Sveriges lantbruksuniversitet
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Faculty of Veterinary Medicine and Animal Science

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Parasitförekomst hos extensivt hållna gotlandsruss

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Abstract

Horses are herbivores that spend almost all day grazing. While grazing they are infected by different endoparasites through ingestion of infective eggs or larvae on pasture. The most significant equine endoparasites in Sweden are the equine roundworm *Parascaris equorum*, small (Cyathostominae) and large strongyles (*Strongylus* spp), the tapeworm *Anoplocephala perfoliata* and the pinworm *Oxyuris equi*. For many years have horses been dewormed on regularly basis, which has resulted in the development of resistance to many anthelmintic classes. Because of the problems with resistance it is since 2007 necessary to have a prescription on anthelmintics and faecal sampling is also recommended. Endoparasites can cause different clinical signs such as weight loss and diarrhea. Therefore it is important to deworm horses with large worm burdens. Depending on what kind of parasite the horse is infected with is there different appropriate anthelmintics. There are three major anthelmintics: benzimidazoles, tetrahydropyrimidines and macrocylic lactones. In this study was the purpose to evaluate how the parasite prevalence is affected by extensive holding of horses on the same pasture for a year. Moreover the horse grazing and defection behavior was evaluated. Twelve one year old stallions of the breed Gotland ponies were held in three enclosures with four horses in each. The horses were kept outside all year round without extra feeding and were included in a bigger project called “russprojektet”. In this master thesis was among others faecal samples analyzed for the equine roundworm *Parascaris equorum*, large and small strongyles as well as the tapeworm *Anoplocephala perfoliata.* The result showed a significant difference between the amount of strongyle eggs during different seasons but no significant differences were seen between horses or enclosures. In addition, it was seen that the horses grazed in an unstructured pattern and that no sign of toilets were observed.

Sammanfattning

Hästar är herbivorer och spenderar större delen av dagen till att beta. Vid betning kan hästen bli infekterad av inälvsparasiter genom intag av infekterade ägg eller larver i betesgräset. Hästens vanligaste inälvsparasiter är spolmask, små och stora blodmaskar samt bandmask och springmask. Under många år har avmaskning av hästar skett på rutin och genom särskilda avmaskningsscheman, vilket har gjort att vissa parasiter har utvecklat resistens mot olika avmaskningsmedel. Med anledning av resistensproblematiken krävs recept sedan 2007 på avmaskningsmedel och träckprov rekommenderas. Inälvsparasiter kan ge en mängd kliniska symptom såsom viktnedgång och diarré. Det är därför viktigt att avmaska om hästen är infekterad med en större mängd parasiter. Beroende på vilken parasit som hästen är infekterad med lämpar sig olika avmaskningsmedel. Det finns idag tre huvudklasser av avmaskningsmedel till häst: benzimidazoler, tetrahydropyrimidiner och makrocykliska laktoner. I denna studie var syftet att undersöka hur parasitprevalensen påverkas av att hästar hålls extensivt på samma bete i ett år och att studera hästarnas betesmönster. Tolv ettåriga hingstar av rasen Gotlandsruss betade i tre hägn med fyra hästar i vardera. Hästarna hölls utomhus året runt utan tillskottsutfodring och ingick i ett större projekt kallat ”russprojektet”. I detta examensarbete analyserades förekomsten av ägg och larver i träck och gräsprover för spolmask, blodmask och bandmask. Resultaten visar att det fanns signifikanta skillnader mellan mängden strongylida ägg under olika årstider men inte mellan hästar och de olika hägnen. Det konstaterades också att hästarna betar enligt ett ostrukturerat mönster och att de inte använder sig av toaletter.

Table of content

1. Introduction

The horse is an herbivore developed to consume a diet consisting of high fiber content (Janis, 1976). It is an excellent grazer because of the moveable upper lip, which makes it possible to graze close to the ground. The horse spends about 16-20 hours grazing every day (Henderson, 2007). During the winter they use their hooves to remove snow before grazing and also the muzzle is used if the amount of snow is low (Salter & Hudson, 1979). During grazing horses may be infected by endoparasites by ingestion of infective parasite eggs or larvae. The most common equine gastrointestinal parasites are the large (*Strongylus* spp.) and small (Cyathostominae) strongyles, the roundworm *Parascaris equorum*, (Kaplan & Vidyashankar, 2012) as well as the tapeworm *Anoplocephala perfoliata* (Dunn, 1978) and the pinworm *Oxyuris equi* (Proudman & Matthews, 2000). Equine endoparasite infections are normally treated with anthelmintics, but a misuse of these drugs have resulted in that both strongyles and roundworms have developed resistance (Herd & Gabel, 1990).

The McMaster technique is a standard method used for quantification of parasite eggs in faecal samples. In the past there has been limited interest among horse owners to rely on this method (Lloyd *et al.*, 2000). Instead the owners have dewormed the horses at regular intervals. Because of the increasing problem with resistance to anthelmintics routine deworming is not advocated today (Kaplan, 2002). To slow down the spread of anthelmintic resistance a well-structured and accurate control program for pasture born parasites are needed. The recommendation in Sweden and in Europe is only to treat heavily infected individuals after faecal diagnosis (Matthee & McGeoch, 2004). From 2007 is it mandatory to have a prescription from the veterinarian before deworming in Sweden (Forshell, 2011; LVFS 2006:11; EU Directive 2001/82/EC).

The aim of this study was to evaluate how parasite exposure is affected by the extensive holding when horses are kept on the same pasture all year round. In addition, the usage of "toilets" during different seasons was studied.

2. Literature study

2.1 Grazing pattern and contaminating of parasite eggs

Horses normally avoid grazing near faeces piles (Ödberg & Francis-Smith, 1977), which decreases the risk to ingest parasite eggs (Medica *et al.*, 1996). A pattern with short grasses without faeces and other areas with higher swards near faeces are noticed (Ödberg & Francis-Smith, 1976). However, in a study by Medica *et al.* (1996) the opposite was seen, where horses seemed to graze and leave their faeces all over the pasture. Lamoot *et al.* (2004) confirmed this more unstructured grazing pattern. If so the exposure to parasite eggs are increased because the horses graze near faeces piles (Medica *et al.*, 1996).

In horses grazing on a contaminated pasture there is normally variations in infection levels between different horses (Duncan & Love, 1991). Even horses that have grazed together since birth can have different numbers of strongyles, even though the horses were exposed on the same pasture (Lyons *et al.*, 2010). In addition, on a pasture with horses of mixed ages do the younger horses normally have more parasites (Duncan & Love, 1991).

2.2 Diagnosis of parasites and deworming practices

The McMaster method for detection of parasite eggs in the faeces is a quantitative counting technique that is routinely used for detection of strongyle and *P. equorum* eggs. This method gives a result in eggs per gram faeces (EPG), where 1000 EPG indicate a heavy infection and 500-1000 EPG a more moderate infection level (Taylor *et al.*, 2007). A cut off level of 200 EPG has been suggested for deworming of Swedish horses (Forshell, 2011). However, even a zero EPG value is no certain sign of a low infection since the horse can be in a developing phase for immunity or newly infected with parasites that have not started to shed their eggs (Taylor *et al.*, 2007). The standard McMaster method is not ideal for detection of tapeworm eggs. Instead a qualitative method based on floatation with a saturated sugar-salt solution with several centrifugations steps is used. There exists several centrifuge/flotation methods but most of them have a low sensitivity (Kjaer *et al.*, 2007; Nielsen, 2012; Williamson *et al.*, 1998). To detect eggs from *Oxyuris equi* a tape test or a scraping around anus can be performed (Reinemeyer & Nielsen, 2013).

The number of anthelmintic classes should ideally be kept down to one drug class per two year. A correct dose is also of major importance to reach the best effect of the anthelmintic drugs. The most important deworming in the year is in May before turn out to keep the contamination of parasite eggs low on pasture. It could be necessary to follow up with deworming during the summer, particularly for younger horses (Forshell, 2011).

2.3 Anthelmintic

There are three major drug classes of anthelmintic commonly used for horses; macrocyclic lactones (ivermectin and moxidectin), benzimidazoles and tetrahydropyrimidines. Resistance to macrocyclic lactones is widespread in the global population of *P. equorum*, including Sweden. Therefore the recommendation is to use fenbendazole or pyrantel pamoat for treatment of parascaris infection (Craig *et al.*, 2007; Ostermann-Lind *et al.*, 2007; Slocombe *et al.*, 2007). On the other hand, benzimidazole resistance is widespread among small strongyles all over the world (Osterman-Lind, 2005; Osterman-Lind *et al.*, 2007). These parasites are also resistant to pyrantel pamoat and the first sign of resistance was seen in 1996 in the USA (Chapman *et al.*, 1996). According to Osterman-Lind *et al.* (2007) does pyrantel still have some efficacy in Sweden, but Höglund *et al.* (2011) discovered resistance against pyrantel in Icelandic horses in 2010. Since Cyathostominae are resistant against benzimidazoles and has reduced efficacy to pyrantel, macrocyclic lactones are the best option for deworming (Craig *et al.*, 2007; Osterman-Lind, 2005; Slocombe *et al.*, 2007). *O. equi* is sensitive against several anthelmintics and can be treated in the same way as strongyles (Proudman & Matthews, 2000). According to Xiao *et al.* (1994) ivermectin is an effective anthelmintic against *O. equi.* No resistance to anthelmintics has been observed for *S. vulgaris* or *A. perfoliata*.

2.4 Equine parasites

2.4.1 Strongyles

Equine strongyles belong to subfamilies Strongylinae and Cyathostominae. There are three larval phases for all strongyles; L_1, L_2 and L_3. The larvae in the two first phases feed on bacteria in soil, while the third stage is infective. The horse is infected by L_3 during grazing (Reinemeyer & Nielsen, 2013).

2.4.2 Strongylus vulgaris

Life cycle

S. vulgaris is a quite large parasite of 1.5-2.5 cm in length as adults. The horse ingests L_3 together with grass on pasture. While L_3 will enter the mucosa of the distal small intestine or large intestine, the adult stage lives in the caecum. After the L_3 has entered the mucosa it will enter the arterioles as L_4 and then migrate below the intimal layer to the cranial mesenteric artery. Sometimes a part of the infected parasites migrate further, as far as the end of the aorta close to the left ventricle (Reinemeyer & Nielsen, 2013). It takes about two weeks for L_4 to migrate to the cranial mesenteric artery where it will stay for at least four months, before it goes back to the large intestine. It takes about 90 days for L_4 to develop into L_5 (Osterman-Lind, 2005). The prevalence of *S. vulgaris* was only 3.6 % in a study by Höglund *et al.* (1997), whereas Osterman-Lind *et al.* (1999) observed a prevalence of 4.8 %. Around 6 months after infection the females start to reproduce, but it can take as long as 10-12 months as well (Osterman-Lind, 2005).

Impact

S. vulgaris is the most pathogenic parasite of horses, due to the location of the late larval stages in the cranial mesenteric artery. This extremely harmful parasite cause arteritis and induce thrombi that may lead to infarction (Osterman-Lind, 2005).

2.4.3 Cyathostominae

Life cycle

Cyathostomes are normally smaller than the *S. vulgaris* but some species can be up to 2.5 cm long (Osterman-Lind, 2005). After the horse has ingested L_3 of Cyathostominae, L_3 enter the mucosa or submucosa of the colon and cecum. Some of the species regarding Cyathostominae does not go any further than the mucosa, while a part of the parasites encyst in the submucosa. The L_3 entering the large intestine are still in an early phase and will after entering the mucosa form a fibrous capsule, and become encysted. L_3 then develop to L_4 which after excystment will migrate back to the intestinal lumen. Here they develop into L_5, which is the adult phase (Osterman-Lind, 2005). Different species are found in the ventral colon, dorsal colon or in the caecum. Parasites in the large intestine are on the way out of the host (Reinemeyer & Nielsen, 2013). Cyathostominae may become arrested in development before reaching the adult stage. As adults Cyathostominae can probably live for three to four months (Reinemeyer & Nielsen, 2013). Most horses that have been on grass are infected with Cyathostominae. Höglund *et al.* (1997) investigated the parasitic burden for 461 horses after slaughter and saw an overall prevalence of 53.7 % in 1-5 year old horses. The highest prevalence was found between July to September and the overall prevalence for strongyle was 78.4 %. After the horse are infected with L_3 it takes around 2-3 months for them to start reproducing (Osterman-Lind, 2005).

More than 40 different species have been described. The most common species in Sweden are *Cylicostephanus longibursatus, Cylicocyclus nassatus, Cyathostomum catinatum, Cylicocyclus leptostomus*, *Cylicostephanus minutus* and *Cylicostephanus calicatus* (Osterman-Lind, 2005).

Impact

Cyathostominae are the most common parasite and of most importance since the number of *Strongylus vulgaris* has been decreased due to good deworming and the remaining parasites that exists is either not so harmful or only exists in youngsters (Love *et al.,* 1999). Deworming practices could result in mass release of encysted larval phases from the gut mucosa. The parasite can cause lesions in the intestines. Most lesions are found in the caecum and ventral colon, while the dorsal colon is often unaffected. A horse can exhibit chronic cyathostominosis with clinical symptoms such as diarrhea, weight loss, colic, pot-belly and gloomy fur (Reinemeyer & Nielsen, 2013). A horse suffering from cyathostominaeosis gets severe enterocolitis, which occasionally may lead to the death of the host (Lichtenfels *et al.,* 2002).

2.4.4 *Parascaris equorum*

Life cycle

The equine roundworm belongs to Ascaridoidea and is the largest equine nematode. It can be as large as 50 cm long (Reinemeyer & Nielsen, 2013). Eggs become infective inside the egg shell after approximately two weeks in the external environment (Taylor *et al.,* 2007). The infective eggs are easily ingested during grazing because they have a sticky outer egg membrane that attach to grass and other surfaces. The sticky protein will be dissolved once the parasite egg has entered the stomach and small intestine because of the acidic and basic environment. After hatching the larvae penetrate the lining of the small intestine and then migrate to the liver. Almost all larvae are found in the liver 2-7 days after infection. After 14 days, the larvae reach the lungs and start to migrate up the bronchi and trachea. The larvae are then swallowed, and thereby return to the small intestine as L_4 (Clayton, 1986). The larvae moult into the final L_5 stage and soon develop to adult worms (Bliss, 2007), which are reproductive after 75 to 80 days (Reinemeyer, 2009). During host migration the larvae cause damage by causing inflammation and lesions (Reinemeyer & Nielsen, 2013). The female worms lay eggs within 7 to 14 days after it has reached the adult stage and the eggs are passed into the environment within the faeces (Taylor *et al.,* 2007). Eggs of *P. equorum* was only found in 8 of 412 horses, which gave a prevalence of 1.9 % among horses 1-30 years old (Höglund *et al.,* 1997). Among foals the prevalence was 48 % on horse farms (Osterman-Lind & Christensson, 2003).

Impact

P. equorum mostly infect the foals and young horses. Most horses develop age resistance against the parasite around an age of 18 months. Even though there are horses over 18 months that have eggs in the faeces and the reason behind this is not yet fully understood (Reinemeyer & Nielsen, 2013). An effect of the infection in younger horses is that they tend to lose weight and become emaciated (Taylor, *et al.,* 2007).

P. equorum can harm several organs due to the tissue migration inside the horse (Reinemeyer & Nielsen, 2013; Taylor *et al.,* 2007) and induce clinical signs such as coughing and nasal discharges (Taylor *et al.,* 2007). Even though the *P. equorum* does not attach to the intestinal mucosa it feed on nutrients in the intestinal contents and this may be associated with dull fur, loose faeces and weight loss (Clayton *et al.,* 1980). Fever and colic can also be seen due to the infection. Horses with a high parasitic burden can develop enteritis associated with diarrhea and constipation (Taylor *et al.,* 2007). Furthermore, massive infections can block the small intestine and thereby cause perforation leading to peritonitis (Taylor *et al.,* 2007). This risk may occur when large burdens of adult worms are killed after deworming (Reinemeyer & Nielsen, 2013).

2.4.5 *Anoplocephala perfoliata*

Life cycle

The equine tapeworm *A. perfoliata* is a cestode that rely on an intermediate host and can as adults in the intestine reach 4-8 cm in length. The eggs are ingested by free-living oribatid mites that lives for more than one season. The horse is infected by ingestion of mites during grazing (Reinemeyer & Nielsen, 2013; Taylor *et al.,* 2007). *A. perfoliata* egg shedding is intermittent (Reinemeyer & Nielsen, 2013). The prepatent period is approximately 6-10 weeks (Williamson *et al.,* 1998). A prevalence of 29 % was found in a Danish study (Kjaer *et al.* 2007) whereas it was 65 % in Sweden (Nilsson *et al.* 1995).

Impact

Horses were examined for *A. perfoliata* after euthanasia but only half of the infected horses shed eggs in the faeces (Kjær *et al.*, 2007). Proudman *et al.* (1998) found a connection with intestinal impaction colic and spasmodic colic and *A. perfoliata* infection. High infection levels are a risk for spasmodic colic (Back *et al.*, 2013; Proudman *et al.*, 1998). According to Williamson *et al.*, (1997) 81 % of worms were found at the predilection site at ileocaecal junction. Here the worms cause ulcerations at the site of scolex attachment. Horses that suffer from infections due to *A. perfoliata* may also suffer from oedema, diphtheritic inflammation and congestion at the worms' attachment place (Williamson *et al.*, 1997).

2.4.6 *Oxyuris equi*

Life cycle

The pinworm *O. equi* is a nematode parasite in the superfamily Oxyuroidea, which can be up to 5-8 cm long. This parasite differs from most other equine nematodes since the worm does not shed any eggs into the faeces, instead the female deposit her eggs at the skin around the anus. Eventually the skin dry and fall of, which will leave the eggs also in the surroundings (Reinemeyer & Nielsen, 2013). Adult *O. equi* normally lives in the lower part of the colon near the rectum. The adults stay close to the anus and it happens that female worms are found hanging out from the anus (Reinemeyer & Nielsen, 2013; Taylor *et al.*, 2007). Only after a few days they will develop to L_3. Eggs stick to the surroundings when the horse rubs its tail. After ingestion of the eggs L_3 are released in the small intestine where it matures to L_4 in the mucosa of the cecum and colon (Taylor *et al.*, 2007). Finally the parasite becomes adult and moves more distally to rectum (Reinemeyer & Nielsen, 2013). It will take about five months after infection until pinworms reach the adult stage (Taylor *et al.*, 2007). The prevalence of *O. equi* was 36 % in a Polish study (Gawor, 1995).

