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Equine strongylidosis on livery yards in Germany

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List of Abbreviations

AR	Anthelmintic resistance
EL3	Early third stage larva
FEC	Fecal egg count
L1	Frist stage larva
L2	Second stage larva
L3	Third stage larva
L4	Fourth stage larva
L5	Fifth stage larva
SAT	Selective anthelmintic therapy

1. Introduction and study objectives

Small strongyles are considered to be the most important helminths of horses (Love et al., 1999). Previously, large strongyle were the focus of research and treatment strategies due to their high pathogenicity; however, with strategic deworming practiced widely the prevalence of large strongyles has diminished (Lyons et al., 1999; Bowman et al., 2003; Nilsson et al., 1989; Becher et al., 2010; Drudge & Lyons, 1996; Gawor, 1995; Poynter, 1969; Fritzen, 2005; von Samson-Himmelstjerna et al., 2007; Hinney et al., 2011; Schneider et al., 2014). With this, the focus shifted to small strongyles as they are highly prevalent throughout the horse population despite regular and frequent anthelmintic treatments (Beelitz et al., 1996; Fritzen, 2005; von Samson-Himmelstjerna et al., 2007; Wirtherle, 2003; Schnerr, 2011; Greite, 2013; Becher et al., 2010; Menzel, 2013). While less pathogenic than the large strongyle, small strogyles can cause both unspecific clinical signs (ill-thrift, poor performance, decreased growth rate, diarrhea, lethargy), as well as larval cyathostominosis which is infrequent but often fatal (Love et al., 1999; Corning, 2009a; Bowman et al., 2003; Lyons et al., 2000; Deplazes et al., 2013).

An additional complication, brought about by the strategic deworming practice, is the development of anthelmintic resistance (AR) of small strongyles against all anthelmintic classes licensed for use in horses (American Association of Equine Practitioners, 2019). The increase in AR incidence has led to the advocacy of selective anthelmintic treatment (SAT) strategies which, besides decreasing the frequency of anthelmintic application, focuses heavily on optimizing management practices (Nielsen et al., 2010b; Kaplan, 2002; Becher, 2010; Greite, 2013; Hinney, 2008; Nielsen, 2012)

The goal of this study is to determine the current prevalence of small strongyles on livery yards in Germany and, via a questionnaire study, assess the effect of internal and external factors on strongyle prevalence and FEC patterns. Additionally, the study will attempt to assess if management practices in Germany have evolved with the intensification of advocacy for SAT implementation.

2. Literature Review

2.1 Strongylids of horses

Equine intestinal parasites are ubiquitous even in well managed populations. Due to their prevalence and pathogenicity the members of the family Strongylidae, and their control strategies hold an especially high importance (Nielsen, 2012). These roundworms, also referred to as strongyles, belong to the phylum Nematoda, Order Strongylida (Taylor et al., 2016).

Based on morphological characteristics, pathogenesis, and pathogenicity, as well as susceptibility to anthelmintics, the Strongylidae family can be divided into two groups: large and small strongyles (Lichtenfels, 1975; Bowman et al., 2003).

Which species belong to the large, and which to the small strongyles differs in literature. Some authors use large strongyles to refer to the entire subfamily Strongylinae with its 14 species belonging to 5 genera (*Strongylus*, *Triodontophorus*, *Oesophagodontus*, *Craterostomum*, and *Bidentostomum*) (Lichtenfels et al., 2008; Maxie, 2016; Lyons et al., 1999; Nielsen et al., 2014b; Marchiando et al., 2019; Saeed et al., 2019). It follows that in this categorization small strongyles are defined as the members of the subfamily Cyathostominae. Other authors consider only the members of the genus *Strongylus* as large strongyles, thus defining small strongyles as all strongylidae of equidae that do not belong to the genus *Strongylus* (Mehlhorn, 2012; Lyons et al., 1999; Deplazes et al., 2013). This paper will refer to large strongyles as the members of the subfamily Strongylinae, while the subfamily. This paper will refer to large strongyles as members of the *Strongylus* genus, while other species of the family Strongylinae will be referred to as small strongyles, or cyathostominae as this genus makes up the most important genus of the small strongyles pathogenic to horses (Deplazes et al., 2013).

2.1.1 Small strongyles

2.1.1.1 Nomenclature of small strogylid species

‘Small strongyles’, also referred to collectively as cyathostomins or cyathostomes, currently includes species of the subfamily Cyathostominae parasitic in Equidae (Lichtenfels et al., 2008; Bredtmann et al., 2017). Discussion of the systematic relationship of these nematodes is ongoing

and intensified with the development of modern molecular methods (Mehlhorn, 2012). However, for accurate re-classification more reliable methods for identification at species level must be developed (Bredtmann et al., 2017). The current classification incorporates over 50 species in 14 genera (Lichtenfels et al., 2008; Taylor et al., 2016; de Vienne, 2016).

Table 1: Predominant cyathostomin species (according to Coring, 2009, Taylor et al., 2016, and Marchiondo et al., de Vienne, 2016)

Genus	Species	
Cyathostomum	*catinatum* *catinatum*	*pateratum* *tetracanthum*
Cylicostephanus	*asymetricus* *bidentatus* *calicatus* *goldi*	*longibursatus* *minutus* *nassatus*
Cylicodontophorus	*bicoronatus*	
Coronocyclus	*coronatus* *labiatus* *labratus*	
Cylicocyclus	*ashworthi* *auriculatus* *brevicapsulatus* *elongatus* *insigne*	*leptostomum* *nassatus* *radiatus* *ultrajectinus*
Paraposteriostomum	*euproctus* *mettami*	
Petrovinema	*poculatus*	

2.1.1.2 Morphology of small strongylid species

Small strongyles are 0.5-2.5cm long, unsegmented worms with a diameter of approximately 1mm as seen in figure 1 (Nielsen et al., 2013a; Mehlhorn, 2012). They vary in color from dark red to yellow white with a shallow, cylindrical buccal capsule that is markedly smaller than that of large strongyles. Adults of most small strongyle species are macroscopically visible on the mucosal surface of the large intestine as well as in digestive and fecal matter. (Taylor et al., 2016; Lichtenfels et al., 2008; Marchiondo et al., 2019; Deplazes et al., 2013; Bowman et al., 2003)

Figure 1:Small strongyles passed with fecal matter (ESCCAP-Empfehlung, 2018)

Stage 3 larvae of small strongyles are approximately 800 µm long with and a body to tail ratio of 1,5:1. Most species have 8 gut cells, but 16 or 20 gut cells can also occur. (Marchiondo et al., 2019; Deplazes et al., 2013)

Eggs shed by cyathostominids are 70-90µm "strongyle-type" eggs meaning they are ellipsoid in shape, thin shelled with a smooth surface and contain a morula embryo as seen in figure 2(Marchiondo et al. 2019; Bowman et al., 2003). These cannot be differentiated from the eggs of large strongyle (Bredtmann et al. 2017; Hummelinck, 1946).

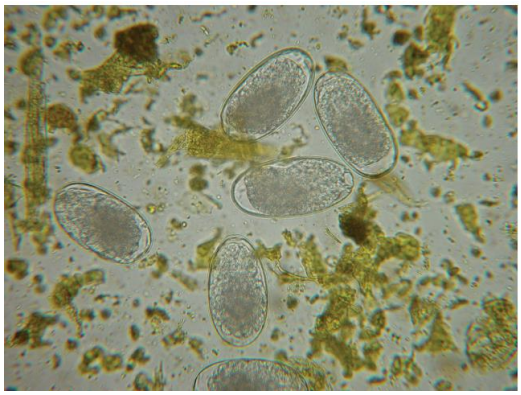


Figure 2: Photomicrograph of eggs from large and small strongyles (Nielsen et al., 2013a)

Detailed identification keys for cyathostomins have been published by Lichtenfels et al. (2008; 1975) and are available in veterinary parasitology textbooks (Taylor et al., 2016; Mehlhorn, 2012; Deplazes et al., 2013; Bowman et al., 2003)

2.1.1.3 Prevalence of small strongylid species

The prevalence of cyathostomins can be viewed at individual level or at herd level. Within one individual small strongyles most frequently cause mixed infections as a number of studies have

shown: Gawor (1995) found a range of 2-16, Anderson and Hasslinger (1982) found 3-11, Ogbourne (1976) found a range between 4-16, Osterman-Lind et al. (2003) found 6-13, Cirak et al. (1996) found 11-17, Reinemeyer et al. (1984) found 2-1,1 and Mfitilodze & Hutchinson (1990) found 2-12. The intraluminal worm population of a host consists of tens to hundreds of thousands ranging from an average of 10.650 worms per horse (Gawor, 1995) to 3,000,000 (Deplazes et al., 2013), infrequently an even larger worm burden can be identified (Reinemeyer et al., 1984; Lichtenfels et al., 2008; Ogbourne, 1976). In addition, the population of larval stages developing in the intestinal walls can be of equal size or even larger (Bucknell et al., 1995; Reinemeyer et al., 1984; Collobert-Laguier, 2002). Numerous studies have also shown that the vast majority of this population, both on an individual and regional level, is made up by only a few specie: In a post-morten investigation, Foster and Oritz (1937) reported that 15 species accounted for 98% of the strongylid population found, while Gawor (1995), Reinemeyer et al., 1984, Mfitilodze and Hutchinson (1990), and Collobert-Laguier (2002) reported 10 species making up 96%, 10 species making up 98.4%, 11 species making up 94%, and 10 species making up 84% of the cyathostome population respectively. More recently, Lichtenfels et al. (2008) observed that 4-14 species make up 50% or more of the population in a host population.

At herd level, numerous prevalence studies have been carried out. The most accurate methodology for these studies is the postmortem evaluation of the worm burden. Prevalence studies at herd level on live horses have also been carried out. These investigate the worm burden by using flotation methods, such as modified McMaster, to evaluate fecal samples. As this methodology only allows for the detection of worm eggs, only infections with adult larvae can be detected, while larval stages as well as encysted stage three larvae go undetected. The studies show that the prevalence of strongyle infections is frequently close to or up to 100%. Results of some prevalence studies conducted in Germany are collected in Table 2.

Table 2: Prevalence of strongyles

Nematodes investigated	Study	Region investigated	Number of stables (n) & number positive (p)	Number of horses and prevalence (p)	Method(s) used
Small (large not found)	Beelitz, 1996	Upper Bavaria, Germany	n = 9 p = 97.3%	n = 74*[6] p = 90.54	Flotation
Small + Large species	Fritzen, 2005	North Rhine-Westphalia, Germany	n = 76 p = 98.7%	n = 2000 p = 49.1% (*S.vulgaris* 1,3%)	Modified McMaster
Small + large species	Hinney, 2008	Brandenburg, Germany	n = 126 p = 98,4%	n = 1407 p = 76%*[5]	Sedimentation and flotation, McMaster, larval culture
Small + large species	Samson-Himmelstjerna et al., 2007	North Rhine-Westphalia, Germany	n = 63 p = *S. vulgaris* 1 stable	n = 2000 p = 49.1%	Fecal egg count examination
No diff. between small and large	Wirtherle, 2003	Niedersachsen, Germany	n = 64 p = 90.6%	n = 1383 p = 39.8 %	Modified McMaster
Identification at species level	Cirak et al, 1996	Germany	n = 3 p = 100%	n = 16 p = 100%*[1]	Postmortem examination
No diff. between small and large	Rehbein et al., 2002	6 states of Germany	n = 49 p = 48	n = 2034 p =76.6%	Flotation-sedimentation
		Austria	n = 7 p = 7	n = 646 p = 61.3%	
No diff. between small and large	Schnerr, 2011	Baden-Württemberg, Germany	n = 4 p = 4	n = 105*[2] p = 20.1%	McMaster and flotation
Strongylus at species level	Greite, 2013	Bavaria, Germany	n = 68 p = 5.9%*	n = 354 p = 1.13%*[3]	McMaster and morphological differentiation of L_3 (Buerger & Stoye, 1968)
No diff. between small and large	Honeder, 2015	40km surrounding ofSaltzburg	n = 35 p = 91.4%	n = 303 (incl. donkeys) *[4] p = 62.4%	McMaster and flotation
No diff. between small and large	Anderson & Hasslinger, 1982	Bavaria, Germany	n = 1 p = 100%	n= 34 p = 100%	Worms eliminated 2-4 days after mebendazole treatment
No diff. between small and large	Menzel, 2011	Baveria, Germany	n = 121 p = 73.5%	n = 518 p = 56.95%*[7]	Flotation – sedimentation, modified McMasters
No diff. between small and large	Becher et al., 2010	40km surroundings of Saltzburg	n = 19	n – 129*[8] p = 59.7%	Modified McMaster
Large + small	Schneider et al., 2014	Germany	n = 192 p = 100% (cyathostomes)	n = 1887 p = 44.6%	Modified McMaster + culture if positive

Large + small	Kaspar et al., 2017	Germany	n = 91 p = 10.9% s. vulgaris one horse per farm only	n = 1455 p = 55.3% Large strongyle: pcr:1.1% (n = 278)	Modified McMaster + larval culture

*[1]Investigated prevalence on a species level (Modified Roberts und OSullivan)
S. vulgaris (12/16), *S. edentatus* (7/16), Small strongyles (16/16)
*[2]sampled each horse 12 times; 1260 samples analyzed in total
*[3] *S. vulgaris* was the only species found
*[4]sampled each horse 1-9 times; 2536 samples analyzed in total; also included donkeys in the study
*[5] *S. vulgaris* was identified at one stable in mixed fecal sample. From the strongyle-type egg positive stables 8% of specimens had 16 gut cells (i.e. unclear if small strongyle or *S. equinus*)
*[6]37 foals and their mothers
*[7] Examined FEC during use of selective therapy
*[8] sampled each horse monthly for 9 months. 1161 samples were analyzed in total

2.1.1.4 Lifecycle of small strongylid species

The life cycle of small stongyles is direct and can be divided into an internal and an external phase as seen in figure 3. The lifecycle up until L3 is equivalent to the external phase and starts when eggs released by an adult female worm into the lumen of the intestines are shed into the environment along with the fecal matter. The first step of the external phase is the embryonation of the egg. Once the first stage larva (L1) has developed inside the egg and environmental conditions are suitable, the L1 larvae hatch from the egg. Typically, this occurs within one to two days. The L1 stage is the first out of three free-living stages. L1 larvae molt to become a second stage larvae (L2). Larvae then mature to the infective, third stage larva (L3). This larval stage does not shed its entire cuticula during molting resulting in a protective covering. Although, this retained cuticle prevents L3 from feeding, the larvae can survive weeks to months on pastures. In warm weather, the development from egg to L3 can take place within 3 days. The L3 larvae then migrate from the fecal matter to herbage. (Nielsen & Lyons, 2017; Nielsen et al., 2007; Corning, 2009a; Maxie, 2016; Bowman et al., 2003; Deplazes et al., 2013)

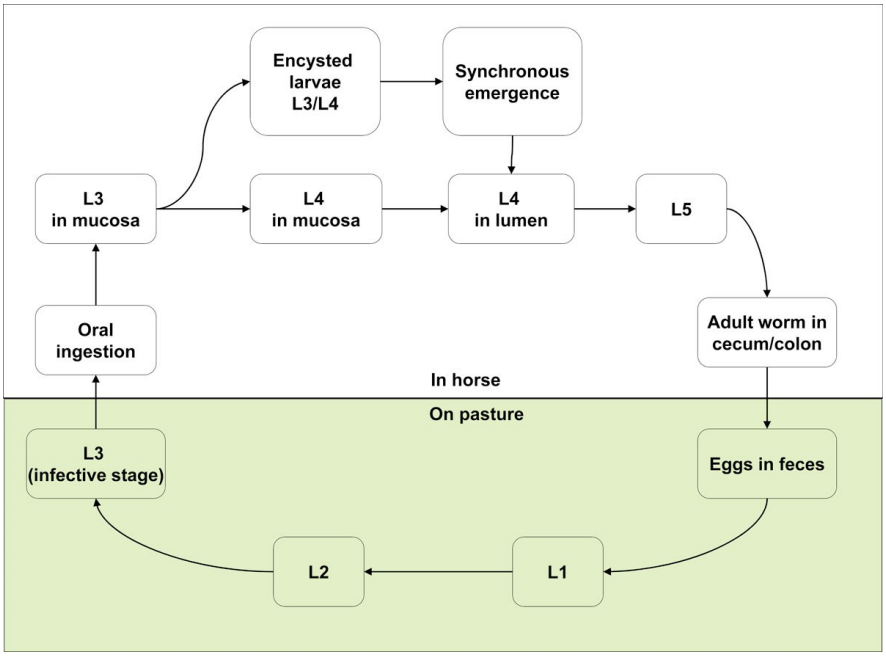


Figure 3: The lifecycle of small strongyles (Corning, 2009b)

When L3 are taken up per os by a host during grazing the internal phase of the lifecycle begins (Deplazes et al., 2013). Once ingested, early third stage larvae (EL3) shed their cuticle and migrate into the Lieberkuehn crypts or the mucous membranes. From here they invade the ceacal or colonic mucosa or, less frequently, the submucosa (Eysker & Merck, 1986; Deplazes et al., 2013). Unlike species of the *Strongylus* genus, cyathostomins do not migrate beyond the intestinal tract. The distribution of the larvae was examined by Reinemeyer and Herd (1986) who on post-mortem investigation found that 98% of encysted small strongyles were location in the cecum and in the proximal ventral colon, whereas only 2% were present in the distal fourth of the ventral colon and dorsal colon combined. They also reported the density of larvae in the dorsal colon only increases with high infection intensity. Eysker and Mirck (1986), Scháňková et al. (2014), and Stancampiano et al. (2010), reported very similar findings with a majority of L3 encysted in the cecum and ventral colon, and fewer L3 in the dorsal colon. 1-2 weeks after the worms are taken by the host, the larvae are encapsulated with connective tissue (Esker & Merck, 1986). Within these cysts, the worms develop into late third stage larvae (LL3) and then fourth stage larvae (L4) within 6-12 days (Deplazes et al., 2013). 1-2 months later, L4 larvae leave the cyst and enter the lumen of the intestine (Ribbeck, 1999; Deplazes et al., 2013). Here they develop into fifth stage larvae (L5) and lastly into adults. This can happen in as little as 5-6 weeks. The adults then loosely attach to the mucosa to a characteristic site in the cecum

and colon depending on the species and produce eggs which the host will excrete with the fecal matter thus contaminating the pasture (Ogbourne, 1976; Gawor, 1995; Corning, 2009a; Nielsen et al., 2013a; Morariu et al., 2016; Stancampiano et al., 2010; Bowman et al., 2003). The pre-patent period (i.e. the time from infection of the host to shedding of eggs commences) varies from 5.5 weeks to 2.5 years, and the maximum life-span of one adult worms is one year (Reinemeyer & Herd, 1986; Round 1969; Love & Duncan, 1992a; Leathwick et al., 2019).

This cycle can be interrupted when EL3, L3, and L4 development becomes arrested for up to 2.5 years or more (Nielsen & Lyons, 2017; Eysker et al., 1983; Matthews et al., 2004; Smith, 1976a; Reinemeyer & Herd, 1986; Round 1969; Love & Duncan, 1992a; Deplazes et al., 2013). This is also referred to as hypobiosis, and as much as 90% of encysted cyathostominae may be arrested (Proudman & Matthews, 2002). Especially in winter, encysted larvae can make up as much as 95% of the total burden (Deplazes et al., 2013). While hypobiosis is incompletely understood, it is generally accepted that this strategy enables the nematodes to avoid environmental conditions not conducive to hatching and larval development (Matthews et al., 2004; Nielsen et al. 2014b; Eysker et al., 1990). In northern temperate climates, hypobiosis is a seasonal phenomenon mainly observed in infections occurring at the end of the summer and autumn with larval development resuming in late winter to early spring (Eysker et al., 1990; Ribbeck, 1999; Love & Duncan, 1992b; Nielsen et al., 2013a). It is also proposed that the removal of adult worms by the use of anthelmintic substances stimulates the resumption of development of arrested L3 (Smith, 1976a; Gibson, 1953) which strengthens the hypothesis that the intra-luminal presence of adult strongyle individual slows the development of L3 (Smith, 1976a). Third stage larva development is also decelerated when consumption of infective L3 on the pasture is high or prolonged (Leathwick et al., 2019; Love & Duncan, 1992b). Love and Duncan (1992a) found that the parasite burden of naïve animals was higher than that of foals previously exposed to these helminths, while the mucosal fraction of the cyathostome population was lower. An experiment conducted by Smith (1978) showed that administration of immunosuppressive drugs did not result in resumption of larval development, thus larval development is not arrested directly by the host's immune system. Rather it suggests that the relationship between the encysted larval population and the host's immune system is multifactorial and more research is needed to fully understand the factors that drive hypobiosis and recommencement of larval development.

2.1.1.5 Epidemiology of small strongyles

The epidemiological aspects of small strongyle infections in horses is largely identical to that of large strongyles (see section 2.1.2.5) (Nielsen et al., 2013a; Mfitilodze & Hutchinson, 1987; Reinemeyer & Herd, 1986). The only major difference is the arrested development of early L3 stages of small strongyle discussed in section 2.1.1.4. The cycle of small strongyle populations is considered to be annual with increasing quantities of eggs are shed in the spring when the dormant adult population increases its reproductive activity and matured larvae that were arrested in hypobiosis start to shed eggs (Kornaś et al., 2006; Nielsen et al., 2013a; Herd 1986a; Ogbourne, 1975; Saeed et al., 2010; McCraw & Slocombe, 1976). This population continues to reproduce and shed eggs. Infection in the early spring, especially under humid conditions, can also result in a second generation of adult small strongyles maturing in the late summer of the same year leading to another rise in egg production (Herd, 1986a; Kornaś et al., 2006; McCraw & Slocombe, 1976). Infections during autumn generally result in arrested larval development, these larvae will enter the gut lumen and mature in adults in the late winter or early spring of the following year, completing the annual cycle (Deplazes et al., 2013).

2.1.1.6 Clinical signs of small strongyle infection

Infection with adult cyathostominae may result in clinical signs if present in the gut in large numbers. In these cases, clinical symptoms are non-specific and may include weight loss, watery diarrhea, lethargy, and failure to thrive (Corning, 2009a; Bowman et al., 2003; Deplazes et al., 2013). Although, clinical signs originating from infestation with cyathostominae occur mainly when many developing larvae emerge from the intestinal mucosa simultaneously, also referred to as larval cyathostominosis (Maxie, 2016; Nielsen, 2014b; Corning, 2009a; Giles et al., 1985; Geldberg, 2017; Bowman et al., 2003). In such cases, an inflammatory enteropathy involving the cecum and colon develops (Giles et al., 1985; Cobb & Boeckh, 2009; Nielsen, 2014b; Geldberg, 2017; Bowman et al., 2003). Clinical signs accompanying this condition are non-specific and may include colic, diarrhea, weight loss, anemia, subcutaneous edema, pyrexia and a rough hair coat (Jasko & Roth, 1984; Cobb & Boeckh, 2009; Archer & Poynter, 1957; Smith 1976a,b; Mair, 1994; Lyons et al., 2000; Nielsen, 2014b; Corning, 2009a; Maxie, 2016 ; Geldberg, 2017; Bowman et al., 2003; Deplazes et al., 2013). Cyathostominae can also result in

specific forms of colic such as cecocolic intussusception, cecal tympany, and non-strangulating infarction. Most frequently clinical signs due to cyathostominosis are seen in horses under 5 years of age in late winter or spring in temperate climates (Geldberg, 2017; Giles et al., 1985; Maxie, 2016; Love et al., 1999; Lyons et al., 2000; Nielsen, 2014b; Corning, 2009a; Bowman et al., 2003).

2.1.1.7 Pathology of small strongyle infection

Gross pathological findings associated with cyathostominid infections are generally nodules, these are better visible with transmural illumination. These are attributable to the encysted larvae developing in the mucosa and are only have a few millimeter-wide diameter (Corning, 2009a; Maxie, 2016). The nodules are surrounded by edema and congestion, and the cut surface of these nodules exposes small grey-red larvae. (Maxie, 2016). Additionally, the cecum and colon may show hyperemia, hemorrhage, ulceration or necrosis (Corning, 2009a).

Histopathologically, a mixed inflammatory reaction with lymphocyte and occasionally eosinophil infiltration is observed around the encysted larvae or more diffusely in the lamina propria and submucosa (Nielsen et al., 2013a; Maxie, 2016; Corning, 2009a). Furthermore, a fibroblastic reaction with goblet cell hyperplasia and hypertrophy may be observed around the encysted larvae (Nielsen et al., 2013a).

2.1.2 Large strongyles

2.1.2.1 Nomenclature of large strongylid species

Large strongyles refers to members of the subfamily *Strongylinae* and the genus *Strongylus* (Nielsen & Lyons, 2017; Lichtenfels et al. 1975). Out of the 5 genera, the genus of *Strongylus* bears the most importance (Maxie, 2016). *Strongylus* species occurring in Central Europe are *Strongylus vulgaris*, *S. equinus*, and *S. edentatus* (Taylor et al., 2016; Lichtenfels et al., 2008; Lichtenfels, 1975). The members members of this genus are considered pathogenic (Lyons et al., 1999; Bowman et al., 2003).

2.1.2.2 Morphology of large strongylid species

Worm belonging to the *Strongylus* genus range from 1.5cm to 4.5cm in length with a diameter of about 2mm. They are greyish-red in color and possess large, globular or funnel shaped buccal capsules (Nielsen et al., 2014b; Lichtenfels et al., 2008; Deplazes et al., 2013).

Unlike cyathostomins, large strongyles vary in morphology enough to allow comparatively easy differentiation at a species level (Lichtenfels et al., 2008).

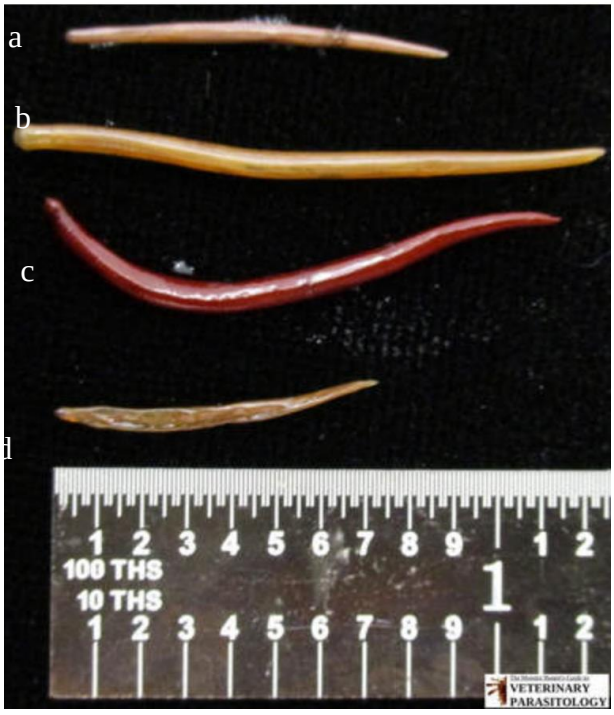


Figure 4: Photograph of (a) a member of the subfamily Cyathostominae, (b) *Strongylus edentatus*, (c*) Strongylus equinus*, and (d) *Strongylus vulgaris* (Wheeler, 2018)

Strongylus edentatus adult males (2.3-2.8cm) and females (3.3-4.4cm) are dark-red to grey (Marchiondo et al., 2019). Worms of this species do not have any teeth at the base of their cup-shaped buccal capsule (Taylor et al., 2016). Stage 3 larvae (L3) measure about 790 μm and have 18-20 weakly defined intestinal cells. The body to tail ratio is 2:1. (Zajac & Conboy, 2012; Russell, 1948)

Strongylus equinus males (2.6-3.5cm) and females (2.6-3.5cm) are usually dark grey in color. This species has four teeth in an oval shaped-shaped buccal capsule (Taylor et al., 2016). The body to tail ratio is 2.8:1 (Russell, 1948).

