

**Intestinal parasite levels of Assateague Island feral
horses: variation within the herd and comparison to
domestic horses**

Joanna McCulloh

Department of Biology, St. Mary's College of Maryland

Mentor: Rachael Myerowitz

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Abstract:

Internal parasites cause many problems in horses from general lack of energy to colic and even death if the infection is severe enough. A factor that may lead to increased parasite infection is a high stocking rate: a large number of horses kept on a small amount of land. In this study the parasite levels of domestic horses kept confined to pastures were compared with the parasite levels of free-roaming feral horses living on Assateague Island, MD. Parasite levels of horses living on the northern end of the island, which is more crowded during the summer, versus the southern end, which is less crowded during the summer, were also compared. My hypothesis was that the domestic horses would have greater overall parasite burdens than the feral horses and that the feral horses living on the more crowded northern end of the island would have greater average parasite burdens than the feral horses living on the southern end. Fecal samples were collected and analyzed using a modified Cornell-McMaster technique to determine numbers of parasite eggs per gram (EPG) passed in the feces of each horse. A high EPG most likely correlates with a high level of sexually reproducing internal parasites. Domestic horses sampled were found to have significantly higher EPGs than Assateague horses. This

finding could be explained by the ability of feral horses to migrate to grazing areas unpolluted with feces, genetic differences among the horse populations, and alternate grazing of feral horses with other species that do not share the same parasites. Northern-dwelling Assateague horses were found to have significantly higher EPG values than the southern-dwelling horses. This observation can be explained by the overcrowded conditions that the northern-dwelling horses face in comparison to the southern-dwelling horses. Unfortunately these findings must be considered preliminary because of small sample size and other problems. Overall the findings of this study are important because they stress the importance of a good parasite control program for horses kept at high stocking rates. Also, results suggest that horses kept at low stocking rates can be treated for parasites less frequently, and this would help to avoid the development of anthelmintic-resistance.

Introduction:

One of the most formidable challenges of horse owners today is controlling internal parasites. Even with the host of anthelmintics on the market today these pests still manage to thrive and cause health and performance problems in horses. As well as contributing to colic, diarrhea, and progressive wasting, internal parasites can kill if the problem is severe enough (King, 1997; Levine, 1978; Bowman, 1999). In a post mortem survey of 480 horses from 1958 to 1980, endoparasitism was one of the top three contributors to animal death (Baker and Ellis, 1981).

Parasitic infection cycles and the damage they can cause:

Three main groups cause the majority of parasitic worm problems in United States domestic horses: cyathostomes, large strongyles, and roundworms (Duncan, 1985).

Internal parasite infection in general begins with ingestion of either third-stage larvae or eggs that are found in the grass on which the horse grazes (Sellnow, 1998). In the case of cyathostomes, or small strongyles, the third stage larvae burrow into the wall of the large intestine where they mature into fourth-stage larvae. These larvae can remain encysted in the intestinal mucosa for as short as several weeks or as long as several years. Once the larvae emerge they mature, mate, and produce eggs, which pass into the environment in feces. The eggs hatch and the larvae mature into the infective third-stage. Then the cycle begins again. The most harmful stage in the life cycle of cyathostomes is the emergence of the encysted larvae, which irritate the lining of the intestine with their metabolic wastes (King, 1997; Levine, 1978; Bowman, 1999). Mass emergence of larvae, or cyathostomiasis, often causes diarrhea, progressive wasting, and hypoalbuminemia. Approximately 75-100% of eggs found in horse feces are cyathostome eggs (Bowman, 1999).

The life cycle of the large strongyle is similar to that of the cyathostome except larvae migrate and mature in the cranial mesenteric artery, in the case of *Strongylus vulgaris* or within the circulatory system of the liver, in the case of *S. equinus*, and *S. edentatus* (Bowman, 1999; Sellnow, 1998). After about four months the mature larvae return to the large intestine to mature, mate, and shed eggs. Migration of the larvae often causes organ damage and blood clot formation (King, 1997; Levine, 1978). A heavy infection can

cause anemia and occasionally intestinal blockage (Bowman, 1999; Duncan, 1985). *S. vulgaris* eggs constitute about 10% or less of the total eggs found in horse feces (Bowman, 1999).

The life cycle of the roundworm, *Parascaris equorum*, is different in that eggs are ingested by the horse and hatch in the small intestine where they burrow into the veins (Sellnow, 1999). From here they travel to the liver, heart, and finally the lungs, where they are coughed up and reswallowed. The worms mature, mate, and produce eggs in the small intestine. Roundworms are mainly a problem in young horses and can stunt growth and occasionally cause ruptures in the small intestine leading to death (Duncan, 1985). Older horses tend to develop immunity to these parasites, although they persist at low levels in horses of all ages (Bowman, 1999; Klei, 2000).