Impact

In most of the cases pinworm infections are subclinical. Still infected horses may suffer from an intense itching around anus, which may lead to loss of hair and inflamed skin. The loss of hair and the dull fur has been called "rat-tail". Because of the itching the horse get restless which is associated with a reduced feed intake, which can result in lower condition. When horses are heavily infected the larvae can cause lesions also in the mucosa, which lead to more severe inflammations (Taylor *et al.*, 2007).

2.5 Seasonal differences

A study in Louisiana, USA, found that the percentage of early third-stage Cyathostominae larvae (EL_3) on pasture were low in the autumn and high in the summer. However, adult worms were found to have the opposite patterns, with the highest percentage in the autumn and lowest in the summer. Horses that were two years and older had a significantly higher amount of EL_3 and a lower number of developing larvae (DL) and adult worms compared to one year old horses. Still, no significant differences were observed in the seasonal distribution of Cyathostominae or large strongyles. On the other hand, when calculating faecal egg counts (FEC) a significant difference was found between different seasons, with the highest values during spring and autumn. Most Cyathostominae species were found all year round although some of them existed in various amount throughout the year. Furthermore, the prevalence of both large strongyles and Cyathostominae differed between older and younger animals, and where younger horses were more frequently infected (Chapman *et al.*, 2003).

3. Materials and Methods

3.1 Field study

The Gotland pony project is a multidisciplinary study where different aspects on the animal and the environment are studied. The study is designed to evaluate how extensively held Gotland ponies, influence the composition of the flora and fauna but also how this affect the wellbeing of the horses. In the study I have concentrated on: 1) the composition and alterations in the infection levels with different parasites during one year, and 2) where faeces were deposited on the pasture. My study was done from May 2014 to April 2015.

3.2 Horses

In the study 12 Gotland ponies were held in groups of four on three different pastures near Krusenberg, outside Uppsala, with access to a shelter, water and limestone. All ponies were stallions born in 2013 and brought up in six different stables. After a period of habituation in the same enclosure, the ponies were divided according to origin into different groups, see table 1. Each group were hold on 3 hectares pasture and 7 hectares of forest, see figure 1 and 2.

Table 1. The horses in the enclosures

Enclosure 1	Breeder	Enclosure 2	Breeder	Enclosure 3	Breeder
Bork	Hjelm	Wiper	Svensson	Messer	Lojsta Hed
Brigg	Lojsta Hed	Hugo	Södergren	Birk	Hjelm
Hampe	Södergren	Qåtten	Lojsta Hed	Tulin	Larsson
Thrim	Larsson	Äskil	Krantz	Picasso	Svensson

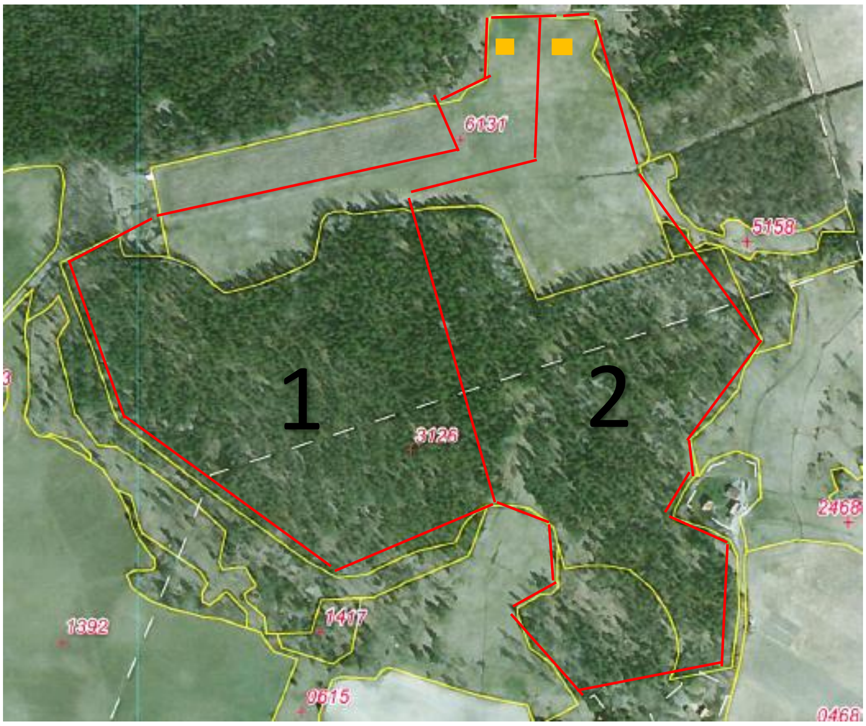

Figure 1. Enclosure 1 and 2. The orange squares show the location of the wind shelters.

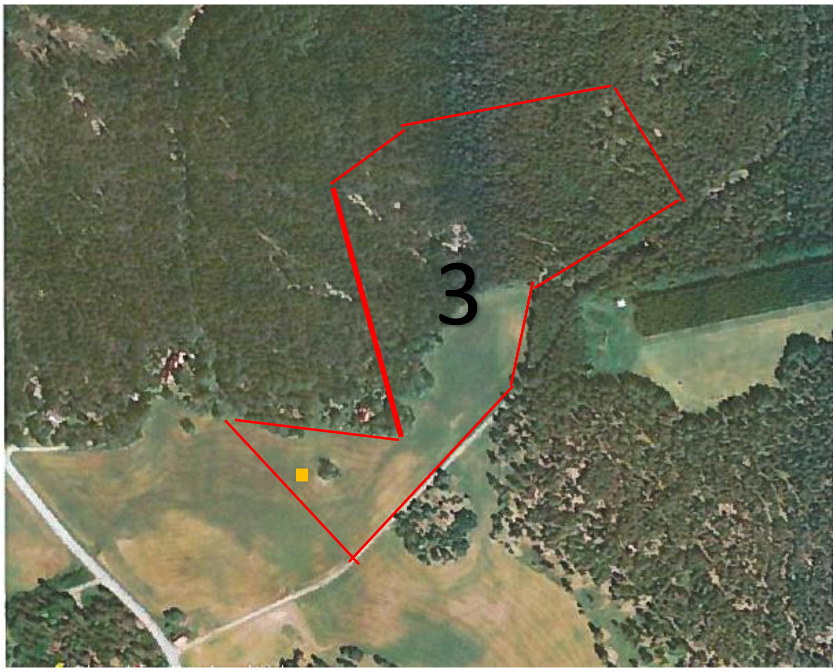

Figure 2. Picture of enclosure 3. The orange square depict the wind shelter.

3.3 GPS-location of faecal pats/piles

The open areas in each enclosure were walked in a "zig-zag" pattern. A GPS-marking was made, with a Garmin Dakota 10, if at least four faeces piles were observed within a 2 meter radius. The number of piles was recorded in numbers of five (five, ten, fifteen and so on). This procedure was done on six occasions in July, August, October, December, February and April.

On each sampling occasion a map was constructed using qGIS 2.6.1 based on the GPS codes. The number of piles in each GPS point was marked with different colors.

- Red=5 faeces piles
- Yellow=10 faeces piles
- Purple=15 faeces piles
- Turquoise=20 faeces piles
- Pink=25 faeces piles
- Green=30 faeces piles

3.4 Pasture sampling

Grass samples were collected in December. In each paddock samples were taken when walking in two w-patterns, every 20th step four samples were taken. Two samples of approximately 270 gram of grass from each paddock were weighed. These samples were put in water in two large Baermann funnels, for each paddock. The funnels were equipped with a fine sieve and before it was filled with water the rubber tube was sealed with a clip, see figure 3. The following morning were 50 ml samples collected from the funnels. The tubes were stored at 6-8 °C until DNA was extracted for PCR-analyze. After sampling the grass were dried for four days at 60 °C and weighed.

Figure 3. Large Baermann funnels for collection of larvae in grass. Photo by Johanna Karlsson.

3.5 Faecal samples

Faeces were collected from most horses at monthly intervals (from May 2014 until April 2015).

3.6 Laboratory methods

3.6.1 McMaster method

Jars were marked with the horse's name and a number for recognition. Faeces in plastic bags were smashed and a subsample of 3 g were taken and mixed with 42 ml of cold water. The samples were then poured through a sieve with an aperture of 150 µm. The sieved fluid was poured into a test tube and centrifuged at 1500 rpm for 3 min. The supernatant was removed using a vacuum pump and replaced with saturated NaCl and mixed by the use of a pipette. Both sides of a McMaster chamber were filled and the number of eggs was counted at a magnification of 40 ×. Eggs per gram (EPG) were counted using the formula: $\frac{n_{eggs} \times 100}{2}$

3.6.2 Flotation method

A sample of 30 g faeces were mixed with 60 ml water and poured through a sieve with 150 µm openings. The liquid were split in four different tubes and centrifuged at 1000 g for 10 min. The supernatant was removed and the pellet was vortexed. The tubes were filled with a saturated sugar-salt solution so a meniscus was formed. A cover glass were placed on the top of the tubes and centrifuged for 5 min at 214 g. The cover glasses with the fluid attached were after 5 min carefully lifted and placed on objects glasses and viewed in a microscope.

3.7 Tape test

Since pinworms were discovered in the faeces on the pasture was a tape test done. This was performed by putting a piece of tape close to anus and then pressed on an object glass. The tape was then examined for the typical eggs under a microscope at 40 × magnification.

3.8 Deworming

All horses were dewormed in May and September with ivermectin at 0.2 mg/kg body weight respectively pyrantel pamoate at 19 mg/kg body weight. These doses are the recommended ones against nematodes and were given to horses weighing 300 kg. The first deworming was conducted during the habituation period and the second after the horses had been separated into the different groups and were grazing in the enclosures. Furthermore, deworming against tapeworms were performed in October with the doubled dose of pyrantel pamoate (eg. 0.4 mg/kg body weight) for Hampe and Äskil. Due to *O. equi*, horses were also dewormed in January and February. The horses in enclosure 1 were dewormed in January with pyrantel pamoat at 19 mg/kg body weight and enclosure 2 with fenbendazole at 7.5 mg/kg body weight. In February was Hampe (enclosure 1) and all horses in enclosure 3 dewormed with pyrantel pamoat at 19 mg/kg body weight. Thrim was dewormed with pyrantel pamoat in February at 19 mg/kg body weight.

3.9 Larval cultures

Larval cultures were done for species identification of strongyle infections. Faeces were put in plastic buckets (Fig 4), and mixed with vermiculite and moisten with water to keep the mixture aerated and humid. Larvae were cultured for one week at room temperature. After one week each bucket was filled with water and put upside down on a glass tray that was filled with water. The following morning or later the same day the liquid with larvae on the glass tray was harvested.

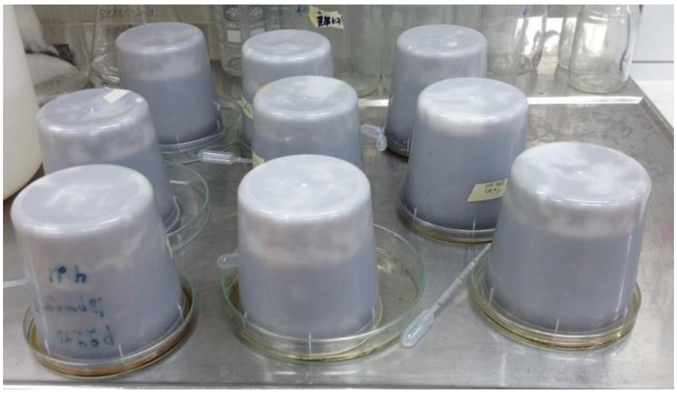

Figure 4. Larvae cultures. Photo by Johanna Karlsson.

3.10 Molecular species identification

To identify strongyle species after polymerase chain reaction (PCR), DNA purification was first performed.

3.10.1 Day 1

Strongyle larvae from the cultures were after thawing centrifuged for a short time and water were sucked out with a pipette. The larvae sample was grinded with a mortar and 180 µl Buffer ATL was added. In the next step 20 µl proteinase K was added and the liquid was vortexed. The tubes were incubated overnight at 56 °C on a rocking platform. For the pasture samples, the water was sucked out before 180 µl Buffer ATL was added. The whole sample was moved to Eppendorf tubes by a pipette and grind with a mortar. All the next steps were the same as for the cultured larvae.

3.10.2 Day 2

First, 200 µl Buffer AL was added to the tubes, mixed by pulse-vortexing and incubated for 10 min at 70 °C. Secondly, 200 µl ethanol was added and pulse-vortexed for 15 sec. The liquid was put into QIAamp mini span column. The tubes were centrifuged at 8000 rpm in one minute and QIAamp mini span column was placed in clean collection tubes. The filtrate was thrown (old collection tube). Added 500 µl Buffer AW1 to the QIAamp mini span column and centrifuged the tubes in one minute at 8000 rpm. Placed the QIAamp mini span column in new collection tubes and discarded the collection tubes. When was 500 µl Buffer AW2 added and centrifuged at full speed in three minutes. Centrifuged the QIAamp mini span column in a new collection tube to get rid of liquids. The QIAamp mini spin column was put in Eppendorf tubes and the collection tubes were thrown. Finally was 50 µl Buffer AE added, incubated for one minute in room temperature and at last centrifuged in 8000 rpm in one minute. The last step was repeated. The Eppendorf tubes were centrifuged between all steps to remove droplets from the inside of the lid.

3.11 Primer design

Primers targeting the Internal Spacer Region 2 (ITS-2) were designed to identify the most common Cyathostominae. Primers were designed for the six most commonly found Cyathostominae. One common forward primer was used for all samples, one general reverse primer together with six unique reverse primers. Primer 47/48 was used for detection of *Cylicostephanus minutus* and *Cylicocyclus nassatus*, 38/39 for *Cylicostephanus calicatus* and *Cyathostomum catinatum*, 45 for *Cylicocyclus leptostomus* and 46 for *Cylicostephanus longibursatus*, see table 2 and appendix 1. Furthermore, primer combinations for detection of *S. vulgaris* were designed according to Nielsen *et al.* (2012).

Table 2. Primers for analyze of Cyathostominae

Cyathostominae	Accession number	Sequence	TM (°C)	PCR product size (µl)
General forward		AAGGTGTTGTATCCAGTAGAG	55.9	-
General reverse		AAAACACCCCCTAGACAGGA	57.3	25
Cylicostephanus minutus & *Cylicocyclus nassatus*	AJ223347.1 & AJ223348.1	CCGCACTTAGCTATTTTTCCT	55.9	25
Cylicostephanus calicatus & *Cyathostomum catinatum*	AJ223338.1 & AJ223339.1	GAACGAAGCAAATACATTTTCTAT	54.2	25
Cylicocyclus leptostomus	AJ223345.1	GAATAGCCCCGTGTTTGGCA	59.4	25
Cylicostephanus longibursatus	AJ223346.1	TCCATTGAAAACACTCACACA	54.0	25

3.12 Polymerase Chain Reaction (PCR)

In the PCR the master mix contained: water, 2.5 µl 10x buff (without $MgCl_2$), 2 µl $MgCl_2$ (25 µM), 0.4 µl BSA (10 mg/ml), 0.65 µl dNTP (10 µM), 0.3 GOLD A. Taq, as well as each of 0.5

µl forward and reverse primer. The amount of water added depended on how much DNA was added to the master mix, but the total volume was always 25 µl. The primers were mixed with water to form a concentration of approximately 20 pmol/µl. The PCR was run for 5 min at 95 °C, denaturation for 30 sec at 95 °C, annealing for 45 sec at 52 °C, extension for 60 sec at 72 °C, and final extension for 3 min at 72 °C and finally at 4 °C. The denaturation, annealing and extension steps were run for 40 cycles. The annealing temperature was raised to 55 °C in the PCR designed for identification of *S. vulgaris*. For all samples except seven, were 5 µl DNA used and in those seven were instead 10 µl DNA used since it was a lower concentration of DNA. The pasture samples were only run with the general reverse primer.

3.13 Gel electrophoresis

Agarose (1.6 %) was prepared from 2.4 g agarose powder mixed with 150 ml TBE and boiled for approximately 1.5 min in a microwave. A gel was formed by mixing about 50 or 75 ml of the agarose solution with 5 or 7.5 µl Gelred and poured into a tray with a comb to produce small wells for the samples (Fig 5). After about 10 min when the gel has solidified the comb was removed. Then 9 µl PCR product was mixed with 3 µl loading buffer (LB) on parafilm before the sample was loaded to the wells in the gel. A 100 bp ladder was used at an amount of 2 µl when the Cyathostomines were examined, whereas both 50 bp and 100 bp ladders were used for *S. vulgaris*. The gel was run for 30 min at 100 V. Finally, the gel was examined with UV-radiation and photographed.

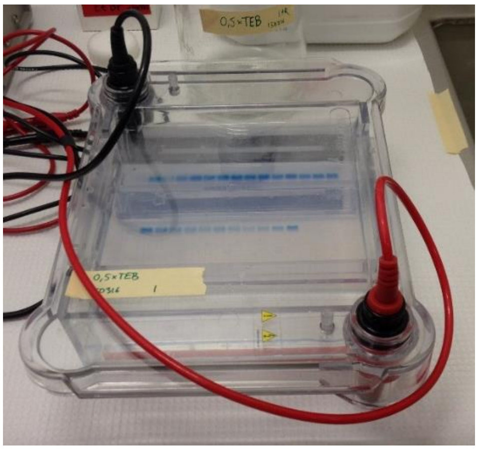

Figure 5. Electrophoresis tray loaded with a gel and connected to 100 volt. Photo by Johanna Karlsson.

3.14 Statistical analysis

Data from the EPG result of strongyle eggs was analyzed with 1way ANOVA in GraphPad Prims version 5. To evaluate the pasture contamination of strongyle FEC from all horses and sampling occasions were compared. EPG data from each enclosure was compared with each other. To evaluate differences in strongyle egg counts between different seasons; May-August, September –December and January-April EPG data from all enclosures were compared. To evaluate if there was a significant difference in egg count among the horses all EPG data for each horse during one year was compared to each other. A 1way ANOVA was made to see if there was significant difference between the horses' EPG values. The significance level was 0.05 and the data was presented as box plots.

