sources that influence Swedish horse owners. The last in this series of studies was a questionnaire study, where 443 respondents out of 627 completed 26 questions on the use of anthelmintics and pastures (V). The results from this study indicate how control practices may be improved in terms of minimising the selection pressure for anthelmintic resistance without jeopardising horse health.

In order to slow the spread of anthelmintic resistance, some recommendations adapted from sheep and goat husbandry have been formulated, namely: (1) minimising the number of doses, (2) slow, usually annual rotation of drug classes, (3) correct dosing, (4) effective treatment of new individuals before introduction to the herd, and (5) regular monitoring for resistance (Lloyd & Soulsby, 1998). Study V showed that pasture hygiene was generally practised infrequently on Swedish horse establishments. Pasture clipping and/or chain harrowing were the most common pasture procedures undertaken (36%), whereas weekly removal of faeces was rare (6%). Moreover, only 10% of respondents practiced mixed or rotational grazing strategies, which was less than reported from surveys in Denmark (Lendal *et al.*, 1998), England (Pascoe, Wilson & Coles, 1999) and Ireland (O'Meara & Mulcahy, 2002). Most likely, pasture hygiene and grazing management practices could be used to a greater extent on Swedish farms, and hence the use of anthelmintics could be reduced.

The number of treatments administered to the horses each year varied from 1-8 with an average of 3.2. Most respondents stated that they dewormed their horses to a lesser extent in the winter, but, remarkably, considered late autumn as the most important time for treatment. The reason for this was thought to be the bot fly, *Gasterophilus intestinalis*. Forty-one percent of the respondents had seen bot flies within the previous two years. A previous Swedish study of slaughtered horses without any clinical signs of parasitism showed that only 9.9% out of 461 horses were infected with larvae of *G. intestinalis* (Höglund *et al.*, 1997). The use of anthelmintics over the year also indicated the concern about this parasite. An increased use of ivermectin in the autumn compared to pyrantel was evident in sales data from Apoteksbolaget (Swedish Pharmacy Chain) as well as in studies I and V (Fig 10a, b).



10a 10b

Fig 10a) Proportional distribution of sales of anthelmintics for horses in Sweden during 2002-2003 according to Apoteksbolaget (Swedish Pharmacy Chain); *b)* the relative use of drugs according to study V. IVO – ivermectin; MOX – moxidectin; PYR – pyrantel and FBZ – fenbendazole.

Routine treatments with macrocyclic lactones against *G. intestinalis* and pyrantel against *Anoplocephala perfoliata*, the equine tapeworm, partly interfere with the recommendation of a slow rotation between drug classes on an annual or bi-annual basis (Lloyd & Soulsby, 1998). Approximately half of the horse owners had used pyrantel and 1% had used praziquantel against the tapeworm, which is highly prevalent in Swedish horses (Nilsson *et al.*, 1995). In view of its low pathogenicity, it seems sensible to exclude routine treatments against larvae of *G. intestinalis* in late autumn, at least in years when pyrantel is used. Instead, the bot fly eggs can be removed manually from the coat. Likewise, pyrantel should not be used against tapeworms in the years that macrocyclic lactones are used. Praziquantel, which does not select for resistance in cyathostomins, would then be a better choice.

To reduce the selection pressure for resistance, there is a growing interest among parasitologists for targeted selective anthelmintic treatments based on FECs of individual animals (Hoste *et al.*, 2002). Thus, only horses with EPG values above a certain threshold would be treated. A limit of 200 EPG has been suggested

(Coles *et al.*, 2003), although it will depend on factors such as type of establishment, stocking rate and time of year. So far, Swedish veterinarians and horse owners are not familiar with this control strategy. Only 1% of the respondents stated that they perform FECs on a regular basis, and the majority had never sent a faecal sample from a healthy horse for parasitological examination. It is plausible that the main reasons for a low frequency of FECs are: 1) lack of motivation, as 72% of the respondents had no personal experience of parasite-related problems in their horses and thus considered parasites to be of minor importance, and 2) the cost of an anthelmintic treatment is considerably lower than the cost of a faecal examination. However, since the questionnaire also revealed that anthelmintic resistance was considered to be an important or very important issue, selective targeted treatments may still be introduced in the future. Based on findings of consistency between results of paired faecal egg counts of the same individuals (Gomez & Georgi, 1991; Döpfer *et al.*, 2004), it has been proposed that some, so-called "wormy", individuals (Coles *et al.*, 2003) serve as reservoirs of infection for the herd. If that hypothesis is correct, it would further motivate selective treatments. However, no long-term field studies have confirmed the existence of "wormy" horses. A number of horses in study I were sampled again after six months and/or one year; however, data were not presented in the paper (Fig 11). Although no detailed information on previous anthelmintic treatments over the year was taken into consideration, Spearman rank correlations showed that there were indeed significant correlations between the FECs obtained from the same individuals on different sampling occasions ($p<0.0001$). The coefficients were 0.5-0.6, but from these figures it still appears to be difficult to predict the EPG levels in individual horses.

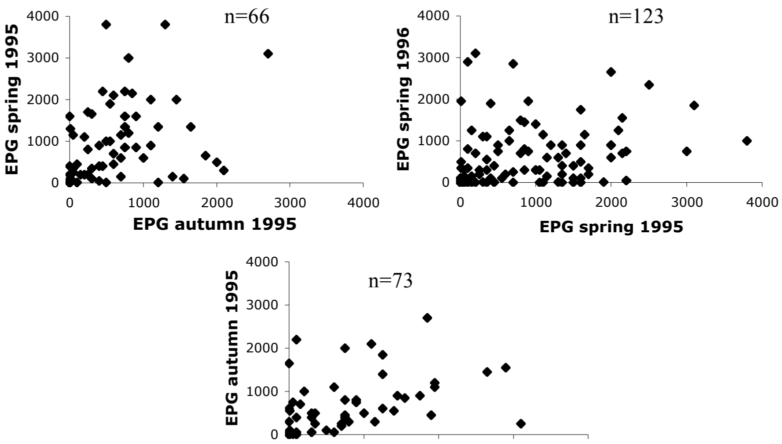


Fig 11. Relationship of EPG values obtained from the same individuals on two or three different occasions – spring 1995, autumn 1995 and spring 1996.

Applications of the results and future perspectives

In our studies, resistance against pyrantel – one of the two most commonly used anthelmintics in horses – was documented for the first time in Sweden. It was also demonstrated that benzimidazole resistance is still highly prevalent in Swedish horse farms (IV). Reversion to susceptibility obviously has not occurred, although the benzimidazole drugs have not been used in Swedish horses for 10-15 years. Although most horse owners considered parasites a minor problem on their horse farms (V), the presence of anthelmintic resistance against two of the three classes of anthelmintics needs to be taken seriously.