Detection of parasitic infection through quantitative and qualitative methods:

In order to detect an internal parasite infection, a fecal sample is collected from the animal in question and tested using one of several methods. Qualitative methods, such as the direct smear technique, the flotation concentration technique, the fecal sedimentation technique, and the Baermann technique can help veterinarians detect an infection and possibly identify the types of parasites involved (Bowman, 1999). Quantitative techniques, such as dilution egg counts and concentration egg counts can be used to determine the severity of infection and possibly to identify the species involved.

The direct smear technique is performed by mixing a very small amount of feces with saline, smearing it onto a slide, adding a coverslip, and examining it under the microscope (Bowman, 1999). Thin feces can even be directly smeared without addition of saline. This method is simple, quick, and requires only a small amount of feces, ideal for situations where the animal is passing very watery diarrhea. Also this method is gentler on fragile larvae and protozoans. The drawback to this method is that negative readings are somewhat inconclusive due to the small amount of feces examined.

The qualitative flotation concentration technique takes advantage of the fact that parasite eggs are less dense than food particles (Bowman, 1999). In this method about a teaspoon of feces is mixed with water to make a thin paste. The mixture is strained using cheesecloth or gauze and the solid wastes discarded. The liquid is centrifuged and the supernatant discarded. Then the pellet is suspended in sodium nitrate solution (specific gravity 1.20), zinc sulfate solution (specific gravity 1.18), or sucrose solution (specific gravity 1.33) and centrifuged again. Then the surface film is carefully removed and examined under 100 x magnification. A simplified variation on this technique is to use gravity instead of centrifugation. Many kits are available in which the feces is mixed with sodium nitrate solution or sucrose solution and a strainer device is used to separate out large particles. The eggs float to the surface and can be examined under the microscope within 10-15 minutes. These methods are more decisive than the smear technique, but they are not as gentle, take longer, and slides must be examined right away or the eggs may become distorted by the flotation solution.

The fecal sedimentation technique is performed by mixing feces with water or formalin and straining through a cheesecloth or gauze (Bowman, 1999). This removes large food particles. The strained liquid is centrifuged and the supernatant discarded. The pellet, containing the eggs, is suspended in water or formalin. This procedure is repeated until the supernatant is clear. The purpose of repeated washing and centrifugation is to separate the eggs from food particles that might make the eggs harder to see under a microscope. Finally the pellet is resuspended in water or formalin and ethyl acetate by shaking vigorously. The mixture is centrifuged again and the pellet examined under the microscope. Obtaining a negative result (no eggs) from this test is more decisive in labeling an animal “parasite free” than the direct smear technique but less so than the flotation concentration method. Of course even an animal with no eggs in the feces may be infected with sexually immature parasites.

Unlike the previous methods, the Baermann technique identifies parasite larvae rather than eggs (Bowman, 1999). This method takes advantage of the fact that nematode larvae cannot swim against gravity. A large fecal mass is broken up and wrapped in cheesecloth. The bundle is secured at the top of a funnel, the bottom of which is clamped off. Inside the funnel is warm water, which stimulates the larvae to migrate. Ideally this setup is left overnight, and then a drop of water from the bottom of the funnel is placed on a slide and examined.

All of the methods mentioned above are useful for detecting infection, but quantitative methods are needed to determine the severity of infection (Bowman, 1999). The most

popular quantitative technique is the dilution egg count Cornell-McMaster method. In this method a defined weight of feces are suspended uniformly in a defined volume of flotation solution, usually sodium nitrate or sucrose. Slides containing chambers of a defined volume with grids of defined size are loaded immediately and examined under the microscope at 100x. The eggs are less dense than the floatation solution and thus they float and stick to the top of the chambers. Using the chamber volume, the weight of feces, and the volume of floatation solution, the number of parasite eggs per gram of feces can be calculated. The biggest plus to this method is that it is simple and fast, ideal for use in a veterinary clinic. Many modifications of this technique have been developed and some kits are available for sale that make the process even easier.

Another quantitative method is an egg extraction technique that relies on concentration of eggs like the method proposed by Rossanigo and Gruner (1991). In this method feces are mixed with water and washed through a series of sieves to separate out large particles. The resulting solution is mixed with hydrated aluminum silicate and centrifuged. The supernatant is then mixed with magnesium sulfate and centrifuged multiple times to collect all of the eggs. The concentrated eggs are then diluted and the number of eggs determined under the microscope. Although this method is much more time-consuming than the Cornell-McMaster technique, 95.9 – 99.5% of the total eggs are extracted using the egg extraction method versus 16.5% with the Cornell-McMaster method.