4 Result

4.1 Faeceal samples - strongyles

The results of the FEC based on McMaster counting are shown in figure 6 and tables 3 to 5. As seen in figure 6 the results vary both between horse and season. The effect of the anthelmintic treatment is clear, as the EPG levels were decreased after deworming. The result from the flotations are shown in table 6. Some of the horses had zero FEC but the results of the flotation show that they were shedding eggs. Typical strongyle eggs are shown in appendix 2, figure 22.

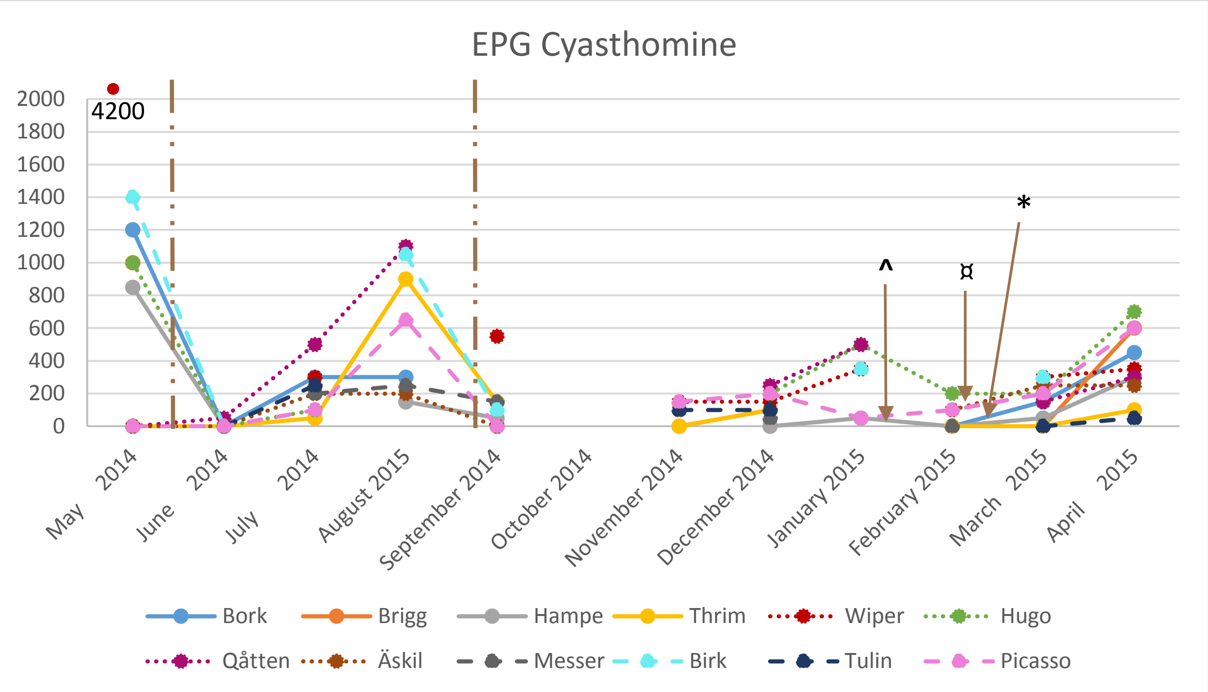

Figure 6. Strongyle egg counts for all horses. The brown line indicates when all horses were dewormed the first time in May with ivermectin and the second time in September with pyrantel pamoate. ^ indicate deworming of horses in enclosure 1 with pyrantel and enclosure 2 with fenbendazole. ¤ indicate deworming of Hampe and all horses in enclosure 3 with pyrantel. *Thrim was dewormed with pyrantel pamoate. Deworming ^ ¤ * was due to infection whit. *O. equi*.

In enclosure 1 (table 3) deworming with ivermectin in May was effective as no strongyle eggs were observed in June. The deworming with pyrantel pamoate in September was not fully effective, but still decreased FEC. The deworming with pyrantel pamoate in January and February against *O. equi* was not completely effective against the strongyles. Bork had the highest mean value egg count (343) while Thrim had the lowest mean value (135).

Table 3. Strongyle egg counts of horses in enclosure 1. Deworming was performed with ivermectin 21/5-2014, pyrantel pamoate 2/9-2014 and in October of Hampe and also the 26/1-2015. In addition, Hampe was dewormed with pyrantel 16/2-2015, and Thrim with pyrantel pamoate 24/2-2015

Horse	May 2014	June 2014	July 2014	Aug 2014	Sep 2014	Oct 2014	Nov 2014	Dec 2014	Jan 2015	Feb 2015	March 2015	April 2015
Bork	1200	0	300	300	*	*	*	*	*	0	150	450
Brigg	1000	*	50	*	0	*	0	100	*	0	100[1]	600
Hampe	850	0	*	150	50	*	*	0	50	50[1]	50	300
Thrim	0	0	50	900	150	*	0	100	*	50[1]	0[1]	100
Mean value	763	0	133	450	67		0	67	50	25	75	363

[1]Temporary excluded from project * No faeces sample analyzed

In enclosure 2 (table 4) the deworming with ivermectin in May was effective against the strongyles except for Qåtten who had an increased FEC in June. The deworming with pyrantel pamoate in September was at least not effective for Wiper. Furthermore, the deworming with fenbendazole in January against *O. equi* was not effective against strongyles. Wiper had the highest mean value egg count (794) mostly due to his high FEC in May and Äskil had the lowest egg count (122). Wiper is the only horse who never had an egg count of zero during these twelve months.

Table 4. Strongyle egg counts of horses in enclosure 2. Deworming with ivermectin 21/5-2014, deworming with pyrantel pamoate 2/9-2014 and in October of Äskil, deworming with fenbendazole 26/1-2015

Horse	May 2014	June 2014	July 2014	Aug 2014	Sep 2014	Oct 2014	Nov 2014	Dec 2014	Jan 2015	Feb 2015	March 2015	April 2015
Wiper	4200	*	300	*	550	*	150	150	350	*	300	350
Hugo	1000	0	100	*	*	*	*	200	500	200	200	700
Qåtten	0	50	500	1100	*	*	*	250	500	*	150	300
Äskil	0	0	200	200	0	*	100	*	*	100	250	250
Mean value	1300	17	275	650	275		125	200	450	150	225	400

*No faeces sample analyzed

As shown in table 5, deworming of the horses in enclosure 3 with ivermectin was effective for treatment of Cyathostominae. Deworming with pyrantel pamoate in beginning of September was also associated with decreased number of eggs but was not as efficient as ivermectin. Similarly, deworming against *O. equi* with pyrantel pamoate in February was inefficient against the strongyles. Birk had the highest mean value FEC (471) and Tulin the lowest (83).

Table 5. EPG Strongyle egg counts of horses in enclosure 3. Deworming with ivermectin 21/5-2014, deworming with pyrantel pamoate 2/9-2014, deworming with pyrantel pamoate 16/2-2015

Horse	May 2014	June 2014	July 2014	Aug 2014	Sep 2014	Oct 2014	Nov 2014	Dec 2014	Jan 2015	Feb 2015	March 2015	April 2015
Messer	0	*	200	250	150	*	*	50	*	*[1]	*	50
Birk	1400	0	*	1050	100	*	100	*	350	*	300	*
Tulin	*	0	250	*	*	*	100	100	*	*	0	50
Picasso	0	0	100	650	0	*	150	200	50	100	200	600
Mean value	467	0	183	650	83		117	117	200	100	167	233

[1]Temporary excluded from project *No faeces sample analyzed

In September Brigg, Äskil and Picasso had no Cyathostominae eggs counts according to the McMaster method but according to the sugar-salt flotation (table 6), they were still infected. In November were both Brigg and Thrim infected with Cyathostominae according to table 6 but had an FEC of 0. In April all tested horses shedded Cyathostominae eggs.

Table 6. Results of flotation of strongyle eggs with saturated sugar-salt

Horse	September 2014	November 2014	April 2015
Bork	*	*	X
Brigg	X	X	X
Hampe	X	*	X
Thrim	X	X	X
Wiper	X	X	X
Hugo	*	*	X
Quåtten	*	*	X
Äskil	X	X	X
Messer	X	*	X
Birk	X	X	*
Tulin	*	X	X
Picasso	X	X	X

*No faecal samples analyzed X Positive sample
Deworming with ivermectin 21/5-2014, deworming with pyrantel pamoate 2/9-2014 and in October of Hampe and Äskil, deworming with pyrantel pamoate 26/1-2015 in enclosure 1, deworming with fenbendazole 26/1-2015 in enclosure 2, deworming of Hampe and enclosure 3 with pyrantel pamoate 16/2-2015, Thrim dewormed with pyrantel pamoate 24/2-2015.

4.1.2 Statistical analyses

Variation between enclosures

There was no differences in strongyle eggs counts ($p = 0.13$) between the different enclosures (Figure 7). Enclosure 2 had the highest mean value egg count (400) and enclosure 3 had the lowest (188).

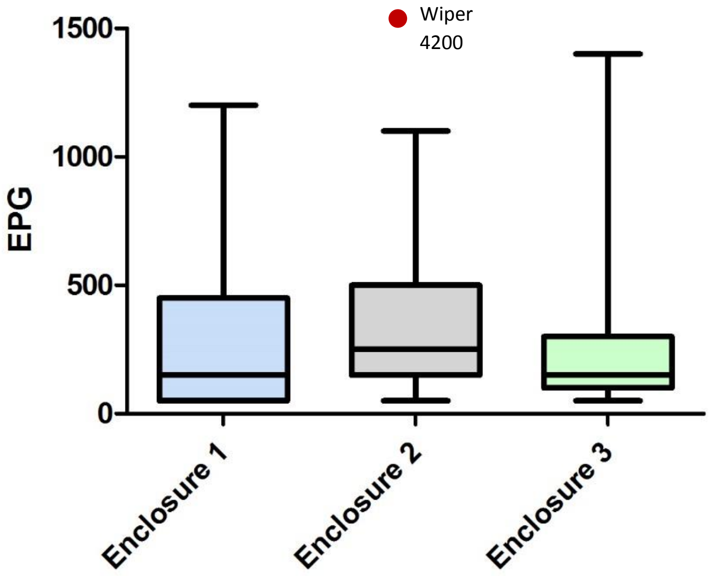

Figure 7. Differences in strongyle egg counts between enclosures. All data collected during the whole year was compared. Wiper's sample from May was excluded from the graph because of the high FEC.

Seasonal variations

There was a significant (p = 0.0197) difference between seasons with the highest egg counts observed between May and August (Figure 8). May-August had the highest egg counts while September-December had the lowest.

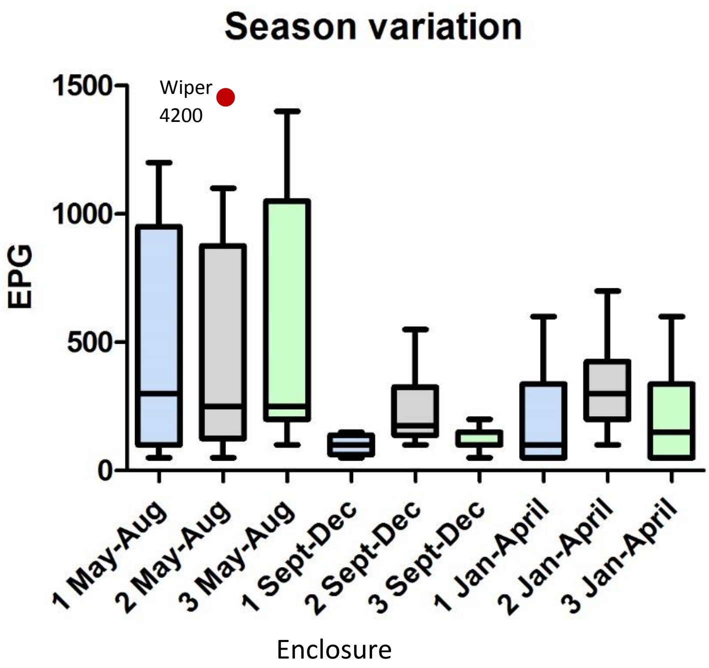

Figure 8. Seasonal differences in strongyle egg counts. Wiper's sample from May was excluded from the graph because of the high FEC.

Variation between horses

The mean EPG values varied between 83 and 471 EPG. The highest values was observed for Wiper in enclosure 2 and the lowest for Tulin in enclosure 3. However, there was no significant (p = 0.1465) difference between the different horses.

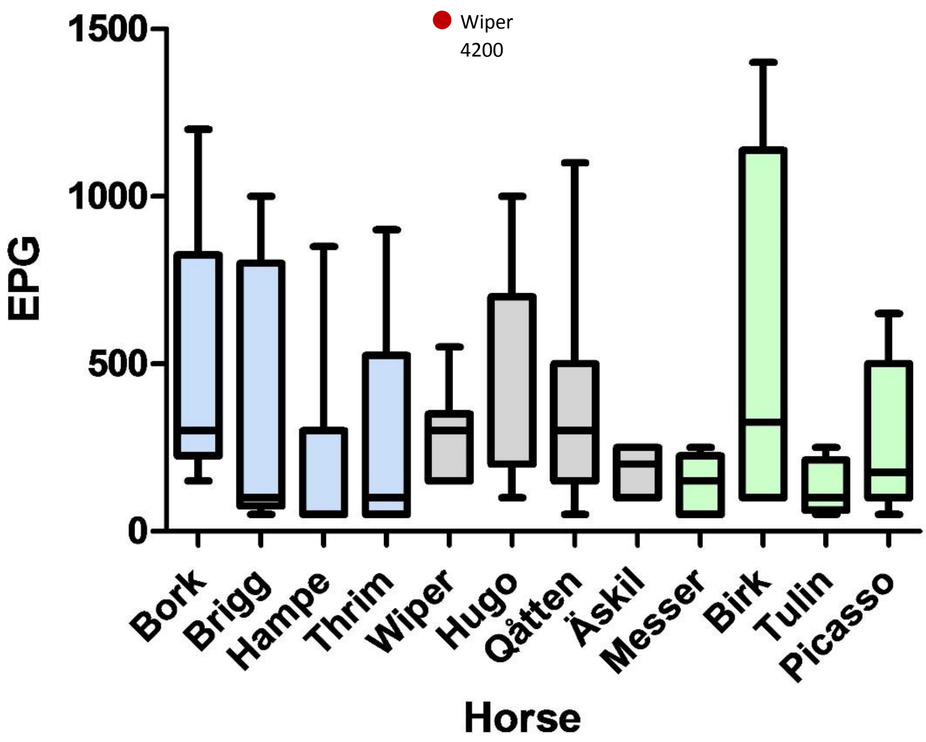

Figure 9. Difference between horses. Wiper's sample from May was excluded from the graph because of the high FEC.

4.2 Faecal samples – *Parascaris equorum*

Only 6 horses (50 %) shedded *P. equorum* eggs at any time during the study. In general, there was a clear trend of decreasing egg counts with increasing age of the horses (Table 7). However, Bork, Qåtten, Wiper and Äskil shedded a few eggs after the initial examination in May 2014. Äskil did still have an egg count of 50 in March. A photo of typical *P. equorum* eggs are shown in appendix 2, figure 21.

Table 7. FEC of *Parascaris equorum*

Horse	May 2014	June 2014	July 2014	Aug 2014	Sep 2014	Oct 2014	Nov 2014	Dec 2014	Jan 2015	Feb 2015	March 2015	April 2015
Bork	200	0	0	50	*	*	*	*	*	0	0	0
Brigg	0	*	0	*	0	*	0	0	*	0	0	0
Hampe	250	0	*	0	0	*	*	0	0	0	0	0
Thrim	0	0	0	0	0	*	0	0	*	0	0	0
Wiper	100	*	0	*	0	*	0	50	0	*	0	0
Hugo	50	0	0	*	*	*	*	0	0	0	0	0
Qåtten	0	0	0	50	*	*	*	0	0	*	0	0
Äskil	0	0	0	0	0	*	0	*	*	50	50	0
Messer	0	*	0	0	0	*	*	*	*	*	*	0
Birk	0	0	*	0	0	*	0	*	0	*	0	*
Tulin	*	0	0	*	*	*	0	*	*	*	0	0
Picasso	0	0	0	0	0	*	0	*	0	0	0	0

*No faecal samples analyzed
Deworming with ivermectin 21/5-2014, deworming with pyrantel pamoate 2/9-2014 and in October of Hampe and Äskil, deworming with pyrantel pamoate 26/1-2015 in enclosure 1, deworming with fenbendazole 26/1-2015 in enclosure 2, deworming of Hampe and enclosure 3 with pyrantel pamoate 16/2-2015, Thrim dewormed with pyrantel pamoate 24/2-2015.

Table 8 shows the results of the flotation test for *P. equorum* eggs. Like with the strongyle eggs some of the horses with a zero egg count according to the McMaster results were egg positive in the flotation. In September Brigg, Hampe, Thrim, Wiper and Birk shedded *P. equorum* eggs despite they were all negative according to the McMaster method. Similarly, in November were Thrim, Äskil, Birk, Tulin and Picasso infected with *P. equorum* (table 8), although no eggs were observed with McMaster (table 7). Furthermore, Wiper and Äskil were infected with *P. equorum* in April (table 8) but had a zero FEC.

Table 8. Results of flotation of *P. equorum* eggs with saturated sugar-salt

Horse	September 2014	November 2014	April 2015
Bork	*	*	-
Brigg	X	-	-
Hampe	X	*	-
Thrim	X	X	-
Wiper	X	-	X
Hugo	*	*	-
Quåtten	*	*	-
Äskil	-	X	X
Messer	-	*	-
Birk	X	X	*
Tulin	*	X	-
Picasso	-	X	-

*No faecal samples analyzed X Positive sample - Negative sample
Deworming with ivermectin 21/5-2014, deworming with pyrantel pamoate 2/9-2014 and in October of Hampe and Äskil, deworming with pyrantel pamoate 26/1-2015 in enclosure 1, deworming with fenbendazole 26/1-2015 in enclosure 2, deworming of Hampe and enclosure 3 with pyrantel pamoate 16/2-2015, Thrim dewormed with pyrantel pamoate 24/2-2015.

4.3 Faecal sample – *Anoplocephala perifoliata*

Table 9 shows the result from the flotation test. Overall, the number of horses with *A. perfoliata* eggs was low. In September it was only two horses. It was Hampe in enclosure 1 and Äskil in enclosure 2. In November no *A. perfoliata* eggs were detected. In contrast, in April three horses Bork, Hampe and Thrim in enclosure 1, were egg positive, and also Äskil in enclosure 2. In enclosure 3 *A. perfoliata* eggs was only detected in one horse (Tulin) in April. For photo of *A. perfoliata* eggs see appendix 2, figure 23.