Strongylus vulgaris adult males (1.1-1.6cm) and females (2-2.5cm) are grey-brown in color. Members of this species have two teeth at the base of an asymmetric, globular buccal capsule (Taylor et al., 2016, Marchiondo et al., 2019). L3 of this species are approximately 1020 μm in size, have 28-32 rectangular intestinal cells (Zajac & Conboy, 2012). The body to tail ratio is 2.5:1 (Russell, 1948).

Figure 5: Photomicrograph depicting morphology the anterior morphology of (a) *Strongylus edentatus*, (b) *Strongylus equinus*, and (c) *Strongylus vulgaris* (Taylor et al., 2016)

Eggs of these worms are 'strongyle type' eggs (70-90 μm, ellipsoid, thin shelled, smooth surface, and contain a morula embryo (Marchiondo et al. 2019; Lichtenfels et al. 1975). These cannot be differentiated from eggs shed by small strongyles (Bredtmann et al. 2017; Hummelinck, 1946).

Detailed identification keys and discussion of morphological features are included in Lichtenfels' et al. (2008; 1975) illustrated guide as well as in veterinary parasitology textbook (Taylor et al., 2016; Mehlhorn, 2012; Foreyt, 2001)

2.1.2.3 Prevalence of large strongylid species

Before the recommendation of systematic anthelmintic use was introduced in the 1970s-80s *Strongylus* species were highly prevalent (Drudge & Lyons, 1996; McCraw & Slocombe, 1976; Tydén et al., 2019). Foster and Oritz (1937) found *S. vulgaris* in 100% of 97 dissected horses, Gawor (1995) recovered *S. vulgaris* from 74% of 50 dissected horses, Poynter (1969) reported a 70% infection rate with *S. vulgaris* when examining fecal samples from 3227 horses,

Slocombe & McCraw (1973) found *S. vulgaris* 85.4% of 48 slaughtered horses known to shed strongye-type eggs, and Reinemeyer (1984) found a prevalence for *S. vulgaris* of 27% in 55 autopsied horses known to shed strongyle-type eggs.

More recent studies reflect a prominent decrease in prevalence of *S. vulgaris.* Studies in 2005 (Fritzen), 2007 (von Samson-Himmelstjerna et al.), 2011 (Hinney et al.), 2012 (Studzińska et al.), and 2014 (Schneider et al) showed prevalence's of only: 1.3%, 0.61%, 0.29%, 22.8%, and 0.11% respectively. It should be noted that prevalence found by von Samson-Himmelstjerna et al. (2007) and Hinney et al. (2011) refers to the prevalence at herd level, whereas the other studies examined prevalence at individual level. Furthermore, it is worth noting that all studies investigated fecal samples, except Studzińska et al. who performed post-mortem investigations. This could explain the significantly higher prevalence found as post-mortem studies have shown to have the highest recovery rates (Chapman et al., 2002, Boxell et al., 2004). Additionally, *S. vulgaris* has a prepatent period of 6-7 months during which fecal examinations cannot detect an infestation (Round, 1969; Duncan & Pirie, 1972). Another possible reason for the difference in infestation incidence is that Studzińska et al. only examined slaughter-horse while all other studies included horses from a variety of yard-types, none explicitly stating slaughter-horses were included. Husbandry practices (e.g. pasturing, anthelmintic strategies, hygiene practices, and quarantine practices of newcomers) under which slaughter-horses are kept are likely to differ from those at other equestrian establishments such as boarding stables, stud-farms, and riding school. This provides significantly different epidemiological conditions for infection and infestation by strongyles. Further investigation into factors influencing the incidence of *S. vulgaris* would be necessary to ascertain the cause of the divergence.

This drastic decrease in prevalence of the highly pathogenic *Strongylus* species within the horse population can be ascribed to the frequent and regular de-worming of horses (Lyons et al., 1999; Nilsson et al., 1989; Becher et al., 2010) as well as the increased usage of long-acting macrocyclic lactones (Bredtmann et al., 2017; Hinney et al. 2011). Although arguments have been made for the re-emergence of large strongyles in the horse population related to the implementation of selective deworming strategies.

In a study conducted in Sweden in 2016 and 2017, Tydén et al. (2019) found that the odds risk of infection with *S. vulgaris* on a farms that based their treatment decisions solely based on fecal

egg counts (FEC) were increased by 2.9 when compared to yards that de-wormed strategically (i.e. at specific intervals without fecal sample results) or based their treatment decisions on FEC and larval culture results. Tydén et al. (2019) investigated fecal samples from 529 horses from 106 yards and found a prevalence of 28% and 61% at horse and farm level, respectively. On a farm level this finding represents a stark increase when compared to prevalence (14%) investigated in 1995 before anthelmintic preparations were made prescription-only in October 2007 (Hedberg-Alm et al., 2020; Osterman Lind et al., 1999), or to the prevalence reported by Höglund et al. (1997) of 6.1% in a post-mortem study of 263 horses. While the studies are not entirely comparable as the experiment designs differ, the evidence of an increase in *S. vulgaris* prevalence is supported by diagnostic worked performed at the Swedish National Veterinary Institute which also reported an increase in incidence of *S. vulgaris* (6% in 2008 to 32-53% between 2013 and 2017 on an individual level) (Tydén et al., 2019).

In Denmark anthelmintic preparations were made prescription-only in August1999 (Hedberg-Alm et al., 2020; Nielsen et al., 2006a; Andersen et al., 2013). As in Sweden, Denmark is also noting a substantial increase in incidence of *S. vulgaris* infections in their horse population. Bracken et al. (2012) examined 331 horses on 18 farms practicing selective anthelmintic strategies for at least 2 years using PCR and found 12.1% of horses positive for *S. vulgaris* and a prevalence of 72% at herd level. Similarly, Nielsen et al. (2012a) examined 663 horses from 42 yards via larval culture from fecal samples. The results showed a prevalence of 12.2% and 64.3% on an individual and herd level, respectively. They then compared the prevalence of *S. vulgaris* between farms using systematic versus farms using selective anthelmintic strategies with the following statistically significant results: Farms with systemic regimes has a prevalence of 7.7% and 38.9% at individual and herd level, respectively; while Farms with selective regimes had a prevalence of 15.5% and 83.3%. These findings were further supported by Nielsen et al. (2012a) who examined 663 horses' fecal samples via modified McMaster and larval culture finding a prevalence of 12.2% and 63.3% on horse and farm level, respectively. While stating that the increased prevalence could possibly be attributed to the difference in methodology (i.e. Nielsen et al. (2012a) identified all larvae from the Baermann sediment, where in many experimental protocol only the first 100-200 larvae are identified), other studies as well as the significant correlation found both at farm and horse level between the use of selective

anthelmintic therapy and the occurrence of *S. vulgaris* support the hypothesis that the prevalence of this nematode in Denmark has increased in recent years.

A questionnaire survey of equine and mixed practitioners was also conducted in Denmark by Nielsen et al. (2006a).This found that while 97% of the practices that responded used FECs to surveil and diagnose strongyle infestation, only 41% used larval cultures and 11% routinely screened for resistance of anthelmintics used. While this does show the significantly higher involvement of veterinarians in helminth control programs and the widespread practice of SAT in Denmark, it also provides a potential explanation of the increase in *S. vulgaris* in the Danish horse population: the lack of surveillance for the presence of *S. vulgaris* in the horse population. This is of vital importance in a selective de-worming regime as the presence of *S. vulgaris* in a horse is not associated with the nematode FEC of that given horse (Tydén et al., 2019). In fact, Tydén et al. (2019) found that 25% of FECs between 0-150 were positive for *S. vulgaris* on PCR analysis.

In Switzerland, the situation resembles that in Germany. A study conducted by Meier and Hertzberg (2005) including 81 stables showed a prevalence of 5% of *Strongylus* species on a herd level. Hertzberg et al. (2014) also report on the situation on several (exact number not given) pilot-trial farms in the greater Zurich area. In a 3-year period (2008-2010) only small strongyles were found in 177 fecal samples, 81% of all fecal samples carried out were under the limit of detection (50 EPG), and only 4% of all fecal samples were over 200 EPG. The direct comparison of these results to other studies is difficult as the report did not elaborate on de-worming intervals, quarantine measures, pasture hygiene procedures, or if a 'safety' annual de-worming was performed on those horses not de-wormed on the basis of FEC throughout the year.

In Germany, Greite (2013) found that the prevalence of small strongyles remained low (1.13% when investigating 354 horses form 68 yards. Although these horses were all in their first year of a selective deworming program. Kaspar et al.'s (2016) findings support the low prevalence of large strongyle on German horse farms. Using PCR and larval culture only 1.9% of 501 fecal samples were positive for *S. vulgaris*. Schneider et al. (2014) further suggest that the occurrence of large strongyles is rare. They found no difference in *S. vulgaris* prevalence in horses being dewormed selectively when compared to horses receiving treatment in strategic intervals. These

authors also found that in Germany only 44% of 195 farms questioned dewormed based on strongyle FECs with a significant proportion of German horses still being dewormed at regular intervals regardless of FEC and, notably, on average at shorter intervals as was the case in Denmark (Schneider et al., 2014).

While the studies performed in Germany indicate that there is no high risk of infection with *S. vulgaris*, studies performed in other countries should be considered as implementation and carrying out of selective anthelmintic schemes proceeds. Although, when looking to Denmark, it should be noted that the prevalence of *S. vulgaris* was higher than in Germany even before the legislation made anthelmintic prescription only (Becher et al., 2018). The current, extremely low *S. vulgaris* prevalence provides excellent circumstances for a more extensive implementation of SATs. Additionally, experiences in countries who already apply this system expansively provide insight into the best possible application methods of selective anthelmintic strategies. Nielsen's et al. (2012b) study, for example, suggest that the lowest prevalence of *S. vulgaris* can be achieved by using both FECs and larval cultivation as the basis of anthelmintic treatment decisions. Bracken's et al. (2012) study further suggested that the monitoring of *S. vulgaris* using PCR rather than larval culture is more reliable and less work intensive. The study also found that pooled and individual PCR samples had a higher sensitivity than larval culture and found no statistical difference between pooled and individual PCR samples. This suggests that pooled PCR samples from farms may be a useful and practical tool for screening horse populations under selective anthelmintic treatment for the presence of *S. vulgaris* (Bracken et al., 2012). Hertzberg et al. (2004) further suggest regular resistance test for active substances used in a herd, as well as quarantine measures for newcomers including an FEC, anthelmintic treatment, and control of the efficacy of the treatment via FEC. The importance of quarantine measures and investigation of worm infestation status of horses that are to be introduced into a herd is highlighted in the study performed by Greite (2013) who found that all *S. vulgaris* positive horses were the only individuals identified as positive in their herd and all 4 were newly introduced into the herd without knowledge of prior quarantine measures or investigations into infestation status. Kaplan (2002) and Sangster (1999) suggest that, to this end, visiting or new horses are treated with a larvicide anthelminthic. Kaplan (2002) also notes that the treatment should not be performed on arrival as this would lead to shedding of only resistant nematode eggs, so far present, for several weeks.

2.1.2.4 Lifecycle of large strongylid species

As is the case for small strongyles, large strongyles have a direct life cycle and consists of an external and an internal phase (Nielsen et al., 2007; Deplazes et al., 2013). Up until the third stage larvae the lifecycle of large strongyles is equivalent to that of small strongyles (refer to section 2.2.4).

The internal phase begins with the early L3 invading the gut tissue. For large strongyles this is also followed by migration to other internal organs. *S. vulgaris* migrates through the vascular system, *S. equinus* migrates through the pancreas, liver, and abdominal cavity, while *S. edentatus* migrates through the retroperitoneum, vena portae and liver.

Strongylus vulgaris larvae migrate into the submucosa of the cecum and colon within 1-7 days after uptake by the host. Here they moult to L4 which then migrate to the arterial walls 1-2 weeks after infection, and travel in the endothelium of these to reach the cranial mesenteric artery where they develop to L5. After about 2-4 months, the majority of the larvae will migrate back to the colon and, preferentially, to the cecum through the bloodstream where they will form nodules in the intestinal walls, before rupturing into the lumen, maturing into reproducing adults that firmly attach to the mucosa. Some larvae do not migrate back become trapped in arterioles of the mesentery and remain here until they die. The prepatent period (i.e. the time between infection and onset of egg laying) is 6 months. (Duncan & Pirie, 1972; Duncan, 1973; Nielsen et al., 2007; Round 1969; Enigk, 1969; Maxie, 2016; McCraw & Slocombe, 1976; DeLay et al., 2001).

Strongylus equinus L3 develop into L4 in the intestinal wall which then migrate from the ileum, cecum and colon to the liver via the abdominal cavity. Later moving to the pancreas and peritoneal cavity where they mature to into L5. Fifth stage larvae return to the cecum and colon 4-5 months after initial infection to mature into adults. Here they attach preferentially at the junction of the cecum and colon, as well as the ventral colon. The prepatent period is 9 months. (Maxie, 2016; Nielsen et al., 2007; McCraw & Slocombe, 1985; Enigk, 1969; Petty et al., 1992)

Strongylus edentatus, too, migrates to the liver, but does this via portal system. Here they mature into L4 after about 30 days post infection. After migrating through the liver for approximately

2 months, the L4 migrate into retroperitoneal tissue, the omentum, the diaphragm, and hepatic ligaments. Although Maxie (2016) notes that the lesions caused by this migration have not decreased in incidence along with the virtually disappearing incidence of *S. edentatus*. A correlation between these lesions and the parasite has thus not been fully confirmed. Once matured to L5, they return to the cecal and colonic mucosa via the hepatic ligaments, where they form nodules before erupting into the lumen to attach at the same locations as *S. equinus*. The pre-patent period of these strongyles is 11 months. (Maxie, 2016; Nielsen et al., 2007; McCraw & Slocombe 1978; Enigk, 1969; Petty et al., 1992)

2.1.2.5 Epidemiology of large strongylidosis

The infection cycle begins with an infected host passing unembryonated strongyle eggs with its feces. Once expelled the eggs become embryonated and L1 hatch. Eggs of equine strongyles require an adequate relative humidity (RH), temperature, and oxygen tension to hatch and develop. A moisture level of over 24% is required for hatching and development to the infective L3 stage, with the optimal RH reported to be 57–63% (Nielsen et al., 2014b; Nielsen et al., 2007; McCraw & Slocombe, 1976). While below 15-20% RH hatching is seized, a rise in RH, due to e.g. rainfall, allows hatching, or larval development depending on the stage of the lifecycle, to resume (Ogbourne, 1972; English, 1979; Fitilodze & Hutchinson, 1987). Ogbourne (1972) also noted that intact fecal balls can protect larvae from desiccation. Besides humidity, temperature also has a substantial influence on the hatching of strongyle larvae. Eggs develop and hatch at temperatures above 8–10°C and below 38°C (Nielsen et al., 2007; Rupasinghe, 1975; Fitilodze & Hutchinson, 1987). The rate of development was found to increase as temperature rises with the highest development rate at between 23 and 33°C (Rupasinghe, 1975; Mfitilodze & Hutchinson, 1987; Ogbourne, 1972). While it is well known that the hatching and development of strongyle larvae requires aerobic conditions, the exact oxygen tension range and optimum at which strongyle develop has not been extensively investigated (Kates, 1965).

The survival time of L1-3 depends on absolute temperature, temperature fluctuations, as well as relative humidity. The impact these factors have on the survival of larvae was summarized by Nielsen et al. (2007) (see table 3). Although, it should be noted that development varies between different climates. In hot climates desiccation of larvae can aid their survival in extremely warm

temperatures (Nielsen et al., 2007). In northern temperate climates the development of the infective L_3 stage mainly occurs during spring, summer and early autumn (Nielsen et al., 2014b; Herd, 1986b).

Table 3: The impact of environmental factors on the survival of larvae

Free-living stages	Frost[1]	Alternation between frost and thaw	Desiccation	Heat[a]
Unembryonated egg	++	++	na	++
Embryonated egg	+	-	na	++
First stage larva	-	-	-	++
Second stage larva	-	-	-	--
Third stage larva	+++	+	+++	-

[1]Indicates very susceptible, + weakly resistant, ++ moderately resistant, +++ very resistant

- a: temperatures in the range of 30-38 °C
- na: no data available

While L1 and L2 consume organic material and bacteria from fecal matter, L3 retain their cuticle which makes them more resistant to adverse environmental factors but prevents the uptake of nutrients (Medica & Sukhdeo, 1997; Nielsen et al. 2007; Nielsen et. al 2014b). The nutrient requirements of L3 are thus provided by fat granules in their intestinal cells (Giovannola, 1936). Baker et al. (1939 cited from Nielsen et al. 2007) found that these stores are faster depleted when temperatures fluctuate between cold (4.5°C) and room temperature. A further study investigating these energy stores was conducted by Medica and Sukhdeo (1997). They reported motility seizing due to storage depletion within 8 days upon incubation at body temperature to stimulate chronic locomotor activity (38°C). They also reported that these exhausted larvae had a weaker penetration ability, emphasizing that survival of larvae does not necessarily mean they retain their infectivity. While Hasslinger & Bittner (1984), Duncan (1974), and Ogbourne (1972) consider the significance of larvae surviving the winter as negligent, Mfitilodze and Hutching (1989) argue that as larvae can survive, and while numbers and infectivity might be lower, horses pastured in the spring can still be (re-)infected (Deplazes et al., 2013).

Once development to L3 larvae is complete, L_3 must leave fecal matter and migrate to herbage to be taken up per os and infect a host (Medica et al.,1996). Ogbourne (1972) found a positive correlation between the amount and frequency of rainfall and the number of larvae migrating from fecal matter to herbage. In 1989 Hutchinson et al. recorded findings that support the hypothesis that herbage contamination is dependent on RH. Based on experiments performed by Grønvold (1987) on L3 of *Ostertagia ostertagi* in cowpats of grazing cattle, who found that the factor increasing the number of L3 on herbage, Nielsen et al. (2007) theorizes that a similar phenomenon influences the number of strongyle larvae on herbage and suggests this be investigated further.

2.1.2.6 Clinical signs of strongylidosis

Strongylosis caused by adult *Strongylus* sp. is associated with non-specific clinical signs such as weight loss, a decreased growth rate, and a rough hair coat (Nielsen et al., 2013a). Large strongyles larvae cause a more severe pathology and graver clinical sings. Especially *S. vulgaris* which is considered the most pathogenic out of the equine nematode parasites (Duncan, 1974; Bowman et al., 2003). When the larvae of *S. vulgaris* migrate through the cranial mesenteric arteria and continue their development, a thromboembolic colic may result as the thrombus material blocks the lumen of the blood vessel leading to ischemia, infarction, and necrosis of the intestinal segment supplied by the vessels (Duncan and Pirie, 1975; Nielsen et al., 2013; Geldberg, 2017; Deplazes et al., 2013). General clinical signs such as depression, pyrexia, periods of anorexia, decreased weight gain are also associated with *S. vulgaris* infections (Hubert et al., 2004). Although a horse infected with large strongyle does not necessarily develop clinical signs. In 2011, Lyons et al. studied an isolated herd of 27 with a herd prevalence of 100% and none showed any clinical symptoms during 11 years under observation.

As Pilo et al. found in a study conducted in 2012, only 39% of horses examined post-mortem had larvae located in their arteries, and all 46 horses examined showed lesions that were plausibly from large strongyle larval migration, but none had been reported to have shown signs of a thromboembolic colic antemortem (Pilo et al., 2012).

2.1.2.7 Pathology of strongylidosis

Gross pathological findings generally include subserosal hemorrhages, of approximately 1cm in diameter, in the ileum referred to as hemomelasma ilei (Nielsen et al., 2013a). *Strongylus* larvae are associated with the thrombi within blood vessels around the migrating larvae. The blood vessels are generally dilated with thickened walls. Older lesions involving fibrosis of vessels walls with dystrophic calcification, or the arterial wall may become lesions free (Nielsen, et al., 2013a). Larvae may migrate elsewhere and cause eosinophilic granulomas in other tissue such as the pancreas, liver, and epicardium (Nielsen et al., 2013a).

Histopathological findings, in the case of large strongyles, are primarily related to the larvae located in the cranial mesenteric artery. Here, typically a chronic and chronic-active endarteritis is seen with an increase in wall thickness (Pilo et al., 2012; Duncan & Pirie, 1975; Morgan, 1999). Mild eosinophil accumulation is seen around lesions containing intact larvae. Lymphocytes, macrophages and multinucleated giant cells also accumulate in these lesions. Around degenerated larvae, a denser cellular infiltration can be observed. Chronic lesions involve fibrosis, while active lesions show necrosis, edema, fibrin deposits, macrophage and neutrophil accumulation (Morgan, 1999).

2.2 Diagnosis of strongylidosis

For diagnosis of a patent *Strongylus* infection fecal matter can be examined for detection for strongyle eggs. Commonly used methods for this are sedimentation-flotation, the McMaster method, mini-FLOTAC, and more recently developed molecular methods.

Sedimentation-flotation allows the detection of strongyle eggs by first mixing the fecal matter with water and allowing time for sedimentation. Then, the sediment is suspended in a liquid with a specific gravity that allows the eggs to float to the top of the suspension while contaminating material sinks to the bottom. A simple flotation (i.e. omitting the sedimentation step and suspending the fecal matter in a flotation suspension immediately) is also a possibility but provides variable and very low result (Rinaldi et al., 2011).

The McMaster method is widely used for detection of strongyle eggs in veterinary practice and research. Besides being quick and not requiring centrifugation, it has the added benefit of

quantitative determination of strongyle eggs (Ballweber et al., 2014; Dias de Castro et al., 2017; Rinaldi et al., 2011). The enumeration of eggs is done by counting a specific amount of fecal matter, counting the eggs in a McMaster chamber, and extrapolating the number of eggs in 1 gram of feces (EPG). According to Noel et al. (2017) and Scare et al. (2017) the McMaster method is less accurate than the mini-FLOTAC, whereas Nápravníková et al. (2019) found that the simple McMaster method was more accurate than the mini-FLOTAC method for counting strongyle eggs. Both agree that it is less precise than the McMaster method. The precision may be improved by examining a larger sample size (Rinaldi et al., 2011, Levecke et al., 2012; Bosco et al., 2014). It should also be noted that there are many other variables, such as time of flotation, technical ability of personnel, and flotation solution used, all contribute to variability in flotation procedures (Ballweber et al., 2014; Egwang and Slocombe, 1982). Despite its shortcomings, it is generally agreed that the method is sufficient for decisions on whether or not to treat (Bosco et al., 2014; Ballweber et al., 2014).

The FECPAK method is an on-farm version of the McMaster method. McCoy et al. (2005) evaluated the utility of this method in the field. They found that the method is both reliable and accurate if performed by highly skilled personnel; however, farmers, even after extensive training, significantly overestimated the FEC. This finding was supported by Gates and Nolan (2009) who compared coprological examination results performed by students with those performed by a diagnostic parasitology laboratory on the same samples. The results only agreed on 62.4% of the samples, emphasizing that on-farm FECs highly depend on training and the skill of the performing personnel. Compared to the McMaster method, the FECPAK method, when performed by skilled persons, has a higher sensitivity than the McMaster method as a larger sample is analyzed (Presland et al., 2005, Mes et a., 2001, Hunter & Quenouille, 1952). Presland et el. 2005, found that when comparing the two methods using the formula provided by Hilborn and Mangel (1997), 37% of FECs performed with McMaster were predicted to give false negative results, whereas only 14% of FECs carried out using FECPAK were predicted to give false negative results (Presland et al., 2005).

The mini-FLOTAC method, an on-farm variation of the FLOTAC method, has a larger area in the counting chamber which results in a lower standard deviation and coefficient of variation (Dias de Castro et al., 2017; Cringoli, 2006). Nápravníková et al. (2019), also reported that the

mini-FLOTAC method was more precise than the McMaster emthod. Especially at low egg counts the mini-FLOTAC was found to be more precise and sensitive leading to less false negative results than McMaster (Bello and Allen, 2009; Levecke et al., 2012; Dias de Castro et al., 2017; Noel et al., 2017; Rinaldi et al., 2011; Neves et al., 2014).

A smartphone-based method (Parasight), for automated counting of eggs in fecal samples is also under development (Slusarewicz et al., 2016). When comparing a prototype of the smartphone-based method to McMaster and mini-FLOTAC, Scare et al. (2017) found that the smartphone-based method had better precision and as it eliminated operator variability but significantly lower specificity. The accuracy, however, was significantly below that of the mini-FLOTAC technique and similar to that of the McMaster method. Other problems Scare et al. (2017) encountered when testing this system was the inability to differentiate close-by and stacked eggs. Although, the further development of the prototype is likely to improve on these errors, possibly providing a standardized, quick, on-site egg counting method (Scare et al., 2017). Since Scare et al.'s review of the prototype, Parasight has reached the market and is available for purchase by veterinary service providers. A literature search provided no further information on sensitivity, specificity, or comparison of this method to conventional methods.

Small and large strongyle eggs cannot be visually differentiated (Nielsen et al., 2008; Traversa et al., 2007; Lichtenfels et al., 2008; Hummelinck, 1946). For differentiation L3 larvae can be cultured, processed, and differentiated based on morphology (Nielsen et al., 2010a, Nielsen, 2012, Lichtenfels et al., 2008; Russel, 1948, Marchiondo et al., 2019; Taylor et al., 2016;). Additionally, new molecular methods have been developed which allowed identification of species. For *S. vulgaris* this can be done from DNA extracted from eggs. After amplification of DNA with primers for the second internal transcribed spacer (IST-2), the DNA is divided on agarose gel and stained to be visible (Nielsen et al. 2008). Bracken et al. (2012) found that the PCR assay developed by Nielsen et al. (2008) was also valuable in analyzing pooled fecal samples. The significance of this is that the re-occurrence of large strongyles in connection with selective deworming strategies that has been reported in recent studies can be monitored more efficiently. For identification of small strongyles, differentiation of 13 different species as well as *S. vulgaris, S. edentatus,* and *S. equinus* a reverse line blot assay is available that uses DNA extracted from any life cycle stage as rDNA does not change throughout the parasites' lifecycle

(Traversa et al., 2007). Hodgkinson et al. (2001) and Hodgkinson et al., (2003) also developed A PCR-ELISA to identify L4 of 6 cyathostominid species with the aim of making the diagnosis of larval cyathostominosis easier.