To slow the spread of anthelmintic resistance in Swedish horse farms, it seems both reasonable and possible to reduce the number of anthelmintic treatments, especially in horses older than four years, since the majority of these have very low levels of faecal egg excretion (I). Preventive treatments with drugs should be more integrated with other measures, such as pasture hygiene and grazing management, which are currently not commonly employed on Swedish horse establishments (V). However, it is not advisable to reduce the use of drugs without also monitoring the farms by FECs. In addition, egg-positive faecal samples should be cultured routinely for the identification of *Strongylus vulgaris*, since this pathogenic parasite still occurs on many Swedish farms (I, IV). An alteration from strategic treatments of all horses to selective targeted treatments of egg-positive individuals would certainly result in reduced numbers of treatments. It would be useful to study and evaluate the targeted treatment strategy under different management conditions. If targeted treatments were to be implemented in the future, it would be of interest to perform thorough long-term field studies on the existence of "wormy" individuals.

Our studies showed that LDA was not reliable for detecting resistance in horse strongyles (III). In a long-term perspective it is important to develop sensitive methods for the detection of anthelmintic resistance, in particular for the macrocyclic lactones. However, for the next few years it is likely that the FECRT will continue to be the test of choice. In the light of the results obtained in study IV, FECRTs of pyrantel and macrocyclic lactones should be performed regularly to monitor the resistance status. Elsewhere it has been suggested that it would be reasonable to monitor premises for resistance every two to three years (Coles *et al.*, 2003). The ideal time of the year for monitoring of resistance remains to be determined as it has been shown in sheep that the results of FECRTs may vary over the year (Anderson, Martin & Jarrett, 1988). Special attention should be paid to stud farms as it is most likely that anthelmintic resistance will emerge on this kind of premises, where the majority of horses are young and where there is a large turnover of horses. It has been shown that the efficacy of anthelmintic treatment is reduced in young individuals (Herd & Gabel, 1990), which was also indicated in study I. In this context it is important to include the equine roundworm *Parascaris equorum*, which is difficult to control on many Swedish stud farms. The magnitude of problems related to *P. equorum* populations refractory to anthelmintic treatment is not known, and this is an urgent matter to investigate in finer detail.

Another important topic that was not within the scope of this thesis is the prevalence of clinical disease caused by strongyle infections in Sweden. With reference to study I, clinical problems caused by strongyle nematodes are most likely to occur in young horses on stud farms. It is well known that larval cyathostominosis is difficult to diagnose clinically. The development of an immunoassay for the detection of mucosal cyathostomin larvae would be a valuable tool for proper diagnosis and treatment of horses suffering from diarrhoea and/or weight loss caused by cyathostomin larvae.

Despite a widespread use of pyrantel and macrocyclic lactones (I, V), infections with multiple species of strongyle nematodes are highly prevalent in Swedish horses (I, II). Currently, there are no realistic alternatives that can replace the use of anthelmintics entirely. Nevertheless, biological control systems based on the use of the nematophagous fungus *Duddingtonia flagrans*, whose effectiveness has been shown only in a few horse studies (Larsen *et al.*, 1996; Baudena *et al.*, 2000b), would be worthwhile to evaluate further, also under Swedish management and environmental conditions. Today, one of the main problems associated with the application of *D. flagrans* is the absence of a commercial product. However, development of resistance against the macrocyclic lactones in horse strongyles would certainly stress the need for alternative control methods.

It will be a challenge to develop and establish integrated control practices where factors such as farm type, management, deworming history etc. are taken into consideration. The goal should be to prevent negative effects of strongyles, as well as of *P. equorum* and *A. perfoliata*, and to slow the spread of anthelmintic resistance. According to the results of the questionnaire study, all categories of horse owners take advice mainly from veterinarians. To increase the knowledge of endoparasites and parasite control in horses, a closer collaboration between parasitologists and veterinary practitioners is desirable.

References

Anderson, N., Martin, P.J. and Jarrett, R.G. 1988. Mixtures of anthelmintics: a strategy against resistance. *Australian Veterinary Journal 65*, 62-64.

Abbott, E., Bairden, K., Cobb, R., Kennedy, T. & Reinemeyer, C. 2004. Anthelmintic resistance and use of anthelmintics in horses. *Veterinary Record 154*, 62-64.

Anderson, I. G. & Hasslinger, M. A. 1982. Cyathostominae and other strongyles of horses in the Federal Republic of Germany. *Journal of the South African Veterinary Association 53*, 195-197.

Anonymous 1986. *Manual of veterinary parasitological laboratory techniques*. 3rd edn. Ministry of Agriculture, Fisheries and Food. London. pp 11-12.

Anonymous 1989. Anthelmintic resistance. *Report of the working party for the Animal Health Committee of the Standing Committee on Agriculture*, *SCA Technical Report Series*, No. 28. 26 pp.

Anonymous 1996. *DrenchRite™, larval development assay, standard operating procedures*. Horizon Technology Pty Limited. Roseville, Australia. 29 pp.

Anonymous 2004. *Hästen i Sverige - betyder mer än du tror.* Nationella Stiftelsen för Hästhållningens Främjande.. 26 pp [In Swedish].

Armour, J. & Duncan, M. 1987. Arrested larval development in cattle nematodes. *Parasitology Today 3*, 171-176.

Baudena, M. A., Chapman, M. R., French, D. D. & Klei, T. R. 2000a. Seasonal development and survival of equine cyathostome larvae on pasture in south Louisiana. *Veterinary Parasitology 88*, 51-60.

Baudena, M. A., Chapman, M. R., Larsen, M. & Klei, T. R. 2000b. Efficacy of the nematophagous fungus *Duddingtonia flagrans* in reducing equine cyathostome larvae on pasture in south Louisiana. *Veterinary Parasitology 89*, 219-230.

Bengtsson, G. 1993. *ABC om hästar*. 9th edn. Natur & Kultur. Stockholm. pp 9-11 [In Swedish].

Bjørn, H., Sommer, C., Schougård, H., Henriksen, S. A. & Nansen, P. 1991. Resistance to benzimidazole anthelmintics in small strongyles *(Cyathostominae)* of horses in Denmark. *Acta Veterinaria Scandinavica 32*, 253-260.

Boersema, J. H., Eysker, M. & Canderaar, W. M. 1998. The reappearance of strongyle eggs in the faeces of horses after treatment with moxidectin. *Veterinary Quarterly 20*, 15-17.

Boersema, J. H., Eysker, M. & Nas, J. W. M. 2002. Apparent resistance of *Parascaris equorum* to macrocyclic lactones. *Veterinary Record 150*, 279-281.

Brinck, P. 1949. Till kännedom om den svenska hästens parasitfauna. *Svenska Militärsällskapets Kvartalsskrift 25*, 43-72 [In Swedish].

Bucknell, D. G., Gasser, R. B. & Beveridge, I. 1995. The prevalence and epidemiology of gastrointestinal parasites of horses in Vicoria, Australia. *International Journal for Parasitology 25*, 711-724.

Chandler, K. J., Collins, M. C. & Love, S. 2000. Efficacy of a five-day course of fenbendazole in benzimidazole-resistant cyathostomes. *Veterinary Record 147*, 661-662.