Table 9. Results for *A. perfoliata* eggs in the floatation test with saturated sugar-salt

Horse	September 2014	November 2014	April 2015
Bork	*	*	X
Brigg	-	-	-
Hampe	X	*	X
Thrim	-	-	X
Wiper	-	-	-
Hugo	*	*	-
Quåtten	*	*	-
Äskil	X	-	X
Messer	-	*	-
Birk	-	-	*
Tulin	*	-	X
Picasso	-	-	-

*No faecal samples analyzed X Positive sample – Negative sample
Deworming with ivermectin 21/5-2014, deworming with pyrantel pamoate 2/9-2014 and in October of Hampe and Äskil, deworming with pyrantel pamoate 26/1-2015 in enclosure 1, deworming with fenbendazole 26/1-2015 in enclosure 2, deworming of Hampe and enclosure 3 with pytantel pamoate 16/2-2015, Thrim dewormed with pyrantel pamoate 24/2-2015.

4.4 Tape test-*Oxyuris equi*

Pin worms were first detected in the faeces in some of the horses grazing in enclosure 1 and 2. As seen in table 10 almost all horses in these enclosures were infected in January. Since the horses in enclosure 3 were less infected only the horses in enclosure 1 and 2 were dewormed with fenbendazole and pyrantel pamoate, respectively. The deworming in enclosure 2 was successful, likewise as the one for the horses (except for Hampe) in enclosure 1. After additional analyses in February it was clear that also two horses in enclosure 3 were infected. Accordingly Hampe and all the horses in enclosure 3 were dewormed with pyrantel pamoate. New samples were collected in April and then it was evident that the horses in enclosure 2 were reinfected. For photo of O. *equi* eggs see appendix 2, figure 24.

Table 10. Tape test; pin worm

Horse	January 2015	^	February 2015	¤	April 2015
Bork	X		-		-
Brigg	X		-		-
Hampe	X		X		X
Thrim	X		-		-
Wiper	X		-		X
Hugo	X		-		X
Qåtten	-		-		-
Äskil	X		-		X
Messer	-		-		X
Birk	*		-		*
Tulin	-		X		X
Picasso	X		X		X

^Deworming in enclosure 1 with pyrantel pamoate and enclosure 2 with fenbendazole. ¤ Deworming of Hampe and all horses in enclosure 3 with pyrantel pamoate. The striped fields show the dewormed horses. * No tape test analyzed.

4.5 GIS maps of faeces piles

The defaecation pattern of the horses faeces was very clear and are shown in figures 11-16. In July, the grass was still growing and thus there was a surplus of feed in all enclosures. Some sections where the grass was around 50 cm, was probably less tasty and therefore not grazed properly by the horses, see figure 11. The horses in enclosures 1 and 2 seemed to have spent most of the time close to the fences, particularly in the beginning of the study. When the grass stopped growing in September and the horses tramped down the grass, faeces were deposited more even, see figure 13 and 14. In October, figure 13, the horses moved around a lot more, which resulted in a larger distribution of faeces all over the enclosures. In February, figure 15, the ground was covered with snow, which is the major reason why there were so few data points. During the winter period when the enclosures were covered with snow the horses seemed to have spent most of their time in the forests. On the last measurement in April, faeces were again observed almost all over the enclosures. From July to April there was no indication whatsoever that the horses used specific areas as toilets. Overall, the horses seemed to deposit their faeces in the same places as where the grazing took place. It seems like the horses graze close to the faeces because of minium amount of grass and do not use toilets.

Explanation to the color of the points in the following figures 10-15.

- Red=5 faeces piles
- Yellow=10 faeces piles
- Purple=15 faeces piles
- Turquoise=20 faeces piles
- Pink=25 faeces piles
- Green=30 faeces piles

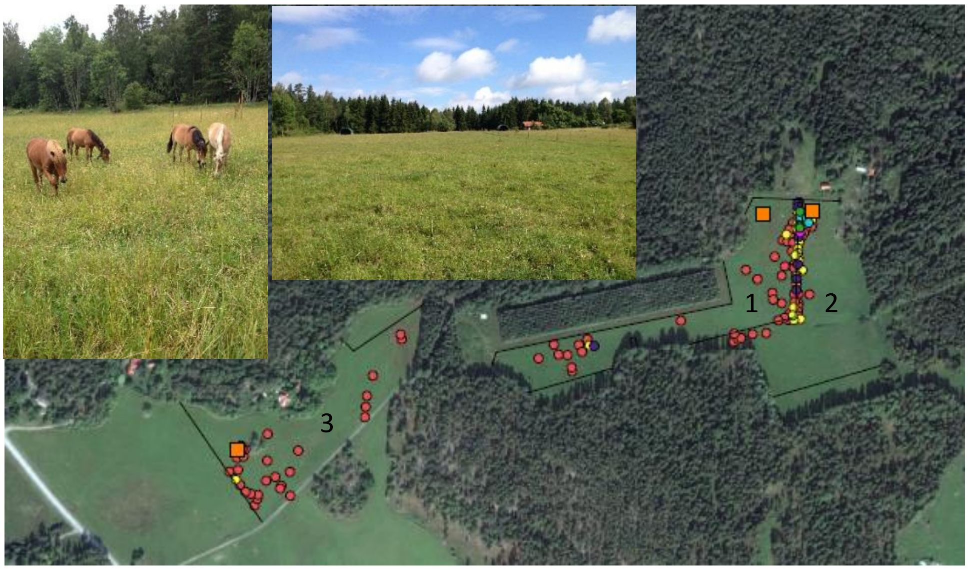

Figure 10. Map of GPS points in July 2014.

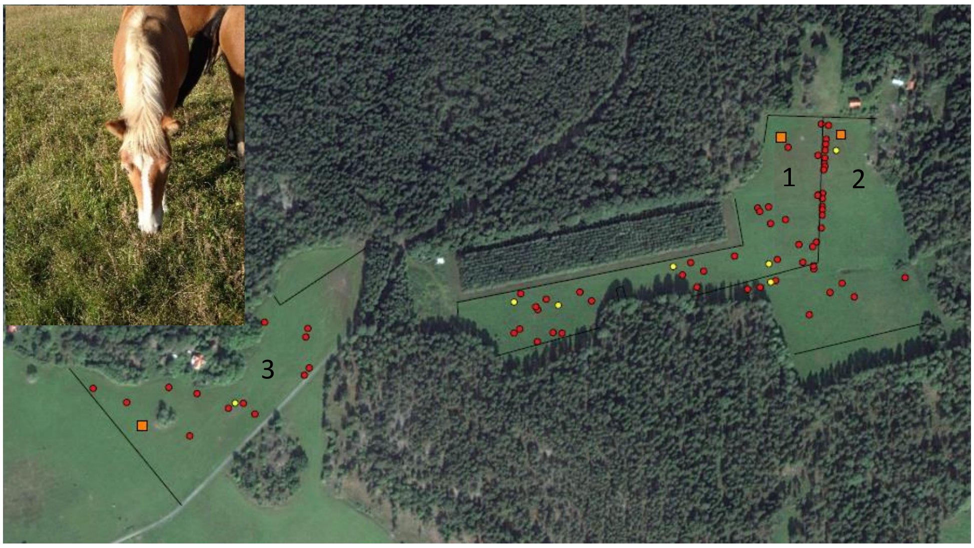

Figure 11. Map of GPS points in August 2014.

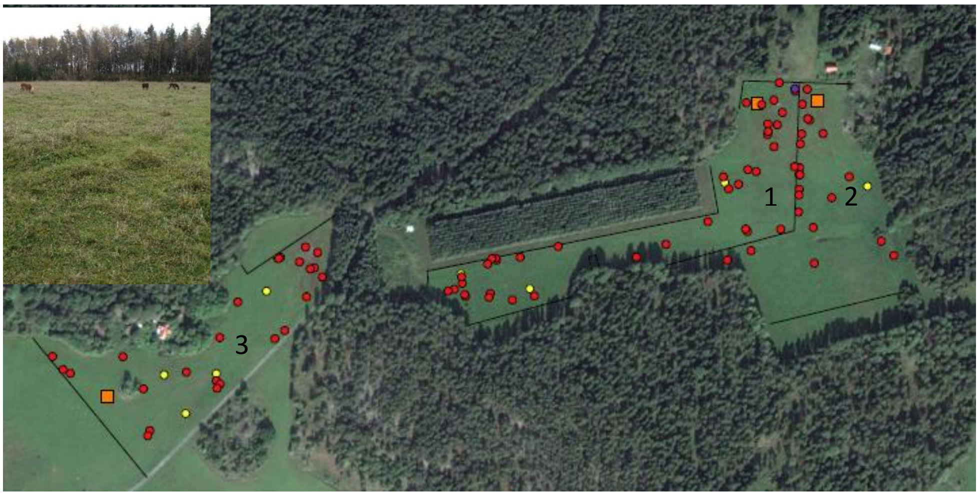

Figure 12. Map of GPS point in October 2014.

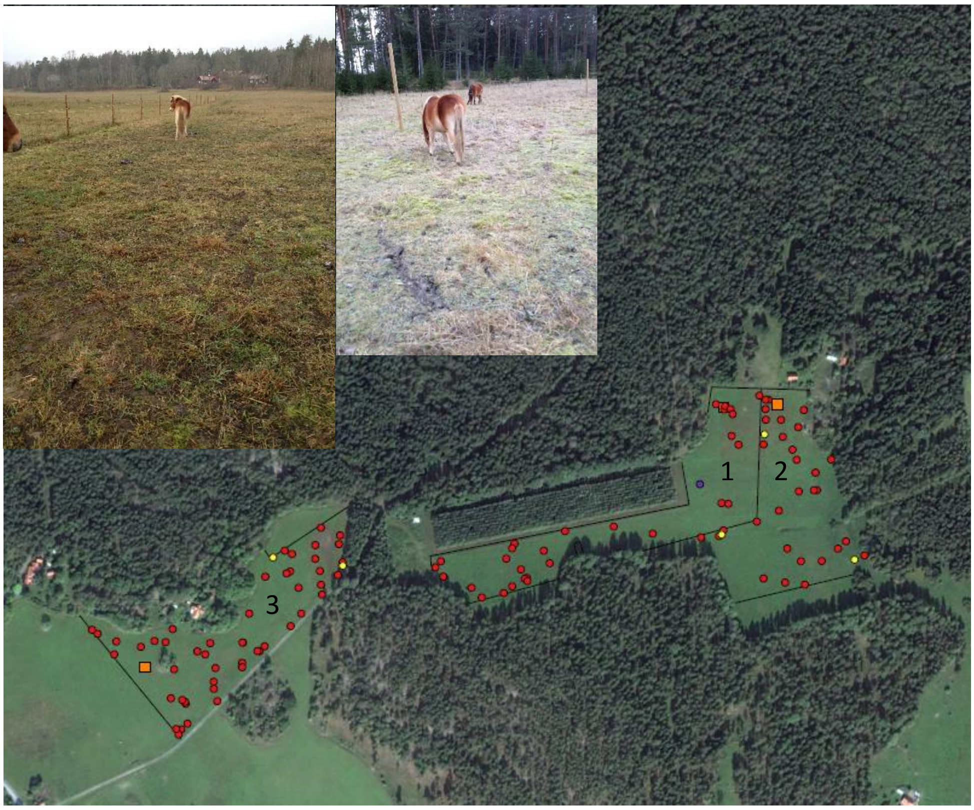

Figure 13. Map of GPS points in December 2014.

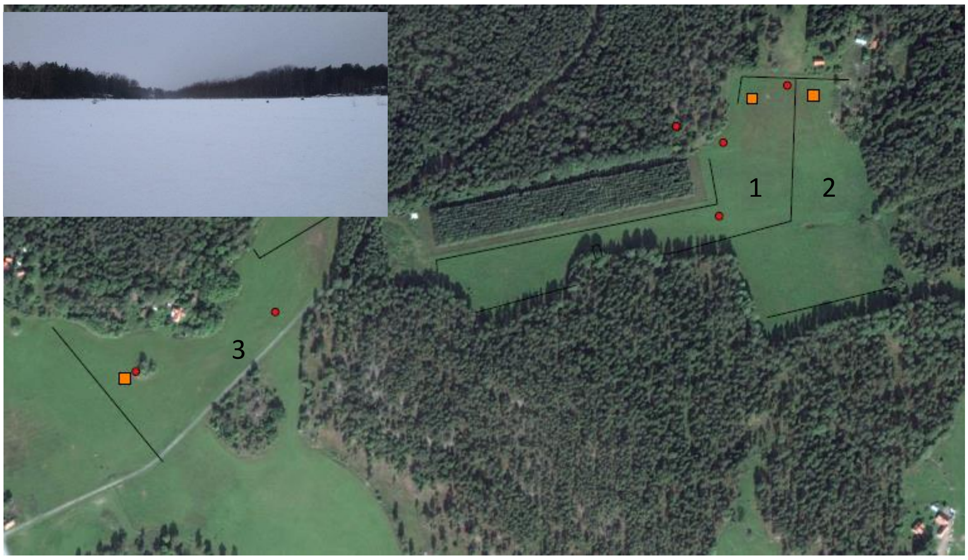

Figure 14. Map of GPS points in February 2015. The red point in the woods should be in enclosure 2.

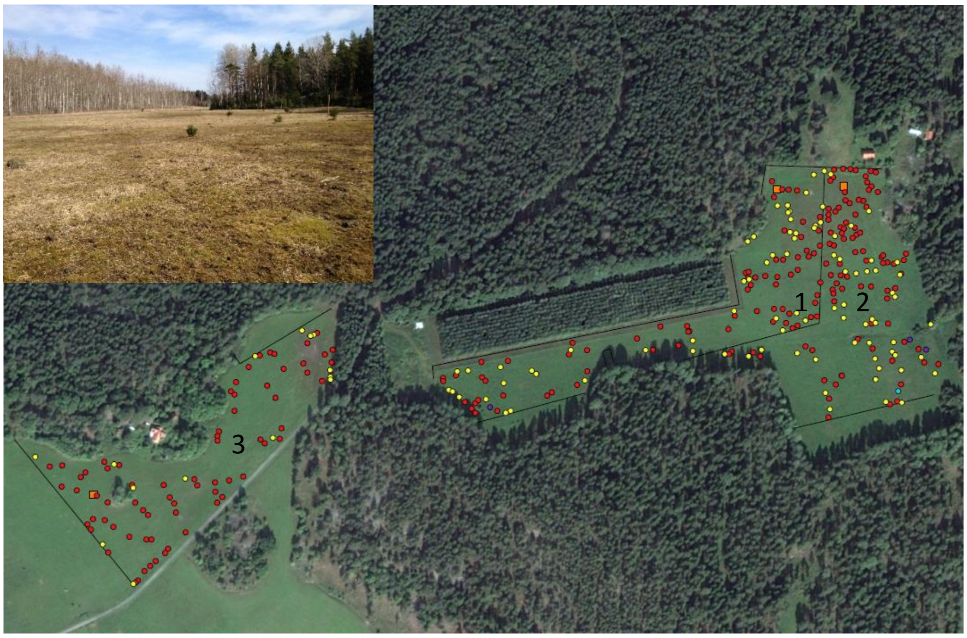

Figure 15. Map of GPS points in April 2015.

4.6 Molecular identification of strongyles

The banding patterns of the gels were examined to reveal the species composition of Cyathostominae.

- R was general
- 47/48 covered *Cylicostephanus minutus* and *Cylicocyclus nassatus*
- 38/39 covered *Cylicostephanus calicatus* and *Cyathostomum catinatum*
- 45 covered *Cylicocyclus leptostomus*
- 46 covered *Cylicostephanus longibursatus*

4.6.1 Gel pictures of Cyathostominae species from January

Bork, Hampe, Wiper and Hugo's samples were taken in May while Tulin, Messer and Picasso's samples were taken in December.

Enclosure 1

Bork was not infected with any specific Cyathostominae, while Hampe was infected with *C. calicatus* or/and *C. catinatum* and *C. longibursatus* (figure 16). For the result in a table see appendix 3.

Enclosure 2

Wiper and Hugo were not infected with any specific Cyathostominae (figure 16). Enclosure 2 did not have any of the most common Cyathostominae, which was found both in enclosures 1 and 3. For the result in table see appendix 3.

Enclosure 3

Tulin was infected with at least four common Cyathostominae, Messer with *C. calicatus* or/and *C. catinatum* and Picasso with the same as Messer and also *C. longibursatus.* Birk on the other hand was infected with *Cylicocyclus leptostomus* (figure 16). For the result in table see appendix 3. Most species were found in the horses from this enclosure.

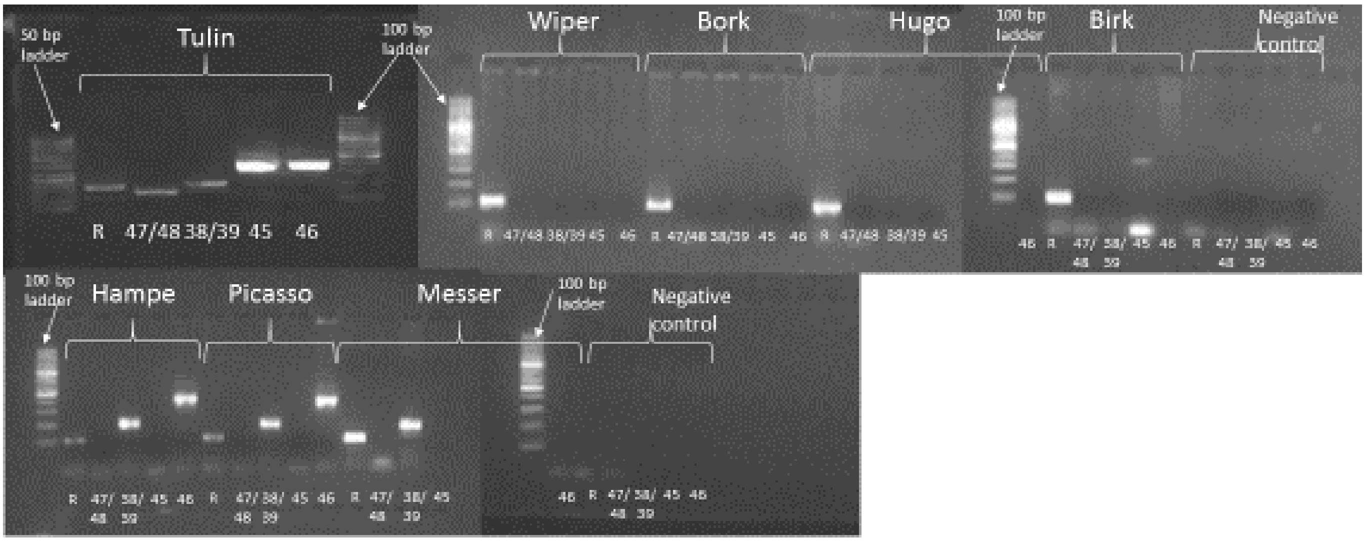

Figure 16. Gel picture from January.