Of course the ultimate method of quantifying parasite infection and also of identifying the parasites plaguing a particular animal is to do a necropsy. However, this is not an

option in most cases unless the animal has died already or experimental animals have been obtained for such a purpose. Next to necropsy, the most effective methods of identifying parasite infection are the egg concentration methods, followed by the dilution methods. In this experiment I chose to use a dilution egg count method because it enabled me to obtain quantitative data and was also fast and required little equipment. Although an egg extraction (concentration) method may have been more reliable, especially with low egg counts, I did not have a centrifuge at my disposal nor a lab full of research assistants to help me examine all of my samples as this method is much more time-consuming.

Anthelmintics and prevention of parasitic disease:

Once an animal is diagnosed with internal parasites it is wise to treat it with anthelmintics, a drug that kill worms that are already in the horse, to avoid further contamination of the pasture and deterioration of the animal's health. Generally it is best to use preventative measures before internal parasite infection becomes an issue. Many methods have been proposed to control internal parasites in horses. The most popular method is to treat horses periodically with anthelmintics. If treatments are given frequently enough contamination of the pasture can be prevented as well. Many anthelmintics are available on the market and some are more broad-spectrum than others. Benzimidazoles effectively kill mature large strongyles and cyathostomes as well as larval cyathostomes that are not encysted (Duncan, 1985). Pyrantel has similar potency and it also kills roundworms effectively. Ivermectin kills mature and larval large strongyles and cyathostomes as well as roundworms. Avermectins, a group of relatively new anthelmintics, kill mature and larval large strongyles, roundworms, and all life

stages of cyathostomes, including encysted larvae (Sellnow, 1998; Rolfe, Dawson, and Holm-Martin, 1998). A study on the efficacy of various anthelmintics showed that moxidectin, an avermectin drug, was the most effective in suppressing fecal eggs counts, followed by ivermectin, morantel, a pyrantel drug, and oxbendazole, a benzimidazole drug (Rolfe, et. al., 1998). Resistance to oxbendazole and morantel were observed in this study.

Other methods of controlling internal parasites involve treatment of the pasture and the horse's living area rather than the horse itself. Feeding areas should be kept separate from manure (Levine, 1978). Hay should be fed from a hayrack and not thrown on the ground so that horses do not ingest eggs or larvae from the ground as they eat (Levine, 1978). Ideally manure should be removed from the pastures and stalls daily and either composted so that the heat of decomposition can kill parasite eggs and larvae or spread on an unused pasture so that the sun can kill the parasites. Even twice-weekly removal of manure has been shown to reduce pasture infectivity and increase grazing area by 50% (Herd, 1986). Alternatively, rotating pastures between sheep and horses has been shown to decrease transmission of large strongyles and cyathostomes (Eysker, Jansen, and Wemmenhove, 1983). However, there is an increased transmission of a less common parasite, *Trichostrongylus axei* under this rotation plan. New horses should be treated for internal parasites before introducing them onto the pasture (Levine, 1978).

A factor that may lead to increased parasite infection and reinfection is a high stocking rate, or a large number of horses kept on a small amount of land (Duncan, 1985). The

reasoning behind this idea is that horses kept on overpopulated pastures have to eat where they defecate because they have no other choice. Therefore they ingest the parasite larvae and eggs spread by their own feces and continually reinfect themselves. In comparison, horses with many acres over which to roam, such as free-ranging feral horses, defecate and then move on to eat somewhere else. Once they come back to the spot where they left their feces initially most of the infective larvae have died (Sellnow, 1998).

Feral Horses of Assateague Island:

It is recommended that all horses kept in paddocks year round be treated with anthelmintics from six to twelve times a year in order to prevent pasture contamination and negative health effects due to parasitism (Duncan, 1985). Free-ranging feral horses of Assateague Island, MD have never received anthelmintic treatment yet they are so reproductively successful that their population size must be controlled using proline zonae pellucida (PZP), a vaccine that causes mares to produce antibodies that prevent fertilization of the egg (Turner, 2001). Most likely the Assateague horses avoid frequent health problems associated with parasitic infections because they are able to roam freely and thus they contract few parasites. The first part of this study examines the parasite levels in the Assateague Island feral horses and compares them to the parasite levels in domestic horses infrequently treated with anthelmintics. My hypothesis was that the Assateague horses should have significantly fewer parasites than the domestic horses. The second part of the study examines the parasite levels of feral horses occupying the northern versus the southern end of Assateague Island. During the winter the horses are fairly spread out and evenly spaced. However, during the summer the northern end of the

island becomes more crowded when many horse bands migrate here. The northern end of the island contains campgrounds popular with the public and thus is sprayed for insects. The horses migrate to the northern end most likely to avoid biting insects (Keiper and Zervanos, 1979). I predicted that horses that spend the whole year on the northern end of the island would have higher parasite levels than those that spend the whole year on the southern end of the island.