Chandrawathani, P., Yusoff, N., Wan, L. C., Ham, A. & Waller, P. J. 2004. Total anthelmintic failure to control nematode parasites of small ruminants on government breeding farms in Sabah, East Malaysia. *Veterinary Research Communications 28*, 479-489.

Chapman, M. R., French, D. D. & Klei, T. R. 2001. Seasonal transmission of gastrointestinal parasites of equids in southern Louisiana. *Journal of Parasitology 87*, 1371-8.

Chapman, M. R., French, D. D., Monahan, C. M. & Klei, T. R. 1996. Identification and characterization of a pyrantel pamoate resistant cyathostome population. *Veterinary Parasitology 66*, 205-212.

Chapman, M. R., French, D. D., Taylor, H. W. & Klei, T. R. 2002. One season of pasture exposure fails to induce a protective resistance to cyathostomes but increases numbers of hypobiotic third-stage larvae. *Journal of Parasitology 88*, 678-683.

Chapman, M. R., Kearney, M. T. & Klei, T. R. 2003. Equine cyathostome populations: accuracy of species composition estimates. *Veterinary Parasitology 116*, 15-21.

Çirak, V. Y., Hermosilla, C. & Bauer, C. 1996. Study on the gastrointestinal parasite fauna of ponies in northern Germany. *Applied Parasitology 37*, 239-244.

Claerebout, E. & Vercruysse, J. 2000. The immune response and the evaluation of acquired immunity against gastrointestinal nematodes in cattle: a review. *Parasitology 120*, 25-42.

Coles, G. C., Bauer, C., Borgsteede, F. H. M., Geerts, S., Klei, T. R., Taylor, M. A. & Waller, P. J. 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology 44*, 35-44.

Coles, G. C., Eysker, M., Hodgkinson, J. E., Matthews, J. B., Kaplan, R. M., Klei, T. R. & Sangster, N. C. 2003. Anthelmintic resistance and use of anthelmintics in horses. *Veterinary Record 153*, 636.

Collobert-Laugier, C., Hoste, H., Sevin, C. & Dorchies, P. 2002. Prevalence, abundance and site distribution of equine small strongyles in Normandy, France. *Veterinary Parasitology 110*, 77-83.

Craig, T. M., Bowen, J. M. & Ludwig, K. G. 1983. Transmission of equine cyathostomes (Strongylidae) in central Texas. *American Journal of Veterinary Research 44*, 1867-1869.

Craven, J., Bjørn, H., Barnes, E. H., Henriksen, S. A. & Nansen, P. 1999. A comparison of *in vitro* tests and a faecal egg count reduction test in detecting anthelmintic resistance in horse strongyles. *Veterinary Parasitology 85*, 49-59.

Craven, J., Bjørn, H., Henriksen, S. A., Nansen, P., Larsen, M. & Lendal, S. 1998. A survey of anthelmintic resistance on Danish horse farms, using 5 different methods of calculating faecal egg count reduction. *Equine Veterinary Journal 30*, 289-293.

Dobson, R. J., Griffiths, D. A., Donald, A. D. & Waller, P. J. 1987. A genetic model describing the evolution of levamisole resistance in *T. colubriformis*, a nematode parasite in sheep. *IMA Journal of Mathematics Applied in Medicine and Biology 4*, 279-293.

Dobson, R. J., Le Jambre, L. F. & Gill, J. H. 1996. Management of anthelmintic resistance: inheritance of resistance and selection with persistent drugs. *International Journal for Parasitology 26*, 993-1000.

Dowdall, S. M. J., Matthews, J. B., Mair, T., Murphy, D., Love, S. & Proudman, C. J. 2002. Antigen-specific IgG(T) responses in natural and experimental cyathostominae infection in horses. *Veterinary Parasitology 106*, 225-242.

Dowdall, S. M. J., Proudman, C. J., Klei, T. R., Mair, T. & Matthews, J. B. 2004. Characterisation of IgG(T) serum antibody responses to two larval antigen complexes in horses naturally - or experimentally - infected with cyathostomins. *International Journal for Parasitology 34*, 101-108.

Dowdall, S. M. J., Proudman, C. J., Love, S., Klei, T. R. & Matthews, J. B. 2003. Purification and analyses of the specificity of two putative diagnostic antigens for larval cyathostomin infection in horse. *Research in Veterinary Science 75*, 223-229.

Drudge, J. H. & Lyons, E. T. 1965. Newer developments in helminth control and *Strongylus vulgaris* research. In: *Proceedings of the 11th Annual Convention of the American Association of Equine Practitioners,* Miami Beach, Florida. pp 381-389.

Drögemüller, M., Failing, K., Schnieder, T. & Samson-Himmelstjerna, G. v. 2004. Effect of repeated benzimidazole treatments with increasing dosages on the phenotype of resistance and beta-tubulin codon 200 genotype distribution in a benzimidazole-resistant cyathostomin population. *Veterinary Parasitology 123*, 201-213.

Duncan, J. L. 1974. Field studies on the epidemiology of mixed strongyle infection in the horse. *Veterinary Record 13*, 337-344.

Duncan, J. L., Bairden, K. & Abbott, E. M. 1998. Elimination of mucosal cyathostome larvae by five daily treatments with fenbendazole. *Veterinary Record 142*, 268-271.

Duncan, J. L. & Love, S. 1991. Preliminary observations on an alternative strategy for the control of horse strongyles. *Equine Veterinary Journal 23*, 226-228.

Duncan, J. L., McBeath, D. G., Best, J. M. J. & Preston, N. K. 1977. The efficacy of fenbendazole in the control of immature strongyle infections in ponies. *Equine Veterinary Journal 9*, 146-149.

Duncan, J. L. & Pirie, H. M. 1973. Strongyle infection in horses. Life cycle, pathogenesis, epidemiology and immunity. In: *Helminth diseases of cattle, sheep and horses in Europe,* Glasgow, UK. pp 115-126.

Duncan, J. L. & Pririe, H. M. 1972. The life cycle of *Strongylus vulgaris* in the horse. *Research in Veterinary Science 13*, 374-379.

Dvojnos, G. M. & Kharchenko, V. A. 1994. *Strongilidy domashnik i dikikh loshadej (Strongylidae in domestic and wild horses)*. Publ. House "Naukova Dumka". Kiev. 234 pp [In Russian].

Döpfer, D., Kerssens, C. M., Meijer, Y. G. M., Boersema, J. H. & Eysker, M. 2004. Shedding consistency of strongyle-type eggs in dutch boarding horses. *Veterinary Parasitology 124*, 249-258.

Elard, L. & Humbert, J. F. 1999. Importance of the mutation of amino acid 200 of the isotype 1 β-tubulin gene in the benzimidazole resistance in the small-ruminant parasite *Teladorsagia circumcincta*. *Parasitology Research 85*, 452-456.

Eysker, M. 1997a. Some aspects of inhibited development of trichostrongylids in ruminants. *Veterinary Parasitology 72*, 265-283.