4.6.2 Pasture samples

Enclosure 1 and 2 were accordingly to the gel picture infected with Cyathostominae, while enclosure 3 was clear, even though enclosure 3 did have the horses with most species of Cyathostominae (figure 17).

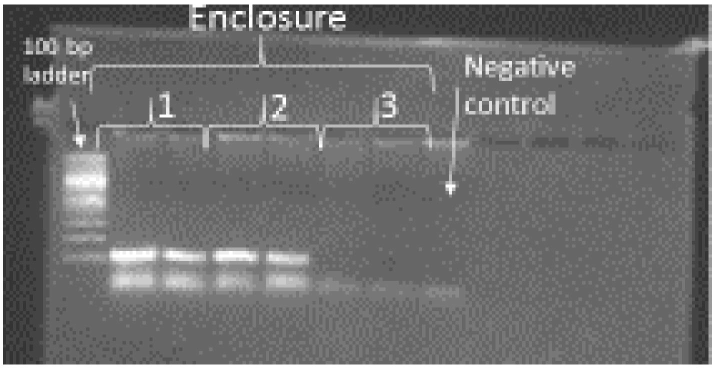

Figure 17. Gel picture from January.

4.6.3 Cyathostominae from April

The horses in all enclosures were infected with all of the most common Cyathostominae in April. All samples from the analysis in April were collected in the end of March and beginning of April, so the season should not have affected the result here.

Enclosure 1

In April was enclosure 1 was grazed by horses infected with *C. minutus* or/and *C. nassatus*, *C. longibursatus* from Bork; *C. calicatus* or/and *C. catinatum*, *C. leptostomus*, *C. longibursatus* from Hampe and Thrim seemed to be infected with at least four of the most common Cyathostominae (figure 18). For the result in table see appendix 3.

Enclosure 2

Äskil was infected with *C. minutus* or/and *C. nassatus*, *C. longibursatus;* Hugo and Qåtten with *C. calicatus* or/and *C. catinatum*, *C. leptostomus*, *C. longibursatus;* Wiper was infected with *C. longibursatus*, *C. calicatus* and/or *C. catinatum*, *C. leptostomus* (figure 18). For the result in table see appendix 3.

Enclosure 3

Picasso was infected with *C. minutus* or/and *C. nassatus*, *C. leptostomus*, *C. longibursatus,* whereas Messer and Tulin had at least four of the most common Cyathostominae and Birk was infected with *Cylicostephanus calicatus* or/and *Cyathostomum catinatum*, *Cylicocyclus leptostomus* and *Cylicostephanus longibursatus* (figure 18). This was the only enclosure which was grazed by horses that had all types of the most common Cyathostominae both in January and in April. However, Picasso did not longer possess *C. calicatus* and *C. catinatum* in April. For the result in table see appendix 3.

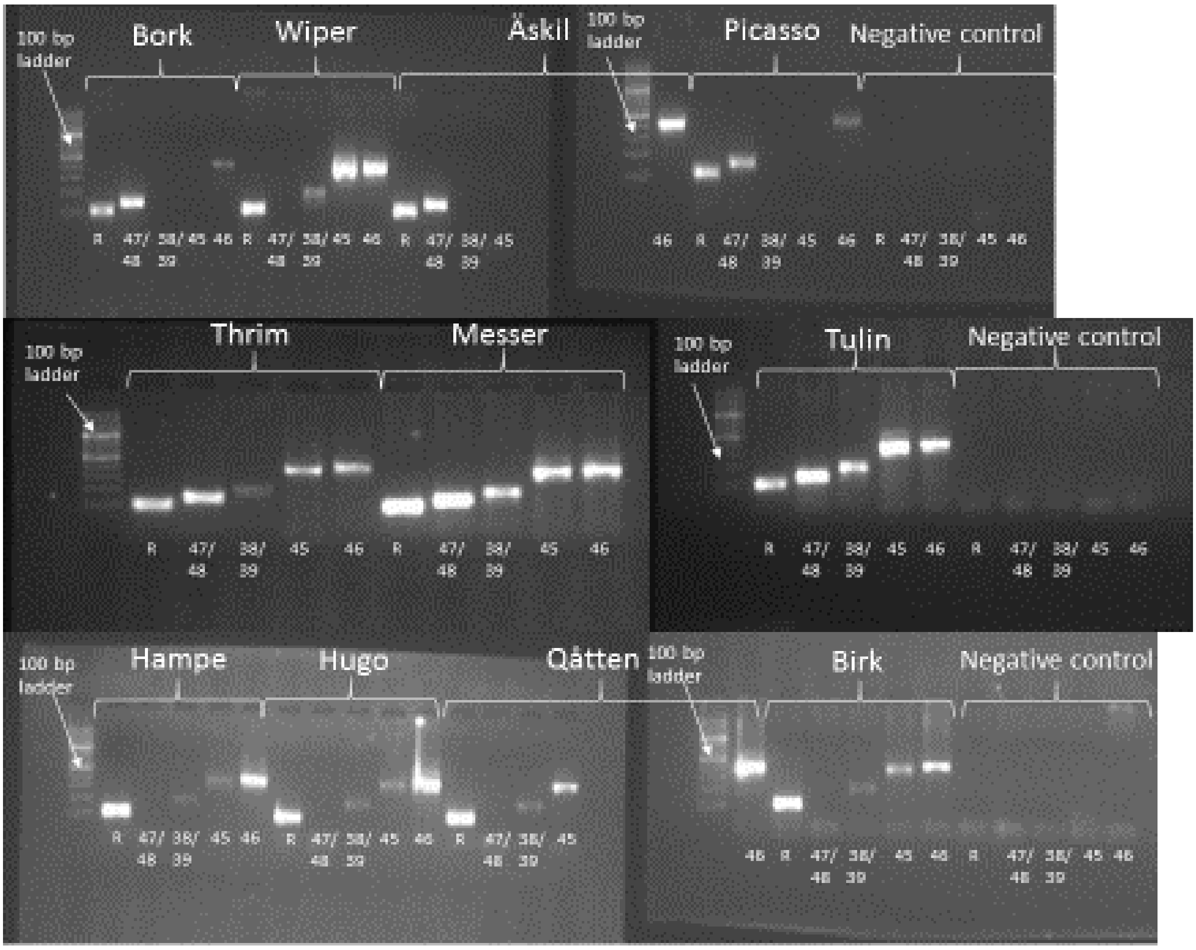

Figure 18. Gel picture from March and April.

4.6.4 *S. vulgaris*

Figure 19 shows the horses that were infected with *S. vulgaris* and it was Thrim, Messer and Tulin. Thrim grazed in enclosure 1 and Messer and Tulin in enclosure 3. Bork (enclosure 1) and Birk (enclosure 3) were thought to have had *S. vulgaris* in May and therefore was a PCR and gel electrophoresis done on them to see if they had brought it in. This is confirmed in figure 20.

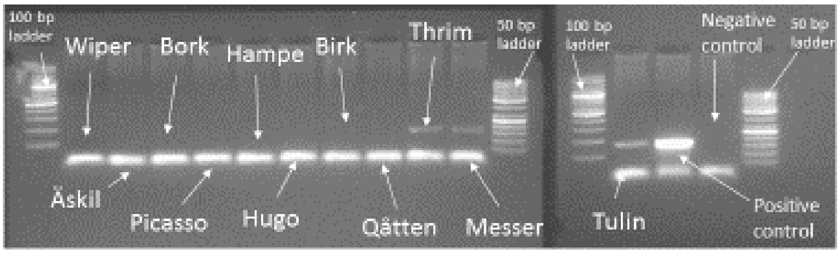

Figure 19. Gel picture from April.

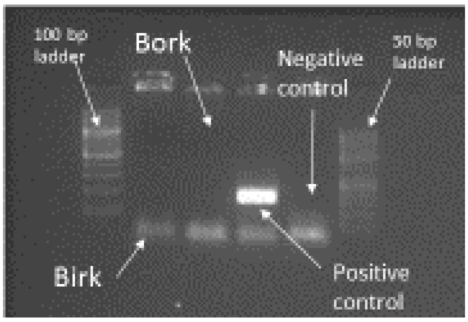

Figure 20. Gel picture from April.

5 Discussion

5.1 Faecal samples

5.1.1 Strongyles

When the horses arrived at least Brigg, Qåtten, Messer, Thrim and Tulin were dewormed. Before the start of the project all horses grazed together in an enclosure for approximately two-three weeks. Faecal samples were collected from all horses in May 2014 and all egg-positive horses were dewormed. Six individuals were heavily infected with an EPG of ≥1000; eg. Bork, Brigg, Wiper, Hugo and Birk, while Hampe had a moderate infection of 850 EPG in May. The rest of the horses had an EPG of 0. The horses were then turned out to the different enclosures, which had not been grazed by horses for many years and thus supposed to be clean pastures.

The strongyle egg counts varied during the study with the highest numbers observed in May for the horses in enclosure 1 and 2 and in August for enclosure 3. The first deworming in May with ivermectin (Noromectin®) was effective since practical all horses had an EPG of 0 when they were re-tested in June, while the deworming in beginning of September with pyrantel (Banminth®) were not as effective since five horses did still shedded eggs 16 days after the deworming. In January and February all horses in enclosures 1 and 2 were dewormed against *O. equi* with pyrantel (Banminth®) and fenbendazol (Axilur®), respectively. These treatment also decreased the strongyle egg counts in most horses the following months. However, in enclosure 3 there was an increase in March after the deworming with pyrantel pamoate in February. Thus, neither pyrantel (Banminth®) nor fenbendazol (Axilur®), that were used against *O. equi* January and February were fully effective against the strongyle infections. This is in agreement with earlier studies, as anthelmintic resistance against fenbendazole (Axilur®), and decreased sensitivity against pyrantel (Banminth®) has been demonstrated before (Osterman-Lind, 2005; Höglund *et al*., 2011). Some horses had zero strongyle egg counts with the McMaster method but were still egg positive according to the flotation test. This highlights the low sensitivity of McMaster method in comparison to the flotation test. An egg count of zero is the value with lowest sensitivity, which could be due to a newly infection or start of immunization (Taylor *et al.,* 2007). Since it was not always possible for practical reasons to obtain faecal samples from all horses, the data set is incomplete. For example, in October only two samples were collected. According to the statistical analyze there was no significant difference in egg counts between the different horses, which suggests that there was no particular horse that was responsible for the contamination. On the other hand, Wiper had a very high FEC (4200) in May, but this value was excluded in the graph. Thus Wiper must have been responsible for a significant part of the egg shedding throughout the whole study. In some horse herds are there normally one or a few horses that shed most of the eggs (Nielsen *et al.*, 2006).

Furthermore, there was no statistical difference between the egg counts from the horses in the different enclosures. By studying the type of pasture in the enclosures could a difference in the type of grass be seen between enclosure 1-2 and enclosure 3, but the non-statistical difference suggest that the type of pasture does not have any effect on the parasite prevalence.

Interestingly there was a significant difference in the egg output between different seasons. The strongyle egg counts were highest from May to August and lowest from September to December. This indicates that the egg laying pattern of strongyle parasite was more active during spring and summer. Likewise, Chapman *et al.* (2003) found a significant difference between season regarding horses from Louisiana, USA. In contrast, Chapman *et al.* (2003) did not show the same result as our study since the highest number of eggs was observed in the spring and autumn, whereas lower egg counts were observed in summer and especially in the winter. One possible reason is the different climate in Louisiana compared to central Sweden. One reason that may explain the significant seasonality is different larval arrestment patterns (Reinemeyer & Nielsen, 2013). Furthermore, the Gotland ponies in this study were always on pasture and they were therefore exposed to parasites throughout the whole study period (Relf *et al.*, 2013).

5.1.2 Parascaris equorum

The egg counts of *P. equorum* were low and never reached the same levels as for the strongyles. At the first sampling occasion in May 2014 only four (Bork, Hampe, Wiper and Hugo) of the 12 horses shedded *P. equorum* eggs according to the results from McMaster method. This is due to an effective deworming and also an increasing immunity against the parasite. In April 2015 two (Äskil and Wiper) of these were still infected according to the flotation results, whereas they were negative with McMaster. Äskil did have an EPG of 50 in March. Likewise as for strongyle eggs, the McMaster counting was less sensitive than flotation for *P. equorum* eggs with saturated sugar-salt solution (see table 6 and 8). This illustrates, that even though the FEC is zero with McMaster some horses may still shed *P. equorum* eggs. Again this shows that the McMaster method is not completely accurate (Taylor *et al.*, 2007). Due to the horse age resistance to *P. equorum* FEC is in general decreasing with increasing age of the horses (Reinemeyer & Nielsen, 2013). Most likely these Gotland ponies already had developed some resistance as the egg counts were low already from the beginning. By the start of the study the horses were around one year old, which is the expected age to start develop immunity and normally is full immunization reached by 18 months.

5.1.3 Anoplocephala perfoliata

All horses were dewormed with pyrantel (Banminth®) before the first analyze in September. Even though two (Hampe and Äskil) horses in enclosure 1 and 2 were shedding tapeworm eggs in September and neither Hampe nor Äskil did shed eggs in November due to deworming with double dose of pyrantel pamoate (Banminth®) in October. Both were on the other hand still egg positive in April despite deworming with pyrantel (Banminth®) and fenbendazole (Axilur®). Reasons for this could either be due to reinfection since the long time interval of five months between the last two sampling occasions or ineffective treatment with pyrantel (Banminth®) and fenbendazole (Axilur®), which was not used against tapeworm from the beginning. One of these horses (Äskil) did not have any tapeworm eggs in November indicating that he was reinfected. Furthermore, three horses in enclosure 1 were shedding *A. perfoliata* eggs in April. In enclosure 3 only one (Tulin) horse was egg positive in April, whereas no horse in enclosure 3 was positive for *A. perfoliata* eggs in September or November. It is impossible to know if the horses in enclosure 3 were infected before due to the sporadic sampling of these horses. In November seven horses was examined and then none of them were *A. perfoliata* egg positive. One horse was removed from the project, and it was then kept together with two (Thrim and Hampe) anoplocephala-infected horses. This is one possible reason for the introduction of *A. perfoliata* into enclosure 3. Messer was on the other hand not infected with the parasite, but the deworming might have worked better on him than on Tulin, but that cannot be confirmed. According to a study done by Kjær *et al.* (2007) and Nilsson *et al.* (1995) *A. perfoliata* eggs are only detected in the most heavily infected horses. Thus, it cannot be excluded that more horses were infected with *A. perfoliata*.

5.2 Tape test - *Oxyuris equi*

Pin worms were found in the faeces of some horses in enclosure 1 and 2 in the January samples. Therefore was a tape test performed in January. Four horses in enclosure 1 were found to be infected, three horses in enclosure 2 and one in enclosure 3. Deworming with pyrantel (Banminth®) or fenbedazole proved to be effective except for Hampe who did not respond to the pyrantel (Banminth®). The horses in enclosure 3 and Hampe in enclosure 1 did not respond to the deworming with pyrantel pamoate (Banminth®) in February. However, horses from all enclosures were infected in April. According to Taylor *et al.* (2007) it is difficult to get rid of pinworms since they deposit their eggs around the anus and due to the itching the horses rub them off in the environment.

5.3 GIS maps

It was difficult to repeat the same pattern in the enclosure since the track was not marked in any way. This could have contributed to that some faeces piles were missed at some measurements. Nevertheless, depending on the amount and height of the grass it seems like the horses utilized different areas in the enclosures. During the period when the ground was snow covered there was practical no faeces found suggesting that the horses spend most of their time in the forest. To get any grass under the snow does it take a lot of work for the horse to dig away the snow (Salter & Hudson, 1979). This probably forced the horse into the forest. Horses are curious and tend to stay for example near fences in particular if there are other horses next door. This behavior was observed in July but also to some extent in August both in enclosure 1 and 2, which were situated next to each other. Horses prefer to eat grass of medium height. Only when there is no access to this type of feed, they will graze in areas with higher grasses that is not as tasty. When there is a shortage of feed horses also graze very close to the faecal pats. This pattern was mainly observed in April even if the grass had begun to grow, but also in October and December. In both October, December and April were there faecal pats all over the pasture, which suggests that the horses move over the whole pastures while feeding. Horses do normally have a grazing pattern where they separate grazing spots from where the faeces is deposited (Ödberg & Francis-Smith, 1977). The defaecation we observed is in agreement both with Medica *et al.* (1996) and Lamoot *et al.* (2004), who both saw an unstructured grazing and faeces deposit pattern. The major conclusion is that no sign of toilets could be observed in any of the enclosures.

5.4 Molecular identification of strongyles

5.4.1 Cyathostominae

Although more than 40 Cyathostominae have been described from horses, it has been shown that Cyathostominae in individual horses often are composed of fewer than ten species. In my study, species specific ITS-2 primers were designed for the most common species previously reported from Sweden (Osterman-Lind, 2005), eg. *Cylicostephanus minutus*, *Cylicocyclus nassatus*, *Cylicostephanus calicatus, Cyathostomum catinatum*, *Cylicocyclus leptostomus* and *Cylicostephanus longibursatus.*

It is hard to compare result from January since Bork, Hampe, Wiper and Hugo's were sampled in May 2014 while Tulin, Messer and Picasso's in December 2014. Since the worms shed more eggs during the warmer period, these observations cannot be properly compared. Interestingly, it was the samples collected in December that had the most different Cyathostominae species. However, when comparing the result from January and April it seems like all horses, which were analyzed on both occasions, have an increased number of Cyathostominae species. All samples from April were collected in beginning of this month, so the season should not have affected the result here. Picasso was the only horse who switched species, the other horses did only increase the number of species. *C. longibursatus* was the most common species as it was fond in all horses in April. However, among those horses that were run with the PCR test, only a few were examined on more than one occasion. Unfortunately, samples from Brigg was never included in the PCR investigation. The PCR results must therefore be regarded as preliminary. In April all of the most common species were found in all enclosures, while in January all species was found in horses from enclosure 3, whereas enclosure 2 did not have any of the most common Cyathostominae species.