Diagnosis of larval cyathostominosis (i.e. enteritis of the cecum and colon due to simultaneous reactivation of a large number of hypobiotic larvae) in practice is difficult as the clinical condition can arise before the cyathostome infestation becomes patent (Lichtenfels et al., 2008; Giles et al., 1985; Love and McKeand, 1997). The only means to establish a definite diagnosis is via a post-mortem examination; however, in a clinical setting the anamnesis, clinical findings, and supplementary examinations can indicate that cyathostominosis is probable. Most often horses are under 6 years old with a history of rare to no de-worming or administration of an anthelmintic in the previous 2 weeks are affected. Epidemiological considerations such as time of year may also be helpful in establishing a diagnosis as most cases occur in late winter and early spring (Giles et al., 1985; Love & McKeand, 1997). Research conducted at the Moredun Research Institute in Scotland has made promising advances. The team of researchers identified a protein (cy-GALA-1) that is expressed only in larvae and specific to cyathostomin species (McWilliam et al., 2010; Andersen et al., 2013). While not yet allowing quantitative diagnostics, this discovery holds potential for being a diagnostic tool of larval cyathostominosis in the future. Another advancement in research that could aid in diagnosis, is molecular identification of small strongyle at a species level. Once this data becomes easier to collect and more reliable, and if certain species can be linked to higher pathogenicity, knowing which species are parasitizing horses on a farm or in a given individual may aid veterinary practitioners in diagnosis, treatment, and prophylaxis (Andersen et al., 2013).

Changes in peripheral blood, while not specific, may support the diagnosis. Frequently anemia, an increase in MCV and MCH, as well as a reduction of the globulin: albumin ratio are seen. In severe, progressed cases hypoalbuminemia is also frequently detected. Occasionally eosinophilia and an increase in beta-globulins are detected. An increase in alpha-globulins and ALKP are seen inconsistently. Together with clinical signs (see section 2.1.1.6) a diagnosis of cyathostominosis is feasible. (Thamsborg et al., 1998; Round, 1968b; Round, 1968a; Giles et al., 1985; Love, 1992; Mair et al., 1990; Mair, 1993; Kelly & Forgarty, 1993; Murphy et al., 1996; Murphy & Love, 1997; Reid et al., 1995; Smets et al., 1999)

Fecal samples from suspected cases of larval cyathostominosis may show excretion of strongly eggs but in a study by Smets et al. (1999) only 36% had patent infections (Giles et al., 1985). This is possible as the symptoms are caused by the mass emergence of immature larvae form the mucosa of the large intestine. Until these fully mature and start to produce eggs the FEC may remain negative or low. One should also note that the magnitude of eggs shed in the feces does not indicate the extend of the luminal burden.

2.3 Treatment of strongylidosis

There are currently three anthelmintic classes licensed for use in equines: benzimidazoles, tetrahydropyridines, and macrocyclic lactones (Kaplan, 2002; Metthews, 2014; Gokbulut & McKellar, 2018; Corning, 2009a).

Benzimidazoles are wide-spectrum anthelmintics with a high therapeutic index (Gokbulut & McKellar, 2018; McKellar & Scott, 1990). They have a higher affinity to nematode than vertebrate tubulin resulting in a multimodal disturbance of cellar hemostasis. Causing the death of cells that are actively dividing or growing, as well as inhibiting cells of adults and encysted larvae (VIN Veterinary Drug Handbook, 2017; Martin et al., 1997; Lacey, 1990, McKellar & Scott, 1990; Corning, 2009a). Efficacy against luminal stages of small strongyles and adult large strongyles is around 90% (Gokbulut & McKellar, 2018). This group of anthelmintics is not effective against encysted larval stages, with the exception of oxfendazole and fenbendazole when administered for 5 consecutive days at a dosage of 7,5-100mg/kg (DiPietro & Todd, 1987; Lacey, 1990; Herd, 1992; Duncan et al., 1998, Duncan et al., 1980). According to Nielsen et al. (2012a) fenbendazole does not show any efficacy against migrating stages of large strongyles. Although Duncan et al. (1980) found that administering 7.5mg/kg of fenbendazole for 5 consequitive days eliminated 80% of migrating *S. vulgaris* larvae, and 100% of migrating *S. edentatus* larvae. Duncan et al. (1977) also reported efficacy of fenbendazole against migrating large strongyle larvae at a dosage of 60 mg/kg. The discrepancies could be due to the development of resistance in the helminth population.

Resistance of cyathostominae against benzimidazoles has frequently been reported and is recognized as a widespread phenomenon (Traversa et al., 2009; Kaplan, 2002; Nielsen, 2012;

Corning, 2009a; Lester et al., 2013; Ostermann Lind et al., 2007; Stratford et al., 2013). An exception is oxfendazole which appears to be efficacious against resistant cyathostomes, although first cases of potential resistance have been reported (Kivipelto & Asquith, 1996). No resistance of large strongyles has been documented (AAEP, 2019).

Pyrantel is the only tetrahydropyrimidine anthelmintic licensed for use in horses (Gokbulut & McKellar, 2018). This anthelmintic has a high therapeutic index and its use has increased since the resistance against benzimidazoles has become widespread (Slocombe & Smart, 1975; Gokulut & McKellar, 2018). Being a selective agonist of acetyl-choline receptors, it causes spastic paralysis and leads to the elimination of luminal stages of cyathostominae effectively (Martin & Roberts, 2007; Gokbulut & McKellar, 2018). Large strongyles are, to a more limited extent, also eliminated from the lumen. Encysted and migrating larval stages are only minimally eliminated when pyrantel is administered (Gokbulut & McKellar, 2018; Nielsen et al., 2012a). Although, according to a study conducted by Reinemeyer et al. (2014), daily administration at a dosage of 2.64mg/kg/day can decrease the quantity of encysted L3 in the gut wall. According to Slocombe & Lake (2007) daily administration can also reduce egg shedding and pasture larval cultures; however, daily administration is not a common practice and is likely to contribute to the development of resistance.

Resistance against pyrantel has been reported in several countries. Kaplan et al. (2004) found decreased FEC reduction on 40.5% of farms investigated in a cross-sectional study including 44 farms in 5 states in the USA. Brazik et al. (2006), Chapman et al. (1996), Lyons et al. (2001), and Tarigo-Martinie et al. (2001) also reported resistance in horses in the United States. Slocombe & deGannes (2006) reported resistance in horses in Canada. Traversa et al. (2009) reported resistance to pyrantel on about 25% of yards in Germany, Italy and the United Kingdom. Milillo et al. (2009), Traversa et al. (2007), Comer et al. (2006), and Stratford et al. (2013) also reported resistance in these countries. Resistance has also been reported, to a lesser extent in Switzerland, Sweden, Norway, France, and to a greater extent in Finland (43%), Denmark (30%), and Brazil (45%) (Meier & Hertzberg, 2005; Osterman Lind et al., 2007; Ihler, 1995; Traversa et al., 2012; Näreaho et al., 2011; Nielsen et al., 2013b; Craven et al., 1998; Molento et al., 2008; Canever et al., 2013)

The macrocyclic lactones, of which ivermectin and moxidectin are licensed for use in horses, are broad spectrum endectocides that eliminate parasites by causing flaccid paralysis and hyperpolarization (Schumacher & Taintor, 2008; Taylor, 2004; Cobb & Boeckh, 2009). These active substances are effective against *Strongylus* sp. and cyathostominae adults as well as migrating *Strongylus* larvae (Eysker et al., 1997; Egerton et al., 1981; Corning, 2009a; Cobb & Boeckh, 2009; Nielsen et al., 2012a). Moxidectin, additionally, shows efficacy against encysted cyathostominae larvae, as well as possessing a higher efficacy against migrating *Strongylus* larvae than ivermectin (Corning, 2009a; Cobb & Boeckh, 2009; Xiao et al., 1994). A further advantage of moxidectin was revealed by a study conducted by Steinbach et al. (2006). In this study one group of ponies was treated with moxidectin (single administration of 0.4mg/kg), while a second was treated with a larvicidal dose of fenbendazole (7.5mh/kg SID for 5 days). The ponies were then sacrificed, and their necropsy revealed that following moxidectin treatment encysted larvae were resorbed without a severe inflammatory response developing. Whereas tissue damage with granuloma formation and accumulation of T-lymphocytes and eosinophils was observed after larvicidal treatment with fenbendazole. In sheep it has been shown that the anthelmintic remains effective even in fecal matter where the development of the third larval stage is inhibited, a literature search revealed no studies investigating similar effects on horse nematodes (Tyrrell et al., 2002).

To date only a limited number of cases of emerging resistance against macrocyclic lactones have been reported (Peregrine et al., 2014). Cases of ivermectin resistance or shortened egg reappearance periods have been reported in the United Kingdom, Brazil, Finland, Italy, Germany, Belgium, Italy, the Netherlands and the United States (Pergerine et al., 2014; Geurden et al., 2014; Schumacher & Taintor, 2008; Relf et al., 2014). Moxidectin resistance has also been reported in Brazil, the Netherlands, Italy, Belgium and the United States (Pergerine et al., 2014; Lyons et al., 2011; van Doorn et al., 2014; Kooyman et al., 2016; Geurden et al., 2014). In the United Kingdom, Trawford et al. (2005) also reported resistance against moxidectin but the study was conducted using injectable cattle moxidectin and administered to donkeys orally (Peregrine et al., 2014). Traversa et al. (2009) found no moxidectin resistance in the United Kingdom (nor in Germany and Italy) in a study they conducted on 1704 horses. Relf et al. (2014) noted a reduced egg reappearance period after administration of moxidectin, possibly an early indication of the development of resistance, supporting the hypothesis of moxidectin resistance

developing in the United Kingdom (van Doorn et al., 2014). Despite lack of compelling evidence for major resistance against macrocyclic lactones being present in the strongyle population, the consensus is that resistance will develop inexorably (Ihler, 2010).

Alternative treatments are being considered with an increasing frequency due to the widespread concern about resistant worm populations. One such alternative is the use of fungal spores of *Duddingtonia flagrans*. This nematophagous fungi germinates in the passed feces and traps nematodes in hyphae thus preventing herbage contamination and infection or re-infection of hosts (Bampidis et al., 2020). The fungal chlamydospores are administered in the form of a feed additive (Bampidis et al., 2020). In a study conducted by Hernández et al. (2016) spores were added to pelleted feed and fed this daily to a group of horses. The FECR of strongyle eggs was determined to be 100% with an ERP of 8 weeks. A further study conducted by Larsen et al. (1995) investigated the fungus *D. flagrans* as a potential therapeutic option for cyathostominae and found that, at dosages of 10^6 and 10^7 fungal units per kg, the number of larvae developing in fecal cultures was reduced. Braga et al. (2009) also carried out an investigation and fed mycelial pellets to horses for 6 months. The authors of this study noted a significant decrease in FEC as well as a 78.5% decrease in level of herbage contamination when compared to the control group. Larsen et al. (2009) also treated a group of yearlings with *D. flagrans* for a 3-month period during which the yearlings were allowed to contaminate a pasture. Following the 3 months, a group of young foals were allowed to graze the contaminated pasture for 4 weeks, housed for 15 more weeks, then sacrificed for post-mortem investigation. During the autopsies, a significantly lower number of cyathostominae larvae were recovered from the mucosa of the colon and cecum (Larsen et al., 2009). While these studies, as well as a systematic review of literature conducted by Canhão-Dias et al. (2020), suggest that *D. flagrans* could function as a supplementary or alternative tool for the control of nematodes, the European Food Safety Authority Panel on Additives and Products or Substances used in Animal Feed points out that currently no adequate studies are available to evaluate the safety of the feed additive for its target species nor for the consumer of horses for slaughter previously treated with a *D. flagrans* feed additive (Bampidis et al., 2020).

2.4. Variability in FECs

2.4.1 Extrinsic factors

2.4.1.1 Pasture hygiene

The main source of infection are contaminated pastures (AAEP, 2019; Herd. 1986b; Matthews, 2008; Nielsen et al., 2018a; Nielsen & Lyons, 2017). It follows that access to pasture and pasture hygiene practices have the potential to influence shedding prevalence and intensity. Access to pasture has been established as a risk factor for high shedding of strongyle type eggs (Nielsen et al., 2018a; Herd, 1986b; Relf et al.,2013; Döpfer et al., 2004). Regarding hygiene practices, it has been demonstrated that regular removal of fecal matter from pastures decreases the risk of having a positive FEC and the mean FEC level, with Herd (1986a) suggesting twice weekly removal being optimal considering eggs require 3-4 days to hatch and develop into infective L3 larvae under optimal conditions (Tzelos et al., 2017; Herd, 1986a; Herd, 1986b; Corbett et al., 2014; Matthews, 2008; Archer, 1980). Becher et al. (2010) confirmed this when reporting that the removals of feces from pasture correlated with a lower shedding intensity. Removal of dung from pastures further increases the grazing area available and thus decreasing infection pressure since adult horses, when given the choice, will graze away from the roughs around which herbage contamination is higher (Ödberg & Smith, 1976; Medica et al., 1996; Herd, 1986b; Herd & Willardson, 1985). Low stocking density has the same effect and is thus also considered a factor to decrease infection pressure and therefor FEC (Matthews, 2008; Fleurance et al., 2007; Chapman, 2013). Removal of roughs and harrowing of pastures were also demonstrated to decrease infection pressure and thus likely decrease shedding intensity (Herd 1986b; Taylor, 1954; Relf et al., 2013; Fritzen et al., 2009; Ertelt et al., 2015). Although, harrowing of pasture is only beneficial when carried out in dry heat, as harrowing during humid conditions would only disperse the larvae across the pasture and increase probably of uptake by the host (Herd, 1986b). Pasture rotation was shown to decrease FEC, although a prolonged de-stocking of the pasture is required, for as long as 1 year, for a prophylactic effect (Herd 1986a; Herd, 1986b; Herd, 1990; Eysker et al., 1986b; Relf et al., 2013; Larsen et al., 2002; Archer, 1980). Rotation with cows or sheep at the start of the grazing seasons is a possibility to use pastures left un-grazed by horses as equine strongyle larvae that overwintered on the pasture would have died out by the time horses are rotated onto the pasture (Eysker et al., 1986a; Herd 1986b). With

rotational grazing the risk of cross-species infection with *Trichostrongylus axei* must be considered (Herd et al., 1986b; Eykser et al., 1983; Eysker et al., 1986a; Leland et al., 1861). Neither Becher et al. (2010) nor Hinney et al. (2011) found that that pasture hygiene measures significantly reduced the risk of infection; however, the authors consider this is not due to the ineffectiveness of such measures, but rather due to the measured being carried out inadequately. Fertilization of pastures with horse manure was demonstrated as a risk factor by Ertelt et al. (2015). Hinney et al. (2011) could not confirm this finding in their study suggesting that horse manure can be used for fertilization if the manure is composted thoroughly (Herd, 1990). Wirtherle (2003) and Fritzen (2005) both note that the use of mineral fertilizer increases the prevalence of strongyle parasites in horses, agreeing that the most probably cause of this correlation is the positive effect the fertilizer has on grass growth thus providing an environment conducive to larval survival. Von Samson-Himmelstjerna et al. (2009) also found that pasture hygiene procedures did not corelated with lower FEC prevalence, although the authors note that cofounding factors as well as limited number of participating farms performed these hygiene measures may have led to this statistical conclusion and may not reflect the truth. It is also important to note that farms were considered to carry out pasture hygienic measures if they are performed once weekly or more which may not be sufficient considering the epidemiology of cyathostominae.

2.4.1.2 Stable hygiene

Although stables are not considered to play a major role in transmission and infection of strongyles and they have not been the focus of research, Hinney et al., (2011) reported that not cleaning out stables daily is a risk factor for high FEC shedding and prevalence. Chapman (2013) also agrees with the significance of stable hygiene, but further studied are needed to confirm the significance and efficacy of different stable hygiene practices such mucking out and disinfection.

2.4.1.3 Anthelmintic strategy

Certain aspects of the de-worming strategy used have also been identified as risk factors for high FECs and prevalence. The choice of anthelmintic is one such factor. Relf et al. (2013)

found that the change of a positive FEC was higher if pyrantel embolite was the last active substance used, whereas the change of an FEC of 200 EPG or more was higher if fenbendazole was the last de-worming substance used. The authors attributed this to the low persistent activity of these active substances as well as a relatively high likelihood of resistance against these substances being present on the premises. This would also explain the findings of Ostermann-Lind et al. (1999) and Kornaś et al. (2010) who found that horses treated with ivermectin and moxidectin showed lower FEC results respectively, as these active substances have a longer duration of anthelmintic activity and no resistance has been reported (although decreased ERP has been observed) against these substances. Regarding timing, simultaneous de-worming of all horses on an establishment increased the change of having a negative FEC result ten-fold (Ertelt et al., 2015). Dosing strategy of anthelmintics also presents a risk as estimation of weight can lead to under- or over-dosing of de-wormer with underdosing presenting a higher risk both for a positive and higher FEC, as well as for the development of anthelmintic resistance (Matthews, 2008; Scott et al., 2015; Nielsen et al., 2010b). Strategies that include weighing the horses, using weighing tapes or body weight estimation formulas are thus related to a reduced risk, with using weighing scales remains the most accurate method (O'meara & Mulcahy, 2002; Relf et al., 2011; Matthews, 2008; Wagner et al., 2011; Reavell, 1999; Hoffmann et al., 2013). A further aspect of anthelmintic use to consider is the rotation of active substances. According to Lloyd et al. (2000) slow (i.e. annual) rotation of anthelmintic classes could decrease the prevalence of helminth infestation, including cyathostominae, by slowing the development of, or possibly even reversing, anthelmintic resistance Barnes et al., 1995; Prichard et al., 1980; Herd, 1986b). Alternatively, treating with two different anthelmintic classes is being considered as an option to slow the development of resistance, which could have the same effect as strongyle prevalence as rotation (Kaplan, 2002; Barnes et al., 1995). Hinney et al., 2011 also identify infrequent de-worming as a risk factor but do not define 'infrequent'. Similarly, Döpfer et al. (2004) found that de-worming at intervals over 6 months is a risk factor for high FEC counts. Although, here too, it should be considered that cofounding factors make it difficult to draw a solid conclusion on the magnitude of risk directly due to frequency of de-worming. Relf et al. (2013), for example, found that treatment frequency was higher in stud farms. Likewise, if resistance against the used active substance is present on a farm, frequent treatment would most likely not decrease the risk of having high shedders.

2.4.1.4 Other extrinsic factors

Other risk factors that have been suggested in studies are quarantine measures, and concomitant illnesses such as PPID (McFarlane et al., 2010; Matthews, 2008; Larsen et a., 2002; Kaplan, 2002.

2.4.2 Intrinsic factors

2.4.2.1 Age

Besides management factors, some intrinsic factors are also hypothesized to carry a higher risk for positive and higher FECs. One of these intrinsic factors is the age of the horse. Becher et al. (2010), Fritzen et al. (2010), Kornaś et al. (2015), as well as Levy et al. (2015) all found that young horses have a higher risk of having a positive FEC as well as having a higher EPG with Becher et al. (2010) using a cut-off of 250ableEPG and Levy et al. (2015) a cut-ff of 500 EPG to define high shedding. The authors cite a decreased acquired immunity, higher infection pressure due to grazing behavior, and different husbandry conditions as possible causes of this increased risk. Hinney et al. (2011), Yadav et al. (2014), and Nielsen et al. (2018a) provide further evidence that age shows a significant negative association with prevalence of positive strongyle FECs. Saeed et al. (2010), Nielsen et al. (2018b), Döpfer et al. (2004), Wood et al. (2012), Klei and Chapman (1999), Osterman Lind et al. (1999), Bucknell et al. (1995), Ertelet et al. (2015), Larsen et al. (2002), Relf et al. (2013), Kornaś et al. (2010), and Love and Duncan (1992a) provide further evidence that young horses tend to have a greater FEC. Although the definition of “young horses” varied widely (ranging from yearlings to under 6 years of age) between the studies making it difficult to define an exact age at which the risk decreases. The only study found during a literature search asserting that adults shed more than young is a study conducted by Eydal and Gunnarsson (1994) who found that strongyle EPG increased significantly with age. Geresu et al. (2014), McFarlance et al. (2010), and Kuzmina et al. (2016) failed to find a significant association of age with high prevalence or FECs.

It is also important to consider how the risk for a positive or high FEC changes for geriatric horses. Adams et al. (2015) notes that old horses (20-33-year-old) again had a significantly

higher FEC than middle ages horses. A similar trend was reported by Döpfer et al. (2004) that found horses over 23 years of age, like horses under 6 years of age, had higher FECs. Wood et al. (2012) also confirmed this in a study where the authors found geriatric animals, and animals under 5 years of age, had higher FECs. Horohov et al. (2010) suggest that the reason for the increase in FECs of geriatric animals is due to immunological senescence.

2.4.2.2 Host Gender

Another intrinsic factor that has been investigated frequently is the sex of the horse. Any studies have reported that females have a higher risk of being high shedders than males (Döpfer et al., 2004; Fransisco et al., 2009; Hinney, 2009; Yadav et al., 2014; and Hautala et al., 2019). Kornaś et al. (2015) found that only young females shed more strongyle eggs than males and that this trend disappears with increasing age. Other studies, however, found that males shed significantly more strongyle eggs (Fabiani et al., 2016; Hinney et al., 2011; Nielsen et al., 2018a; and Debeffe et al., (2016). Bucknell et al. (1995) and Kornaś et al. (2010) found that geldings shed more than stallion and mares. These contradictory findings are, in this authors opinion, likely due to cofounding factors such as use of horse and husbandry practices. Stud mares, for example, are much more likely to have considerably more exposure to pasture than stallions. Thus, to designate a certain sex as a risk factor for a positive or high FEC, more controlled studies or a larger data set would need to be investigated.

2.4.2.3 Breed

Breed is a further intrinsic factor that has been investigated as a potential risk factor. Kornaś et al. (2010) found that thoroughbreds in Poland are likely to have higher FECs. Hinney (2009) reported heavy breeds had a higher risk. Kuzmina et al. (2016) found pure certain pure breeds (Thoroughbreds, Ukrainian Saddlers and Russian Racers) had a higher risk, and Francisco et al. (2009) found that Pura Raza Galega horses were at higher risk. What is important to consider is that as the authors of Nielsen et al. (2018a) and Franscisco et al. (2009) point out, there is often a certain use and husbandry system connected to certain breeds making it very difficult to investigate breed as an isolated risk factor. Uses that have been associated with a higher risk are

studfarms and riding schools (Larsen et al., 2002; Ostermann Lind et al., 1999).Whereas, Nielsen et al. (2008) found that horses at boarding and training stables, and horses kept for personal use were at lower risk.

2.4.3 Other sources of FEC variability

The main use of FECs is in the context of selective anthelmintic therapy (i.e. detecting high shedders and evaluating the efficacy of anthelmintics). SAT relies on the FEC remaining consistent to **a.:** identify high egg shedders, **b.:** reduce the frequency and number of samples necessary to make implementation of an SAT program feasible, and **c.:** to ensure compatibility of FECs during and FECRT (Gomez and Georgi, 1991; Döpfer et al., 2004; Nielsen et al., 2006b; Eysker et al., 2008; Duncan & Love, 1991). Numerous studies have evaluated the repeatability and variability of equine FECs.

2.4.3.1 Long term variance

In the long term, whether a horse is a high or low shedder is a stable characteristic that is unlikely to change over time give that it is otherwise healthy, management practices remain unchanged, and the horse does not move to another farm (AAEP, 2019; Scheuerle et al., 2016). Becher et al. (2010) samples 129 horses from 19 farms at 4-week intervals from March to November resulting in 9 samples per horse. The authors found that the FEC results from the first and second sampling predicted the FEC results in the following 7 months with the maximum FEC during the following 7 months having an 82% probability of remaining below 250 EPG if the first two samples were below 250 EPG. A similar study conducted in Denmark by Nielsen et al. (2006b) using an interval of 7 months for sampling, revealed an 84% probability of having an FEC below 200 if the previous two FECs were also under 200 EPG. Lester et al. (2018) conducted a longitudinal cohort study with 573 horses and found that 94% of horses remained in the same treatment category (based on FECs) for the duration of the study (February until October/December). Scheuerle et al. (2016) also collected monthly samples for nine months (March-November) and found moderately high repeatability of FEC providing a reasonably well predictability for future FECs. Wood et al. (2012) failed to observe a strong consistency in FEC

but this study investigated horses kept for conservational purposes and which had not received anthelmintic treatment suggesting that there is a higher infection pressure than in most domestic equines. A recognize pattern of variance in FEC is a seasonal one. Nielsen et al. (2018a) as well as Relf et al. (2013) regard the pasture season, i.e. summer and fall, to corelate to higher FECs. Accordingly, Wood et al. (2012) suggest to take FEC samples during the pasture season in order to identify high shedders for SAT. Herd (1986b) found that FEC peaked in april-may and august-september, whereas Eydal and Gunnarsson (1994) found FECs to peak in summer and be lower in the autumn and winter. A possible explanation of the disagreement in peak seasons is the climate dependency of the strongyle lifecycle. Wood et al. (2012) and Bucknell et al. (1995) suggest that rather than a concrete time of year, the FEC magnitude correlates to the weather, in particular rainfall and temperature. Due to the lag effect due to the prepatent period and the variable hypobiotic stage of the strongyle lifecycle, it is difficult to draw obvious conclusions between the quantity of eggs shed and the weather.

2.4.3.1 Short term variance

Short term variance, besides influencing usability of FEC data, also influences optimal timing of sampling for FECs. Denwood et al. (2012) detected no diurnal variance in FEC counts of four adult horses sampled at 9 am and 3 pm for five days, suggesting that the time of day does not influence the outcome of the FEC for a given horse. Carstensen et al. (2013) samples six horses four times daily for 5 days and confirmed there is no significant variance between the samples from different time points or from consecutive days, supporting the hypothesis that short term variance does not influence FECs.

Denwood et al. (2012) found that a major source of variability in FEC originated from the variation between piles of fecal matter from a single animal, followed by the variability due to aggregation of eggs within fecal matter. To minimize this variability, the authors suggest increasing sampling size to at least 20g of fecal matter or counting a larger number of eggs during the McMaster procedure.

3. Own study

3.1 Method and Materials

3.1.1 Sample population

The target population of this study was privately owned and riding school horses on livery yards in Germany. Due to logistical reasons, the geographical area for recruitment of livery yards was focused on Wolfsburg and the surrounding are, as well as the North-Schwarzwald region (see figure 6). The study was advertised through social media by persons actively involved in the riding scene, as well as directly approaching owners at stables from which participants had already been recruited.