Eysker, M., Boersema, J. H. & Kooyman, F. N. J. 1990. Seasonally inhibited development of cyathostomine nematodes in Shetland ponies in the Netherlands. *Veterinary Parasitology 36*, 259-264.

Eysker, M., Boersema, J. H. & Kooyman, F. N. J. 1991. Effect of early season ivermectin and pyrantel treatments on strongylid infections in young Shetland ponies in The Netherlands. *Veterinary Parasitology 38*, 33-39.

Eysker, M., Boersema, J. H. & Kooyman, F. N. J. 1992. The effect of ivermectin against inhibited early third stage, late third stage and fourth stage larvae and adult stages of the cyathostomes in Shetland ponies and spontaneous expulsion of these helminths. *Veterinary Parasitology 42*, 295-302.

Eysker, M., Boersema, J.H., Grinwis, G.C.M., Kooyman, F.N.J. & Poot, J. 1997b. Controlled dose confirmation study of a 2% moxidectin equine gel against equine internal parasites in The Netherlands. *Veterinary Parasitology 70*, 165-173.

Eysker, M., Jansen, J. & Mirck, M. H. 1984. Inhibited development of Cyathostominae in the horse in the early third stage. *Research in Veterinary Science 37*, 355-356.

Eysker, M., Jansen, J. & Mirck, M. H. 1986. Control of strongylosis in horses by alternate grazing of horses and sheep and some other aspects of the epidemiology of strongylidae infections. *Veterinary Parasitology 19*, 103-115.

Fernández, A. S., Larsen, M., Nansen, P., Grønvold, J., Henriksen, S.A. & Wolstrup, J. 1997. Effect of the nematode-trapping fungus *Duddingtonia flagrans* on the free-living stages of horse parasitic nematodes: a plot study. *Veterinary Parasitology 73*, 257-266.

French, D. D. & Klei, T. R. 1983. Benzimidazole-resistant strongyle infections: A review of significance, occurrence, diagnosis and control. In: *Proceedings of the 29th Annual Meeting of the American Association of Equine Practitioners,* Las Vegas, Nevada. pp 313-317.

Gasser, R. B., Hung, G.-C., Chilton, N. B. & Beveridge, I. 2004. Advances in developing molecular-diagnostic tools for strongyloid nematodes of equids: fundamental and applied implications. *Molecular and Cellular Probes 18*, 3-16.

Gasser, R. B., Stevenson, L. A., Chilton, N. B., Nansen, P., Bucknell, D. G. & Beveridge, I. 1996. Species markers for equine strongyles detected in intergenic rDNA by PCR-RFLP. *Molecular and Cellular Probes 10*, 371-378.

Gawor, J. J. 1995. The prevalence and abundance of internal parasites in working horses autopsied in Poland. *Veterinary Parasitology 58*, 99-108.

Gibson, T. E. 1953. The effect of repeated anthelmintic treatment with phenothiazine on the faecal egg counts of housed horses, with some observations on the life cycle of *Trichonema spp.* in the horse. *Journal for Helminthology 27*, 29-40.

Giles, C. J., Urquhart, K. A. & Longstaffe, J. A. 1985. Larval cyathostomiasis (immature trichonema-induced enteropathy): A report of 15 clinical cases. *Equine Veterinary Journal 17*, 196-201.

Gomez, H. H. & Georgi, J. R. 1991. Equine helminth infections: control by selective chemotherapy. *Equine Veterinary Journal 23*, 198-200.

Hartwich, G. 1986. Zum Strongylus tetracanthus - problem und zur systematik der Cyathostominea (Nematoda: Strongyloidea). *Mitteilungen aus dem Zoologischen Museum in Berlin 62*, 61-102. [In German].

Hearn, F. P. & Peregrine, A. S. 2003. Identification of foals infected with *Parascaris equorum* apparently resistant to ivermectin. *Journal of the American Veterinary Medical Association 15*, 482-485.

Herd, R. P. 1986. Epidemiology and control of equine strongylosis at Newmarket. *Equine Veterinary Journal 18*, 447-452.

Herd, R. P. 1990. The changing world of worms: The rise of the cyathostomes and the decline of *Strongylus vulgaris*. *The Compendium on Continuing Education for the Practicing Veterinarian 12*, 732-736.

Herd, R. P. 1992. Performing equine fecal egg counts. *Veterinary Medicine 87*, 240-244.

Herd, R. P. & Gabel, A. A. 1990. Reduced efficacy of anthelmintics in young compared with adult horses. *Equine Veterinary Journal 22*, 164-169.

Herd, R. P. & Majewski, G. A. 1994. Comparison of daily and monthly pyrantel treatment in yearling Thoroughbreds and the protective effect of strategic medication of mares and their foals. *Veterinary Parasitology 55*, 93-104.

Herd, R. P. & Willardson, K. L. 1985. Seasonal distribution of infective strongyle larvae on horse pastures. *Equine Veterinary Journal 17*, 235-237.

Herd, R. P., Willardson, K. L. & Gabel, A. A. 1985. Epidemiological approach to the control of horse strongyles. *Equine Veterinary Journal 17*, 202-207.

Hodgkinson, J. E., Lichtenfels, J. R., Mair, T. S., Cripps, P., Freeman, K. L., Ramsey, Y. H., Love, S. & Matthews, J. B. 2003. A PCR-ELISA for the identification of cyathostomin fourth-stage larvae from clinical cases of larval cyathostominosis. *International Journal for Parasitology 33*, 1427-1435.

Hodgkinson, J. E., Love, S., Lichtenfels, J. R., Palfreman, S., Ramsey, Y. H. & Matthews, J. B. 2001. Evaluation of the specificity of five oligoprobes for identification of cyathostomin species from horses. *International Journal for Parasitology 31*, 197-204.

Hoste, H., Chartier, C., Lefrileux, Y., Goudeau, C., Broqua, C., Pors, I., Bergeaud, J. P. & Dorchies, P. 2002. Targeted application of anthelmintics to control trichostrongylosis in dairy goats: result from a 2-year survey in farms. *Veterinary Parasitology 110*, 101-108.

Hung, G.-C., Chilton, N. B., Beveridge, I. & Gasser, R. B. 2000. Molecular systematic framework for equine strongyles based on DNA sequence data. *International Journal for Parasitology 30*, 95-103.

Hung, G.-C., Gasser, R. B., Beveridge, I. & Chilton, N. B. 1999. Species-specific amplification by PCR of ribosomal DNA from some equine strongyles. *Parasitology 119*, 69-80.

Höglund, J., Ljungström, B.-L., Nilsson, O., Lundquist, H., Osterman, E. & Uggla, A. 1997. Occurrence of *Gasterophilus intestinalis* and some parasitic nematodes of horses in Sweden. *Acta Veterinaria Scandinavica 38*, 157-166.

Ihler, C.F. 1995a. A field survey on anthelmintic resistance in equine small strongyles in Norway. *Acta Veterinaria Scandinavica 36*, 135-143.