Four different species; *C. nassatus*, *C. catinatum*, *C. leptostomus* and *C. longibursatus* have been observed to increase with increasing age (Chapman *et al.*, 2003). This could be one possible reason why the number of Cyathostominae species increased between sampling occassions. *C. calicatus* does according to Chapman *et al.* (2003) differ significantly between seasons, with peak numbers in winter and autumn. All six of the most common Cyathostominae do exist in all season, but in different amounts. The number of horses infected with *C. calicatus* increased from the analysis in January to April. In January did most of the horses who were sampled in December have *C. calicatus,* which is in accordance with Chapman *et al.* (2003).

5.4.2 Pasture samples

The PCR of the larvae harvested from the grass samples shows that both enclosure 1 and 2 were infected with Cyathostominae, but this was never verified with additional sample from the same enclosures. This cannot be complete trusted since we know that also the horses in enclosure 3 were infected with Cyathostominae and according to the PCR of the faeces samples do enclosure 3 have most Cyathostominae species in January. Again sampling was too limited in order make any conclusions.

5.4.3 Strongylus vulgaris

Three horses (Thrim, Messer and Tulin) were infected with *S. vulgaris.* It is impossible to state which horses that were infected with S. *vulgaris* already from the project start. However we believe that it in this case were two horses (eg. Birk and Bork) which came from the same breeder. Larval cultures from both of these horses in May 2014 was also analyzed but then no *S. vulgaris* were detected. One horse (Thrim) was from the same enclosure (enclosure 1) as Bork, whereas Birk grazed in the same enclosure as Messer and Tulin (enclosure 3). Finding of *S. vulgaris* requires attention since it is the most pathogenic intestinal parasite in horses (Osterman-Lind, 2005).

6 Conclusion

Despite deworming before all ponies were turned out on in three different enclosures most ponies had a wide range of different parasites. This was despite that the enclosures had never before been grazed by horses. Since the horses were kept in the same enclosures for one year it can be assumed that pasture contamination built up gradually. Despite repeated deworming with ivermectin but also with fenbendazole and/or pyrantel pamoate several horses remained parasite infected, mostly with Cyathostominae. Thus all horses were re-infected with larvae from the pasture. Finally, there was no sign of toilets in any enclosure, which suggests that the Gotland ponies have an uneven grazing pattern.

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Appendix 1: Clustal 2.1 multiple sequence alignment