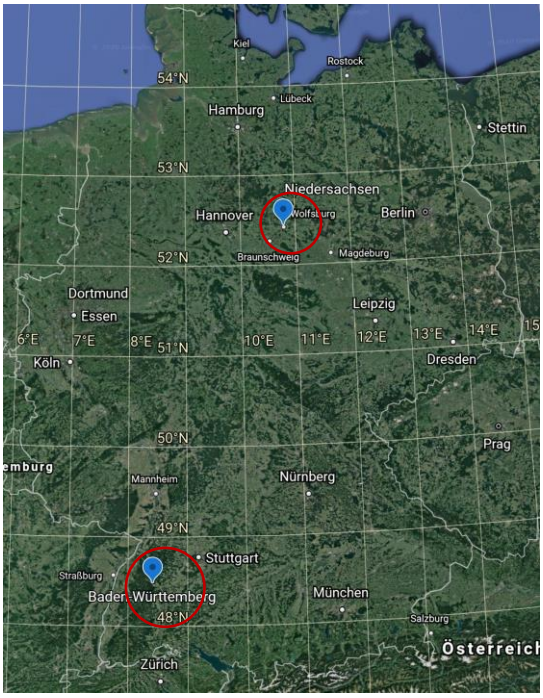


Figure 6: Sampling area (*https://earth.google.com/web/)*

The sampling population was recruited based on the following criteria: owners' consent, horses had not received anthelmintic treatment in the previous 8 weeks (or 12 weeks if macrocyclic lactones were the last anthelmintic class used), horses have regular access to pasture, and yard managers were willing to fill out the questionnaire. A total of 85 horses on 6 livery yards in Germany were recruited, out of which 3 yards provided samples for all horses on the premises. The number of residents on the yards ranged from 10 to 45 and all horses were 3 years of age or older with a 13% being older than 20 years.

3.1.2 Questionnaire

A questionnaire was designed to provide information on husbandry practices, demographics of the yard, and the anthelmintic program followed (Appendix 1.1). The questionnaire consisted of 21 open-ended and 2 close-ended questions and was filled out together with the yard manager on the day of sample collection.

3.1.3 Fecal sample collection

The fecal samples were collected between August 25th and October 29th. A minimum of 150g of feces was collected from several fecal boluses from one fecal pile of each horse. The feces collected was passed less than 12 hours ago and fecal matter contaminated with bedding was avoided. Only fecal matter of "normal" consistency was collected, and no dried feces was sampled. The samples were collected with a gloved hand and placed in individual, labeled zip-loc bags from which all air was pressed out before closure to achieve anaerobic storage conditions. The samples were immediately placed inside a cool box at 4-6°C, equipped with icepacks and a thermometer. The samples were then stored at 4-6°C until re-packaging into Styrofoam boxes, also equipped with icepacks, immediately before being shipped on the day of collection to reach the veterinary laboratory in Hannover the following day. Samples were tested within 7 days of collection.

3.2.4 FEC

The FEC was determined with a modified McMaster procedure. This involves weighing out a previously specified amount of feces, mixing this with a flotation solution, and straining the sample. After mixing the sample again, an aliquot of the suspension is withdrawn and filled into a counting chamber of the McMaster slide. After mixing the sample again, another aliquot is withdrawn and filled into the second counting chamber. In the case of air bubbles in the

chambers, the chamber must be emptied and filled once more. The slide is allowed to stand for a predetermined time (usually 2-5 minutes and up to 60 minutes if sodium nitrate flotation solution is used) to allow the eggs to float to the top of the chamber. The eggs inside the grid of the two chambers are then counted and the eggs per gram feces calculated with the formula in figure 7 (AAEP, 2019).

$$\text{EPG} = \frac{\text{counted eggs(n) x } \left(\frac{\text{flotation solution(ml)}}{\text{volume examined in slide(ml)}}\right)}{\text{fecal matter (g)}}$$

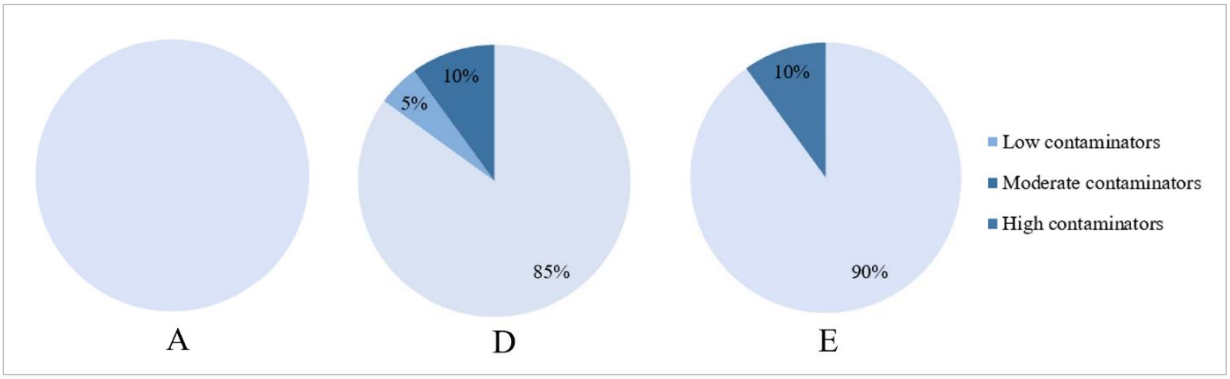
Figure 7: Formula for calculating eggs per gram from eggs counted on McMaster slide

The detailed method used to evaluate samples collected for this study is privileged information of the Institute for Parasitology (Dept. of Infectious Diseases) of the University of Veterinary Medicine Hannover Foundation.

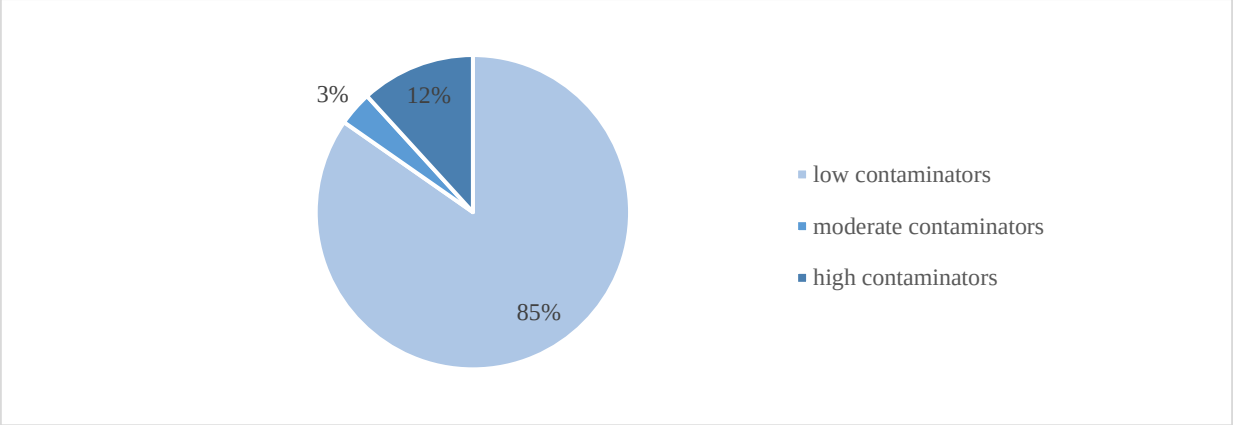
3.2 Data analysis and results

Excel and Program R were used to analyze and graph the questionnaire and FEC data.

For each yard, the horses were divided into levels of egg shedding intensity according to the AAEP guidelines (2019): low contaminators (<200 EPG), moderate contaminators (200-500 EPG), and high contaminators (>500 EPG) to evaluate the distribution of horses among the categories. The percent of individuals in each category were graphed on yard level for the three yards providing FECs for all horses on the premises (Yard A, D, and E) (Graph 1), as well as on sample population level (Graph 2).



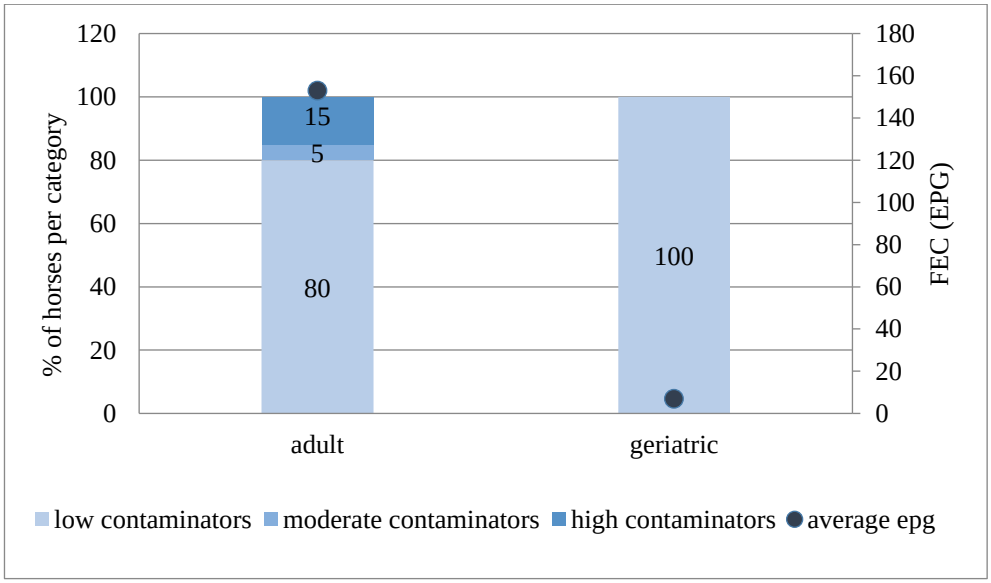
Graph 1: Distribution of egg shedding categories at yards A, D, and E



Graph 2: Shedding category distribution of the study population as a whole

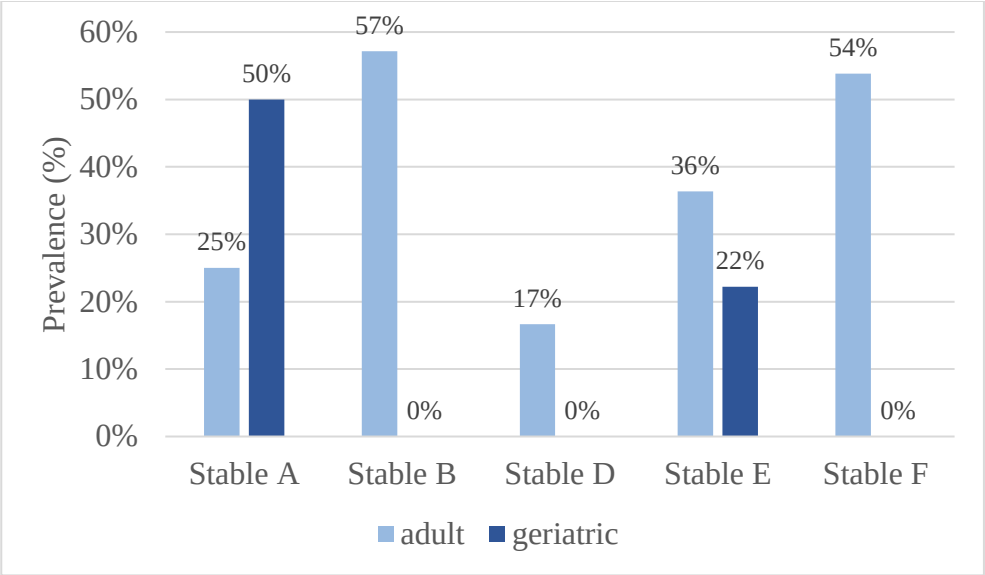
The stocking density was calculated by dividing the total number of horses by the total pasture area available on the yard. The pasture area was determined using the website https://www.mapdevelopers.com/area_finder.php. To ensure all pastures were identified and the area defined correctly, a contact person from each yard was asked to control the selection made on the satellite map (Appendix 1.2)

To evaluate the effect of intrinsic factors on the FEC count, horses were grouped into age categories and the average FECs of adult horses (here defined as 3-20 years old) were compared to the FECs of geriatric horses (here defined as over 20 years old) (Graph 3).



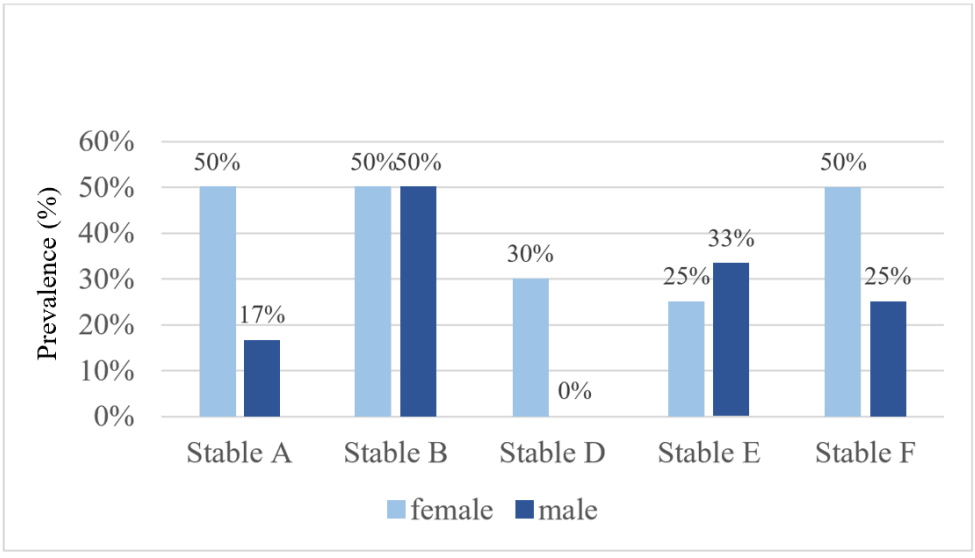
Graph 3: Average FEC (fecal egg count) and FEC shedding categories of adult vs. geriatric horses

The prevalence of positive FECs per age group was also separately determined for stable A, B, D, E, and F as this allowed evaluation of sex on FEC between individuals subject to the same husbandry conditions (i.e. extrinsic risk factors were controlled variables) (Graph 4).

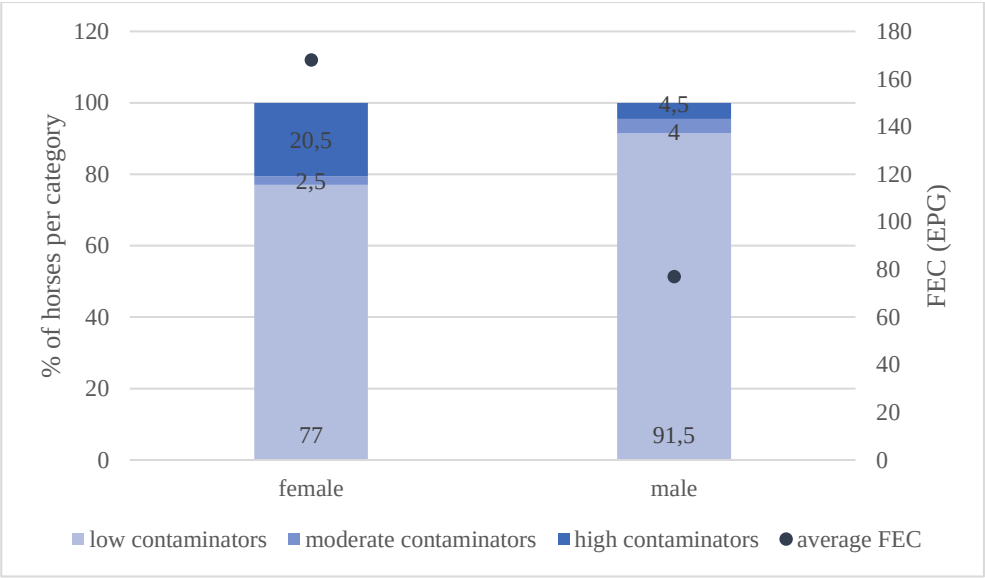


Graph 4: Prevalence of positive FECs (fecal egg count) of adult vs. geriatric horses on yard-level

The FEC pattern of geldings was compared to that of mares in a similar manner (Graph 5 and 6).

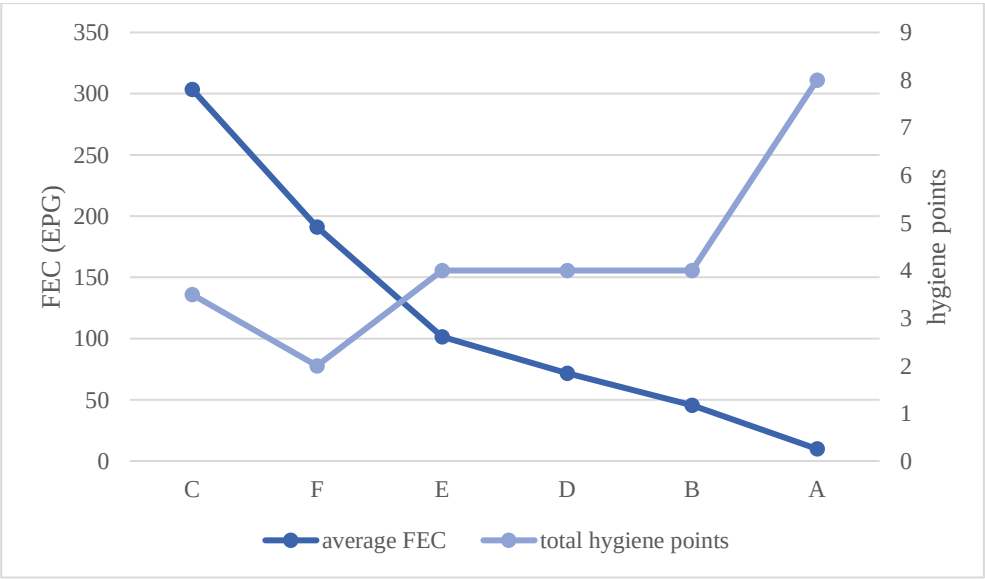


Graph 5: Comparison of prevalence of positive FECs (fecal egg counts) of female vs. male horses on yard-level



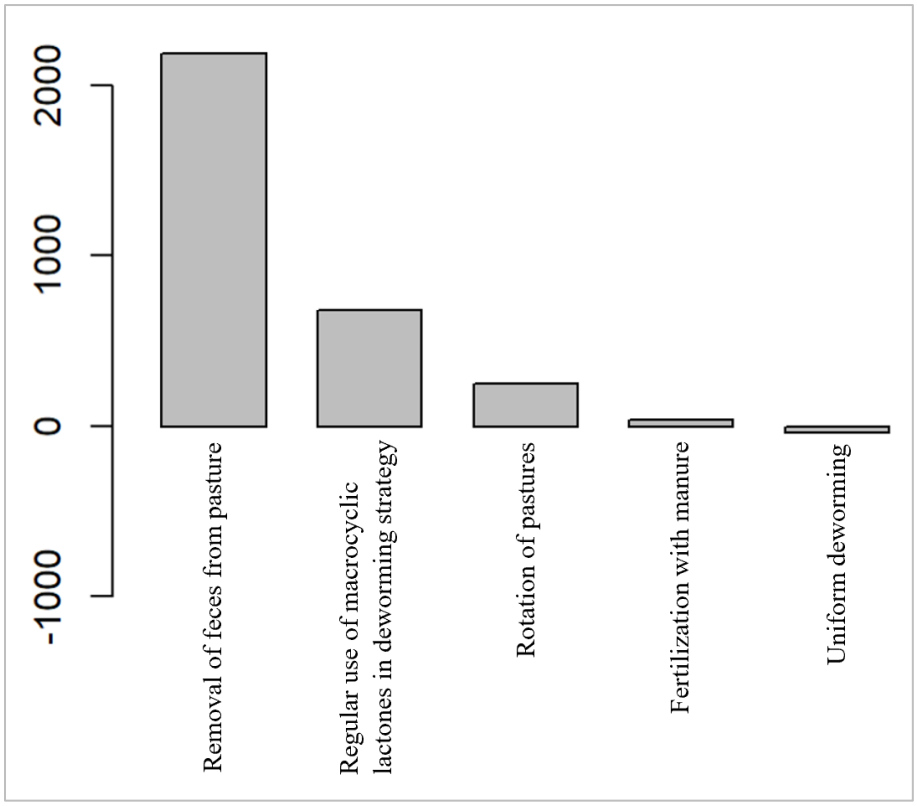
Graph 6: Average FEC (fecal egg count) and FEC shedding categories of female vs. male horses

Management practices on each yard were evaluated by calculating a hygiene score. This was done by awarding one point for practices that from a biological and epidemiological standpoint decrease the risk of high FEC results (Appendix 1.3). The hygiene scores were then calculated and graphed against the average FECs of the horses on that yard (Graph 7).



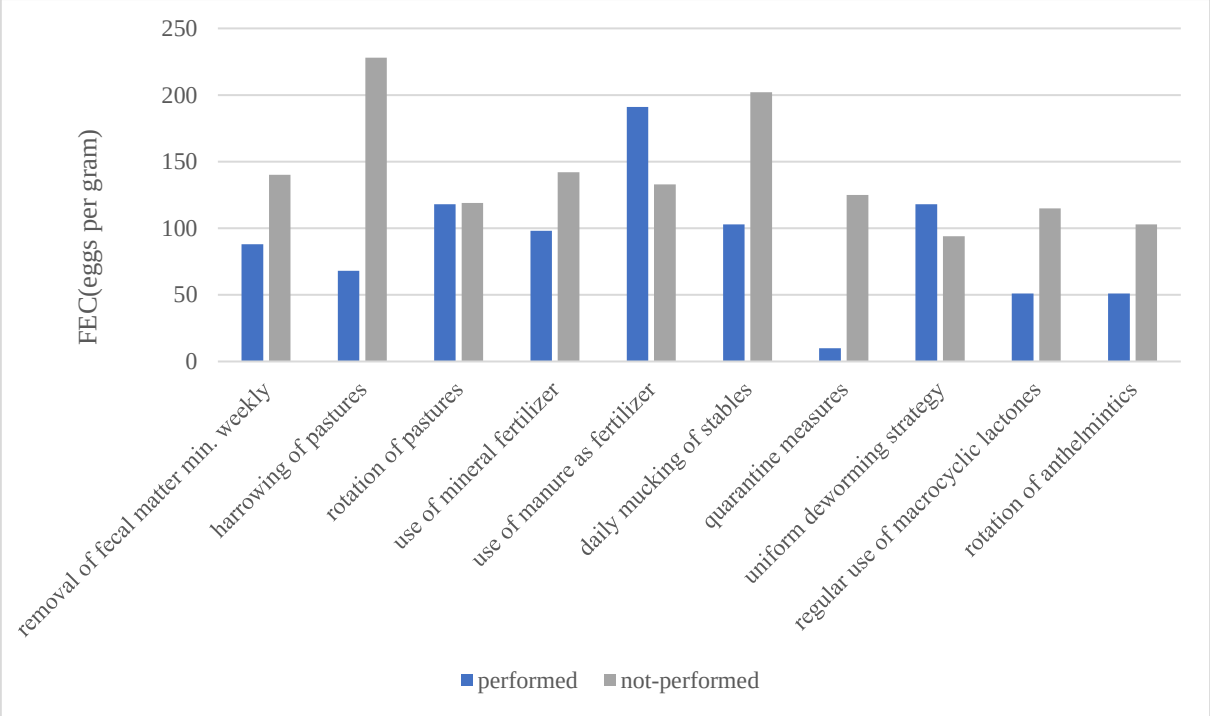
Graph 7: Yard average FEC (fecal egg count) and hygiene points received

Subsequently, a decision tree was used to predict the degree of influence selected factors had on the FEC of a horse (Graph 8).

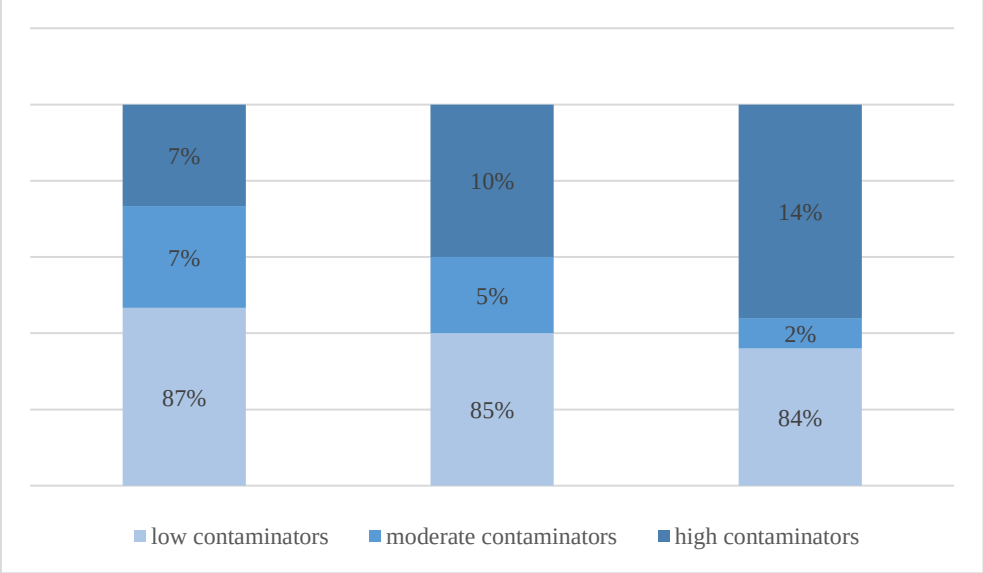


Graph 8: Order of variable importance in prediction of FEC (fecal egg count)

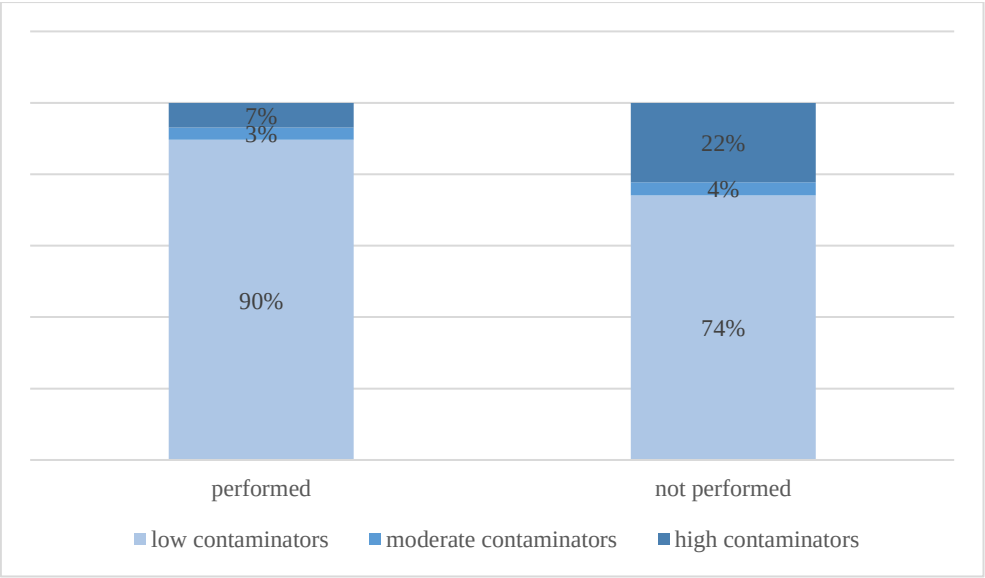
To evaluate the individual effects of different extrinsic factors on FECs, the FEC average and shedding category distribution of the sample population not subject to a management practice was compared to the that of the sample population subject to that management practice (graphs 9 through 17).