The importance of this study:

This study is important to gain a better understanding of the feral Assateague horses. Also, this study is important for the horse-owning community because resistance of internal parasites to anthelmintics is a growing problem. For example, benzimidazoles like fenbendazole and oxibendazole, have generally been found to be ineffective against many strongyle infections because of resistance (Rolfe, Dawson, and Holm-Martin, 1998; Parry, Fisher, Grimshaw, and Jacobs, 1993). The more often the parasites are exposed to a particular anthelmintic the faster a resistant strain can develop through natural selection. Therefore one of the best ways to prevent resistance formation is to control parasites through non-chemical methods and treat horses less often. Although it may not always be possible for horses owners to expand their pastures to control parasites, those horse owners who already have a large amount of land with very few horses might consider treating their horses for parasites less often than is currently recommended. Also it might be advisable to rotate pastures when possible to imitate the migration of feral horses.

MATERIALS AND METHODS:

Domestic Horses:

Environment and Lifestyle of Horses:

Fecal samples were collected from horses on 11 Amish and Mennonite farms in St.

Mary's and Charles County in southern Maryland, USA from June 20 to July 26, 2001.

The time period since the last anthelmintic treatment had been given to the horses ranged from 6 months to over a year. Horses at one farm had never been treated conventionally for internal parasites but were fed a home remedy of ash and sulfur every other month.

Various types of anthelmintic treatments had been used on the other farms. Most horses were used for either plowing or pulling a buggy. A few horses were stalled unless they were working and were seldom turned out on a pasture, but most horses were turned out on a pasture at least four days a week. Horses were pastured on anywhere from an eighth of an acre to twenty acres and were kept at densities of 4 acres per horse to 0.125 acres per horse. Some horses were pastured with cows. Most horses were fed corn or grain, hay, and were allowed to graze. The life stages of horses tested ranged from weanlings to aged adults.

Fecal sample collection:

Fecal samples were collected at varying times of day from the ground in stalls or pastures. If manure was not typically removed from stalls a sample was taken from the freshest-looking part of the manure pile. A sample was taken from every fresh manure pile regardless of horse identity. Samples were collected only from horses that had been

without anthelmintic treatment for at least six months. After collection, samples were kept in an ice cooler until they could be analyzed.

Assateague Horses:

Environment and Lifestyle of Horses:

Fecal samples were collected from feral horses at Assateague Island National Seashore on Assateague Island, Maryland, USA from June 6 to July 31, 2001. Assateague is a barrier island on the Atlantic coast that is approximately 61 km long (latitude 38° 2', longitude 75° 12') (Rudman and Keiper, 1991). The average summer temperature is 24° C (July and August), and the average winter temperature is 3-4° C (January and February). Average precipitation is 11.91 cm for July, 14.07 cm for August, 9.50 cm for January, and 8.38 cm for February.

Feral horses in Assateague feed primarily on saltmarsh cordgrass (*Spartina alterniflora*), American three-square sedge (*Scirpus americanus*) and American beachgrass (*Ammophila breviligulata*) (Zervanos and Keiper, 1979; Rudman and Keiper, 1991). The horses associate in bands, groups of two to eight generally comprised of one dominant stallion and his mares. Sometimes bands contain a younger subordinate colt or stallion, usually the offspring of one of the mares. "Bachelor" males also roam by themselves or with other lone males. The horses are wild and do not receive veterinary care except in cases where euthanasia is necessary. They receive no anthelmintic treatment.

Most samples were obtained from the developed area, which is accessible by car.

Samples collected in developed areas were found in the marshes on the bay side of the

island, in the campgrounds, and on the beach on the ocean side of the island. Samples were also collected in the undeveloped areas (ORV zone), accessible only by off-road vehicle, by foot, and by canoe.

Fecal sample collection for comparison to domestic horses:

Fecal samples were collected from the area around grazing horse bands at varying times of the day. Each band present during sample collection was videotaped and identified. An attempt was made not to collect in an area containing a band that had been present during a previous collection session. Samples were collected from every fresh manure pile sited in the area near the horses. The exact identity of the horse that left the sample pile was not determined. Samples were kept in an ice cooler until they could be analyzed.