Yellow = general forward primer
Green = reverse primer 47/48
Blue = reverse primer 38/39
Purple = reverse primer 45
Red = reverse primer 46
Grey = general reverse primer

```
CLUSTAL 2.1 multiple sequence alignment


gi|2791657|emb|AJ223340.1|      --------------------------------------------------
gi|2791660|emb|AJ223345.1|      --------------------------------------------------
gi|2791573|emb|AJ223334.1|      GAGCTGGGTTTAGACCGTCGTGAGACAGGTTAGTTTTACCCTACTGTTGA 50
gi|2791650|emb|AJ223343.1|      --------------------------------------------------
gi|2791659|emb|AJ223344.1|      --------------------------------------------------
gi|2791574|emb|AJ223335.1|      --------------------------------------------------
gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      GAGCTGGGTTTAGACCGTCGTGAGACAGGTTAGTTTTACCCTACTGTTGA 50
gi|2791661|emb|AJ223346.1|      GAGCTGGGTTTAGACCGTCGTGAGACAGGTTAGTTTTACCCTACTGTTGA 50
gi|2791658|emb|AJ223342.1|      GAGCTGGGTTTAGACCGTCGTGAGACAGGTTAGTTTTACCCTACTGTTGA 50
gi|2791662|emb|AJ223347.1|      --------------------------------------------------
gi|2791663|emb|AJ223348.1|      GAGCTGGGTTTAGACCGTCGTGAGACAGGTTAGTTTTACCCTACTGTTGA 50
gi|2791575|emb|AJ223336.1|      --------------------------------------------------
gi|2791651|emb|AJ223341.1|      --------------------------------------------------


gi|2791657|emb|AJ223340.1|      --------------------------------------------------
gi|2791660|emb|AJ223345.1|      --------------------------------------------------
gi|2791573|emb|AJ223334.1|      CTAATTGTTGCGATAGTAATCCTGCTTAGTACGAGAGGAACAGCAGGTTT 100
gi|2791650|emb|AJ223343.1|      --------------------------------------------------
gi|2791659|emb|AJ223344.1|      --------------------------------------------------
gi|2791574|emb|AJ223335.1|      --------------------------------------------------
gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      CTAATTGTTGCGATAGTAATCCTGCTTAGTACGAGAGGAACAGCAGGTTT 100
gi|2791661|emb|AJ223346.1|      CTAATTGTTGCGATAGTAATCCTGCTTAGTACGAGAGGAACAGCAGGTTT 100
gi|2791658|emb|AJ223342.1|      CTAATTGTTGCGATAGTAATCCTGCTTAGTACGAGAGGAACAGCAGGTTT 100
gi|2791662|emb|AJ223347.1|      --------------------------------------------------
gi|2791663|emb|AJ223348.1|      CTAATTGTTGCGATAGTAATCCTGCTTAGTACGAGAGGAACAGCAGGTTT 100
gi|2791575|emb|AJ223336.1|      --------------------------------------------------
gi|2791651|emb|AJ223341.1|      --------------------------------------------------


gi|2791657|emb|AJ223340.1|      --------------------------------------------------
gi|2791660|emb|AJ223345.1|      --------------------------------------------------
gi|2791573|emb|AJ223334.1|      AGACATTTGGTTCATGGACTTGACCGATCGGTCAATGGTCCGAAGCTAAC 150
gi|2791650|emb|AJ223343.1|      --------------------------------------------------
gi|2791659|emb|AJ223344.1|      --------------------------------------------------
gi|2791574|emb|AJ223335.1|      --------------------------------------------------
gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      AGACATTTGGTTCATGGACTTGACCGATCGGTCAATGGTCCGAAGCTAAC 150
gi|2791661|emb|AJ223346.1|      AGACATTTGGTTCATGGACTTGACCGATCGGTCAATGGTCCGAAGCTAAC 150
gi|2791658|emb|AJ223342.1|      AGACATTTGGTTCATGGACTTGACCGATCGGTCAATGGTCCGAAGCTAAC 150
gi|2791662|emb|AJ223347.1|      --------------------------------------------------
gi|2791663|emb|AJ223348.1|      AGACATTTGGTTCATGGACTTGACCGATCGGTCAATGGTCCGAAGCTAAC 150
gi|2791575|emb|AJ223336.1|      --------------------------------------------------
gi|2791651|emb|AJ223341.1|      --------------------------------------------------


gi|2791657|emb|AJ223340.1|      --------------------------------------------------
gi|2791660|emb|AJ223345.1|      --------------------------------------------------
gi|2791573|emb|AJ223334.1|      ATCTAGGGGATTATGACTGAACGCCTCTAAGTCAGAATCCAACCTAGTCA 200
gi|2791650|emb|AJ223343.1|      --------------------------------------------------
gi|2791659|emb|AJ223344.1|      --------------------------------------------------
gi|2791574|emb|AJ223335.1|      --------------------------------------------------
gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
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gi|2791656|emb|AJ223339.1|      ATCTAGGGGATTATGACTGAACGCCTCTAAGTCAGAATCCAACCTAGTCA 200
gi|2791661|emb|AJ223346.1|      ATCTAGGGGATTATGACTGAACGCCTCTAAGTCAGAATCCAACCTAGTCA 200
gi|2791658|emb|AJ223342.1|      ATCTAGGGGATTATGACTGAACGCCTCTAAGTCAGAATCCAACCTAGTCA 200
gi|2791662|emb|AJ223347.1|      --------------------------------------------------
gi|2791663|emb|AJ223348.1|      ATCTAGGGGATTATGACTGAACGCCTCTAAGTCAGAATCCAACCTAGTCA 200
gi|2791575|emb|AJ223336.1|      --------------------------------------------------
gi|2791651|emb|AJ223341.1|      --------------------------------------------------


gi|2791657|emb|AJ223340.1|      --------------------------------------------------
gi|2791660|emb|AJ223345.1|      --------------------------------------------------
gi|2791573|emb|AJ223334.1|      AGGTAGTAATAACTTTGTTTCCCGGTGTCGGGAGGCAACTCTATCTCGTG 250
gi|2791650|emb|AJ223343.1|      --------------------------------------------------
gi|2791659|emb|AJ223344.1|      --------------------------------------------------
gi|2791574|emb|AJ223335.1|      --------------------------------------------------
gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      AGGTAGTAATAACTTTCTTTCCCGGTGTCGGGAGGCAACTCTATCTCGTG 250
gi|2791661|emb|AJ223346.1|      AGGTAGTAATAACTTTGTTTCCCGGTGTCGGGAGGCAACTCTATCTCGTG 250
gi|2791658|emb|AJ223342.1|      AGGTAGTAATAACTTTCTTTCCCGGTGTCGGGAGGCAACTCTATCTCGTG 250
gi|2791662|emb|AJ223347.1|      --------------------------------------------------
gi|2791663|emb|AJ223348.1|      AGGTAGTAATAACTTTCTTTCCCGGTGTCGGGAGGCAACTCTATCTCGTG 250
gi|2791575|emb|AJ223336.1|      --------------------------------------------------
gi|2791651|emb|AJ223341.1|      --------------------------------------------------


gi|2791657|emb|AJ223340.1|      --------------------------------------------------
gi|2791660|emb|AJ223345.1|      --------------------------------------------------
gi|2791573|emb|AJ223334.1|      GTAACGCGAGAGCTTATGCCCCAGTAAATGGCCTTGCCGGTATGGATAAT 300
gi|2791650|emb|AJ223343.1|      --------------------------------------------------
gi|2791659|emb|AJ223344.1|      --------------------------------------------------
gi|2791574|emb|AJ223335.1|      --------------------------------------------------
gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      GTAACGCGAGAGCTTATGCCCCAGTAAATGGCCTTGCCGGTATGGATAAT 300
gi|2791661|emb|AJ223346.1|      GTAACGCGAGAGCTTATGCCCCAGTAAATGGCCTTGCCGGTATGGATAAT 300
gi|2791658|emb|AJ223342.1|      GTAACGCGAGAGCTTATGCCCCAGTAAATGGCCTTGCCGGTATGGATAAT 300
gi|2791662|emb|AJ223347.1|      --------------------------------------------------
gi|2791663|emb|AJ223348.1|      GTAACGCGAGAGCTTATGCCCCAGTAAATGGCCTTGCCGGTATGGATAAT 300
gi|2791575|emb|AJ223336.1|      --------------------------------------------------
gi|2791651|emb|AJ223341.1|      --------------------------------------------------


gi|2791657|emb|AJ223340.1|      --------------------------------------------------
gi|2791660|emb|AJ223345.1|      --------------------------------------------------
gi|2791573|emb|AJ223334.1|      CCCTGCTGGTTGCTAAAGCCAGATCACTCTGGTTCAATGTCGGGGCGCAA 350
gi|2791650|emb|AJ223343.1|      --------------------------------------------------
gi|2791659|emb|AJ223344.1|      --------------------------------------------------
gi|2791574|emb|AJ223335.1|      --------------------------------------------------
gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      CCCTGCTGGTTGCTAAAGCCAGATCACTCTGGTTCAATGTCGGGGCGCAA 350
gi|2791661|emb|AJ223346.1|      CCCTGCTGGTTGCTAAAGCCAGATCACTCTGGTTCAATGTCGGGGCGCAA 350
gi|2791658|emb|AJ223342.1|      CCCTGCTGGTTGCTAAAGCCAGATCACTCTTGTTCAATCTCGGGGCGCAA 350
gi|2791662|emb|AJ223347.1|      --------------------------------------------------
gi|2791663|emb|AJ223348.1|      CCCTGCTGGTTGCTAAAGCCAGATCACTCTGGTTCAATGTCGGGGCGCAA 350
gi|2791575|emb|AJ223336.1|      --------------------------------------------------
gi|2791651|emb|AJ223341.1|      --------------------------------------------------


gi|2791657|emb|AJ223340.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
gi|2791660|emb|AJ223345.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
gi|2791573|emb|AJ223334.1|      AATCACATGTCTACGACATGTATACTGGTCAAGGTGTTGTATCCAGTAGA 400
gi|2791650|emb|AJ223343.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
gi|2791659|emb|AJ223344.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
gi|2791574|emb|AJ223335.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
gi|2791572|emb|AJ223337.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
gi|2791576|emb|AJ223338.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
gi|2791656|emb|AJ223339.1|      AATCACATGTCTACGACATGTATACTGGTCAAGGTGTTGTATCCAGTAGA 400
gi|2791661|emb|AJ223346.1|      AATCACATGTCTACGACATGTATACTGGTCAAGGTGTTGTATCCAGTAGA 400
gi|2791658|emb|AJ223342.1|      AATCACATGTCTACGACATGTATACTGGTCAAGGTGTTGTATCCAGTAGA 400
gi|2791662|emb|AJ223347.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
gi|2791663|emb|AJ223348.1|      AATCACATGTCTACGACATGTATACTGGTCAAGGTGTTGTATCCAGTAGA 400
gi|2791575|emb|AJ223336.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
gi|2791651|emb|AJ223341.1|      --------------------------GGTCAAGGTGTTGTATCCAGTAGA 24
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gi|2791657|emb|AJ223340.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
gi|2791660|emb|AJ223345.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
gi|2791573|emb|AJ223334.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 450
gi|2791650|emb|AJ223343.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
gi|2791659|emb|AJ223344.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
gi|2791574|emb|AJ223335.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
gi|2791572|emb|AJ223337.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
gi|2791576|emb|AJ223338.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
gi|2791656|emb|AJ223339.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 450
gi|2791661|emb|AJ223346.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 450
gi|2791658|emb|AJ223342.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 450
gi|2791662|emb|AJ223347.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
gi|2791663|emb|AJ223348.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 450
gi|2791575|emb|AJ223336.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
gi|2791651|emb|AJ223341.1|    GCAGTGTTTGTATACTGCGATCTGTTGAGACTATCCTATGATCGGGTGTT 74
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gi|2791657|emb|AJ223340.1|    TTGTCTTCTCCTGTCTAGGGGGTGTTTTAATTCACTTTCTAGCGTGGA-- 122
gi|2791660|emb|AJ223345.1|    TTGTCTTCTCCTGTCTAGGGGGTGTTTTAATTCACTTTCTAGCGTGGG-- 122
gi|2791573|emb|AJ223334.1|    TTGTCTTCTCCTGTCTAGGGGGTGTTTTAATTCACTTTCTAGCGTGGATA 500
gi|2791650|emb|AJ223343.1|    TTGTCTTCTCCTGTCCAGGGGGTGTTTTAATTCACTCTCTAGCGTGGA-- 122
gi|2791659|emb|AJ223344.1|    TTGTCTTCTCCTGTCAAGGGGGTGTTTTAATTCACTCTCTAGCGTGGA-- 122
gi|2791574|emb|AJ223335.1|    TTGTCTTCTCCTGTCCAGGGGGTGTTTTTTTTCACTCTCTAGCGCGGA-- 122
gi|2791572|emb|AJ223337.1|    TTGTCTTCTCCTGTCTAGGGGGTGTTTTAAATCACTTCTTAGCGTTGG-- 122
gi|2791576|emb|AJ223338.1|    TTGTCTTCTCCTGTCTAGGGGGTGTTTTAAATCACTTCTTAGCGTTGG-- 122
gi|2791656|emb|AJ223339.1|    TTGTCTTCTCCTGTCTAGGGGGTGTTTTAAATCACTTCTTAGCGTTGG-- 498
gi|2791661|emb|AJ223346.1|    TTGTCTTCTCCTGTCTAGGGGGTGTTTTAAATCACTTCTTAGCGTTGG-- 498
gi|2791658|emb|AJ223342.1|    TTGTCTTCTCCTGTCTAGGGGGTGTTTTAAATCACTTCTTAGCATCAG-- 498
gi|2791662|emb|AJ223347.1|    TTGTCTTCTACTGTCTAGGGGGTGTCTTAATTCACTCTCTAGCGTGATG- 123
gi|2791663|emb|AJ223348.1|    TTGTCTTCTACTGTCTAGGGGGTGTTTTAATTCACTCTCTAGCGTGATG- 499
gi|2791575|emb|AJ223336.1|    TTGTCTTCTGCTGTCTAGGGGGTGTCTTAATTCACTCTTCAGTACGGAG- 123
gi|2791651|emb|AJ223341.1|    TTGTCTTCTGCTGTCTAGGGGGTGTTTTAAATCACCTCCTAGCGCTGAG- 123
                              ********* ***** ********* **:::****   ** . .

gi|2791657|emb|AJ223340.1|    GAAAATTTTAGCCAAGTGAGGTGACCAAGTGTACATCATATTGGTCTCAC 172
gi|2791660|emb|AJ223345.1|    GAAAACTTTAGCCAAGTGTGGTGACCAAGTGTACATCATATTGGTCTCAC 172
gi|2791573|emb|AJ223334.1|    GAAAAATTTAGCCAAGTGTGGTGACTAACTGTACATCATATTGGTCTTAC 550
gi|2791650|emb|AJ223343.1|    GCGAAATTTAGCT--GTGTGGTG-ACAAGTGTAAATCGTATTTGTCCCAC 169
gi|2791659|emb|AJ223344.1|    GCGAAATTTAGCTAAGTGTGGTGACCAAGTGTAAATCGTATTGGTCCCAC 172
gi|2791574|emb|AJ223335.1|    GCGAAATTTAGCCAAGTGTGGTGACTAAGTGTACATCGCATTGGTCCTAC 172
gi|2791572|emb|AJ223337.1|    GAGAAATTTAGCCAAGTGTGG-GACTAGGCGTATATCATACG-GTCCC-C 169
gi|2791576|emb|AJ223338.1|    GAGAAATTTAGCCAAGTGTGGCGACTAGGCGTACATCATACG-GTCCCAC 171
gi|2791656|emb|AJ223339.1|    GAGAAATTTAGCCAAGTGTGGCGACTAGGCGTACATCATACG-GTCCTAC 547
gi|2791661|emb|AJ223346.1|    GAGAAATTGG--------TGGCGACTAGGCGTACATCATACG-GTCCCAC 539
gi|2791658|emb|AJ223342.1|    GAGAAATTTAGCCAAG--TAACGACTAGCCGTAAATCATATG-GTCCTAC 545
gi|2791662|emb|AJ223347.1|    AGGAAAAATAGCTAAGTGCGGTGAATAAGTG--CATCATACTGGTCCACA 171
gi|2791663|emb|AJ223348.1|    AGGAAAAATAGCTAAGTGCGGTGAATAAGTG--CATCATACTGGTCCACA 547
gi|2791575|emb|AJ223336.1|    G-AAAATATAGCTAAGTGTGGTGACTGGATGCACATG-TATAGGTCCCAC 171
gi|2791651|emb|AJ223341.1|    GAAAAATACAGCTAAGTCTAGTGACTAGGTGTARATTCTATAGGTCTTAC 173
                              . .** :: .     .. * . ..  *   **   *   ***   .

gi|2791657|emb|AJ223340.1|    ACAGAAAATGTATTTGCTTCATTTTT------GGTGGCCTCT--AGTTTC 214
gi|2791660|emb|AJ223345.1|    ATAGAAAATGTATTTGCTTCATTTTT------GGTGGCCTCT--AGTTTC 214
gi|2791573|emb|AJ223334.1|    ATAGAAAATGTATTTGCTTCATTTT-------GGTGGCATCT--AGTTTC 591
gi|2791650|emb|AJ223343.1|    ATAGAAAATGTATTTGCATCGTTTTCGAGT--GGTGACATCT--AGTTTC 215
gi|2791659|emb|AJ223344.1|    ATAGAAAATGTATTTGCTTCGTTTTCGAGT--GGTGACATCT--AGTTTC 218
gi|2791574|emb|AJ223335.1|    ATAGAAAATGTATTTGTTG---TTTCGAGT--GGTGACATCTCTAATTTC 217
gi|2791572|emb|AJ223337.1|    ATAGAAAATGTATTTGCTTCGTTCTG------AATAGCTAGT----TTTC 209
gi|2791576|emb|AJ223338.1|    ATAGAAAATGTATTTGCTTCGTTCTG------AATAGCTAGT----TTTC 211
gi|2791656|emb|AJ223339.1|    ATAGAAAATGTATTTGCTTCGTTCTG------AATAGCTGGT----TTTC 587
gi|2791661|emb|AJ223346.1|    ATTGAAAATGTATTTGCTTCGTTCTG------AGTAGCAA------TTTC 577
gi|2791658|emb|AJ223342.1|    ATAG-AAATGTACTTGCTTCGCTCTG------AGTTAGCAAG----TTCC 584
gi|2791662|emb|AJ223347.1|    GTAACATTGATTATAACTTATGCAGTGTAA--CGTGGGTCGG----TTTC 215
gi|2791663|emb|AJ223348.1|    GTAACATTGATTATAATTTATGCAGTGTAA--CGTGGGTCGG----TTTC 591
gi|2791575|emb|AJ223336.1|    ATAGAAAATGTAATCTT-CTGTTTTCTGGTGCAACAGCATGT--TGTTTC 218
gi|2791651|emb|AJ223341.1|    ATGGGATTTGTATTTGTGTCATTTTCGTGTGCWATAGCACCT--TGGTTG 221
                              .  . *:: .*: *           . .      *

gi|2791657|emb|AJ223340.1|    AAGG--TTTTAATCGCATAATGCTG---ACATATGTATGCCATT--CTTT 257
gi|2791660|emb|AJ223345.1|    AAGG--TTTTAATCGCATAATGCTG---ACATATGTATGCCATT--CTTT 257
gi|2791573|emb|AJ223334.1|    ATGG--TTTCAATCGCATAATGCTG---ACATATGTGCGTCATT--CTTT 634
gi|2791650|emb|AJ223343.1|    AAGG--ATGCACTCACATATTGACA---ACAAACGTATGTCAAT--CTTT 258
gi|2791659|emb|AJ223344.1|    AAGG--ATTCACTCACATATTGACA---ACAAACGTATGTCAAT--CTTT 261
gi|2791574|emb|AJ223335.1|    AAGG--ATTCACTCACATTTTGCTA---ACAAACGTATGTCAAT--CTTT 260
gi|2791572|emb|AJ223337.1|    AATG--TTGCAGTCACATAATGCTA---TTGA--GTATGGCATT--CTCT 250
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gi|2791576|emb|AJ223338.1| AATG--TTGCGGTCACATAATGCTA---TTGA--GTATGGCATT--CTTT 252
gi|2791656|emb|AJ223339.1| AATG--TTGCAGTCACATAATGCTA---ATGA--GTATGGCATT--CTTT 628
gi|2791661|emb|AJ223346.1| AAGG--TTTCAATCACATAATGCTA---TTGATTGTATGGCATT--CTTT 620
gi|2791658|emb|AJ223342.1| ACGG--TTTCAATCACATAATGCTA---TTGATCATATGGCATT--CTCA 627
gi|2791662|emb|AJ223347.1| GGTATAAATGACTTATACAGTGCTGG-TCAAAACTTTAGCCTAAGCCTTG 264
gi|2791663|emb|AJ223348.1| GGTAAAAATGACTTATACAGTGCTGG-TCAAAACTTTAGCCTAAGCCTTG 640
gi|2791575|emb|AJ223336.1| AATG--TGTT-ATAAGGTAATGCCAATAATTATGCTATAGTGCTGGCCAA 265
gi|2791651|emb|AJ223341.1| AAGG--TTTTGAACATATAATGCCAC-AGTTGTGGTTGTTTTATGG---A 265
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gi|2791657|emb|AJ223340.1| TATAGAAGCATTAATTCTACTT-----CATGCAG-TGTAAAATTCTCG-A 300
gi|2791660|emb|AJ223345.1| TATAAAGGCATTAATTCTACTT-----CATGCAG-TGTAAAATTCTCG-T 300
gi|2791573|emb|AJ223334.1| TATAGAAGCATTAGTTCTACTT-----TATGCAG-TGTAAAATTCTCG-T 677
gi|2791650|emb|AJ223343.1| CATAGAATCATTGATTCTACA------TATACAA-TGTAACATTCTTGTT 301
gi|2791659|emb|AJ223344.1| CATAGAATCATTGATTCTACA------TATACAA-TGTAACATTCTTGTT 304
gi|2791574|emb|AJ223335.1| TAAAGAATTATTGATTCTAT-------TATACAA-TGTAACATTCTTGTT 302
gi|2791572|emb|AJ223337.1| TATGAAAGCACTGYTTTCACTT-----CATACAGTTGTAACATTCTCG-- 293
gi|2791576|emb|AJ223338.1| TATGAAAGCACTGTTTTCACTT-----AATACAATTGTAACATTCTCA-- 295
gi|2791656|emb|AJ223339.1| TATGAAAGCACTGTTTTCACTT-----CATACAATTGTAACATTTTCG-- 671
gi|2791661|emb|AJ223346.1| TATGGAAGCATTGTTTTCACTT-----CATACAATTGTAGCATTCTCA-- 663
gi|2791658|emb|AJ223342.1| CATGGAAACATTGTTTTCACTT-----CATACAATTGTAACA-------- 664
gi|2791662|emb|AJ223347.1| TGCTTAGGCTTAGGCTTAGGCT-----TAGGCTTACGGAAAAGCCACA-C 308
gi|2791663|emb|AJ223348.1| GGCTTAGGCTTAGGCTTAGGCT-----TAGGCTTACGGAAAAGCCACA-C 684
gi|2791575|emb|AJ223336.1| AACTATAGCCTTACTTGCTTCAGTTCGCATGCCAGTGAGTTAACTTAGGA 315
gi|2791651|emb|AJ223341.1| TGCATTGACTCTACTTCTTGCACTGTAACTTCCTACTTG--GAAGTAG-- 311
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gi|2791657|emb|AJ223340.1| TTGGGTATAAATGCTTTACAATTCTATGTATGTATGTGTAGTCAATGTTT 350
gi|2791660|emb|AJ223345.1| TTGGGTATAAATGCTTTACAATTCTATGAATGTATGTGTAGTCAATGTTT 350
gi|2791573|emb|AJ223334.1| TTGGGTATAAATGCTTTACAATTCTATGTATGTATGTGTAGTCAATGTTT 727
gi|2791650|emb|AJ223343.1| TTGGGTATAAATGAATTACAATATTGTGTGTGTATATGTA-TCAATGTCT 350
gi|2791659|emb|AJ223344.1| TTGGGTATAAATGAATTACAATATTGTGTATGTATATGTA-TCAATGTCT 353
gi|2791574|emb|AJ223335.1| TTGAGTATGAATGAATCACAGTTTTATGCATGTATGTGTA-TCAGTGTCT 351
gi|2791572|emb|AJ223337.1| TATGGTATAAATGGCTTACAGCTCTGTATATGTAAGTGTAATCAGTGTTT 343
gi|2791576|emb|AJ223338.1| TTTGGTATAAACGGCTTACAACTCTGTATATGTAAGTGTAATCAGTGTTT 345
gi|2791656|emb|AJ223339.1| TTTGGTATAAATGGCTTACAACTCTGTATATGTAAGTGTAATCAGTGTTT 721
gi|2791661|emb|AJ223346.1| TTTGGTATAAATGGCTTACAACTTTGTATATGTAAGTGTAATCAATGTTT 713
gi|2791658|emb|AJ223342.1| ----GTATAAATGGCTTACAACTCTGTATATGTAAATGTAATCAATGTTT 710