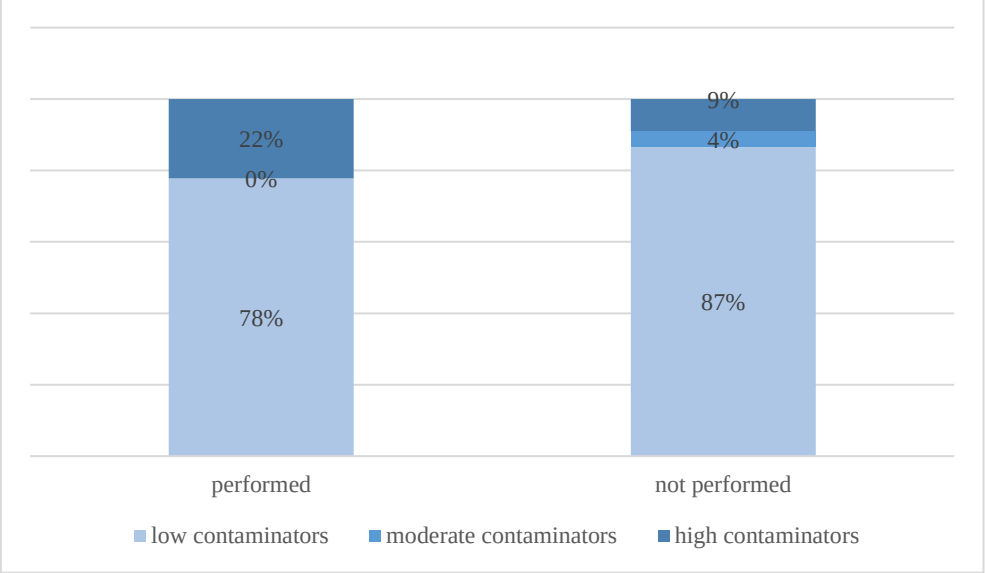
Graph 9: Effects of certain husbandry procedures on the average FECs (fecal egg counts) of horses subjected vs. not subjected to a particular practice



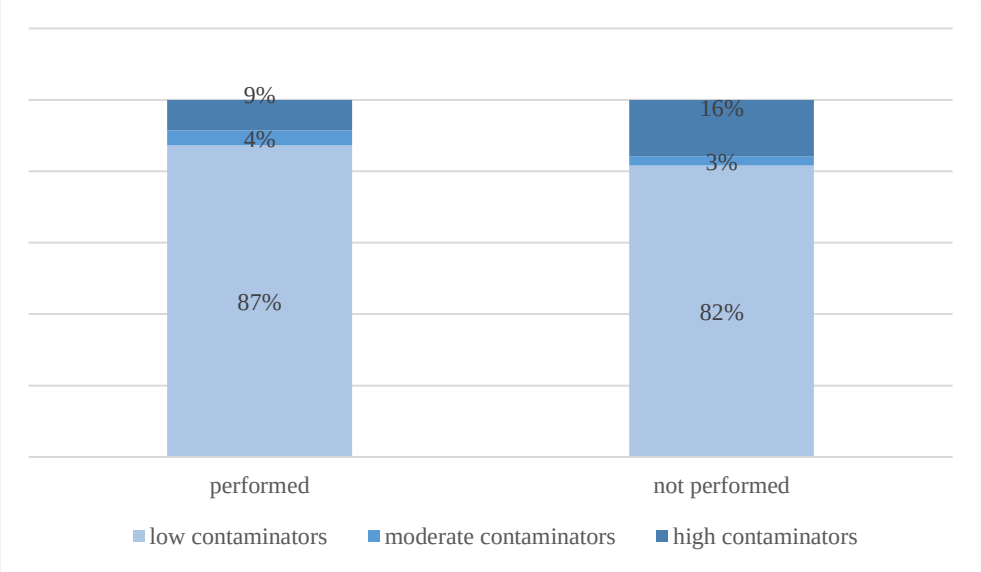
Graph 10: Effect of frequency of fecal matter removal from pastures on shedding category distribution



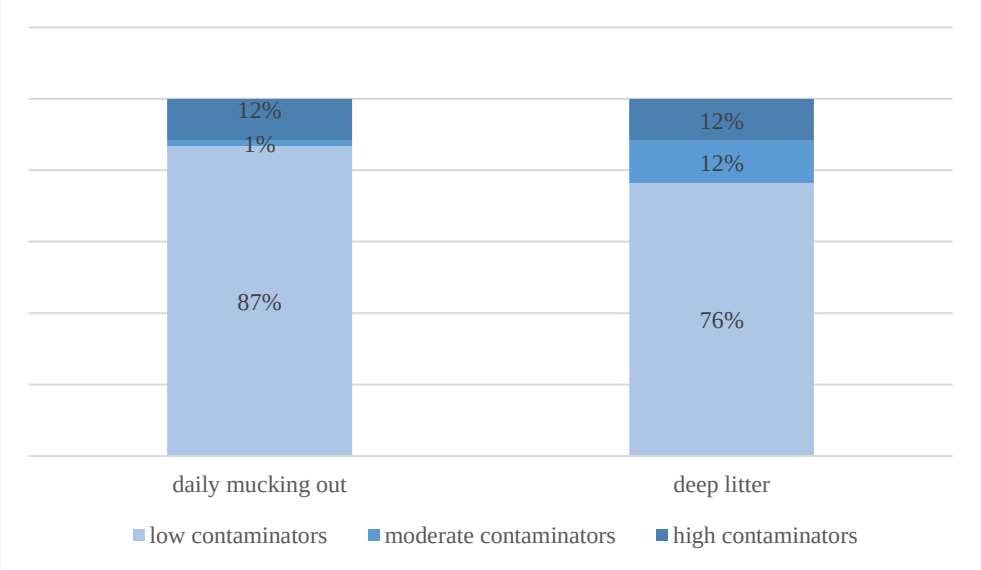
Graph 11: Effect of harrowing of pastures on shedding category distribution



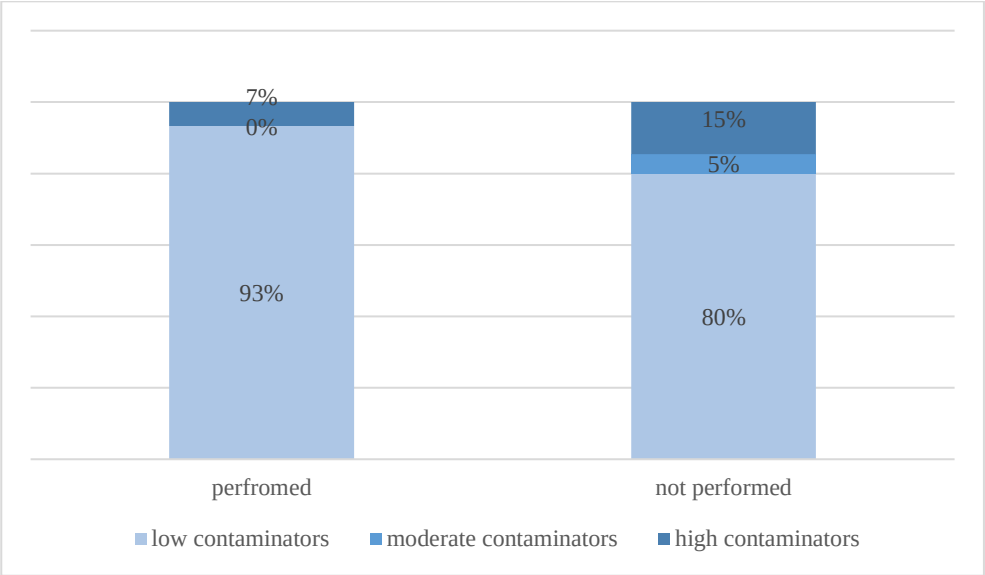
Graph 12: Effect of manure fertilization of pastures on shedding category distribution



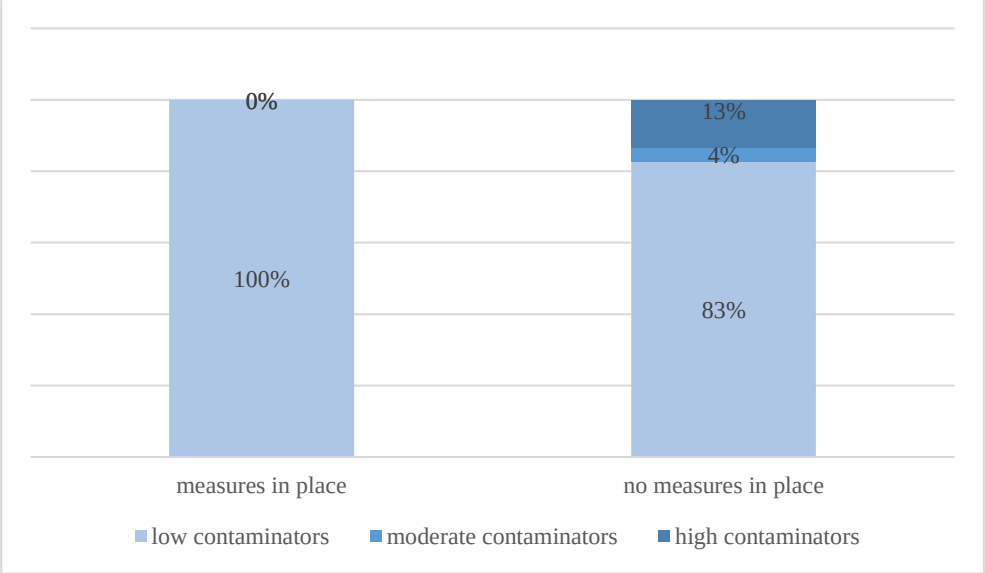
Graph 13: Effect of mineral fertilization of pastures on shedding category distribution



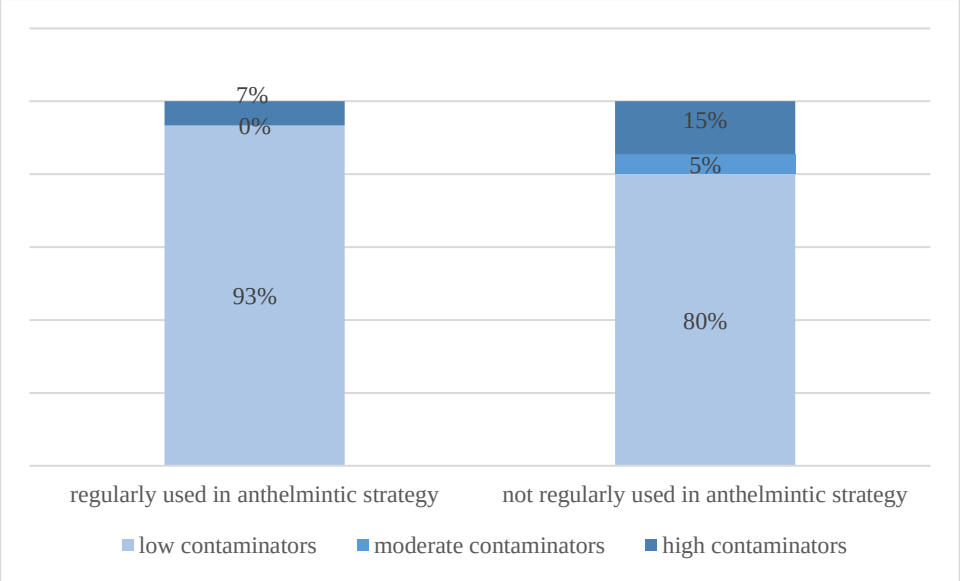
Graph 14: Effect of stable hygiene on shedding category distribution



Graph 15: Effect of rotation of anthelmintics on shedding category distribution



Graph 16: Effect of quarantine measures on shedding category distribution



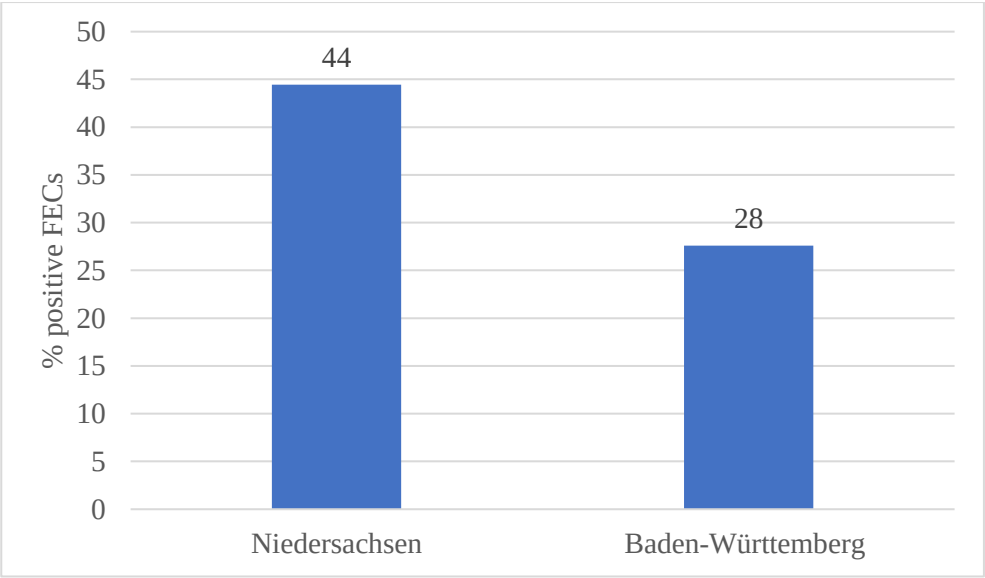
Graph 17: Effect of regular use of macrocyclic lactones on shedding category distribution

Variables that all yards have in common were collected. Their impact on FEC could not be evaluated in this study. These variables are: no young animals housed on the premises, no alternate grazing with another species, no removal of roughs, no additional measures are carried out when de-worming the resident horses (e.g. change in pasture, cleaning of stables), and no disinfection of stables. The impact of selective deworming could also not be evaluated as too few horses included in the sample are dewormed selectively.

3.3 Discussion

3.3.1 Prevalence

As expected, at a yard level the prevalence of strongyles is 100%. At an individual level the prevalence of patent strongyle infection is 33%. This lays between the prevalence reported by previous studies conducted in the same German states, Baden-Württemberg and Niedersachsen, where strongyle prevalence was observed to be 26.7% (n=105) and 39.8% (n=1383) respectively (Schnerr, 2011; Wirtherle, 2003). When comparing the two geographical regions (Graph 18) the results of this study correspond well to the findings of Schnerr (2011) and Wirtherle (2003).



Graph 18: comparison of the prevalence of positive FECs (fecal egg counts) in Niedersachsen to that found in Baden-Württemberg

Possible reasons for a lower prevalence in Baden-Württemberg as compared to Niedersachsen are a difference in climate (Appendix 1.4), difference in demographics of the horse population, and different trends in husbandry practices. Regarding climate, it must be considered that the samples from Niedersachsen were collected between the 25th and 30th of August, whereas the samples from Baden-Württemberg were collected between the 27th and 29th of October. While this both falls within the grazing seasons, and sampling windows used in longitudinal studies conducted in Germany from which no substantial difference was reported between the time points, it cannot be excluded that the seasonal pattern of strongyle egg shedding biased the data (Scheuerle et al., 2016; Hinney et al., 2011; Schnerr, 2011). This bias could originate from two sources. Firstly, samples transported in August were exposed to higher ambient temperatures during their transport, providing conditions more conducive to egg hatching (thus falsely decreasing FECs). Secondly, the effect of weather conditions in the previous months could have influenced the FEC magnitude; however, due to the variable prepatent period and the hypobiotic stage of the strongyle life cycle, these effects are near impossible to define precisely and likely contribute to the overall unexplained variance. An additional bias the time difference in sample introduced was the added time that horses in Baden-Württemberg had access to pasture before being samples. While the infections newly acquired during this additional grazing time would not yet be patent at the time of sampling, female larvae ingested in July and August could have

matured and started to shed eggs, adding to the magnitude of the FEC. When summed up these effects would favor a higher prevalence in Baden-Württemberg. As this is not the case, and prevalence in Baden- Württemberg is in fact lower, it can be concluded that FEC patterns are more strongly influenced by intrinsic and extrinsic factors other than geographic difference.

Compared to results from postmortem studies, the prevalence reported here is expectedly lower as the examination of fecal samples unavoidably misses pre-patent infections as well as the male of the worm burden and the proportion of larvae encysted in the intestinal mucosa.

3.3.2 Distribution of egg shedding intensity

If the population included in this study is considered as a whole, approximately 12% of the horses were responsible for 80% of the total eggs shed. Generally, the "20/80" rule, i.e. 20% of the horses are responsible for 80% of the total egg output, is accepted and cited as an argument for the implementation of SAT. Although the findings in this study do not exactly coincide with the 20/80 rule, they do show the expected over-dispersion and support the plausibility of SAT.

For the yards where all horses participated, the distribution of low (< 200 EPG), moderate (200-500 EPG), and high (>500 EPG) contaminators was graphed (Graph 3). Stables D and E fit the distribution reported in other studies and described in the AAEP review (2019) moderately well. All horses at stable A fall into the low shedding category, which does not match the general conception that there are a few high shedding horses in a given population, with the rest shedding low number of parasite eggs. This is perhaps explained by the management practices which eliminate more risk factors for high FECs when compared to the other participating yards (Graph 9). This suggests that it is possible to influence the level of contamination originating from a horse beyond what is dictated by intrinsic factors by using husbandry practices, especially considering that stable A's population is 20% geriatric, which would presumably have a senescent immunity.

3.3.3 Intrinsic factors

To evaluate the intrinsic factors of age and sex the sample population was looked at as a whole, as well as comparing geldings to mares per yard for yards A, B, D, E, and F. For yard C this was not done for sex, as geldings and mares are subject to different management practices.

As Graph 8 shows, the overall FEC average was lower for male horses, as was the percent of males belonging to the moderate and high shedding category. On a yard level, however, no consistent trend could be observed for the effect of sex on age (Graph 7). On yards A, D, and F males horses had a lower prevalence, for yard B the prevalence was equal, while on yard E females showed a higher prevalence. This supports the hypothesis that there is no significant effect of sex on age, and that in those studies were a statistical significance was found, the correlation is likely due to covariances.

Surprisingly, the average FECs of adult horses (3-20 years old) was higher than that of geriatric horses (>20 years old). Furthermore, all geriatric horses included in the study, shed either no eggs or were classified as high shedders (Graph 5). As discussed in section 2.4.2.1, both young and old age are considered risk factors for high FECs. Here, however, old horses had a strikingly lower FEC average. When comparing the age groups on a yard level to eliminate covariance, yards B, D, E, and F had a lower prevalence of positive FECs for geriatric horses. Only yard A had a higher prevalence for geriatric horses (Graph 6). Possible explanations for this are that the age groups defined in the study do not correlate with the age at which a horse is truly biologically geriatric, or that age may have an effect on FEC prevalence and magnitude but with less significance than other factors and thus its effect can be overpowered.

3.3.4. Extrinsic factor

Graph 9 shows that a general conclusion can be drawn about the impact of management practices on the average FEC of a farm. Overall, hygiene measures are negatively correlated to the average FEC on a farm, with good husbandry decreasing the average FEC. A decision tree analysis of certain management factors allowed for evaluation of the extent to which a certain practice effect the FEC (Graph 10). Removal of feces from pasture evidently had the greatest impact on FEC. Regular use of macrocyclic lactones in the deworming strategy, rotation of

pastures, and fertilization with horse manure also appear to effect FEC but to a lesser extent. This confirms that a comprehensive pasture hygiene practices decrease the contamination with strongyle eggs, and that pasture practices should be a focus of SAT.

To evaluate the effects of individual husbandry practices on FEC, the average FEC of the population fraction subjected was graphed against the population not subjected to that management practices (Graph 11).

Graph 11 shows the effects of individual husbandry practices on the average FEC. Removal of fecal matter, pasture harrowing, daily mucking of stables, quarantine measures, regular use of macrocyclic lactones, and rotation of anthelmintics appear to have a positive effect (i.e. lower average FEC) on FECs. The use of mineral fertilizer, too, seems to lower the FEC. The literature review revealed that mineral fertilizer use is likely to increase infection pressure as lush grass provides a better environment for survival and maturation of strongyle larvae. This study suggests otherwise, which could be explained by looking at the grazing behavior of horses rather than the epidemiology of strongyles. That is if the pasture is not fertilized and grass becomes scarce, horses are more likely to graze near roughs as well as grazing the proportion of grass very low to the ground. More infective third stage strongyle larvae are near roughs as well as the lower proportions of grass, thus this grazing behavior would increase infectious pressure while fertilizing the pasture might prevent this behavior from occurring. The use of manure fertilizer, however, seems to have a negative effect (i.e. higher average FEC) on FECs. This finding coincides with the theory that fertilization with fecal matter poses a risk when already contaminated with strongyle eggs. Although this finding agrees with previous studies, it is important to note that only one stable fertilized their pastures with manure and a larger sample size (on a yard level) would be needed to draw a reliable conclusion. Uniform de-worming strategy, too, seemed to have a negative effect on FECs. Here, it is important to note that none of the stables in Baden-Württemberg have a uniform de-worming program, while all stables in Niedersachsen do. While both regions have a very similar FEC average (Baden-Württemberg: 118.8 EPG; Niedersachsen: 118.4 EPG), it is highly likely that the effect of having a uniform deworming program was biased by covariances. Rotation of pastures seems to have neither a positive nor a negative effect on average FEC. This is most likely explained by the rotation

strategy used. None of the yards conducting pasture rotation leave pastures empty long enough for strongyles to be eliminated from the pastures, thus making the practice redundant.

Another way to evaluate the influence of extrinsic factors on FECs, is by examining the effect on the distribution of horses between the shedding categories (Graphs 12-19). Current recommendations are to remove fecal matter from pasture twice weekly based on the epidemiology of strongyle larvae development. This study found that weekly fecal matter removal already contributed to having a smaller proportion of horses in the moderate and high shedding categories. Although, the study also supports that a more frequent (here daily) removal is even more beneficial. No yards were included in the study that performed this practice twice weekly, thus making it impossible to evaluate if daily fecal matter removal is more beneficial than twice weekly removal. Therefore, the conclusion that can be drawn is that weekly removal is beneficial but for optimal results fecal matter removal should be conducted at a higher frequency. As seen from the graphs harrowing of pastures, daily mucking of stables, quarantine measures, regular use of macrocyclic lactones and rotation of anthelmintics also had a positive effect (i.e. reduced the proportion of moderate and high shedders) on FEC shedding patterns. Although quarantine measures are only in place at yard A, which has the overall highest hygiene standard. Thus, while it is believable that quarantine measures decrease the risk of having positive and/or high FECs, no reliable conclusion on this can be drawn from the data collected here. As expected, fertilizing pastures with manure has a negative effect not only on the average FEC, but also is linked to a higher proportion of horses falling into the moderate and high shedding categories. Uniform deworming strategy was not looked at further for previously discussed reasons. Pasture rotation was also not analyzed in this way as pasture rotation strategies were not biologically and epidemiologically sound.

3.3.5 Conclusions to be drawn from this for designing anthelmintic strategies

The focus of anthelmintic strategies should, based on this data, focus on removal of fecal matter from pasture, harrowing of pasture, stable hygiene, rotation of anthelmintic classes and avoidance of using manure to fertilize pastures. Out of these, pasture hygiene appears to deserve the most attention and should, in practice, receive the highest priority when designing anthelmintic strategy and consideration when owners' compliance and resources are limited.

While speculations can also be made about the use of mineral fertilizer and quarantine measures, the data collected here does not allow clear support for recommendations. The data also suggest that the effect of age on the level of contamination originating from a horse ought to be more thoroughly investigated.

3.3.6 Trends in opinion and awareness

Conclusions about current trends in the equine industry, with a focus on livery yards, can also be drawn from this study. There still seems to be a lack of awareness about anthelmintic resistance and SAT considering only 6 out of the 85 horses included in the study were de-wormed selectively. Furthermore, during completion of the questionnaire many owners were surprised at the questions included as they were not aware that the investigated husbandry practices bear any significance for worm control. It was also difficult to find a person aware of the exact timing of and active substance used in the last anthelmintic treatment carried out. Interestingly, when asked about the weather conditions while harrowing of pastures, the yards that do harrow their pastures all said their harrowing pastures during a relatively dry period was to avoid damaging the ground and were unaware of the risk of spreading parasitic eggs and hence increasing pressure of infection. All this is testimony to the lack of information reaching the horse owners and yard managers, as well as to the little importance assigned to a comprehensive anthelmintic strategy. Interestingly, 4 of the horse owners in stable A were already performing fecal examinations yearly, but these did not include quantitative egg counts nor did they influence the choices made regarding deworming the tested horses. This shows that there is willingness to invest money and time, but also that involvement of veterinary practitioners is either lacking or not requested but required for the promotion and proper implementation of comprehensive anthelmintic strategies.

3.3.7. Considerations

The results from this study must be evaluated with the sample populations selection in mind. The yards and horses investigated were not picked based on random sampling. Instead, those yards were included where either horse owners or the yard manager responded to the

advertisement through social media. This selection bias could have led to an increased response from farms that either already have a high interest and information level on anthelmintic control (and good husbandry practices and deworming protocols as a consequence), or farms that have a history of clinical cases related to helminth infections (and inadequate husbandry and deworming protocol). Considering all yards answered question 23 of the questionnaire with no, the former is more likely suggesting that the population examined here is receiving above average care in terms of anthelmintic strategies. While acknowledging that this selection bias may have influenced the overall prevalence and distribution of shedding pattern, it does not fundamentally influence the evaluation of the risks in their effect on FEC outcomes.

3.3.8 Limitations

Although samples were all analyzed within the recommended 7 days, a subset of samples from Baden-Württemberg failed to be delivered the day after postage and was only received by the laboratory 46 hours after dispatch. Despite transport in a Styrofoam box with icepacks on days with air temperatures ranging from 1 to 11.5°C, it can be expected that the temperature rose above 6 °C (dwd.de).

It must also be emphasized that the results obtained are only a snapshot of the FEC status of the included horses. A longitudinal study would be required to fully characterize the egg shedding potential of the horses, just as several samples would need to be taken to characterize the horses for the implementation of an SAT strategy.

3.3.9 Potential areas for expansion of scope

Information that would have been of additional value is results of larval cultures. Eggs were assumed to be of small strongyles due to the disappearing low prevalence of large strongyle in Germany. Larval cultures would have allowed the identification of large strongyle, not only ensuring that all eggs counted originated from small strongyles but also providing, in some part, the answer to the question whether large strongyle prevalence remains low. Further, small strongyle could have been identified on a species level, allowing investigation of small strongyle species prevalence and distribution and the impact this has on FEC patterns. Evaluating the

efficacy of anthelmintics on the studied yards would have added value, not only as additional information to aid in the evaluation of anthelmintic programs used and the spread of anthelmintic resistance in Germany, but also by providing a possibility to show yard managers and owners the risks of anthelmintic over-use.