Ihler, C. F. 1995b. The distribution of *Parascaris equorum* eggs in the soil of bare paddocks in some Norwegian studs. *Veterinary Research Communications 19*, 495-501.

Ihler, C. F. & Bjørn, H. 1996. Use of two *in vitro* methods for the detection of benzimidazole resistannce in equine small strongyle (*Cyathostoma* spp.). *Veterinary Parasitology 65*, 117-125.

Jubb, K. V. F., Kennedy, P. C. & Palmer, N. 1985. *Pathology of domestic animals*. 3th edn. Academic Press Inc. Orlando, USA. Volume 2, p 178.

Kaplan, R. M. 2002. Anthelmintic resistance in nematodes of horses. *Veterinary Research 33*, 491-507.

Kaplan, R. M. 2004. Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitology 20*, 477-481.

Kaplan, R. M., Klei, T. R., Lyons, E. T., Lester, G., Courtney, C. H., French, D. D., Tolliver, S. C., Vidyashankar, A. N. & Zhao, Y. 2004. Prevalence of anthelmintic-resistant cyathostomes on horse farms. *Journal of the American Veterinary Medical Association 225*, 903-910.

Kelly, J. C. & Fogarty, M. G. 1993. Outbreak of larval cyathostomiasis on a Thoroughbred stud farm. *Irish Veterinary Journal 46*, 133-136.

Klei, T. R. 1992. Recent observations on the epidemiology, pathogenesis and immunology of equine helminth infections. In: *Proceedings of the 6th International Conference on Equine Infectious Diseases*. Cambridge, UK. pp 129-136.

Klei, T. R. & Chapman, M. R. 1999. Immunity in equine cyathostome infections. *Veterinary Parasitology 85*, 123-136.

Klei, T. R., Chapman, M. R., French, D. D. & Taylor, H. W. 1993. Evaluation of ivermectin at an elevated dose against encysted equine cyathostome larvae. *Veterinary Parasitology 47*, 99-106.

Klei, T. R., French, D. D., Chapman, M. R., McClure, J. R., Dennis, V. A., Taylor, H. W. & Hutchinson, G. W. 1989. Protection of yearling ponies against *Strongylus vulgaris* by foalhood vaccination. *Equine Veterinary Journal Supplement*, 2-7.

Klei, T. R., Torbert, B. J., Chapman, M. R. & Ochoa, R. 1982. Irradiated larval vaccinations of ponies against *Strongylus vulgaris*. *Journal of Parasitology 68*, 561-569.

Krecek, R. C., Guthrie, A. J., Van Nieuwenhuizen, L. C. & Booth, L. M. 1994. A comparison between the effects of conventional and selective antiparasitic treatments on nematode parasites of horses from two management schemes. *Journal of the South African Veterinary Association 65*, 97-100.

Krecek, R. C., Reinecke, R. K. & Horak, I. G. 1989. Internal parasites of horses on mixed grassveld and bushveld in Transvaal, Republic of South Africa. *Veterinary Parasitology 34*, 135-143.

Kwa, M. S., Veenstra, J. G. & Roos, M. H. 1994. Benzimidazole resistance in Haemonchus contortus is correlated with a conserved mutation at amino acid 200 in β-tubulin isotype 1. *Molecular and Biochemical Parasitology 63*, 299-303.

Köhler, P. 2001. The biochemical basis of anthelmintic action and resistance. *International Journal for Parasitology 31*, 336-345.

Lacey, E., Redwin, J. M., Gill, J. H., Demargheriti, V. M. & Waller, P. J. 1990. A larval development assay for the simultaneous detection of broad spectrum anthelmintic resistance. In: *Resistance of parasites to antiparasitic drugs*. J. C. Boray, P. J. Martin and R. T. Roush (eds.). MSD AGVET, Rathway, New Jersey. pp 177-184.

Larsen, M. 1999. Biological control of helminths. *International Journal for Parasitology 29*, 139-146.

Larsen, M., Nansen, P., Grøndahl, C., Thamsborg, S. M., Grønvold, J., Wolstrup, J., S.A., H. & Monrad, J. 1996. The capacity of the fungus *Duddingtonia flagrans* to prevent strongyle infections in foals on pasture. *Parasitology 113*, 1-6.

Lendal, S., Larsen, M. M., Bjørn, H., Craven, J., Chriél, M. & Olsen, S. N. 1998. A questionnaire survey on nematode control practices on horse farms in Denmark and the existence of risk factors for the development of anthelmintic resistance. *Veterinary Parasitology 78*, 49-63.

Lichtenfels, J. R. 1975. Helminths of domestic equids. Illustrated keys to genera and species with emphasis on North American forms. *Proceedings of the Helminthological Society of Washington. 42*, 1-92.

Lichtenfels, J. R., Gibbons, L. M. & Krecek, R. C. 2002. Recommended terminolgy and advances in the systematics of the Cyatostomiea (Nematoda: Strongyloidea) of horses. *Veterinary Parasitology 107*, 337-342.

Lichtenfels, J. R., Kharchenko, V. A., Krecek, R. C. & Gibbons, L. M. 1998. An annotated checklist by genus and species of 93 species level names for 51 recognized species of small strongyles (Nematoda: Strongyloidea: Cyathostominea) of horses, asses and zebras of the world. *Veterinary Parasitology 79*, 65-79.

Lichtenfels, J. R., Kharchenko, V. A., Sommer, C. & Ito, M. 1997. Key characters for the microscopical identification of *Cylicocyclus nassatus* and *Cylicocyclus ashworthi* (Nematoda: Cyathostominae) of the horse, *Equus caballus*. *Journal of the Helminthological Society of Washington 64*, 120-127.

Lichtenfels, J. R., McDonnell, A., Love, S. & Matthews, J. B. 2001. Nematodes of the Tribe Cyathostominea (Strongylidae) collected from horses in Scotland. *The Compendium on Parasitology 68*, 265-269.

Lindberg, R. 1976. Överlevnad av infektiösa larver av hästens strongylida nematoder i betesgräs. *Svensk Veterinärtidning 28*, 509-515 [In Swedish].

Little, D., Flowers, J. R., Hammerberg, B. H. & Gardner, S. Y. 2003. Management of drug-resistant cyathostominosis on a breeding farm in central North Carolina. *Equine Veterinary Journal 35*, 246-251.

Lloyd, S. & Soulsby, E. J. L. 1998. Is anthelmintic resistance inevitable: back to basics? *Equine Veterinary Journal 30*, 280-283.

Love, S. 1995. Recognizing disease associated with strongyles in horses. *The Compendium on Continuing Education for the Practicing Veterinarian 17*, 564-567.

Love, S. & Duncan, J. L. 1992a. Development of cyathostome infection of helminth-naive foals. *Equine Veterinary Journal Supplement 13*, 93-98.

Love, S. & Duncan, J. L. 1992b. The development of naturally acquired cyathostome infection in ponies. *Veterinary Parasitology 44*, 127-142.

Love, S., Mair, T. S. & Hillyer, M. H. 1992. Chronic diarrhoea in adult horses: a review of 51 referred cases. *Veterinary Record 14*, 217-219.