gi|2791662|emb|AJ223347.1| TGTGATTTTGCTATATCTTACTCAGTTTCGAAG-AGAGAAACCAAATKCC 357
gi|2791663|emb|AJ223348.1| TGTGATTTTGCTATATCTTACTCAGTTTCGAAARAGAGAAACCAAATTCC 734
gi|2791575|emb|AJ223336.1| TTTGGCTAAAATGTGTATACAATCTATAACATTGTACTAAGTAGTTAGTT 365
gi|2791651|emb|AJ223341.1| --TGACTTATAATTGAATAAATGTAGTTG--ATGTTCTATGCTTTCCCCA 357
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gi|2791657|emb|AJ223340.1| ---CATTTTATCAGCACTTTGCTGTGCCAAACACGGAGATTTTC------ 391
gi|2791660|emb|AJ223345.1| ---CATTATATCAGCACTTTGCTGTGCCAAACACGGGGCTATTC------ 391
gi|2791573|emb|AJ223334.1| ---CATTATATCAGCACTTTGCTGTGCCAAACACGGGGATATTC------ 768
gi|2791650|emb|AJ223343.1| CAACTTTAG-ACAGCACTT-GCTGTGCCAA-----TGGAAGTCT------ 387
gi|2791659|emb|AJ223344.1| TAACTTTAG-ACAGCACTT-GCTGTGCCAA-----TGGAAGTCT------ 390
gi|2791574|emb|AJ223335.1| CAACTTTAGGTCAGCACTT-GCTGTGCCAAACAC-TGGAAGTTT------ 393
gi|2791572|emb|AJ223337.1| CAACTTTATATCAGCACTTCTATGTGCCAAACACTGAGAAATAATGTGCT 393
gi|2791576|emb|AJ223338.1| CAACTTT-TATCAGCACTTCTATGTGCCAAACACTGAGAAATAATGTGCT 394
gi|2791656|emb|AJ223339.1| CAACTTTGTATCAGCACTTCTATGTGCCAAACACTGCGAAATAATGTGCT 771
gi|2791661|emb|AJ223346.1| CAACTTTATATCAGCACTTCGATGTGCCAAACACTGAGAAATTATGCGCT 763
gi|2791658|emb|AJ223342.1| AAACTTTATCTCAGCACTTCGATGTGCCAAACACTGAAAAATATTATGCT 760
gi|2791662|emb|AJ223347.1| GATATTGCGTGAATCACACTGCTTAGCGTTAAGKCAAGAAATAC------ 401
gi|2791663|emb|AJ223348.1| GATATTGCGTGAATCACACTGCTTAGCGTTTAGTCAAGAAATAC------ 778
gi|2791575|emb|AJ223336.1| TGTTTTTGAGTCATAGATTAATTCATTGATAGACAAATGCATTGCAGCG- 414
gi|2791651|emb|AJ223341.1| CATTTATGT-ACGAAACATCATTTTGAAATAATTTCCAACGTTAAAATG- 405
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gi|2791657|emb|AJ223340.1| -------------------------CAAAATATGCTCATATAAAT-ATTT 415
gi|2791660|emb|AJ223345.1| -------------------------CAAAATATGCTTAAACGAAT-ATTT 415
gi|2791573|emb|AJ223334.1| -------------------------CAAAATATGCTTAAACGAAT-ATTT 792
gi|2791650|emb|AJ223343.1| -------------------------CAAAATATGTTTATAGGAAT-AATT 411
gi|2791659|emb|AJ223344.1| -------------------------CAAAATATGTTTATAGGAAT-AATT 414
gi|2791574|emb|AJ223335.1| -------------------------TGAAATATGTTTATATGAAT-AATT 417
gi|2791572|emb|AJ223337.1| TATGTGAATGTTTTCGGTGGAAAAGCAAAATATGCTTATATGAA--TATT 441
gi|2791576|emb|AJ223338.1| TATGTGAATGTTTTCGGTGGAAAAGCAAAATATGCTTATATGAA--TATT 442
gi|2791656|emb|AJ223339.1| TATGTGAATGTTTTCGGTGGAAAAGCAAAATATGCTTATATTAAGGTTTT 821
gi|2791661|emb|AJ223346.1| TGTGTGAGTGTTTTCAATGGAAAAGCAAAATATGCTTATATGAA--TATT 811
gi|2791658|emb|AJ223342.1| TATGTGAATGTTTTCAATGGAAAAGCAAAATATGCTTATATGAA--TATT 808
gi|2791662|emb|AJ223347.1| -------------------------TAAAGTTTCATTACACTTG------ 420
gi|2791663|emb|AJ223348.1| -------------------------TAAAGTTTCATTACACTTG------ 797
gi|2791575|emb|AJ223336.1| -----------------------ATTTACAAATAGAACTCAAAAAAGTTC 441
gi|2791651|emb|AJ223341.1| -----------------------TGCTCTTTATACAATTTCAAAATGTGC 432

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                          . ::* :     :.

gi|2791657|emb|AJ223340.1|     TCAA--TAG-------AAAAGCAACATCTGGACATAATA-CT-TGAAGTG 454
gi|2791660|emb|AJ223345.1|     TCAA--TCG-------AAAAGCAACATCTAGACATAATA-CTGTGAAATA 455
gi|2791573|emb|AJ223334.1|     TTAA--TAG-------AAAAGCAACATCTAGACATAATA-CCGTGAAATA 832
gi|2791650|emb|AJ223343.1|     TCAA--TAG-------AAAAGCAACATTCAGACATAATG-TTGTGGAACA 451
gi|2791659|emb|AJ223344.1|     TCAA--TAG-------AAAAGCAACATTCAGACATAATG-TTGTGGAACA 454
gi|2791574|emb|AJ223335.1|     TCAA--TAG-------AAAAGTAACATTCAGACATAATGGCTGTGAATTA 458
gi|2791572|emb|AJ223337.1|     TCAG--TAG-------AAATGCAACATTTAGACGCAATG-TGGTGAGATA 481
gi|2791576|emb|AJ223338.1|     TCAG--TGG-------AAATGCAACATTTAGACGCAATG-TGGTGAGATA 482
gi|2791656|emb|AJ223339.1|     TCAAGTAGA-------AAATGCAACATTCAGAAACAATG-TGGTGAGATA 863
gi|2791661|emb|AJ223346.1|     TCAG--CAG-------AAAAGCAAAATTTAGACGTAATG-TCGTGAAATA 851
gi|2791658|emb|AJ223342.1|     TTAT---AG-------AAAAGCAACATTTGGACGCAATT-TCGTGAGATA 847
gi|2791662|emb|AJ223347.1|     -CGA--AGG-------CTTTGGGAATAATAATAGTAATAATAAATAT--- 457
gi|2791663|emb|AJ223348.1|     -CGA--AGG-------CTTTGGGAATAATAATAGTAATAATAAATATTAA 837
gi|2791575|emb|AJ223336.1|     ACTTTCAAGAAATGCTCATAAGAGCATTTTATACAGAAAGTCAACAATTT 491
gi|2791651|emb|AJ223341.1|     TCATATAGG-------CATTCAAGCATTTCGAATAACAAAGCAACATTTT 475
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gi|2791660|emb|AJ223345.1|     TG-TTATGTTATCGAGATATTACATAGAAATTGCTGTTCTTTAATG-TAA 503
gi|2791573|emb|AJ223334.1|     TG-TTATGTTATCG----ATTACATAGAAATTACTGTTCTTCAATG-TAA 876
gi|2791650|emb|AJ223343.1|     TG-TTATGATTT-GAGATATTACGTAGAAATTACTGTTATTCAAAG-TAA 498
gi|2791659|emb|AJ223344.1|     TG-TTATGATTT-GAGATATTACGTAGAAATTACTGTTATTCAAAG-TAA 501
gi|2791574|emb|AJ223335.1|     TGGGTATGTTATCGAAATATTACATAGAAATTACT--TATTTAATG-TAA 505
gi|2791572|emb|AJ223337.1|     TG-TTATGTTATCGAAGTATTACATAGAAATTACTGCTCACCGATA-AAA 529
gi|2791576|emb|AJ223338.1|     TG-TTATGTTATCGAAGTATTACATAGAAATTACTGCTCACCGATA-CAA 530
gi|2791656|emb|AJ223339.1|     TG-TTATGTTATCGAAGTATTACATAGAAATTACTGCTCACCGATA-CAA 911
gi|2791661|emb|AJ223346.1|     TG-TTATGTTATCAAAGTATTACATAGAAATTACTGCTTTTCGATA-CAT 899
gi|2791658|emb|AJ223342.1|     TG-TTATGTTGTCGAAGTATTACATAGAAATTACTGCTCTCCGATA-CAA 895
gi|2791662|emb|AJ223347.1|     TAATAATAATAAATAATAATAATAATAATAATAATAAATAATAATAATT-TAA 506
gi|2791663|emb|AJ223348.1|     TAATAATAATAAATAATAATAATAATAATAAAAATAAATAATAATAATT-TAA 886
gi|2791575|emb|AJ223336.1|     CTCTTATAATGTAAGATATTACGTGAAAATTTGGTGATTTCTAARR-CAA 540
gi|2791651|emb|AJ223341.1|     G-CTTC-AAAGTGAAATAGTAC--AAAGAGTTTCTACATTCTAAACGTAA 521
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gi|2791573|emb|AJ223334.1|     TCTGTAACATT--CTGATTCG-----TAAAACATTTTTT-CCACATAAAT 918
gi|2791650|emb|AJ223343.1|     TCTATAACATT--TTGAATTG-----AAGAGCATGTTTC--CACTTAATT 539
gi|2791659|emb|AJ223344.1|     TCTATAACATT--TTGAATTG-----AAGAGCATGTTTC--CACTTAATT 542
gi|2791574|emb|AJ223335.1|     TTCATAGCATT--TTGAATTA-----AAGAACATTTTTC--CACATAAAT 546
gi|2791572|emb|AJ223337.1|     TCTATAACATT--TTCACTT-----TAACAGCATTTTCC--CTTGTAAAT 570
gi|2791576|emb|AJ223338.1|     TCTATAACATT--TTCACTT-----TAACAGCATTTTCC--CTTGTAAAT 571
gi|2791656|emb|AJ223339.1|     TCTATAACATT--TTCACTT-----TAACAGCATTTTCC--CTTGTAAAT 952
gi|2791661|emb|AJ223346.1|     ---ATAACATT--TTCACTT-----CAACAGCATTTTCC--CTTGTAAAT 937
gi|2791658|emb|AJ223342.1|     TTTATAAAATT--TTCATTTGCATTCAACAGCATTCTCC--CTTGTAAAT 941
gi|2791662|emb|AJ223347.1|     TTTATAGCATT--CTGACYTG-------AAATGTTTTCC-------ACAT 540
gi|2791663|emb|AJ223348.1|     TTTATAGCATT--CTGACTTG-------AAATGTTTTCC-------ACAT 920
gi|2791575|emb|AJ223336.1|     ACTATAATATT-CCTAGCTTAT----GAAAACATCACGT-TCTCATAAGC 584
gi|2791651|emb|AJ223341.1|     TCTATAACATTTTCTAGCTTAT----GAAAACATCGTTT-CTTCATAAGT 566
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gi|2791573|emb|AJ223334.1|     GCT-AAAGAACGTTGGAAATTGCAAGAATCT---------CGCTCAAATG 958
gi|2791650|emb|AJ223343.1|     GCT-TAAAAATATTAGAATTAGCAAAAATCT---------CGCTGAAACA 579
gi|2791659|emb|AJ223344.1|     GCT-TAAAAATATTAGAATTAGCAAAAATCT---------CGCTGAAACA 582
gi|2791574|emb|AJ223335.1|     GCT-TAAGAATGTTGGATATGGCAAGATTCT---------CACTGAAGCG 586
gi|2791572|emb|AJ223337.1|     GTT-CGAAAATGTTGGAAAATGCAAG-----------------CAAAACG 602
gi|2791576|emb|AJ223338.1|     GTT-CGAAAATGTTGGAAAACGCAAG-----------------CAAAACG 603
gi|2791656|emb|AJ223339.1|     GTT-CGAAAATGTTGGAAAATGCAAG-----------------CAAAACG 984
gi|2791661|emb|AJ223346.1|     GTT-CGAGGATGTTGAAAAATGCAAG-----------------CAAAACG 969
gi|2791658|emb|AJ223342.1|     GTTGAGTATATGTTGGAAAATGCAAG-----------------CAAAACG 974
gi|2791662|emb|AJ223347.1|     ATG-AAAGAACTTTGGAAATTGCAAGAATTT---------CGCTAAAATG 580
gi|2791663|emb|AJ223348.1|     ATG-AAAGAACTTTGGAAATTGCAAGAACTT---------CGCTGAAATG 960
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gi|2791651|emb|AJ223341.1|     CAG-AGAAAATGTTGCGATATATAAG-----------------TMTAACG 598
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gi|2791573|emb|AJ223334.1|     CCTTCGATCAATG-CATTTCGTAATGTTCAGGGG---------------- 991
gi|2791650|emb|AJ223343.1|     CCTGTGAACAATT-CATTTCATGGTGTTTAGGGA---------------- 612
gi|2791659|emb|AJ223344.1|     CCTGTGAACAATT-CATTTCATGGTGTTTAGGGG---------------- 615
gi|2791574|emb|AJ223335.1|     CCTGTGATCAAGT-CATCTCATGGTGTTTAGGGG---------------- 619
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gi|2791656|emb|AJ223339.1|     CTTCAAATCAATG-CATTTCGTGGTGTTCAGGGG---------------- 1017
gi|2791661|emb|AJ223346.1|     CCTCTGATCAATG-CATTTCGTGGTGTTCAGGGG---------------- 1002
gi|2791658|emb|AJ223342.1|     CCTCTGATCAGTG-AATTTCGTGGTGTTCAGGGG---------------- 1007
gi|2791662|emb|AJ223347.1|     CCTGTAATCAGTG-CATTTCGTGATGTTCAGGGGGTGTTTTAATTCACTA 629
gi|2791663|emb|AJ223348.1|     CCTGTAATCAGTG-CATTTCGTGATGTTCAGGGGGTGTTTTAATTCACTA 1009
gi|2791575|emb|AJ223336.1|     CCTTTCAAAAACG-CATCTCCAGGTATCCAGGGGGTGTCTTAATTCACTA 662
gi|2791651|emb|AJ223341.1|     TCTCTAAACAATG-CATTTCTTGGCRTCTAGGGGGTGTCTTAATTCACTG 647
                               *   *:.*.   .** ** :..  *  ****.

gi|2791657|emb|AJ223340.1|     ----------------GTGTTTTAATTCACTAGCATTG-CAGACCGTTAT 650
gi|2791660|emb|AJ223345.1|     ----------------GTGTTTTAATTCACTAGCATTG-CAGACCGTTAT 662
gi|2791573|emb|AJ223334.1|     ----------------GTGTTTTAATTCACTAGCATTG-CAGACCGTTAT 1024
gi|2791650|emb|AJ223343.1|     ----------------GTGTTTTAATTCACTTGCATTG-CAGAGCGTTAT 645
gi|2791659|emb|AJ223344.1|     ----------------GTGTTTTAATTCACTTGCATTG-CAGAGCGTTAT 648
gi|2791574|emb|AJ223335.1|     ----------------GTGCTTTAATTCACTAGCATTG-CCGAACGTTAT 652
gi|2791572|emb|AJ223337.1|     ----------------GTGTTTTAATTCACCAGCATTG-CATATCGTTAT 668
gi|2791576|emb|AJ223338.1|     ----------------GTGTTTTAATTCACTAGCATTG-CATATCGTTAT 669
gi|2791656|emb|AJ223339.1|     ----------------GTGTTTTAATTCACTAGCATTG-CATATCGTTAT 1050
gi|2791661|emb|AJ223346.1|     ----------------GTGTTTTAATTCACTAGCATTG-CATATCGTTAT 1035
gi|2791658|emb|AJ223342.1|     ----------------GTGTTTTAATTCACTAGCATTG-CATATCGTTAT 1040
gi|2791662|emb|AJ223347.1|     GCAATGAG----GGGGGTGTTTTAATTCACTAGCAATGACAGACCGTTAT 675
gi|2791663|emb|AJ223348.1|     GCAATGCAGACAGGGGGTGTTTTAATTCACTAGCAATG-CAGACCGTTAT 1058
gi|2791575|emb|AJ223336.1|     TCCAGGGG--------GTGTCTTAATTCACTAGCATTG-CAGAACGTTAT 703
gi|2791651|emb|AJ223341.1|     -ACAGGGG--------GTGTTTTAATTCACTGGCAGTG-CAGAGCGCTAT 687
                                    ***  *********  *** ** *. * ** ***

gi|2791657|emb|AJ223340.1|     GTCGAGTAGAAGTCTTTTTGTACCG------------------------- 675
gi|2791660|emb|AJ223345.1|     GTCGAGTAGAAGTCTTTTTGTACCG------------------------- 687
gi|2791573|emb|AJ223334.1|     GTCGAGTAGAAGTCTTTTTGTACCGATATCACTTGGGAAAAATCGCAGCG 1074
gi|2791650|emb|AJ223343.1|     GTCGAGTAGAAGTCTTTTTGTACCG------------------------- 670
gi|2791659|emb|AJ223344.1|     GTCGAGTAGAAGTCTTTTTGTACCG------------------------- 673
gi|2791574|emb|AJ223335.1|     GTCGAGTAGAAGTCTTTTTGTACCG------------------------- 677
gi|2791572|emb|AJ223337.1|     GTCGAGTAGAAGTCTTTTTGTACCG------------------------- 693
gi|2791576|emb|AJ223338.1|     GCGGAGTAGAAGTCTTTTTGTACCG------------------------- 694
gi|2791656|emb|AJ223339.1|     GTCGAGTAGAAGTCTTTTTGTACCGATATCACTTGGGAAAAATCGCAGCG 1100
gi|2791661|emb|AJ223346.1|     GTCGAGTAGAAGTCTTTTTGTACCGATATCACTTGGGAAAAACCGCAGCG 1085
gi|2791658|emb|AJ223342.1|     GTCGAGTAGAAGTCTTTTTGTACCGATATCACTTGGGGAAAACTCCGCCA 1090
gi|2791662|emb|AJ223347.1|     GTCGAGTAGAAGTCTTTTTGTACCG------------------------- 700
gi|2791663|emb|AJ223348.1|     GTCGAGTAGAAGTCTTTTTGTACCGATATCACTTGGGAAAAATCGCAGCG 1108
gi|2791575|emb|AJ223336.1|     GTCGAGTAGAAGTCTTTTTGTACCG------------------------- 728
gi|2791651|emb|AJ223341.1|     GTCGAGTAGAAGTCTTTTTGTACCG------------------------- 712
                               *  ***********************

gi|2791657|emb|AJ223340.1|     --------------------------------------------------
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gi|2791650|emb|AJ223343.1|     --------------------------------------------------
gi|2791659|emb|AJ223344.1|     --------------------------------------------------
gi|2791574|emb|AJ223335.1|     --------------------------------------------------
gi|2791572|emb|AJ223337.1|     --------------------------------------------------
gi|2791576|emb|AJ223338.1|     --------------------------------------------------
gi|2791656|emb|AJ223339.1|     GC--CATCGTAAGGGTGGGATGAAATTTTTCTAGATTCGCTAGAAATCTT 1148
gi|2791661|emb|AJ223346.1|     GC--CATCGTAAGGGTGGGATGAAATTTTTCTAGATTCGCTAGAAATCTT 1133
gi|2791658|emb|AJ223342.1|     GCGGCGTCGTGAGGGTGGGATGAAATTTTTCTAGATTCGCTAGAATTCTT 1140
gi|2791662|emb|AJ223347.1|     --------------------------------------------------
gi|2791663|emb|AJ223348.1|     --GCGATCGTAAGGGTGGGATGAAATTTTTCTAGATTCGCTAGAAATCTT 1156
gi|2791575|emb|AJ223336.1|     --------------------------------------------------
gi|2791651|emb|AJ223341.1|     --------------------------------------------------


gi|2791657|emb|AJ223340.1|     --------------------------------------------------
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gi|2791573|emb|AJ223334.1|     TCTGAGATCCCAACGTTTCCTTGCATACTGGTGTCGTCGTGTGAAAATCT 1172
gi|2791650|emb|AJ223343.1|     --------------------------------------------------
gi|2791659|emb|AJ223344.1|     --------------------------------------------------
gi|2791574|emb|AJ223335.1|     --------------------------------------------------
gi|2791572|emb|AJ223337.1|     --------------------------------------------------
gi|2791576|emb|AJ223338.1|     --------------------------------------------------
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gi|2791658|emb|AJ223342.1|     TCTGCGATTCCAACATTTCCTTGCAAACTTGTGTCGTCGTGTGAAAATCT 1190
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gi|2791663|emb|AJ223348.1|     TCTGAGATCCCAACATTTCCTTGCATACTGGTGTCGTCGTGTGAAAATCT 1206
gi|2791575|emb|AJ223336.1|     --------------------------------------------------
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gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      GCATTTCT-CTTACATCTGTTGGAGTTTTGCGAAAATTCAAACGTTTGGA 1247
gi|2791661|emb|AJ223346.1|      GCATTTCTTCTTACATCTGTTGGAGTTTTGCGAAAATTCAAACGTTTGGA 1233
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gi|2791651|emb|AJ223341.1|      --------------------------------------------------

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gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      AGAAACCATCATTCGTGATTCGCTGGTTTCGCAGCCATTCGCGTCGATTG 1297
gi|2791661|emb|AJ223346.1|      AGAAACCATCATTCGTGATTCGCTGGTTTCGCAACCATTCGCGTCGATTG 1283
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gi|2791663|emb|AJ223348.1|      AGAAACCATCATTCGTGATTCGCTGGTTTCGTAACCATTCGCGTCGATTG 1305
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gi|2791650|emb|AJ223343.1|      --------------------------------------------------
gi|2791659|emb|AJ223344.1|      --------------------------------------------------
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gi|2791572|emb|AJ223337.1|      --------------------------------------------------
gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      TGGTCCTTATTCGTAGCATTTGCGGCGAATTTGCGGTTGCCTTCGCGTTT 1347
gi|2791661|emb|AJ223346.1|      TGGTCCTTATTCGTAGCATTTGCGGCGAATTTGCGGTTGCCTTCGCGTTT 1333
gi|2791658|emb|AJ223342.1|      TGGTCCTTATTCGTAGCATTTGCGGCGAATTTGCGGTTGCCTTCGCGTTT 1340
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gi|2791663|emb|AJ223348.1|      TGGTCCTTATTCGTAGCATTTGCGGCGAATTTGCGGTTGCCTTCGCGTTT 1355
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gi|2791659|emb|AJ223344.1|      --------------------------------------------------
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gi|2791576|emb|AJ223338.1|      --------------------------------------------------
gi|2791656|emb|AJ223339.1|      TGGTGGTCGAGCCACGCGTTATCGTCGTATGTGGTCTCTCATACGCAAAT 1397
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gi|2791658|emb|AJ223342.1|      TGGTGGTCGAGCCACGCGTTATCGTCGTATGTGGTCTCTCATACGCAAAT 1390
gi|2791662|emb|AJ223347.1|      --------------------------------------------------
gi|2791663|emb|AJ223348.1|      TGGTGGTCGAGCCATGCGTTATCGTCGTATGTGGTCTCTCATACGCAAAT 1405
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gi|2791651|emb|AJ223341.1|      --------------------------------------------------

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gi|2791660|emb|AJ223345.1|      --------------------------------------------------
gi|2791573|emb|AJ223334.1|      ACCTGATTGATTCTGTCAGCGCTATATGCTCAGTTTAAAGATTAAGCCAT 1420
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gi|2791659|emb|AJ223344.1|      --------------------------------------------------
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gi|2791572|emb|AJ223337.1| --
gi|2791576|emb|AJ223338.1| --
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gi|2791661|emb|AJ223346.1| ACCTGATTGATTCTGTCAGCGCTATATGCTCAGTTTAAAGATTAAGCCAT 1433
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gi|2791662|emb|AJ223347.1| --
gi|2791663|emb|AJ223348.1| ACCTGATTGATTCTGTCAGCGCTATATGCTCAGTTTAAAGATTAAGCCAT 1455
gi|2791575|emb|AJ223336.1| --
gi|2791651|emb|AJ223341.1| --

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gi|2791659|emb|AJ223344.1| -----------
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gi|2791572|emb|AJ223337.1| -----------
gi|2791576|emb|AJ223338.1| -----------
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gi|2791658|emb|AJ223342.1| GCATGTCTAAG 1451
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gi|2791663|emb|AJ223348.1| GCATGTCTAAG 1466
gi|2791575|emb|AJ223336.1| -----------
gi|2791651|emb|AJ223341.1| -----------

Appendix 2: Pictures of parasite eggs

Figure 21. *Parascaris equorum egg.*

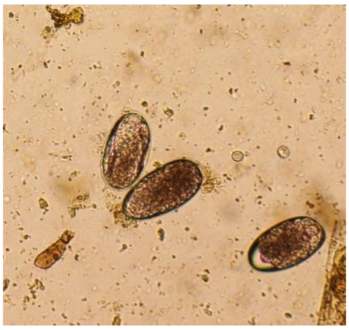

Figure 22. Strongyle eggs.

Figure 23. *Anoplocephala perfoliata* egg.

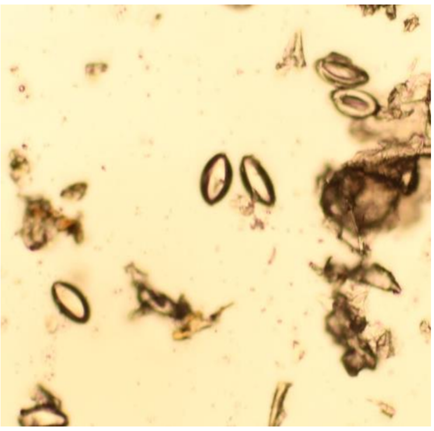

Figure 24. *Oxyuris equi* eggs.

Appendix 3: Result of gel electrophoresis

Table 11. Result from January

Horse	R	47/48	38/39	45	46
Bork	x	-	-	-	-
Hampe	x	-	x	-	x
Hugo	x	-	-	-	-
Wiper	x	-	-	-	-
Birk	x	-	-	x	-
Messer	x	-	x	-	-
Picasso	x	-	x	-	x
Tulin	x	x	x	x	x

X=Positive result - =negative result

Table 12. Result from April

Horse	R	47/48	38/39	45	46
Bork	x	x	-	-	x
Hampe	x	-	x	x	x
Thrim	x	x	x	x	x
Hugo	x	-	x	x	x
Qåtten	x	-	x	x	x
Wiper	x	-	x	x	x
Äskil	x	x	-	-	x
Birk	x	-	x	x	x
Messer	x	x	x	x	x
Picasso	x	x	-	-	x
Tulin	x	x	x	x	x

X=Positive result - =negative result