Besides analyzing the included intrinsic and extrinsic factors in terms of risk factors for positive and high FECs, it would be interesting to evaluate their effect on the development of anthelmintic resistance. This could help in identifying those management practices that both decrease egg contamination and slow the development of resistance, as well as define the magnitude by which they effect the two, which would help design efficient and practical SAT strategies.

4. Summary

Fecal samples of eighty-five horses in two German states were examined for Fecal Egg Counts (FEC) of strongyle nematodes from August to October of 2020. The prevalence of positive FECs was 100% on yard level and 33% on horse level with the magnitude ranging from 33 – 1600. The prevalence in Baden-Württemberg was 28% while in Niedersachsen a prevalence of 44% was found.

From the positive shedders, 85% were in the low contaminator category (<200 EPG), 3% in the moderate contaminator category (200-500 EPG), and 12% in the high contaminator category (>500 EPG). Approximately 12% of horses were responsible for shedding 80% of the total strongyle eggs.

A questionnaire study was simultaneously conducted. The results from the questionnaire were used to analyze the effect of different management practices (extrinsic factors) and host characteristics (intrinsic factors) on contamination level category distribution. Host sex did not show a significant impact on prevalence or egg shedding category distribution. In this study horses over 20 years old appeared to have both a lower prevalence and were more likely to belong to the low shedding category. Pasture harrowing, daily mucking of stables, quarantine measures, use of mineral fertilizer, regular use of macrocyclic lactones and rotation of anthelmintics were linked to a shift towards low contamination. Fertilization with manure was linked to a shift towards high contamination. Removal of fecal matter from pastures was linked to a lower percentage of high and moderate shedders, with fecal matter removal daily having a better effect than weekly removal.

It was also evident that the awareness and knowledge of horse owners and yard managers regarding selective anthelmintic strategies (SAT) is very limited. Further it was evident that there was no to very limited involvement of veterinary practitioners in the planning or conducting of anthelmintic therapy strategies. None of the farms conducted fecal egg reduction tests in order to evaluate anthelmintic efficacy and obtaining data about the timing and active substance of the last anthelmintic treatment carried out on the farm was not easily obtained indicating that there is little attention payed to comprehensive deworming strategies or development of resistance.

4. Összefoglaló

Németország két tartományában 2020 augusztus és október között gyűjtött 85 ló bélsár mintájában strongilida petéket vizsgáltunk. A résztvevő lovardák mindegyikében megtalálhatóak voltak a strongylida-pozitív egyedek. Összességében a lovak 33%-a volt pozitív, ahol a peteszám a 33-1600 között mozgott. Baden-Württemberg-ben 28% és Niedersachsen 44%-os prevalenciával voltak a strongylida peték megtalálhatóak.

A petéket ürítő lovak 85% a “kis” (<200 Pete Per Gram), 3% a “közepes” (200-500 PPG), és 12% a “nagyszámú” petét ürítő csoportba (>500 PPG) tartozott. A lovak 12%-a volt felelős az ürített stongylida peték 80%-áért.

A peteszám vizsgálat mellett kérdőívekkel is kiegészítettük a vizsgálatunkat. A kérdőívek segítségével a lovardákban alkalmazott különböző módszereknek (extrinsic faktorok) és a lovak tulajdonságainak (intrinsic faktorok) hatását vizsgáltuk a peteürítési kategóriák eloszlására. A lovak nemének nem volt hatása a peték ürítésére. A vizsgálatban résztvevő lovak között kis prevalenciával voltak idős lovak (20 évnél idősebb) és ezek ezen felül nagy valószínűséggel a kis peteszámot ürítő kategóriába estek. A legelőkön a bélsár megfelelő kezelése, az istállók napi takarítása, karanténozás, ásványi műtrágyázás, rendszeres féreghajtás macrociklikus laktonokkal és a féreghajtók rendszeres rotációja csökkentheti a kibocsájtott peteszámot. Azokon a legelőkön ahol a lovak bélsarát használják trágyázásra ott a nagy ürített peteszám volt megfigyelhető. A legelőről a lovak bélsarának rendszeres eltávolítása jótékony hatással van az ürített peték számára,ezen felül a napi hullaték eltávolításának is kedvezőbb hatása volt a peteszámra, mint a heti eltávolításnak.

A gazdák és a lovarda tulajdonosok tájékozottsága a szelektív féreghajtási stratégiákról (SAT) sajnos nagyon limitált. Sajnos a lovak féreghajtását és annak megtervezését is szakember (állatorvos) bevonása nélkül végzik. A lovardák közül egyiksem fordított figyelmet arra, hogy megbizonyosodjanak arról, hogy a féreghajtás hatásos volt-e, megfelelő időben történt-e és hogy a féreghajtás hatása tartós-e lóállományban. Ezen felül nem fordítottak megfelelő figyelmet az átfogó féreghajtási stratégiákra vagy éppen az féreghajtók elleni rezisztencia kialakulására.

5. Bibliography

AAEP, 2019. *AAEP Internal Parasite Control Guidelines*. [online] AAEP Parasite Control Subcommittee of the AAEP Infectious Disease Committee. Available at: https://aaep.org/sites/default/files/Documents/InternalParasiteGuidelinesFinal5.23.19_0.pdf> [Accessed 5 October 2020].

Adams, A., Betancourt, A., Barker, V., Siard, M., Elzinga, S., Bellaw, J., Amodie, D. and Nielsen, M., 2015: Comparison of the Immunologic Response to Anthelmintic Treatment in Old Versus Middle-Aged Horses. *Journal of Equine Veterinary Science*, 35. 11-12. p.873-881.

Andersen, U., Howe, D., Olsen, S. and Nielsen, M., 2013: Recent advances in diagnosing pathogenic equine gastrointestinal helminths: The challenge of prepatent detection. *Veterinary Parasitology*, 192.1-3. p.1-9.

Anderson, I. and Hasslinger, M., 1982: Cyathostominae and other strongyles of horses in the Federal Republic of Germany. *Journal of the South African veterinary association*, 53.3. p.195-197.

Archer, M., 1980: Grassland management for horses. *Veterinary Record*, 107.8. p.171-174.

Archer, R. and Poynter, D., 1957: Anaemia and eosinophilia associated with helminthiasis in young horses. *Journal of Comparative Pathology and Therapeutics*, 67.2. p.196-207.

Ballweber, L., Beugnet, F., Marchiondo, A. and Payne, P., 2014: American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use—Is there really one best technique?. *Veterinary Parasitology*, 204.1-2. p.73-80.

Bampidis, V., Azimonti, G., Bastos, M., Christensen, H., Dusemund, B., Durjava, M., Kouba, M., López-Alonso, M., Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R., Woutersen, R., Chesson, A., Cocconcelli, P., Rychen, G., Wallace, J., Galobart, J., Innocenti, M., Brozzi, R. and Saarela, M., 2020: Safety And Efficacy Of Bioworma® (Duddingtonia Flagrans NCIMB 30336) As A Feed Additive For All Grazing Animals. [article] URL: https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.6208. Accessed: 14 October 2020.

Barnes, E., Dobson, R. and Barger, I., 1995: Worm Control and Anthelmintic Resistance: Adventures with a Model. *Parasitology Today*, 11.2. p.56-63.

Becher, A., Mahling, M., Nielsen, M. and Pfister, K., 2010. Selective anthelmintic therapy of horses in the Federal states of Bavaria (Germany) and Salzburg (Austria): An investigation into strongyle egg shedding consistency. *Veterinary Parasitology*, 171(1-2), pp.116-122.

Becher, A., van Doorn, D., Pfister, K., Kaplan, R., Reist, M. and Nielsen, M., 2018: Equine parasite control and the role of national legislation – A multinational questionnaire survey. *Veterinary Parasitology*, 259. p.6-12.

Beelitz P, Göbel E, Gothe R. 1996: Artenspektrum und Befallshäufigkeit von Endoparasiten bei Fohlen und ihren Mutterstuten aus Zuchtbetrieben mit und ohne Anthelminthika Prophylaxe in Oberbayern. Tierarztl Prax 24.48-54.

Bello, T. and Allen, T., 2009: Comparison of two fecal egg recovery techniques and larval culture for cyathostomins in horses. *American Journal of Veterinary Research*, 70.5. p.571-573.

Bosco, A., Rinaldi, L., Maurelli, M., Musella, V., Coles, G. and Cringoli, G., 2014: The comparison of FLOTAC, FECPAK and McMaster techniques for nematode egg counts in cattle. *Acta Parasitologica*, 59.4. p.625–628.

Bowman, D., Lynn, R., Eberhard, M. and Alcaraz, A., 2003: Gerogi's Parasitology For Veterinarians. 8th ed. St. Louis, Saunders. p.115-180,287-257.

Boxell, A., Gibson, K., Hobbs, R. and Thompson, R., 2004: Occurrence of gastrointestinal parasites in horses in metropolitan Perth, Western Australia. *Australian Veterinary Journal*, 82.1-2. p.91-95.

Bracken, M., Wøhlk, C., Petersen, S. and Nielsen, M., 2012: Evaluation of conventional PCR for detection of Strongylus vulgaris on horse farms. *Veterinary Parasitology*, 184.2-4. p.387-391.

Braga, F., Araújo, J., Silva, A., Araujo, J., Carvalho, R., Tavela, A., Campos, A. and Carvalho, G., 2009: Biological control of horse cyathostomin (Nematoda: Cyathostominae) using the nematophagous fungus Duddingtonia flagrans in tropical southeastern Brazil. *Veterinary Parasitology*, 163.4.p.335-340.

Brazik, E., Luquire, J. and Little, D., 2006: Pyrantel pamoate resistance in horses receiving daily administration of pyrantel tartrate. *Journal of the American Veterinary Medical Association*, 228.1. p.101-103.

Bredtmann, C., Krücken, J., Murugaiyan, J., Kuzmina, T. and von Samson-Himmelstjerna, G., 2017: Nematode Species Identification—Current Status, Challenges and Future Perspectives for Cyathostomins. *Frontiers in Cellular and Infection Microbiology*, 7.

Bucknell, D., Gasser, R. and Beveridge, I., 1995. The prevalence and epidemiology of gastrointestinal parasites of horses in Victoria, Australia. *International Journal for Parasitology*, 25.6. p.711-724.

Canever, R., Braga, P., Boeckh, A., Grycajuck, M., Bier, D. and Molento, M., 2013: Lack of Cyathostomin sp. reduction after anthelmintic treatment in horses in Brazil. *Veterinary Parasitology*, 194.1. p.35-39.

Canhão-Dias, M., Paz-Silva, A. and Madeira de Carvalho, L., 2020: The efficacy of predatory fungi on the control of gastrointestinal parasites in domestic and wild animals—A systematic review. *Veterinary Parasitology*, 283.

Carstensen, H., Larsen, L., Ritz, C. and Nielsen, M., 2013: Daily Variability of Strongyle Fecal Egg Counts in Horses. *Journal of Equine Veterinary Science*, 33.3. p.161-164.

Chapman, M., French, D. and Klei, T., 2002: Gastrointestinal Helminths of Ponies in Louisiana: A Comparison of Species Currently Prevalent With Those Present 20 Years Ago. *Journal of Parasitology*, 88.6. p.1130-1134.

Chapman, M., French, D. and Klei, T., 2003: Prevalence of Strongyle Nematodes in Naturally Infected Ponies of Different Ages and durign Different Seasons of the Year in Louisiana. *Journal of Parasitology*, 89.2. p.309-314.

Chapman, M., French, D., Monahan, C. and Klei, T., 1996: Identification and characterization of a pyrantel pamoate resistant cyathostome population. *Veterinary Parasitology*, 66.3-4. p.205-212.

Chapman, S., 2013: Control of gastrointestinal nematode species in horses: an evidence-based approach. *Livestock*, 18.5. p.195-200.

Cirak, V., Hermosilla, C. and Bauer, C., 1996: Study on the gastrointestinal parasite fauna of ponies in northern Germany. *Applied parasitology*, 37. p.239-244.

Cobb, R. and Boeckh, A., 2009: Moxidectin: a review of chemistry, pharmacokinetics and use in horses. *Parasites & Vectors*, 2.Suppl 2.

Collobert-Laugier, C., Hoste, H., Sevin, C. and Dorchies, P., 2002: Prevalence, abundance and site distribution of equine small strongyles in Normandy, France. *Veterinary Parasitology*, 110.1-2. p.77-83.

Comer, K., Hillyer, M. and Coles, G., 2006: Anthelmintic use and resistance on thoroughbred training yards in the UK. *Veterinary Record*, 158.17. p.596-598.

Corbett, C., Love, S., Moore, A., Burden, F., Matthews, J. and Denwood, M., 2014: The effectiveness of faecal removal methods of pasture management to control the cyathostomin burden of donkeys. *Parasites & Vectors*, 7.

Corning, S., 2009a: Equine cyathostomins: a review of biology, clinical significance and therapy. *Parasites & Vectors*, 2(Suppl 2).

Corning, S., 2009b: *Life Cycle Of Cyathostomins.*. [image] Available at: <https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-2-S2-S1/figures/1> [Accessed 8 November 2020].

Cringoli, G., 2006: FLTOAC, a novel apparatus for multivalent faecal egg count technique. *Parassitologia*, 48.3. p.381-384.

de Vienne, D., 2016: Lifemap: Exploring the Entire Tree of Life. *PLOS Biology*, 14.12.

Debeffe, L., McLoughlin, P., Medill, S., Stewart, K., Andres, D., Shury, T., Wagner, B., Jenkins, E., Gilleard, J. and Poissant, J., 2016: Negative covariance between parasite load and body condition in a population of feral horses. *Parasitology*, 143.8. p.983-997.

DeLay, J., Peregrine, A. and Parsons, D., 2001: Verminous arteritis in a 3-month-old Thoroughbred foal. *The Canadian Veterinary Journal*, 42.4. p.289-291.

Denwood, M., Love, S., Innocent, G., Matthews, L., McKendrick, I., Hillary, N., Smith, A. and Reid, S., 2012: Quantifying the sources of variability in equine faecal egg counts: Implications for improving the utility of the method. *Veterinary Parasitology*, 188.1-2. p.120-126.

Deplazes, P., Eckert, J., von Samson-Himmelstjerna, G. and Zahner, H., 2013: Lehrbuch Der Parasitologie Für Die Tiermedizin. 3rd ed. Stuttgart. Enke Verlag. p.235-261,507-564,581-586.

Dias de Castro, L., Abrahão, C., Buzatti, A., Molento, M., Bastianetto, E., Rodrigues, D., Lopes, L., Silva, M., de Freitas, M., Conde, M. and Borges, F., 2017: Comparison of McMaster and Mini-FLOTAC fecal egg counting techniques in cattle and horses. *Veterinary Parasitology: Regional Studies and Reports*, 10. p.132-135.

DiPietro, J. and Todd, K., 1987: Anthelmintics Used in Treatment of Parasitic Infections of Horses. *Veterinary Clinics of North America: Equine Practice*, 3.1. p.1-14.

Döpfer, D., Kerssens, C., Meijer, Y., Boersema, J. and Eysker, M., 2004: Shedding consistency of strongyle-type eggs in dutch boarding horses. *Veterinary Parasitology*, 124.3-4. p.249-258.

Drudge, J. and Lyons, E., 1966: Control of internal parasites of the horse. *Journal of the American Veterinary Medical Association*, 148.4. p.378-383.

Duncan, J. and Love, S., 1991: Preliminary observations on an alternative strategy for the control of horse strongyles. *Equine Veterinary Journal*, 23.3. p.226-228.

Duncan, J. and Pirie, H., 1972: The Life Cycle of Strongylus vulgaris in the Horse. *Research in Veterinary Science*, 13. p.374-379.

Duncan, J. and Pirie, H., 1975: The pathogenesis of single experimental infections with Strongylus vulgaris in foals. *Research in Veterinary Science*, 18. pp.82-93.

Duncan, J., 1974: Strongylus vulgaris infection in the horse. *Veterinary Record*, 95.2. p.34-37.

Duncan, J., Arundel, J., Drudge, J., Malczewski, A. and Slocombe, J., 1988: World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of equine anthelmintics. *Veterinary Parasitology*, 30.1. p.57-72.

Duncan, J., Bairden, K. and Abbott, E., 1999: Elimination of mucosal cyathostome larvae by five daily treatments with fenbendazole. *Veterinary Record*, 142. p.268-271.

Duncan, J., McBeath, D. and Preston, N., 1980: Studies on the efficacy of fenbendazole used in a divided dosage regime against strongyle infections in ponies. *Equine Veterinary Journal*, 12.2. p.78-80.

Duncan, J., McBeath, D., Best, J. and Preston, N., 1977: The Efficacy of Fenbendazole in the Control of Immature Strongyle Infections in Ponies. *Equine Veterinary Journal*, 9.3. p.146-149.

Duncan, J.L., 1973: Strongylus vuglaris Infection in the Horse. PhD Thesis. Department of Veterianry Pathology, University of Glasgow.

Egerton, J., Brokken, E., Suhayda, D., Eary, C., Wooden, J. and Kilgore, R., 1981: The antiparasitic activity of ivermectin in horses. *Veterinary Parasitology*, 8. p.83-88.

Egwang, T. and Slocombe, J., 1982: Evaluation of the Cornell-Wisconsin Centrifugal Flotation technique for Recovering Trichostrongylid Eggs from Bovine Feces. *Canadian Journal of Comparative Medicine*, 46. p.133-137.

English, A., 1979: The Epidemiology of Equine Strongylosis in Southern Queensland 1. The Bionomics of the Free-Living Stages in Faeces and on Pasture. *Australian Veterinary Journal*, 55.7. p.299-305.

Enigk, K., 1969: The development of the three species of Strongylus of the horse during the prepatent period. In: Proc. 2nd Int. Conf. on Equine Infectious Diseases. Paris: Equine infectious diseases. pp.259-268.

Ertelt, A., Merle, R., von Samson-Himmelstjerna, G., Wulke, N., Demeler, J. and Gehlen, H., 2015: Management factors and their impact on helminthic fecal egg count in horses. *Pferdeheilkunde*, 31.4. p.332-340.

ESCCAP, 2019: *Pferdekot Mit Typischen Roten Stadien Bestimmter Kleiner Strongyliden-Arten.*. [image] Available at: <https://www.esccap.de/v2/wp-content/uploads/2020/06/2019_Pferd-Guideline-Web.pdf> [Accessed 8 November 2020].

Eydal, M. and Gunnarsson, E., 1994: Helminth infections in a group of Icelandic horses with little exposure to anthelmintics. *Icelandic Agricultural Science*, 8. p.86-91.

Eysker, M. and Mirck, M., 1986: The distribution of inhibited early third stage Cyathostominae larvae in the large intestine of the horse. *Zeitschrift für Parasitenkunde*, 72.6. p.815-820.

Eysker, M., Bakker, J., van den Berg, M., van Doorn, D. and Ploeger, H., 2008: The use of age-clustered pooled faecal samples for monitoring worm control in horses. *Veterinary Parasitology*, 151.2-4. p.249-255.

Eysker, M., Boersema, J. and Kooyman, F., 1990: Seasonally inhibited development of cyathostomine nematodes in shetland ponies in The Netherlands. *Veterinary Parasitology*, 36.3-4. p.259-264.

Eysker, M., Boersema, J., Grinwis, G., Kooyman, F. and Poot, J., 1997: Controlled dose confirmation study of a 2% moxidectin equine gel against equine internal parasites in The Netherlands. *Veterinary Parasitology*, 70.1-3. p.165-173.

Eysker, M., Jansen, J. and Mirck, M., 1986a: Control of strongylosis in horses by alternate grazing of horses and sheep and some other aspects of the epidemiology of strongylidae infections. *Veterinary Parasitology*, 19.1-2. p.103-115.

Eysker, M., Jansen, J., Kooyman, F., Mirck, M. and Wensing, T., 1986b: Comparison of two control systems for cyathostome infections in the horse and further aspects of the epidemiology of these infections. *Veterinary Parasitology*, 22.1-2. p.105-112.

Eysker, M., Jansen, J., Wemmenhove, R. and Mirck, M., 1983: Alternate grazing of horses and sheep as control for gastro-intestinal helminthiasis in horses. *Veterinary Parasitology*, 13. p.273-280.

Fabiani, J., Lyons, E. and Nielsen, M., 2016: Dynamics of Parascaris and Strongylus spp. parasites in untreated juvenile horses. *Veterinary Parasitology*, 230. p.62-66.

Fleurance, G., Duncan, P., Fritz, H., Cabaret, J., Cortet, J. and Gordon, I., 2007: Selection of feeding sites by horses at pasture: Testing the anti-parasite theory. *Applied Animal Behaviour Science*, 108.3-4. p.288-301.

Foreyt, W., 2001: Veterinary Parasitology Reference Manual. 5th ed. Iowa: Blackwell Publishing.

Foster, A. and Ortiz, P., 1937: A Further Report on the Parasites of a Selected Group of Equines in Panama. *The Journal of Parasitology*, 23.4. p.360-264.

Francisco, I., Arias, M., Cortiñas, F., Francisco, R., Mochales, E., Dacal, V., Suárez, J., Uriarte, J., Morrondo, P., Sánchez-Andrade, R., Díez-Baños, P. and Paz-Silva, A., 2009: Intrinsic Factors Influencing the Infection by Helminth Parasites in Horses under an Oceanic Climate Area (NW Spain). *Journal of Parasitology Research*.

Fritzen, B., Rohn, K., Schneider, T. and Von Samson-Himmelstjerna, G., 2009: Endoparasite control management on horse farms – lessons from worm prevalence and questionnaire data. *Equine Veterinary Journal*, 42.1. p.79-83.

Fritzen, G.M., 2005: Untersuchungen zum Vorkommen von Anthelminthika-Resistenz in nordrhein-westfälischen Pferdebeständen. D.V.M. Dissertation. Tierärztliche Hochschule Hannover.

Gates, M. and Nolan, T., 2009: Comparison of Passive Fecal Flotation Run by Veterinary Students to Zinc-Sulfate Centrifugation Flotation Run in a Diagnostic Parasitology Laboratory. *Journal of Parasitology*, 95.5. p.1213-1214.

Gawor, J., 1995: The prevalence and abundance of internal parasites in working horses autopsied in Poland. *Veterinary Parasitology*, 58.1-2. p.99-108.

Geldberg, H.B., 2017: Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity. In: Zachary, J., (edt.) 2017. Pathologic Basis Of Veterinary Disease. 2nd ed. St. Louis, Elsevier. p.372-374, 387-388.

Geresu, A., Abera, Z. and Gizachew, A., 2014: Prevalence of Strongyle Parasites in Working Horses in Goba Woreda, Bale Zone, Ethiopia. *European Journal of Biological Science*, 6.3. p.66-70.

Geurden, T., van Doorn, D., Claerebout, E., Kooyman, F., De Keersmaecker, S., Vercruysse, J., Besognet, B., Vanimisetti, B., di Regalbono, A., Beraldo, P., Di Cesare, A. and Traversa, D., 2014: Decreased strongyle egg re-appearance period after treatment with ivermectin and moxidectin in horses in Belgium, Italy and The Netherlands. *Veterinary Parasitology*, 204.3-4. p.291-296.

Gibson, T., 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 of Helminthology*, 27.1-2. p.29-40.

Giles, C., Urquhart, K. and Longstaffe, J., 1985. Larval cyathostomiasis (immature trichonema-induced enteropathy): A report of 15 clinical cases. *Equine Veterinary Journal*, 17.3. p.196-201.

Giovannola, A., 1936: Energy and Food Reserves in the Development of Nematodes. *The Journal of Parasitology*, 22.2. p.207.

Gokbulut, C. and McKellar, Q., 2018: Anthelmintic drugs used in equine species. *Veterinary Parasitology*, 261. p.27-52.

Gomez, H. and Georgi, J., 1991: Equine helminth infections: control by selective chemotherapy. *Equine Veterinary Journal*, 23.3. p.198-200.

Greite, L., 2013: Untersuchungen zur Verbreitung von Strongylus vulgaris im Rahmen der Selektiven Entwurmung bei Pferden in Süddeutschland. D.V.M. Dissertation. Ludwig-Maximilians-Universität München.

Grønvold, J., 1987: A field experiment on rain splash dispersal of infective larvae of Ostertagia ostertagi (Trichostrongylidae) from cow pats to surrounding grass. *Acta Veterinaria Scandinavica*, 28.3-4. p.459-461.

Hasslinger, M. and Bittner, G., 1984: Zur Saisondynamik der Larven von Pferdestrongyliden und deren Beziehung zum Infektionsrisiko auf der Weide. *Zentralblatt für Veterinärmedizin Reihe B*, 31.1-10. p.25-31.

Hautala, K., Näreaho, A., Kauppinen, O., Nielsen, M., Sukura, A. and Rajala-Schultz, P., 2019: Risk factors for equine intestinal parasite infections and reduced efficacy of pyrantel embonate against Parascaris sp. *Veterinary Parasitology*, 273. p.52-59.

Hedberg-Alm, Y., Penell, J., Riihimäki, M., Osterman-Lind, E., Nielsen, M. and Tydén, E., 2020: Parasite Occurrence and Parasite Management in Swedish Horses Presenting with Gastrointestinal Disease—A Case–Control Study. *Animals*, 10.4. p.638.

Herd, R. and Willardson, K., 1985: Seasonal distribution of infective strongyle larvae on horse pastures. *Equine Veterinary Journal*, 17.3. p.235-237.

Herd, R., 1986a: Epidemiology and control of equine strongylosis at Newmarket. *Equine Veterinary Journal*, 18.6. p.447-452.

Herd, R., 1986b: Epidemiology and Control of Parasites in Northern Temperate Regions. *Veterinary Clinics of North America: Equine Practice*, 2.2. p.337-355.

Herd, R., 1990: Equine parasite control - solutions to anthelmintic associated problems. *Equine Veterinary Education*, 2.2. p.86-91.

Herd, R., 1992: Choosing the optimal equine anthelmintic. *Veterinary Medicine*, 87.3. p.231-239.

Hernández, J., Arroyo, F., Suárez, J., Cazapal-Monteiro, C., Romasanta, Á., López-Arellano, M., Pedreira, J., de Carvalho, L., Sánchez-Andrade, R., Arias, M., de Gives, P. and Paz-Silva, A., 2016: Feeding horses with industrially manufactured pellets with fungal spores to promote nematode integrated control. *Veterinary Parasitology*, 229. p.37-44.

Hertzberg, H., Schwarzwald, C., Grimm, F., Frey, C., Gottstein, B. and Gerber, V., 2014: Helminthenmanagement beim adulten Pferd: Notwendigkeit einer Neuorientierung. *Schweizer Archiv für Tierheilkunde*, 156.2. p.61-70.