Fecal sample collection for comparison of north versus south dwelling horses:

Samples were collected from bands in the ORV zone and in the developed zone from January 2- 8, 2002. Once a band was sited, its members were followed until they were observed defecating, and then a fecal sample was collected. As many samples as possible were collected from each band in the time allowed by daylight and the moving patterns of the horses. Pictures were taken of the band members in order to facilitate identification by Assateague National Seashore Park Ranger Allison Turner.

Categorization of north and south dwelling horse bands

Each month, except for June and January, Ms. Turner completes a survey listing the location of each Assateague Island horse. The location of each band sampled was identified for the months of May, July, August, and September using the surveys from

2001. The bands that were found in zone 11, which marks the end of the developed area, or more south for the majority of those months was labeled “south-dwelling.” The bands found north of zone 11 for the majority of those months were labeled “north-dwelling.” If a band was found in the northern area and the southern area equally during those months, Ms. Turner was asked where this band was most likely found, and this became the tiebreaker.

EPG Testing:

Samples were usually analyzed within a few hours of collection. A modified Cornell-McMaster technique using a Paracount-EPG analysis kit was used to find EPG (Olympic Equine Products, Issaquah WA). The mixing vial in the kit was filled to the line indicated with sodium nitrate solution with a specific gravity of 1.200 (26 mL) (Fecasol by Evsco Pharmaceuticals, Buena NJ). Then feces were added to the vial until the solution reached the second line (30 mL total). The mixture was stirred until an even consistency was reached and then the chambers of the counting slide were filled immediately. The samples were analyzed at 100x magnification. The number of eggs within the lines in the chambers was determined. If the last of three readings had a deviance greater than 10% from the average another reading was taken until the last reading taken had a deviance of 10% or less from the average (Levine, 1978). A maximum of six replicates were performed. The average value of all of the readings was multiplied by 50, and this number was the EPG recorded.

Statistical Analysis:

In both parts of the experiment F-test results suggested that the variance between the two sets of data were not equal so t-tests assuming unequal variance were performed as well as descriptive statistics (Excel 1997; Microsoft; Redmond, WA). Categories were created to characterize EPG values in terms of severity of parasite infection. A high level infection was described as 1001 + EPG, a medium level infection was described as 501 – 1000 EPG, and a low level infection was described as 500 EPG or less.

RESULTS:

Comparison of parasite levels of domestic horses versus feral horses:

Statistically the Assateague horses have lower EPG values than the domestic horses ($p = 0.000128$). The mean EPG of Assateague horses is 612 while the mean EPG of domestic horses is 1601 (see Table 1). The median EPG of Assateague horses is 573 and the median EPG of domestic horses is 1058 (see Table 1). The sample variance in both sets of data is very high (see Table 1). Although the standard error of both samples is very high, Figure 1 shows that the data sets are still statistically different with domestic EPG being higher than Assateague EPG. Most Assateague horses tested had a low parasite infection while most domestic horses had high level infections (see Table 2). After low level infections, Assateague horses had medium and then high level infections (see Table 2). The levels of domestic horses with medium and low level infections were very similar, 21% versus 23% (see Table 2).

Table 1: Mean, median, and sample variance calculated from EPG counts of Assateague and domestic horses.

	Assateague horses	Domestic horses
Sample size	56	48
Mean EPG	612	1601
Median EPG	573	1058
EPG Sample Variance	225047	2562319

Table 2: Percent of Assateague and domestic horses with high, medium or low parasite levels. The parasite infection level that has the largest percent for each horse category is highlighted yellow.

Type of horse	Percent in each parasite infection level		
	High	Medium	Low
Assateague	21	36	43
Domestic	56	21	23

Comparison of parasite levels of southern-dwelling horses versus northern-dwelling horses:

Overall the northern-dwelling horses sampled have higher EPG values than the southern-dwelling horses ($p = 0.0113$). Figure 2 shows that the standard error bars of each population do not overlap. It is important to note that there are only eleven horses in the northern horse category and twenty in the southern horse category. Only a small number of horses were sampled due to lack of time and the fact that the horse bands tend to spread out over the island during the winter and are more difficult to locate. Out of the horses sampled, not all could be identified, and so these EPG values were not used. The mean EPG value for the northern horses is 248 while that of the southern horses is 78 (see Table 3). Both of these means fall under the low level infection category (less than 500 EPG). Only one horse sampled has an EPG count in the moderate infection category (650 EPG). This was a northern-dwelling animal. The medians for northern and

southern horses are 217 and 25 respectively (see Table 3). The sample variances for both categories are high, with that of the northern horse population being about three times that of the southern horse category.