Love, S., Murphy, D. & Mellor, D. 1999. Pathogenicity of cyathostome infection. *Veterinary Parasitology 85*, 113-122.

Mair, T. S. 1993. Recurrent diarrhoea in aged ponies associated with larval cyathostomiasis. *Equine Veterinary Journal 25*, 161-163.

Mair, T. S. & Pearson, G. R. 1995. Multifocal non-strangulating intestinal infarction associated with larval cyathostomiasis in a pony. *Equine Veterinary Journal 27*, 154-155.

Martin, P. J. & Jarrett, R. G. 1989. Detecting benzimidazole resistance with faecal egg count reduction tests and *in vitro* assays. *Australian Veterinary Journal 66*, 236-240.

Martin, P. J., Le Jambre, L. F. & Claxton, J. H. 1981. The impact of refugia on the development of thiabendazole resistance in *Haemonchus contortus*. *International Journal for Parasitology 11*, 35-41.

Martin, R. J. 1997. Modes of action of anthelmintic drugs. *Veterinary Journal 154*, 11-34.

Martin, R. J., Robertson, A. P. & Bjørn, H. 1997. Target sites of anthelmintics. *Parasitology 114*, 111-124.

Martin-Downum, K., Yazwinski, T., Tucker, C., Fincher, M., Ralph, J. & Hamilton, J. 2001. Cyathostome fecal egg count trends in horses treated with moxidectin, ivermectin or fenbendazole. *Veterinary Parasitology 101*, 75-79.

Matthee, S., Krecek, R. C. & Gibbons, L. M. 2002. *Cylicocyclus asinus* n.sp. (Nematoda: Cyathostominae) from donkeys, *Equus asinus*, in South Africa. *Systematic Parasitology 51*, 29-35.

Matthews, J. B., Hodgkinson, J., Dowdall, S. M. J. & Proudman, C. J. 2004. Recent developments in research into the Cyathostominae and *Anoplocephala perfoliata*. *Veterinary Research 35*, 371-381.

McDonnell, A., Love, S., Tait, A., Lichtenfels, J. R. & Matthews, J. B. 2000. Phylogenetic analysis of partial mitochondrial cytochrome oxidase c subunit I and large ribosomal RNA sequences and nuclear internal transcribed spacer I sequences from species of Cyathostominae and Strongylinae (Nematoda, order Strongylida), parasites in the horse. *Parasitology 121*, 649-659.

Mfitilodze, M. W. & Hutchinson, G. W. 1987. Development and survival of free-living stages of equine strongyles under laboratory conditions. *Veterinary Parasitology 23*, 121-133.

Mfitilodze, M. W. & Hutchinson, G. W. 1990. Prevalence and abundance of equine strongyles (Nematoda: Strongyloidea) in tropical Australia. *International Journal for Parasitology 76*, 487-494.

Mirck, M. H. 1981. An investigation into the epidemiology of Strongylidae infections in the horse in the Netherlands. *Veterinary Quarterly 3*, 98-100.

Monahan, C. M., Chapman, M. R., Taylor, H. W., French, D. D. & Klei, T. R. 1996. Comparision of moxidectin oral gel and ivermectin oral paste against a spectrum of internal parasites of ponies with special attention to encysted cyathostome larvae. *Veterinary Parasitology 63*, 225-235.

Monahan, C. M., Chapman, M. R., Taylor, H. W., French, D. D. & Klei, T. R. 1998. Experimental cyathostome challenge of ponies maintained with or without benefit of daily pyrantel tartrate feed additive: comparison of parasite burdens, immunity and colonic pathology. *Veterinary Parasitology 74*, 229-241.

Monahan, C. M., Chapman, M. R., Taylor, H. W. & Klei, T. R. 1997. Foals raised on pasture with or without daily pyrantel tartrate feed additive: comparison of parasite burdens and host responses following experimental challenge with large and small strongyle larvae. *Veterinary Parasitology 73*, 277-289.

Monahan, C. M., Taylor, H. W., Chapman, M. R. & Klei, T. R. 1994. Experimental immunization of ponies with *Strongylus vulgaris* radiation-attenuated larvae or crude soluble somatic extracts from larval or adult stages. *Journal of Parasitology 80*, 911-923.

Nautrup Olsen, S., Schumann, T., Pedersen, A. & Eriksen, L. 2003. Recovery of live immature cyathostome larvae from faeces of horses by Baermann technique. *Veterinary Parasitology 116*, 259-263.

Nilsson, O. & Andersson, T. 1979. *Strongylus vulgaris* hos häst - epizootologi och profylax. *Svensk Veterinärtidning 31*, 148-156 [In Swedish].

Nilsson, O., Hellander, J. & Jerneld, P. 1990. Effekten av subkliniska strongylid-infektioner på unghästars tillväxt på bete. *Svensk Veterinärtidning 42*, 167-171 [In Swedish].

Nilsson, O., Lindholm, A. & Christensson, D. 1989. A field evaluation of anthelmintics in horses in Sweden. *Veterinary Parasitology 32*, 163-171.

Nilsson, O., Ljungström, B.-L., Höglund, J., Lundquist, H. & Uggla, A. 1995. *Anoplocephala perfoliata* in horses in Sweden: prevalence, infection levels and intestinal lesions. *Acta Veterinaria Scandinavica 36*, 319-328.

O'Meara, B. & Mulcahy, G. 2002. A survey of helminth control practices in equine establishments in Ireland. *Veterinary Parasitology 109*, 101-110.

Ogbourne, C. P. 1972. Observations on the free-living stages of strongylid nematodes of the horse. *Parasitology 64*, 461-477.

Ogbourne, C. P. 1975a. Epidemiological studies on horses infected with nematodes of the family Trichonematidae (Witenberg, 1925). *International Journal for Parasitology 5*, 667-672.

Ogbourne, C. P. 1975b. Studies on the epidemiology of *S. vulgaris* infection of the horse. *International Journal for Parasitology 5*, 423-426.

Ogbourne, C. P. 1976. The prevalence, relative abundance and site distribution of nematodes of the subfamily *Cyathostominae* in horses killed in Britain. *Journal for Helminthology 50*, 203-214.

Ogbourne, C. P. 1978. *Pathogenesis of cyathostome (Trichonema) infections of the horse. A review.* Commonwealth Agricultural Bureaux. UK. 25 pp.

Osterman, E., Nilsson, O., Höglund, J. & Uggla, A. 1996. Avmaskningsintervall för ivermektin och pyrantel. *Svensk Veterinärtidning 48*, 281-284. [In Swedish].

Pascoe, R. J., Wilson, T. J. & Coles, G. C. 1999. Nematode control in eventer horses. *Veterinary Record 145*, 200-201.

Paul, W. J. 1998. Equine larval cyathostomosis. *The Compendium on Continuing Education for the Practicing Veterinarian 20*, 509-514.

Polley, L. 1986. Strongylid parasites of horses: Experimental ecology of free-living stages on the Canadian prairie. *American Journal of Veterinary Research 47*, 1686-1693.