Hilborn, R., Mangel, M., 1997: Probability and probability models: know your data. In: The Ecological Detective: Confronting Models with Data. Princeton, Princeton University Press. p 70

Hinney, B., 2008: Prävalenz von Helminthen und Risikofaktoren für ihre Befallsstärke bei Pferden in Brandenburg. D.V.M. Dissertation. Freie Universität Berlin.

Hinney, B., Wirtherle, N., Kyule, M., Miethe, N., Zessin, K. and Clausen, P., 2011: A questionnaire survey on helminth control on horse farms in Brandenburg, Germany and the assessment of risks caused by different kinds of management. *Parasitology Research*, 109. p.1625-1635.

Hodgkinson, J., Lichtenfels, J., Mair, T., Cripps, P., Freeman, K., Ramsey, Y., Love, S. and Matthews, J., 2003: A PCR–ELISA for the identification of cyathostomin fourth-stage larvae from clinical cases of larval cyathostominosis. *International Journal for Parasitology*, 33.12. p.1427-1435.

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

Hoffmann, G., Bentke, A., Rose-Meierhöfer, S., Ammon, C., Mazetti, P. and Hardarson, G., 2013: Estimation of the Body Weight of Icelandic Horses. *Journal of Equine Veterinary Science*, 33.11. p.893-895.

Höglund, J., Ljungström, B., Nilsson, O., Lundquist, H., Osterman, E. and Uggla, A., 1997: Occurence of Gasterophilus intestinalis and some parasitic nematodes of horses in Sweden. *Acta Vet Scand.*, 38.2. p.157-165.

Honeder, A., 2015: Selektive anthelmintische Therapie bei Pferden im Raum Salzburg und Oberbayern. D.V.M. Dissertation. Ludwig-Maximilians-Universität München.

Horohov, D., Adams, A. and Chambers, T., 2010: Immunosenescence of the Equine Immune System. *Journal of Comparative Pathology*, 142. p.78-S84.

Hubert, J., Seahorn, T., Klei, T., Hogswood, G., Horohov, D. and Moore, R., 2004: Clinical signs and hematologic, cytokine, and plasma nitric oxide alterations in response to Strongylus vulgaris infection in helminth-naïve ponies. *Canadian Journal of Veterinary Research*, 68.3. p.192-200.

Hummelinck, P., 1946: Investigation of the eggs of horse strongyles. Tijdschr Diergeneeskd, 71. p. 411-427.

Hunter, G. and Quenouille, M., 1952: A Statistical Examination of the Worm Egg Count Sampling Technique for Sheep. *Journal of Helminthology*, 26.4. p.157-170.

Hutchinson, G., Abba, S. and Mfitilodze, M., 1989: Seasonal translation of equine strongyle infective larvae to herbage in tropical Australia. *Veterinary Parasitology*, 33. p.251-263.

Ihler, C., 1995: A field survey on anthelmintic resistance in equine small strongyles in Norway. *Acta Veterinaria Scadinavica*, 36.1. p.135-143.

Ihler, C., 2010: Anthelmintic resistance. An overview of the situation in the Nordic countries. *Acta Veterinaria Scandinavica*, 52.

Jasko, D. and Roth, L., 1984: Granulomatous colitis associated with small strongyle larvae in a horse. *Journal of the American Veterinary Medical Association*, 185.5. p.553-553.

Kaplan, R., 2002: Anthelmintic resistance in nematodes of horses. *Veterinary Research*, 33.5. p.491-507.

Kaplan, R., 2004: Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitology*, 20.10, p.477-481.

Kaspar, A., Pfister, K., Nielsen, M., Silaghi, C., Fink, H. and Scheuerle, M., 2016: Detection of Strongylus vulgaris in equine faecal samples by real-time PCR and larval culture – method comparison and occurrence assessment. *BMC Veterinary Research*, 13.

Kates, K., 1965: Ecological Aspects of Helminth Transmission in Domesticated Animals. *American Zoologist*, 5. p.95-130.

Kelly, J. and Fogarty, U., 1993: Outbreak of larval cyathostomiasis on a Thoroughbred stud farm. *Irish Veterinary Journal*, 46. p.133-136.

Kivipelto, J. and Asquith, R., 1997: Efficacy of pyrantel pamoate against small strongyle populations. *Veterinary Clinics of North America: Equine Practice*, 19.2. p.14-18.

Klei, T. and Chapman, M., 1999: Immunity in equine cyathostome infections. *Veterinary Parasitology*, 85.2-3. p.123-136.

Kooyman, F., van Doorn, D., Geurden, T., Mughini-Gras, L., Ploeger, H. and Wagenaar, J., 2016: Species composition of larvae cultured after anthelmintic treatment indicates reduced moxidectin susceptibility of immature Cylicocyclus species in horses. *Veterinary Parasitology*, 227. p.77-84.

Kornaś, S., Cabaret, J., Skalska, M. and Nowosad, B., 2010: Horse infection with intestinal helminths in relation to age, sex, access to grass and farm system. *Veterinary Parasitology*, 174.3-4. p.285-291.

Kornaś, S., Nowosad, B. and Skalska, M., 2006: Dynamics of small strongyle (cyathostominae) infection in horses under different management systems. *Annals of Animal Science*, 6.1. p.129-138.

Kornaś, S., Sallé, G., Skalska, M., David, I., Ricard, A. and Cabaret, J., 2015: Estimation of genetic parameters for resistance to gastro-intestinal nematodes in pure blood Arabian horses. *International Journal for Parasitology*, 45.4. p.237-242.

Kuzmina, T., Dzeverin, I. and Kharchenko, V., 2016: Strongylids in domestic horses: Influence of horse age, breed and deworming programs on the strongyle parasite community. *Veterinary Parasitology*, 227. p.56-63.

Kuzmina, T., Kuzmin, Y. and Kharchenko, V., 2006: Field study on the survival, migration and overwintering of infective larvae of horse strongyles on pasture in central Ukraine. *Veterinary Parasitology*, 141.3-4. p.264-272.

Kuzmina, T., Lyons, E., Tolliver, S., Dzeverin, I. and Kharchenko, V., 2012: Fecundity of various species of strongylids (Nematoda: Strongylidae)—parasites of domestic horses. *Parasitology Research*, 111.6. p.2265-2271.

Lacey, E., 1990: Mode of action of benzimidazoles. *Parasitology Today*, 6.4. p.112-115.

Larsen, M., Lendal, S., Chriél, M., Olsen, S. and Bjørn, H., 2002: Risk Factors for High Endoparasitic Burden and the Efficiency of a Single Anthelmintic Treatment of Danish Horses. *Acta Veterinaria Scandinavica*, 43. p.99-106.

Larsen, M., Nansen, P., Grøndahl, C., Thamsborg, S., Grønvold, J., Wolstrup, J., Henriksen, S. and Monrad, J., 1996: The capacity of the fungus Duddingtonia flagrans to prevent strongyle infections in foals on pasture. *Parasitology*, 113.1. p.1-6.

Larsen, M., Nansen, P., Henriksen, S., Wolstrup, J., Grønvold, J., Zorn, A. and Wedø, E., 1995: Predacious activity of the nematode-trapping fungus Duddingtonia flagrans against cyathostome larvae in faeces after passage through the gastrointestinal tract of horses. *Veterinary Parasitology*, 60.3-4. p.315-320.

Leathwick, D., Sauermann, C., Reinemeyer, C. and Nielsen, M., 2019: A model for the dynamics of the parasitic stages of equine cyathostomins. *Veterinary Parasitology*, 268. p.53-60.

Leland, S., Drudge, J., Wyant, Z. and Elam, G., 1961: Studies on Trichostrongylus axei (Cobbold 1879). VII. Some quantitative and pathologic aspects of natural and experimental infections in the horse. *American Journal of Veterinary Research*, 22.86. p.128-138.

Lester, H., Morgan, E., Hodgkinson, J. and Matthews, J., 2018: Analysis of Strongyle Egg Shedding Consistency in Horses and Factors That Affect It. *Journal of Equine Veterinary Science*, 60.

Lester, H., Spanton, J., Stratford, C., Bartley, D., Morgan, E., Hodgkinson, J., Coumbe, K., Mair, T., Swan, B., Lemon, G., Cookson, R. and Matthews, J., 2013: Anthelmintic efficacy against cyathostomins in horses in Southern England. *Veterinary Parasitology*, 197.1-2. p.189-196.

Levecke, B., Rinaldi, L., Charlier, J., Maurelli, M., Bosco, A., Vercruysse, J. and Cringoli, G., 2012: The bias, accuracy and precision of faecal egg count reduction test results in cattle using McMaster, Cornell-Wisconsin and FLOTAC egg counting methods. *Veterinary Parasitology*, 188.1-2. p.194-199.

Lichtenfels, J., Gibbons, L. and Krecek, R., 2002: Recommended terminology and advances in the systematics of the Cyathostominea (Nematoda: Strongyloidea) of horses. *Veterinary Parasitology*, 107.4. p.337-342.

Lichtenfels, J., Kharchenko, V. and Dvojnos, G., 2008: Illustrated identification keys to strongylid parasites (strongylidae: Nematoda) of horses, zebras and asses (Equidae). *Veterinary Parasitology*, 156.1-2. p.4-161.

Lichtenfels, J., Kharchenko, V., Krecek, R. and Gibbons, L., 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*, 79.1. p.65-79.

Lichtenfels, R., 1975. Helminths of Domestic Equids. Illustrated Keys to Genera and Species with Emphasis on North American Forms. In: *Proceedings of the Helminthological Society of Washington*. Lawrence, Kansas: Helminthological Society of Washington, p.1-69, 83-92.

Lloyd, S., Smith, J., Connan, R., Hatcher, M., Hedges, T., Humphrey, D. and Jones, A., 2000: Parasite control methods used by horse owners: factors predisposing to the development of anthelmintic resistance in nematodes. *Veterinary Record*, 146.17. p.487-492.

Love, S. and Duncan, J., 1992a: The development of naturally acquired cyathostome infection in ponies. *Veterinary Parasitology*, 44.1-2. p.127-142.

Love, S. and Mckeand, J., 1997: Cyathostomosis: Practical issues of treatment and control. *Equine Veterinary Education*, 9.5. p.253-256.

Love, S., 1999: Review Article: The role of equine strongyles in the pathogenesis of colic and current options for prophylaxis. *Equine Veterinary Journal*, Suppl.13. p.5-9.

Love, S., Duncan, J. L., 1992b: Development of cyathostome infection of helminth-naive foals. Equine Veterinary Journal, 24. 13. p.93-98

Love, S., Mair, T. and Hillyer, M., 1992: Chronic diarrhoea in adult horses: a review of 51 referred cases. *Veterinary Record*, 130. p.217-219.

Love, S., Murphy, D. and Mellor, D., 1999: Pathogenicity of cyathostome infection. *Veterinary Parasitology*, 85.2-3.p.113-122.

Lyons, E., Swerczek, T., Tolliver, S., Bair, H., Drudge, J. and Ennis, L., 2000: Prevalence of selected species of internal parasites in equids at necropsy in central Kentucky (1995–1999). *Veterinary Parasitology*. 92. p.51-62.

Lyons, E., Tolliver, S. and Drudge, J., 1999: Historical perspective of cyathostomes: prevalence, treatment and control programs. *Veterinary Parasitology*, 85.2-3. p.97-112.

Lyons, E., Tolliver, S., Collins, S. and Drudge, J., 2001: Transmission of endoparasites in horse foals born on the same pasture on a farm in central Kentucky (1996–1999). *Veterinary Parasitology*, 97. p.113-121.

Lyons, E., Tolliver, S., Collins, S., Ionita, M., Kuzmina, T. and Rossano, M., 2011: Field tests demonstrating reduced activity of ivermectin and moxidectin against small strongyles in horses on 14 farms in Central Kentucky in 2007–2009. *Parasitology Research*, 108. p.355-360.

Mair, T., 1993: Recurrent diarrhoea in aged ponies associated with larval cyathostomiasis. *Equine Veterinary Journal*, 25.2. p.161-163.

Mair, T., 1994: Outbreak of larval cyathostomiasis among a group of yearling and two-year-old horses. *The Veterinary Record*, 135.25. p.598-600.

Mair, T., de Westerlaken, L., Cripps, P. and Love, S., 1990: Diarrhoea in adult horses: a survey of clinical cases and an assessment of some prognostic indices. *The Veterinary Record*, 126.19. p.479-481.

Martin, R. and Robertson, A., 2007: Mode of action of levamisole and pyrantel, anthelmintic resistance, E153 and Q57. *Parasitology*, 134.8.

Martin, R., Robertson, A. and Bjorn, H., 1997: Target sites of anthelmintics. *Parasitology*, 114.7. p.111-124.

Matthews, J., 2008: An update on cyathostomins: Anthelmintic resistance and worm control. *Equine Veterinary Education*, 20.10. p.552-560.

Matthews, J., 2014: Anthelmintic resistance in equine nematodes. *International Journal for Parasitology: Drugs and Drug Resistance*, 4.3.p.310-315.

Matthews, J., Hodgkinson, J., Dowdall, S. and Proudman, C., 2004: Recent developments in research into the Cyathostominae and Anoplocephala perfoliata. *Veterinary Research*, 35.4. p.371-381.

Maxie, M. (Edt.), 2016: Jubb, Kennedy, And Palmer's Pathology Of Domestic Animals. 6th ed. St. Louis, Elsevier. p.204-226.

McCoy, M., Edgar, H., Kenny, J., Gordon, A., Dawson, L. and Carson, A., 2005: Evaluation of on-farm faecal wormm egg counting sheep. *Veterianry Record*, 156. p.21-23.

McCraw, B. and Slocombe, J., 1978: Strongylus edentatus: Development and Lesions from Ten Weeks Postinfection to Patency. *The Canadian Journal of Comparative Medicine*, 42. p.340-356.

McCraw, B. and Slocombe, J., 1985: Strongylus equinus: Development and Pathological Effects in the Equine Host. *The Canadian Journal of Comparative Medicine*, 49. p.372-383.

McCraw, B., Slocombe, J., 1976: Strongylus vulgaris in the horse: a review. *the Canadian Veterinary Journal*, 17.6. p.150-157.

McFarlane, D., Hale, G., Johnson, E. and Maxwell, L., 2010: Fecal egg counts after anthelmintic administration to aged horses and horses with pituitary pars intermedia dysfunction. *Journal of the American Veterinary Medical Association*, 236.3. p.330-334.

McKellar, Q. and Scott, E., 1990: The benzimidazole anthelmintic agents-a review. *Journal of Veterinary Pharmacology and Therapeutics*, 13. p.223-247.

McWilliam, H., Nisbet, A., Dowdall, S., Hodgkinson, J. and Matthews, J., 2010: Identification and characterisation of an immunodiagnostic marker for cyathostomin developing stage larvae. *International Journal for Parasitology*, 40.3. p.265-275.

Medica, D. and Sukhdeo, M., 1997: Role of Lipids in the Transmission of the Infective Stage (L3) of Strongylus vulgaris (Nematoda: Strongylida). *The Journal of Parasitology*, 83.5. p.775-779.

Medica, D., Hanaway, M., Ralston, S. and Sukhdeo, M., 1996: Grazing behavior of horses on pasture: Predisposition to strongylid infection?. *Journal of Equine Veterinary Science*, 16.10. p.421-427.

Mehlhorn, H., 2012: Animal Parasites. Diagnosis, Treatment, Prevention. 7th ed. Cham, Springer. p. 1-13, 23-32, 251-463, 663-680.

Meier, A. and Hertzberg, H., 2005: Strongyliden beim Pferd. II. Vorkommen von Anthelminthika-Resistenzen in der Schweiz. *Schweizer Archiv für Tierheilkunde*, 147.9. p.389-396.

Menzel, M.A., 2013: Selektive Entwurmung der Pferde in einer Pferdepraxis: Einführung sowie wissenschaftliche und betriebswirtschaftliche Analyse. D.V.M. Dissertation. Ludwig-Maximilians-Universität München.

Mes, T., Ploeger, H., Terlou, M., Kooyman, F., Van der Ploeg, M. and Eysker, M., 2001: A novel method for the isolation of gastro-intestinal nematode eggs that allows automated analysis of digital images of egg preparations and high throughput screening. *Parasitology*, 123.3. p.309-314.

Mfitilodze, M. and Hutchinson, G., 1987: Development and survival of free-living stages of equine strongyles under laboratory conditions. *Veterinary Parasitology*, 23.1-2. p.121-133.

Mfitilodze, M. and Hutchinson, G., 1990: Prevalence and Abundance of Equine Strongyles (Nematoda: Strongyloidea) in Tropical Australia. *The Journal of Parasitology*, 76.4. p.487-494.

Milillo, P., Boeckh, A., Cobb, R., Otranto, D., Lia, R., Perrucci, S., di Regalbono, A., Beraldo, P., von Samson-Himmelstjerna, G., Demeler, J., Bartolini, R. and Traversa, D., 2009: Faecal Cyathostomin Egg Count distribution and efficacy of anthelmintics against cyathostomins in Italy: a matter of geography?. *Parasites & Vectors*, 2.Suppl 2).

Molento, M., Antunes, J., Bentes, R. and Coles, G., 2008: Anthelmintic resistant nematodes in Brazilian horses. *Veterianry Record*, 162. p.384-385.

Morariu, S., Mederle, N., Badea, C., Dărăbuş, G., Ferrari, N. and Genchi, C., 2016: The prevalence, abundance and distribution of cyathostomins (small stongyles) in horses from Western Romania. *Veterinary Parasitology*, 223. p.205-209.

Morgan, E., Cavill, L., Curry, G., Wood, R. and Mitchell, E., 2005: Effects of aggregation and sample size on composite faecal egg counts in sheep. *Veterinary Parasitology*, 131.1-2. p.79-87.

Morgan, S., Stromberg, P., Storts, R., Sowa, B. and Lay, J., 1991: Histology and morphometry of Strongylus vulgaris-mediated equine mesenteric arteritis. *Journal of Comparative Pathology*, 104, p.89-99.

Murphy, D. and Love, S., 1997: The pathogenic effects of experimental cyathostome infections in ponies. *Veterinary Parasitology*, 70.1-3. p.99-110.

Murphy, D., Edwards, S., Russell, T., Sammin, D. and Love, S., 1996: Diagnosis, Management and Outcome of 18 cases of chronic diarrhoea in adult horses. *Irish Veterinary Journal*, 49. p.216-220.

Nápravníková, J., Petrtýl, M., Stupka, R. and Vadlejch, J., 2019: Reliability of three common fecal egg counting techniques for detecting strongylid and ascarid infections in horses. *Veterinary Parasitology*, 272. p.53-57.

Näreaho, A., Vainio, K. and Oksanen, A., 2011: Impaired efficacy of ivermectin against Parascaris equorum, and both ivermectin and pyrantel against strongyle infections in trotter foals in Finland. *Veterinary Parasitology*, 182.2-4. p.372-377.

Neves, J., Carvalho, N., Rinaldi, L., Cringoli, G. and Amarante, A., 2014: Diagnosis of anthelmintic resistance in cattle in Brazil: A comparison of different methodologies. *Veterinary Parasitology*, 206.3-4. p.216-226.

Nielsen, M. and Lyons, E., 2017: Encysted cyathostomin larvae in foals – progression of stages and the effect of seasonality. *Veterinary Parasitology*, 236. p.108-112.

Nielsen, M. K., Reinemeyer, C. R., Sellon, D.C., 2013a: Nematodes. In: Sellon, D.C., Long, M., (eds.) 2013a: Equine Infectious Diseases. 2nd ed. St. Louis, Saunders Elsevier. p.475-489.

Nielsen, M., 2012. Sustainable equine parasite control: Perspectives and research needs. *Veterinary Parasitology*, 185.1. p.32-44.

Nielsen, M., Baptiste, K., Tolliver, S., Collins, S. and Lyons, E., 2010a: Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of feces are reliable indicators of numbers of strongyles and ascarids present. *Veterinary Parasitology*, 174.1-2. p.77-84.

Nielsen, M., Branan, M., Wiedenheft, A., Digianantonio, R., Garber, L., Kopral, C., Phillippi-Taylor, A. and Traub-Dargatz, J., 2018b: Parasite control strategies used by equine owners in the United States: A national survey. *Veterinary Parasitology*, 250, pp.45-51.

Nielsen, M., Branan, M., Wiedenheft, A., Digianantonio, R., Scare, J., Bellaw, J., Garber, L., Kopral, C., Phillippi-Taylor, A. and Traub-Dargatz, J., 2018a: Risk factors associated with strongylid egg count prevalence and abundance in the United States equine population. *Veterinary Parasitology*, 257. p.58-68.

Nielsen, M., Fritzen, B., Duncan, J., Guillot, J., Eysker, M., Dorchies, P., Laugier, C., Beugnet, F., Meana, A., Lussot-Kervern, I. and Von Samson-Himmelstjerna, G., 2010b: Practical aspects of equine parasite control: A review based upon a workshop discussion consensus. *Equine Veterinary Journal*, 42.5. p.460-468.

Nielsen, M., Haaning, N. and Olsen, S., 2006b: Strongyle egg shedding consistency in horses on farms using selective therapy in Denmark. *Veterinary Parasitology*, 135.3-4. p.333-335.

Nielsen, M., Kaplan, R., Thamsborg, S., Monrad, J. and Olsen, S., 2007: Climatic influences on development and survival of free-living stages of equine strongyles: Implications for worm control strategies and managing anthelmintic resistance. *The Veterinary Journal*, 174. p.23-32.

Nielsen, M., Monrad, J. and Olsen, S., 2006a: Prescription-only anthelmintics—A questionnaire survey of strategies for surveillance and control of equine strongyles in Denmark. *Veterinary Parasitology*, 135. p.47-55.

Nielsen, M., Olsen, S., Lyons, E., Monrad, J. and Thamsborg, S., 2012b: Real-time PCR evaluation of Strongylus vulgaris in horses on farms in Denmark and Central Kentucky. *Veterinary Parasitology*, 190.3-4. p.461-466.

Nielsen, M., Peterson, D., Monrad, J., Thamsborg, S., Olsen, S. and Kaplan, R., 2008: Detection and semi-quantification of Strongylus vulgaris DNA in equine faeces by real-time quantitative PCR. *International Journal for Parasitology*, 38.3-4. p.443-453.

Nielsen, M., Pfister, K. and von Samson-Himmelstjerna, G., 2014a: Selective therapy in equine parasite control—Application and limitations. *Veterinary Parasitology*, 202.3-4. p.95-103.

Nielsen, M., Reinemeyer, C., Donecker, J., Leathwick, D., Marchiondo, A. and Kaplan, R., 2014b: Anthelmintic resistance in equine parasites—Current evidence and knowledge gaps. *Veterinary Parasitology*, 204.1-2. p.55-63.

Nielsen, M., Vidyashankar, A., Andersen, U., DeLisi, K., Pilegaard, K. and Kaplan, R., 2010c: Effects of fecal collection and storage factors on strongylid egg counts in horses. *Veterinary Parasitology*, 167.1. p.55-61.

Nielsen, M., Vidyashankar, A., Hanlon, B., Diao, G., Petersen, S. and Kaplan, R., 2013b: Hierarchical model for evaluating pyrantel efficacy against strongyle parasites in horses. *Veterinary Parasitology*, 197.3-4. p.614-622.

Nielsen, M., Vidyashankar, A., Olsen, S., Monrad, J. and Thamsborg, S., 2012a: Strongylus vulgaris associated with usage of selective therapy on Danish horse farms—Is it reemerging?. *Veterinary Parasitology*, 189.2-4. p.260-266.

Nilsson, O., Lindholm, A. and Christensson, D., 1989: A field evaluation of anthelmintics in horses in Sweden. *Veterinary Parasitology*, 32.2-3. p.163-171.

Noel, M., Scare, J., Bellaw, J. and Nielsen, M., 2017: Accuracy and Precision of Mini-FLOTAC and McMaster Techniques for Determining Equine Strongyle Egg Counts. *Journal of Equine Veterinary Science*, 48. p.182-187.

Ödberg, F. and Francis-Smith, K., 1976: A Study on Eliminative and Grazing Behaviour - The Use of the Field by Captive Horses. *Equine Veterinary Journal*, 8.4. p.147-149.

Ogbourne, C., 1972: Observations on the free-living stages of strongylid nematodes of the horse. *Parasitology*, 64.3. p.461-477.

Ogbourne, C., 1975: Epidemiological studies on horses infected with nematodes of the family Trichonematidae (Witenberg, 1925). *International Journal for Parasitology*, 5.6. p.667-672.

Ogbourne, C., 1976: The prevalence, relative abundance and site distribution of nematodes of the subfamily Cyathostominae in horses killed in Britain. *Journal of Helminthology*, 50.3. p.203-214.

O'Meara, B. and Mulcahy, G., 2002: 89. Helminth control practices in equine establishments in Ireland. *Research in Veterinary Science*, 109. p.101-110.

Osterman Lind, E., Eysker, M., Nilsson, O., Uggla, A. and Höglund, J., 2003: Expulsion of small strongyle nematodes (cyathostomin spp) following deworming of horses on a stud farm in Sweden. *Veterinary Parasitology*, 115.4. p.289-299.

Osterman Lind, E., Höglund, J., Ljungström, B., Nilsson, O. and Uggla, A.,1999: A field survey on the distribution of strongyle infections of horses in Sweden and factors affecting faecal egg counts. *Equine Veterinary Journal*, 31.1. p.68-72.

Osterman Lind, E., Kuzmina, T., Uggla, A., Waller, P. and Höglund, J., 2006: A Field Study on the Effect of Some Anthelmintics on Cyathostomins of Horses in Sweden. *Veterinary Research Communications*, 31,1. p.53-65.

Osterman Lind, E., Rautalinko, E., Uggla, A., Waller, P., Morrison, D. and Höglund, J., 2007: Parasite control practices on Swedish horse farms. *Acta Veterinaria Scandinavica*, 49.

Peregrine, A., Molento, M., Kaplan, R. and Nielsen, M., 2014: Anthelmintic resistance in important parasites of horses: Does it really matter?. *Veterinary Parasitology*, 201.1-2. p.1-8.

Petty, D., Lange, A., Verster, A. and Hattingh, J., 1992: Necropsies of Eight Horses Infected with Strongylus Equinus and Strongylus edentatus. *Journal of the South African Veterinary Association*, 62.2. p.66-69.

Pilo, C., Altea, A., Pirino, S., Nicolussi, P., Varcasia, A., Genchi, M. and Scala, A., 2012: Strongylus vulgaris (Looss, 1900) in horses in Italy: Is it still a problem?. *Veterinary Parasitology*, 184.2-4. p.161-167.

Poynter, D., 1969: Some observations on the nematode parasites of horses. In: Proc. 2nd Int. Conf. on Equine Infectious Diseases. Paris: Equine infectious diseases. pp.269-289.

Presland, S., Morgan, E. and Coles, G., 2005: Counting nematode eggs in equine faecal samples. *Veterinary Record*, 156.7. p.208-210.

Prichard, R., Hall, C., Kelly, J., Martin, I. and Donald, A., 1980: The Problem of Anthelmintic Resistance in Nematodes. *Australian Veterinary Journal*, 56. p.239-250.