Table 3: Mean, median, and sample variance calculated from EPG counts of northern and southern-dwelling Assateague horses.

	Northern horses	Southern horses
Sample size	11	20
Mean EPG	248	78
Median EPG	217	25
EPG Sample Variance	31077	12448

Discussion:

Comparison of parasite levels of domestic horses versus feral horses:

Data suggests that during the summer months the Assateague horses on average have lower EPG values than domestic horses treated with anthelmintics twice a year or less. Lower EPG values most likely correlate with a lower level of sexually mature parasites (Bowman 1999). During the summer most Assateague horses have EPG values that suggest low level infections while most domestic horses treated twice a year or less have high level infections. There are several possible explanations for this observation, including the ability of feral horses to migrate, genetic differences among the horse populations, and alternate grazing of horses with other species that do not share the same parasites.

Most likely the reason for the low parasite levels among Assateague horses is that the feral horses have the ability to migrate, leaving behind infective feces. In doing so the

horses break the parasite life cycle and thus are infected by fewer parasites. In general horses kept confined to a small pasture or stall are more likely to encounter larvae and eggs than horses allowed to roam over hundreds of acres (Duncan, 1985; Sellnow, 1998). Horses have evolved a natural avoidance of feces, but confined horses have little choice but to eat where they defecate (Herd, 1996). Researchers mimicked the conditions of feral horses by removing feces twice a week from the pasture and found that this measure was even more effective than anthelmintics in controlling parasites. Frequent removal of feces is a common preventative measure against horse parasites in Ethiopia, Zimbabwe, and South Africa (Krecek and Guthrie, 1999).

Another possible explanation for the disparity between Assateague horses and domestic horses is genetic differences. Horses have run wild on Assateague Island since the mid-1600's so there is a possibility that the genetic makeup of this population has deviated from that of domestic horses. A population bottleneck might have occurred in which horses originally introduced to the island had a greater immunity to parasites than the average horse and this trait is found in the feral horses of Assateague today. Also over time the Assateague line could have evolved a greater immunity through natural selection. This theory is not the best explanation, however, because over the years new horses, and thus new genes, have been introduced to the Assateague herd. One study done on the Chincoteague, Virginia herd, which was derived from the same original horses as the Assateague group, found that these horses were not genetically unique at the loci sampled (Goodloe, Warren, Cothran, Bratton, and Trembicki, 1991).

Additionally the low parasite levels in the Assateague horses could be the result of alternate and mixed grazing of the horses and other wildlife that do not share the same parasites, such as the Sika deer (*Cervus nippon*) or the Whitetail deer (*Ovis virginianus*), that also occupy Assateague National Seashore. One study on domestic horses found that alternate grazing of sheep and horses results in a reduced transmission of nematodes in the subfamilies Cyathostominae and Strongylinae (Eysker, Jansen, and Wemmenhove, 1983). Mixed and rotational grazing with cattle have also been shown to reduce pasture infectivity (Duncan, 1985). (Several domestic horses in this study were kept on a pasture with one or two cattle; the EPG counts of these horses were not remarkably different from those of the other horses. Possibly there were not enough cattle present to affect parasite levels.)

Little to no research has been done on the parasites infecting the species of Assateague National Seashore. However, preliminary studies on Assateague Sika deer killed by hunters showed that this species was infected with four different parasites, none of which were found in the Assateague horses: *P. equorum*, *Fasciola hepatica*, *Ascaris* spp., and *Trichuris* spp (Keiper and Gavaghan, 1989). The latter three parasites usually do not infect horses (Bowman, 1999). *P. equorum*, however, is not a natural parasite of cervids like the Sika deer, but does infect young horses (Samuel, Pybus, and Kocan, 2001; Duncan, 1985; Bowman, 1999). Older horses tend to develop immunity to *P. equorum*. The feral horses must have introduced this parasite to the area, and over time it has infected a different species. Even though no *P. equorum* eggs were found in the Assateague population, some of them must be infected with this parasite. Strongyles and

cyathostomes are the main parasites found in these horses. Although the sika deer have become a reservoir for *P. equorum*, they are not infected by large strongyles or cyathostomes, the primary parasites of the horses, so their alternate grazing with the horses could negatively affect parasite levels. In theory, when the deer graze they ingest horse-parasite larvae and break the infection cycle because they cannot become infected with these parasites.

Whitetail deer are another species that could potentially lower parasite levels in the horses through alternate grazing. The only parasite that they share with horses is *Trichostrongylus axei*, which was not found in the samples taken from Assateague horses (Samuel, 2001; Bowman, 1999). Most likely the horses are occasionally infected with *T. axei*, but again, this does not appear to be one of their primary parasites.