Pook, J. F., Power, M. L., Sangster, N. C., Hodgson, J. L. & Hodgson, D. R. 2002. Evaluation of tests for anthelmintic resistance in cyathostomes. *Veterinary Parasitology 106*, 331-343.

Popova, T. I. 1955 [Translated from Russian, Israel Program for Scientific Translations, 1964]. *Strongyloids of animals and man*. Jerusalem, Israel pp 121-130.

Popova, T. I. 1958 [Translated from Russian, Israel Program for Scientific Translations, 1965]. *Strongyloids of animals and man. Trichonematidae.* Jerusalem, Israel. pp 366-375.

Posedi, J., Drögemüller, M., Schnieder, T., Höglund, J., Lichtenfels, J. R. & Samson-Himmelstjerna, G. v. 2004. Microchip capillary electrophoresis-based comparison of closely related cyathostomin nematode parasites of horses using randomly amplified polymorphic DNA polymerase chain reaction. *Parasitology Research 92*, 421-429.

Poynter, D. 1954. Seasonal fluctuation in the number of strongyle eggs passed by horses. *Veterinary Record 66*, 74-78.

Prichard, R. K., Hall, C. A., Kelly, J. D., Martin, I. C. A. & Donald, A. D. 1980. The problem of anthelmintic resistance in nematodes. *Australian Veterinary Journal 56*, 239-250.

Proudman, C. J. & Matthews, J. B. 2000. Control of intestinal parasites in horses. *In Practice 22*, 90-97.

Ramsey, Y. H., Christley, R. M., Matthews, J. B., Hodgkinson, J. E., McGoldrick, J. & Love, S. 2004. Seasonal development of Cyathostominae larvae on pasture in a northern temperate region of the United Kingdom. *Veterinary Parasitology 119*, 307-318.

Reid, S. W. J., Mair, T. S., Hillyer, M. H. & Love, S. 1995. Epidemiological risk factors associated with a diagnosis of clinical cyathostomiasis in the horse. *Equine Veterinary Journal 27*, 127-130.

Reilly, G. A. C., Cassidy, J. P. & Taylor, S. M. 1993. Two fatal cases of diarrhoea in horses associated with larvae of the small strongyles. *Veterinary Record 13*, 267-268.

Reinemeyer, C. R. 1986. Small strongyles: Recent advances. In: *The Veterinary Clinics of North America: Equine Practice.* W. B. Saunders Company. Philadelphia. pp 281-312.

Reinemeyer, C. R., Smith, S. A., Gabel, A. A. & Herd, R. P. 1984. The prevalence and intensity of internal parasites of horses in the U.S.A. *Veterinary Parasitology 15*, 75-83.

Reinemeyer, C. R., Smith, S. A., Gabel, A. A. & Herd, R. P. 1986. Observations on the population dynamics of five cyathostome nematode species of horses in northern USA. *Equine Veterinary Journal 18*, 121-124.

Samson-Himmelstjerna, G. v., Witzendorff, C., Sievers, G. & Schnieder, T. 2002. Comparative use of faecal egg count reduction test, egg hatch assay and beta-tubulin codon 200 genotyping in small strongyles (cyathostominae) before and after benzimidazole treatment. *Veterinary Parasitology 108*, 227-235.

Sangster, N. C. 1999. Pharmacology of anthelmintic resistance in cyathostomes: will it occur with the avermectin/milbemycins? *Veterinary Parasitology 85*, 189-204.

Sangster, N. C. 2003. A practical approach to anthelmintic resistance. *Equine Veterinary Journal 35*, 218-219.

Silva, A. V. M., Costa, H. M. A., Santos, H. A. & Carvalho, R. O. 1999. Cyathostominae (Nematoda) parasites of *Equus caballus* in some Brazilian states. *Veterinary Parasitology 86*, 15-21.

Slocombe, J. O. D. 1992. Anthelmintic resistance in strongyles of equids. In: *Proceedings of the 6th International Conference on Equine Infectious Diseases,* Cambridge, UK. pp 137-143.

Slocombe, J. O. D., Valenzuela, J. & Lake, M. C. 1987. Epidemiology of Strongyles in Ponies in Ontario. *Canadian Journal of Veterinary Research 51*, 470-474.

Smith, H. J. 1978. Experimental *Trichonema* infections in mature ponies. *Veterinary Parasitology 4*, 265-273.

Soulsby, E. J. L. 1986. *Helminths, arthropods and protozoa of domesticated animals*. 7th edn. Ballière Tindall. Eastbourne. p 809.

Statens Jordbruksverk, Informationsavdelningen. Antal hästar och hästhållare i Sverige. http://www.sjv.se/startsida/amnesomraden/statistikfakta (accessed 28-Feb-2005).

Tandon, R. & Kaplan, R. M. 2004. Evaluation of a larval development assay (DrenchRite®) for the detection of anhelmintic resistance in cyathostomin nematodes of horses. *Veterinary Parasitology 121*, 125-142.

Tarigo-Martinie, J. L., Wyatt, A. R. & Kaplan, R. M. 2001. Prevalence and clinical implications of anthelmintic resistance in cyathostomes of horses. *Journal of the American Veterinary Medical Association 218*, 1957-1960.

Thamsborg, S. M., Leifsson, P. S., Grøndahl, C., Larsen, M. M. & Nansen, P. 1998. Impact of mixed strongyle infections in foals after one month on pasture. *Equine Veterinary Journal 30*, 240-245.

Thienpont, D., Rochette, F. & Vanparijs, O. F. J. 1986. *Diagnosing helminthiasis through coprological examination*. 2nd edn. Janssen Research Foundation. Beerse, Belgium. pp 85-87.

Tizard, I. R. 2000. *Veterinary immunology: an introduction*. 6th edn. W.B. Saunders Company. Philadelphia. pp 280-294.

Tolling, S. 1976. Bekämpning av *Strongylus vulgaris* hos häst genom systematisk avmaskning. *Svensk Veterinärtidning 28*, 287-289 [In Swedish].

Torbert, B. J., Klei, T. R., Lichtenfels, J. R. & Chapman, R. 1986. A survey in Louisiana of intestinal helminths of ponies with little exposure to anthelmintics. *International Journal for Parasitology 72*, 926-930.

Turk, M. A. M. & Klei, T. R. 1984. Effect of ivermectin treatment on eosinophilic pneumonia and other extravascular leisons of late *Strongylus vulgaris* larval migration in foals. *Veterinary Pathology 21*, 87-91.

Uhlinger, C. 1990. Effects of three anthelmintic schedules on the incidence of colic in horses. *Equine Veterinary Journal 22*, 251-254.

Uhlinger, C. A. 1991. Equine small strongyles: epidemiology, pathology and control. *The Compendium on Continuing Education for the Practicing Veterinarian 13*, 332-338.

Uhlinger, C. A. 1992. Preliminary studies into factors affecting the variability of egg reappearance period and anthelmintic treatment intervals in the control of equine cyathostomes. In: *Proceedings of the 6th International Conference on Equine Infectious Diseases,* Cambridge, UK. pp 157-161.

Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M. & Jennings, F. W. 1996. *Veterinary parasitology*. 2nd edn. Blackwell Science Ltd. Oxford. pp 4-10, 42-47.

van Wyk, J. A. 2001. Refugia - overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. *Onderstepoort Journal of Veterinary Research 66*, 55-67.

Velichkin, P. A. 1952. Uzelkovyj trikhonematoz tolstikh kishok u tabunnykh loshadej (Nodular trichonematosis of the large intestine in a horse herd). *Izvestiya Moskovskogo Zootekhnicheskogo Instituta Konevodstva 7*, 18-25 [In Russian].

Warnick, L. D. 1992. Daily variability of equine faecal strongyle egg counts. *Cornell Veterinarian 82*, 453-463.

Wescott, R. B. 1986. Anthelmintics and drug resistance. In: *Veterinary Clinics of North America: Equine Practice.* W.B. Saunders Company. Philadelphia. 367-380.

Wolstenholme, A. J., Fairweather, I., Prichard, R., Samson-Himmelstjerna, G. v. & Sangster, N. C. 2004. Drug resistance in veterinary helminths. *Trends in Parasitology 20*, 469-476.

Woods, T. F., Lane, T. J., Zeng, Q.-Y. & Courtney, C. H. 1998. Anthelmintic Resistance on horse farms in North Central Florida. *Equine Practice 20*, 14-17.

Young, K. E., Garza, V., Snowden, K., Dobson, R. J., Powell, D. & Craig, T. M. 1999. Parasite diversity and anthelmintic resistance in two herds of horses. *Veterinary Parasitology 85*, 205-214.

Acknowledgements

This project was carried out at the Department of Biomedical Sciences and Veterinary Public Health, Division of Parasitology and Virology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. Financial support was obtained from the Swedish Horse Race Totalisator Board (ATG) and Agria Insurance Co, Stockholm, Sweden.

I want to thank all of you who in various ways have given their support to this work over the years and thus contributed to this thesis. In particular I would like to acknowledge:

Arvid Uggla, my scientific supervisor, for the initiation of this project, and for encouragement and patience during a decade. In the end, I managed to produce more papers than boys! You are a great source of inspiration in the process of scientific writing. I appreciate your concern about the language in our papers.

Johan Höglund, my associate scientific supervisor, with whom I have worked closest in the project, especially in the beginning, before the ruminants also needed your expertise. Thank you for taking great interest in my work, and for stimulating discussions and clever ideas in designing interesting studies.

Peter Waller, a most efficient and organised associate scientific supervisor. I am grateful for your quick response on my drafts. I have been fortunate to have had you in the group of supervising men, and enjoyed your company during the late lunches.

Olle Nilsson, whose genuine interest in parasites has resulted in unlimited numbers of ideas and hypotheses over the years. It has been a challenge for me to give talks where a charming person like you has lectured previously!

Jens Mattsson and *Dan Christensson*, for your continuous encouragement, for facilitating the progress and completion of this work, and for giving me an idea of what I would like to do after the dissertation.

David Morrison, the tremendous baker at SWEPAR, for taking interest in the control practices applied on Swedish horse farms. I am very thankful for valuable assistance and your proof-reading of the last paper and the thesis.

Tania Kuzmina, the most recent Ukraine doctor of horse strongyles, my friend and co-author. Thank you for all your efforts in critically reading my drafts. I am looking forward to continuing our scientific and non-scientific relationship.

Erik Rautalinko, co-author of the last paper, and *Stina Osterman*, my dear sister, for valuable ideas and thoughts during the development of the questionnaire.

Lasse Lind, for your enthusiasm while "bathing" in the faecal samples.

Anna Lundén and *Eva Wattrang*, for taking the time to comment bits and pieces of my work, and for sharing the interest in horses.

Ingrid Leemans, for being such a generous person who is always prepared to listen to other people. Only you would suggest spending the evening at SVA and going through my thesis instead of having a nice meal somewhere else.

Jan Chirico, my office neighbour. Did I overdo it? I would like to thank a big doctor for cheering up a tiny little "doktorand".

My fellow PhD students at SWEPAR: *Anna Larsson*, for being so encouraging and giving me self-confidence; *Erland Ljunggren*, the athletic next-to-be, for friendship and support while we were both writing our books; *Malin Hagberg*, for fruitful immunology conversations; *Karin Troell*, for aesthetic advice on my diagrams; *Jackie Hrabok*, the pseudo-sami ice-hockey player, for your warm heart and positive attitude to most things in life; *Sara Arvidsson*; *Anita Haug*; *Sissay Menkir*; *Eva Pettersson*; *Sunan Pinitkiatisakul*; *Seng Sokerya* for your friendship.

Doctors: *Stene Dimander*; *John Githiori*; *Lotta Gunnarsson*; *Katarina Gustafsson*; *Joakim Holmdahl*; *Lam Thi Thu Huong; Helena Nordenfors*; *Chandrawathani Panchadcharam*; *Susanne Stenlund*; *Lotta Waldenstedt*, former fellow PhD students at SWEPAR. You are all lovely and humorous people, who have contributed to many good times at the department.

The Section for Diagnostics: *Ann-Charlotte Andersson*; *Birgitta Andersson*; *Kenneth Backström*; *Jenny Blom*; *Set Bornstein; Bodil Christensson; Maria Dahlén; Carina Häll; Susanne Johansson; Göran Zakrisson,* for making me realise how interesting non-strongyle parasites can be. You have taught me a lot!

Katrin Bergström and *Sten Olof Fredriksson*, for excellent computer support whenever needed.

Gunilla Lindgren and *Anna Rothman* for your efficient help in dealing with practical matters, and Anna also for your "encouraging" and "calming" pre-dissertation comments.

Annie Engström; Eva-Britt Jakubek; Owe Jansson; Katarina Näslund; Per Thebo, Anna Rydzik, for contributing to the friendly atmosphere at the department, helping to make it such an enjoyable place to work.

Gunnel Erne and *Agneta Lind* at the library of SVA, for helping me to find old and less old literature.

Bengt Ekberg, for continuously providing me with outstanding photographs.

Peter Jerneld; Malin Johansson; Bitte Ljungström; Ebba Nilsson; Merike Ronéus; Petra Jernfält Wingefors, and many more colleagues, friends and horse owners, for interesting and stimulating discussions on horse parasites.

Stefanos-kören, for your friendship and for always being there Tuesday evenings (and some Saturdays and Sundays). During the hours of singing it has really been possible to recover from intensive hours of thinking and writing.

My mother, *Karin*, who came like an angel from Åre to help us during the most hectic weeks in the autumn. Thank you also for showing genuine interest in my work and reading the first draft of the thesis.

And most of all, *Thomas, Hjalmar, Olle* and *Manfred*, my beloved family. Thank you for your support and for giving me true perspective of life. Thomas knows a lot about horse strongyles – believe me!