Proudman, C. and Matthews, J., 2000: Control of intestinal parasites in horses. *In Practice 2000*, 22. p.99-97.

Reavell, D., 1999: Measuring and estimating the weight of horses with tapes, formulae and by visual assessment. *Equine Veterinary Education*, 11.6. p.314-317.

Rehbein, S., Visser, M. and Winter, R., 2002: Koproskopische Untersuchungen bei Pferden in Deutschland und Österreich. *Pferdeheilkunde*, 18.5. p.439-449.

Rehbein, S., Visser, M. and Winter, R., 2013: Prevalence, intensity and seasonality of gastrointestinal parasites in abattoir horses in Germany. *Parasitology Research*, 112.1. p.407-413.

Reid, S., Mair, T., Hillyer, M. and Love, S., 1995: Epidemiological risk factors associated with a diagnosis of clinical cyathostomiasis in the horse. *Equine Veterinary Journal*, 27.2. p.127-130.

Reinemeyer, C. and Herd, R., 1986: Anatomic distribution of encysted cyathostome larvae in the horse. *American Journal of Veterinary Research*, 47.3. p.510-513.

Reinemeyer, C., Prado, J., Andersen, U., Nielsen, M., Schricker, B. and Kennedy, T., 2014: Effects of daily pyrantel tartrate on strongylid population dynamics and performance parameters of young horses repeatedly infected with cyathostomins and Strongylus vulgaris. *Veterinary Parasitology*, 204.3-4. p.229-237.

Reinemeyer, C., Smith, S., Gabel, A. and Herd, R., 1984: The prevalence and intensity of internal parasites of horses in the U.S.A. *Veterinary Parasitology*, 15.1. p.75-83.

Relf, V., Lester, H., Morgan, E., Hodgkinson, J. and Matthews, J., 2014: Anthelmintic efficacy on UK Thoroughbred stud farms. *International Journal for Parasitology*, 44.8. p.507-514.

Relf, V., Morgan, E., Hodgkinson, J. and Matthews, J., 2011: A questionnaire study on parasite control practices on UK breeding Thoroughbred studs. *Equine Veterinary Journal*, 44.4. p.466-471.

Relf, V., Morgan, E., Hodgkinson, J. and Matthews, J., 2013: Helminth egg excretion with regard to age, gender and management practices on UK Thoroughbred studs. *Parasitology*, 140.5. p.641-652.

Ribbeck, R., 1999: Klinik und Epidemiologie der Infektion mit Kleinen Strongyliden. *Pferdeheilkunde*, 15.2. p.155-158.

Rinaldi, L., Coles, G., Maurelli, M., Musella, V. and Cringoli, G., 2011: Calibration and diagnostic accuracy of simple flotation, McMaster and FLOTAC for parasite egg counts in sheep. *Veterinary Parasitology*, 177.3-4. p.345-352.

Round, M., 1968: The diagnosis of helminthiasis in horses. *Veterinary Record*, 82.2. p.39-43.

Round, M., 1968a: The Course of Naturally Acquired Helminth Infections of Horses Given Regular Anthelmintic Treatment. *Research in Veterinary Science*, 9.6. p.583-590.

Round, M., 1969: The Prepatent Period of Some Horse Nematodes Determined by Experimental Infection. *Journal of Helminthology*, 43.1-2. p.185-192.

Rupasinghe, D., 1975: Developmental, Physiolocial And Morphological Observations On The Free-Living And Parasitic Stages Of Some Strongylid Nematodes Of The Horse. Ph.D thesis. University of London.

Russell, A., 1948: The Development of Helminthiasis In Thoroughbred Foals. *Journal of Comparative Pathology and Therapeutics*, 58., p.107-127.

Saeed, K., Qadir, Z., Ashraf, K. and Ahmad, N., 2010: Role of Intrinsic and Extrinsic Epidemiological Factors on Strongylosis in Horses. *The Journal of Animal and Plant Science*, 204. p.277-280.

Saeed, M., Beveridge, I., Abbas, G., Beasley, A., Bauquier, J., Wilkes, E., Jacobson, C., Hughes, K., El-Hage, C., O'Handley, R., Hurley, J., Cudmore, L., Carrigan, P., Walter, L., Tennent-Brown, B., Nielsen, M. and Jabbar, A., 2019: Systematic review of gastrointestinal nematodes of horses from Australia. *Parasites & Vectors*, 12.

Sangster, N., 1999: Pharmacology of anthelmintic resistance in cyathostomes: will it occur with the avermectin/milbemycins?. *Veterinary Parasitology*, 85.2-3. p.189-204.

Scare, J., Slusarewicz, P., Noel, M., Wielgus, K. and Nielsen, M., 2017: Evaluation of accuracy and precision of a smartphone based automated parasite egg counting system in comparison to the McMaster and Mini-FLOTAC methods. *Veterinary Parasitology*, 24. p.85-92.

Scháňková, Š., Maršálek, M., Wagnerová, P., Langrová, I., Starostová, L., Stupka, R., Navrátil, J., Brožová, A., Truněčková, J., Kudrnáčová, M., Jankovská, I., Vadlejch, J., Čadková, Z. and Křivská, D., 2014: Arrested development of experimental Cyathostominae infections in ponies in Czech republic. *Veterinary Parasitology*, 206.3-4. p.328-332.

Scheuerle, M., Stear, M., Honeder, A., Becher, A. and Pfister, K., 2016: Repeatability of strongyle egg counts in naturally infected horses. *Veterinary Parasitology*, 228. p.103-107.

Schneider, S., 2015: Einfluss von Entwurmungsmethoden auf die Strongylidenpopulation bei Pferden in Deutschland. D.V.M. Dissertation. Ludwig-Maximilians-Universität München.

Schneider, S., Pfister, K., Becher, A. and Scheuerle, M., 2014: Strongyle infections and parasitic control strategies in German horses — a risk assessment. *BMC Veterinary Research*, 10.

Schnerr, C.U., 2011: Feldstudie zur Epidemiologie und Bekämpfung von Strongyliden in Pferdebeständen im Raum Baden- Württemberg . D.V.M. Dissertation. Ludwig-Maximilians-Universität München.

Schumacher, J. and Taintor, J., 2008: A review of the use of moxidectin in horses. *Equine Veterinary Education*, 20.10. p.546-551.

Scott, I., Bishop, R. and Pomroy, W., 2015: Anthelmintic resistance in equine helminth parasites – a growing issue for horse owners and veterinarians in New Zealand?. *New Zealand Veterinary Journal*, 63.4. p.188-198.

Slocombe, J. and McCraw, B., 1973: Gastrointestinal Nematodes in Horses in Ontario. *The Canadian Veterinary Journal*, 14.5. p.101-105.

Slocombe, J., deGannes, R., 2006: Cyathostomes in horses in Canada resistant to pyrantel salts and effectively removed by moxidectin. *Veterinary Parasitology*, 140.1-2. p.181-184.

Slocombe, J., Lake, M., 2007: Efficacy of Daily Pyrantel Tartrate (Strongid C) against Strongyles in Ponies on Pasture. *Journal of Equine Veterinary Science*, 27.10. p.439-445.

Slocombe, J., Smart, J., 1975: Evaluation of Pyrantel Pamoate Against Strongyles in Horses. *The Canadian Veterinary Journal*, 16.10. p.310-312.

Slusarewicz, P., Pagano, S., Mills, C., Popa, G., Chow, K., Mendenhall, M., Rodgers, D. and Nielsen, M., 2016: Automated parasite faecal egg counting using fluorescence labelling, smartphone image capture and computational image analysis. *International Journal for Parasitology*, 46.8. p.485-493.

Smets, K., Shaw, D., Deprez, P. and Vercruysse, J., 1999: Diagnosis of alrval cyathostominosis in horses in Belgium. *Veterinary Record*, 144. p.655-668.

Smith, H., 1976a: Strongyle Infections In Ponies I. Response to Intermittent Thiabendazole Treatments. *The Canadian Journal of Comparative Medicine*, 40. p.327-333.

Smith, H., 1976b: Strongyle Infections in Ponies II. Reinfection of Treated Animals. *The Canadian Journal of Comparative Medicine*, 40. p.334-340.

Smith, H., 1978: Experimental Trichonema infections in mature ponies. *Veterinary Parasitology*, 4.3. p.265-273.

Stancampiano, L., Gras, L.,Poglayen, G., 2010: Spatial niche competition among helminth parasites in horse's large intestine. *Veterinary Parasitology*, 170.1-2. p.88-95.

Steinbach, T., Bauer, C., Sasse, H., Baumgärtner, W., Rey-Moreno, C., Hermosilla, C., Damriyasa, I. and Zahner, H., 2006: Small strongyle infection: Consequences of larvicidal treatment of horses with fenbendazole and moxidectin. *Veterinary Parasitology*, 139.1-3. p.115-131.

Stratford, C., Lester, H., Pickles, K., McGorum, B. and Matthews, J., 2013: An investigation of anthelmintic efficacy against strongyles on equine yards in Scotland. *Equine Veterinary Journal*, 46.1. p.17-24.

Stratford, C., McGorium, B., Pickles, K. and Matthews, J., 2011: An update on cyathostomins: Anthelmintic resistance and diagnostic tools. *Equine Veterinary Journal*, 43. p.133-139.

Studzińska, M., Tomczuk, K., Demkowska-Kutrzepa, M. and Szczepaniak, K., 2012: The Strongylidae belonging to Strongylus genus in horses from southeastern Poland. *Parasitology Research*, 111.4. p.1417-1421.

Tarigo-Martinie, J., Wyatt, A. and Kaplan, R., 2001: Prevalence and clinical implications of anthelmintic resistance in cyathostomes of horses. *Journal of the American Veterinary Medical Association*, 218.12. p.1957-1960.

Taylor, E., 1954. Grazing behaviour and helminthic disease. *British Journal of Animal Behaviour*, 2.2. p.61-62.

Taylor, M., 2004: Macrocyclic Lactones in Antiparasitic Therapy. J. Vercruysse and R.S. Rew, CABI Publishing, 2002. 432pp. £75 (hard) ISBN 08511996175. *The Veterinary Journal*, 167.2. p.120.

Taylor, M.A., Coop, R.L., Wall, R.L., 2016: Veterinary Parasitology. 4th ed. Sussex, Wiley Blackwell. p. vii-312, 525-564.

Thamsborg, S., Leifsson, P., Grøndahl, C., Larsen, M. and Nansen, P., 1998: Impact of mixed strongyle infections in foals after one month on pasture. *Equine Veterinary Journal*, 30.3. p.240-245.

Tirosh Levy, T., Kaminiski-Perez, Y., Mandel Horn, H., Sutton, G., Markovics, A. and Steinman, A., 2015: Prevalence and Risk Factor Analysis of Equine Infestation with Gastrointestinal Parasites in Israel. *Israel Journal of Veterinary Medicine*, 70.3. p.32-40.

Traversa, D., Castagna, G., von Samson-Himmelstjerna, G., Meloni, S., Bartolini, R., Geurden, T., Pearce, M., Woringer, E., Besognet, B., Milillo, P. and D'Espois, M., 2012: Efficacy of major anthelmintics against horse cyathostomins in France. *Veterinary Parasitology*, 188.3-4. p.294-300.

Traversa, D., Iorio, R., Klei, T., Kharchenko, V., Gawor, J., Otranto, D. and Sparagano, O., 2007: New Method for Simultaneous Species-Specific Identification of Equine Strongyles (Nematoda, Strongylida) by Reverse Line Blot Hybridization. *Journal of Clinical Microbiology*, 45.9. p.2937-2942.

Traversa, D., Milillo, P., Barnes, H., von Samson-Himmelstjerna, G., Schurmann, S., Demeler, J., Otranto, D., Lia, R., Perrucci, S., Frangipane di Regalbono, A., Beraldo, P., Amodie, D., Rohn, K., Cobb, R. and Boeckh, A., 2010: Distribution and species-specific occurrence of cyathostomins (Nematoda, Strongylida) in naturally infected horses from Italy, United Kingdom and Germany. *Veterinary Parasitology*, 168.1-2. p.84-92.

Traversa, D., von Samson-Himmelstjerna, G., Demeler, J., Milillo, P., Schürmann, S., Barnes, H., Otranto, D., Perrucci, S., Fragipane di Regalbono, A., Beraldo, P., Boeckh, A. and Cobb, R., 2009: Anthelmintic resistance in cyathostomin populations from horse yards in Italy, United Kingdom and Germany. *Parasites and Vectors*, 2.Suppl 2.

Tydén, E., Enemark, H., Franko, M., Höglund, J. and Osterman-Lind, E., 2019: Prevalence of Strongylus vulgaris in horses after ten years of prescription usage of anthelmintics in Sweden. *Veterinary Parasitology: X*, 2.

Tyrrell, K., Dobson, R., Stein, P. and Walkden-Brown, S., 2002: The effects of ivermectin and moxidectin on egg viability and larval development of ivermectin-resistant Haemonchus contortus. *Veterinary Parasitology*, 107.1-2. p.85-93.

Tzelos, T., Barbeito, J., Nielsen, M., Morgan, E., Hodgkinson, J. and Matthews, J., 2017: Strongyle egg reappearance period after moxidectin treatment and its relationship with management factors in UK equine populations. *Veterinary Parasitology*, 237. p.70-76.

van Doorn, D., Ploeger, H., Eysker, M., Geurden, T., Wagenaar, J. and Kooyman, F., 2014: Cylicocyclus species predominate during shortened egg reappearance period in horses after treatment with ivermectin and moxidectin. *Veterinary Parasitology*, 206.3-4. p.246-252.

Vin Veterinary Drug Handbook, 2017: [online] URL: https://www.vin.com/members/cms/project/defaultadv1.aspx?pId=13468&catId=75984&id=7404968&pcatid=70374&alpha=A&ind=24. Accessed 13 October 2020.

von Samson-Himmelstjerna, G., 2012: Anthelmintic resistance in equine parasites – detection, potential clinical relevance and implications for control. *Veterinary Parasitology*, 185.1. p.2-8.

von Samson-Himmelstjerna, G., 2016: Wurminfektionen beim Pferd: Aktuelle Problematik und Empfehlungen für eine nachhaltige sowie gesundheitserhaltende Kontrolle. *Tierärztliche Umschau*, 71. p.247-256.

von Samson-Himmelstjerna, G., Fritzen, B., Demeler, J., Schürmann, S., Rohn, K., Schnieder, T. and Epe, C., 2007: Cases of reduced cyathostomin egg-reappearance period and failure of Parascaris equorum egg count reduction following ivermectin treatment as well as survey on pyrantel efficacy on German horse farms. *Veterinary Parasitology*, 144.1-2. p.74-80.

von Samson-Himmelstjerna, G., Ilchmann, G., Clausen, P., Schein, E., Fritzen, B., Handler, J., Lischer, C., Schnieder, T., Demeler, J., Reimers, G. and Mehn, P., 2011: Empfehlungen zur nachhaltigen Kontrolle von Magen-Darmwurminfektionen beim Pferd in Deutschland. *Pferdeheilkunde*, 27.2. p.127-140.

von Samson-Himmelstjerna, G., Traversa, D., Demeler, J., Rohn, K., Milillo, P., Schurmann, S., Lia, R., Perrucci, S., di Regalbono, A., Beraldo, P., Barnes, H., Cobb, R. and Boeckh, A., 2009: Effects of worm control practices examined by a combined faecal egg count and questionnaire survey on horse farms in Germany, Italy and the UK. *Parasites & Vectors*, 2.Suppl 2.

Wagner, E. and Tyler, P., 2011: A Comparison of Weight Estimation Methods in Adult Horses. *Journal of Equine Veterinary Science*, 31.12. p.706-710.

Wheeler, L., 2018: *Equine Parasitic Strongyles (From Top To Bottom: Member Of Subfamily Cyathostominae, Strongylus Edentatus, Strongylus Equinus, And Strongylus Vulgaris)*. [image] Available at: <https://www.veterinaryparasitology.com/strongylus.html> [Accessed 8 November 2020].

Wirtherle, N.C., 2003: Untersuchugnen zu Verbreitung von Anthelminthikaresistenzen bei Pferden in Niedersachsen. D.V.M. Dissertation. Tierärztliche Hochschule Hannover.

Wood, E., Matthews, J., Stephenson, S., Slote, M., Nussey, D., 2012: Variation in fecal egg counts in horses managed for conservation purposes: individual egg shedding consistency, age effects and seasonal variation. *Parasitology*, 140.1. p.115-128.

Xiao, L., Herd, R. and Majewski, G., 1994: Comparative efficacy of moxidectin and ivermectin against hypobiotic and encysted cyathostomes and other equine parasites. *Veterinary Parasitology*, 53.1-2. p.83-90.

Yadav, K., Shukla, P., Gupta, D. and Mishra, A., 2014: Prevalence of Gastrointestinal Nematodes in Horses of Jabalpur Region. *Research Journal for Veterinary Practitioners*, 2.3. p.44-48.

Zajac, A. and Conboy, G., (eds.) 2012: Veterinary Clinical Parasitology. 8th ed. Ames, Wiley Blackwell. p.30, 114-127.

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Appendix

1.1 Questionnaire

Umfrage – Eiauscheidungszahlen und Entwurmung

Seite **1** of **2**

1 – 5) Teilnehmende Pferde

Stall: _____

Pferd(e)	Geschlecht	Alter	Letzte Entwurmung Datum + Substanz	Selektiv? ☑ja ☒nein
				☐
				☐
				☐
				☐
				☐
				☐

Weidezugang + hygienische Maßnahmen

6) Weidegang: _____
Stunden/Tag + Monate/Jahr

7) Weiden Rotation: _____
Wann, wie oft, mit anderen Spezies?

8) Weiden abäppeln: _____
Wie, wie oft?

9) Weide Abschleppung: _____
Wann, wie oft, in welchen Wetterbedingungen?

10) Geilstellen Entfernung: _____
Wann, wie oft?

11) Düngen der Weiden: _____
Wann, wie oft, mit welchem Dünger?

Stall Hygiene

12) Ausmisten: _____

13) Desinfektion: _____

14) Einstreu Art: _____

Entwurmungsprotokolle

15) Einheitlich für den ganzen Stall? _____
Wenn nein, Beschreibung des Programmes

16) Häufigkeit und Zeitpunkt des Entwurmens: _____

17) Verbunden mit koprologischen Untersuchungen? Resistenz Tests? _____

18) Rotation von Wirkstoffen? _____

19) Verbunden mit Stall- oder Weiden-hygienischen Maßnahmen? _____

20) Schätzung/Messung des Gewichtes für die Dosierung? _____

Quarantäne Maßnahmen

21) Für Neuzugänge: _____

22) Für temporäre Besuchspferde? _____

Starker Wurm Befall bekannt?

23) Klinische Fälle in den letzten 5 Jahren? : ☐ ja ☐ nein

1.2 Satellite maps used for calculation of stocking density

1.2.1 Stable A

1.2.2 Stable B

1.2.3 Stable C

1.2.4 Stable D

1.2.5 Stable E

1.2.5 Stable F

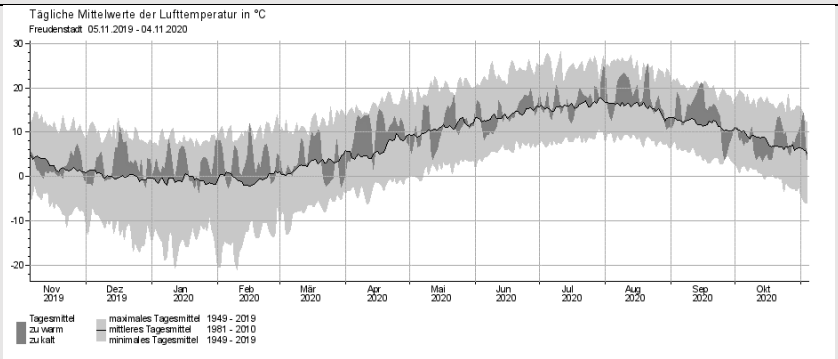
1.3 Hygiene point calculations

Based on the consensus of the literature review and findings of this study, yards received 1 point for management practices deemed sufficient in decreasing the infection pressure originiating from small strongyles as follows:

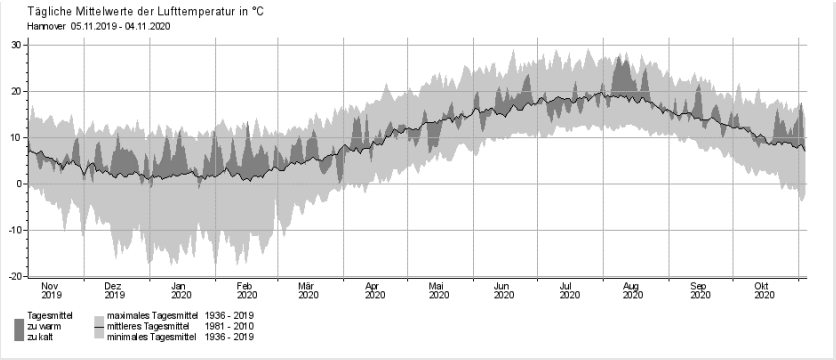
Management practice	**Explanation**	**Points**
Pasture access	Source of infection	0
Removal of fecal matter from pasture	Decreases infectious pressure (min. weekly removal)	1 for performing
Harowing of pastures	Performed in dry weather decreases infectious pressure	1 for performing
Removal of roughs	Increases grazing area thus decreasing rough grazing and infectious pressure	1 for performing

Rotation of pastures	Decreases infectious pressure if pastures left empty for enough time for larvar to die (weather dependent)	1 for performing
Rotation of pasutres with other species	Decreases infectious pressure as strongyle larvae find no hosts and die	1 for performing
Fertilization of pastures with manure	Manure may contain strongyle eggs and larve if not processed sufficiently	1 for not performing
Fertilization of pastures with mineral fertilizer	As contradictory findings in study and literature review not included in this analysis	n.a.
Daily mucking out of stabels	Larvae can develop in a stable environment too thus daily mucking out decreases the contamination and infectious pressure	1 for performing

1.4 Comparison of important climatic parameters for strongyle development and infection sourced from https://www.dwd.de/DE/wetter/wetterundklima_vorort_node.html

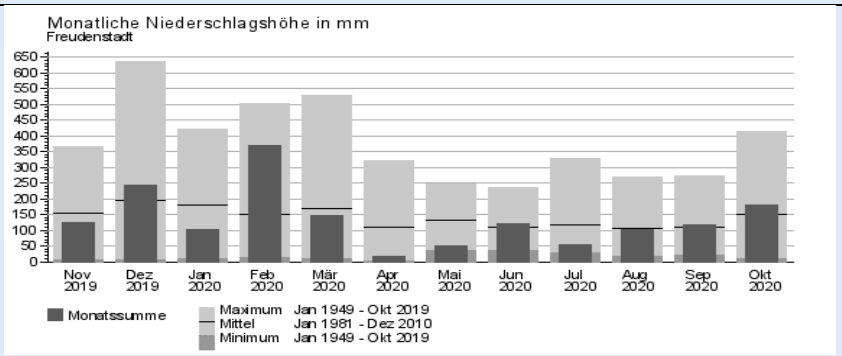
Daily temperature fluctuations in degrees Celsius	
Baden-Württemberg	

Niedersachsen



Monthly rainfall in millimeters

Baden-Württemberg



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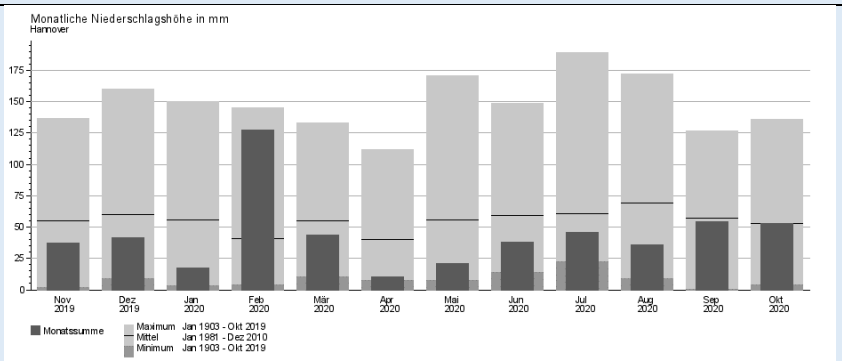
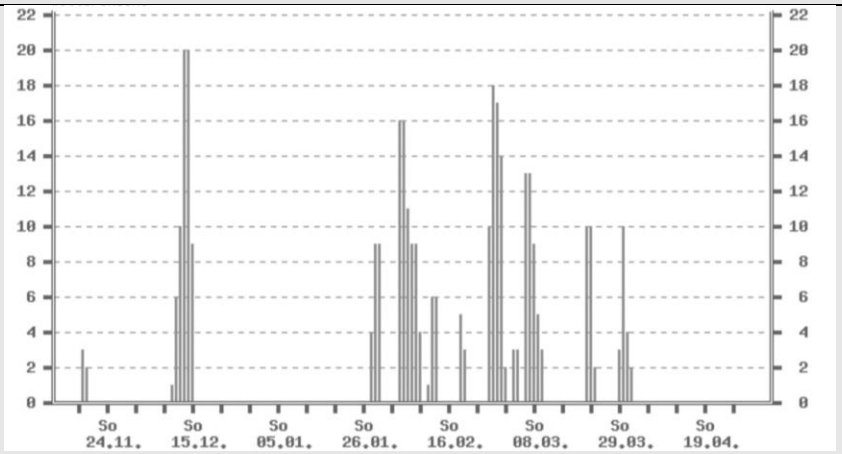


Days of snow cover (cm) over the winter of 2019/2020

Baden-Württemberg



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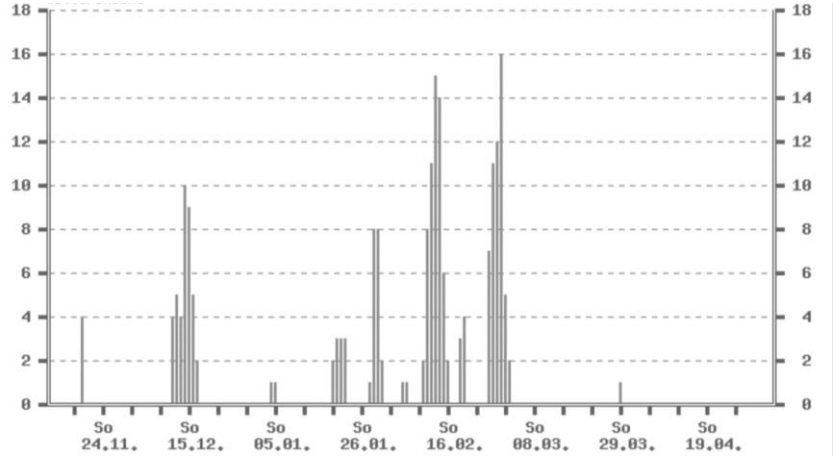

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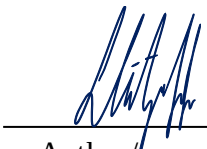

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