Although EPG counts have been used extensively in veterinary medicine to determine intensity of parasite infection there are many possible sources of error in this method. In theory internal parasites would release eggs at a constant rate throughout the day and the seasons. These eggs would then be completely and randomly mixed throughout the feces by peristaltic contractions of the intestines. However, studies have shown that some of these assumptions are false. Warnick found that there is a significant daily variation in strongyle EPG values (1992). A larger variation was found in horses with low EPGs. This variation was attributed to differences in rate of egg release, incomplete mixing of the feces, and changes in fecal output. There was no correlation found between time of day and EPG levels (Bennett, 1990). Size of the host as well as fecal consistency may

affect EPG counts (Hall, 182). Although all of the Assateague horses were about equal in size, a lot of variability existed in the size of the domestic horses sampled. Most of them were larger than the Assateague horses as well. Samples collected from any foals may have been artificially high. None of the samples examined were diarrhetic, so fecal consistency was not excessively variable. Seasonal variation was seen in fecal egg counts, with a peak from June to October and a dip from November to May (Poynter, 1954). All of the samples in this study were collected between June and August, so they can be compared validly.

The best way to determine parasite load is to examine the intestines of slaughtered horses. Obviously this was not possible, so most practical method was to use EPG counts. Any factor that could possibly affect EPG was controlled for if possible: samples were collected at the same time of year, from areas of similar elevation weather patterns. All other factors were beyond human control but must be considered nonetheless in determining the validity of the study.

Comparison Between Northern-dwelling and Southern-dwelling Assateague Horses

Preliminary data suggest that the Assateague horses that spend most of the summer on the northern part of the island have higher EPG values than those that spend their summers on the southern part of the island. Higher EPG values most likely correlate with a higher level of sexually mature parasites (Bowman 1999). The reason for this difference is most

likely related to an unequal distribution of horses on the island during the summer months.

During the summer months, starting about late May or early June and ending in late August or early September, horses migrate from the southern part of Assateague and spend more time grazing on the northern part of the island. This is most likely related to the fact that the northern part of the park, containing the campsites accessible by vehicle, is sprayed for insects and therefore the biting insects are less severe (Keiper and Zervanos 1979). Also campers frequently feed the horses and horses forage on the garbage left by the campers.

This migration leaves the horses on the northern part of the island more crowded than those on the southern part of the island. Multiple bands are frequently observed grazing in the same areas at the same time. In contrast, during the summer, the southern-dwelling bands hardly ever graze in the same areas. As in the situation of the domestic horse, the northern-dwelling Assateague horse has more of a chance of coming across an infective parasite larvae or egg than a southern-dwelling horse because the greater concentration of horses creates a greater concentration of feces. This would explain the higher EPG values of the northern-dwelling horses. Unfortunately there are several reasons why the findings in this study must be considered preliminary.

The most important reason why findings must be regarded as preliminary is that the sample sizes of both southern and northern-dwelling horses are very small. Eleven

horses in the northern-dwelling horse category and twenty in the southern-dwelling horse category were sampled in contrast to the fifty-six Assateague horses sampled in the first part of the project. The technique used to determine parasite load is not sensitive enough for these small sample sizes to back any hypothesis. As mentioned earlier, there is a significant daily variation in strongyle EPG values (Warnick 1992). A larger variation is generally found in horses with low EPGs. All of the horses sampled except for one north-dwelling horse have low EPGs. With such small sample sizes, the higher EPG values of the northern-dwelling horses could be the result of a chance situation where all of these horses released more parasite eggs than average on the day sampled.

Another reason why the data in this part of the project should be considered preliminary is that it was not collected at the ideal time of year. There is a general rise in parasite egg production in the spring, and a maximum is reached around August or September (Poynter, 1954). In the winter parasite levels drop and the minimum occurs in January and February. These fluctuations are the result of seasonal variation in rate of infection and rapid maturation of encysted larvae in the intestinal mucosa (Ogbourne, 1975; Reinemeyer, Smith, Gabel, and Herd, 1986). The seasonal fluctuation in EPG was evident in this study as well; the EPG levels in the samples collected from the Assateague horses during the summer were much higher than the EPG levels in the winter samples (collected in January). As a measure of parasite infection, EPG levels are much less variable and therefore more accurate when they are higher (Warnick 1992). The best time of year to get the most accurate results would be at the peak of the rise, in August or September.

Additionally the southern-dwelling horses from which the samples were collected were not the ideal horses to sample. Ideally the “southern-dwelling horses” sampled would have been spotted south of zone 11 May through September. However, every horse labeled “southern-dwelling” was found north of zone 11 at least once during these months and most were found in the northern zones twice out of the four months surveyed. Unfortunately time constraints did not allow for collection from bands never found in the northern zones.

Preliminary research is promising in that the northern-dwelling horses sampled did have significantly higher EPGs than the southern-dwelling horses. More extensive data collection, preferably in late summer or early fall would yield more conclusive results. Horses are infected with the greatest number of egg-laying adults as opposed to larvae or spent adults from spring into fall (April to December) (Reinmeyer, et. al., 1986). Taking the samples in early fall would also allow some of the larvae ingested over the summer, when the northern-dwelling horses are most crowded and the southern-dwelling horses the least crowded, to reach maturity (Round, 1969). The shortest periods between ingestion of infective nematode larvae and appearance of eggs in the feces (prepatent period) ranges from 35-42 days for trichonemid species and 63-70 days for *Triodontophorus* spp. and *Poteriostomum* spp. to 149-156 days for *S. vulgaris*. Also, for the most conclusive results the southern-dwelling horse group should be comprised of horses found further south than zone 11 May through August.

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References:

Baker, J.R. and C. E. Ellis. 1981. A survey of post mortem findings in 480 horses 1958 to 1980: (1) Causes of death. *Equine Veterinary Journal*. 13(1): 43-46.

Bowman, D. D. 1999. *Georgis' Parasitology for Veterinarians*, 7th ed. W. B. Saunders Company, U.S.A.

Duncan, J. L. 1985. Internal parasites of the horse and their control. *Equine Veterinary Journal*. 17(2): 79-82.

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

Gavaghan, Patrick and R.R. Keiper. 1988. Determining Parasite Levels in Assateague White-tailed and Sika Deer. United States Department of the Interior, National Park Service. Form 10-226: Investigator's Annual Report (Natural Sciences Research).

Goodloe, R.B., R.J. Warren, E.G. Cothran, S.Pratton, and K.A. Trembicki. 1991. Genetic variation and its management applications in eastern U.S. feral horses. *Journal of Wildlife Management*. 55(3): 412-421.

Hall, Andrew. 1982. Intestinal helminths of man: interpretation of egg counts. *Parasitology*. 85: 605-613.

Herd, Rupert. 1986. Pasture hygiene: a nonchemical approach to equine endoparasite control. *Modern Veterinary Practice*. 67: 36-38.

Keiper, Ronald R. and Stam M. Zervanos. 1979. Ecological Impact and Carrying Capacity of Feral Ponies on Assateague Island National Seashore. The National Park Service, Philadelphia, PA.

King, Marcia. June 1997. The Newest Dewormers.
<http://www.thehorse.com/0697/newestdewormers0697.html>. Accessed March 1, 2001.

Krecek, R.C. and A.J. Guthrie. 1999. Alternative approaches to control of cyathostomes: an African perspective. *Veterinary Parasitology*. 85: 151-162.

Levine, N. D. 1978. *Textbook of Veterinary Parasitology*. Burgess Publishing Company, U.S.A.

Ogbourne, Colin P. 1975. Epidemiological studies on horses infected with nematodes of the family trichonemidae (Witenburg, 1925). *International Journal for Parasitology*. 5:667-672.

Parry, J.M., Fisher, Grimshaw, and Jacobs. 1993. Anthelmintic dosing intervals for horses: comparison of three chemical groups. *Veterinary Record*. 133: 346-347.

Poynter, D. 1954. Seasonal fluctuation in the number of strongyle eggs passed by horses. *The Veterinary Record*. 66(5): 74-78.

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

Reinmeyer, C.R., S.A. Smith, A.A. Gabel, and R.P. Herd. 1986. Observations on the population dynamics of five cyathostome nematode species of horses in the northern USA. *Equine Veterinary Journal*. 18(2): 121-124.

Rolfe, P. F., Dawson, and Holm-Martin. 1998. Efficacy of moxidectin and other anthelmintics against small strongyles in horses. *Australian Veterinary Journal*. 76(5): 332-334.

Rossanigo, C. E. and L. Gruner. 1991. Accuracy of two methods for counting eggs of sheep nematode parasites. *Veterinary Parasitology*. 39: 115-121.

Round. 1969. The Prepatent Period of Some Horse Nematodes Determined by Experimental Infection. *Journal of Helminthology*. 43:185-192.

Samuel, W.M., M.J. Pybus, and A.A. Kocan. 2001. *Parasitic Diseases of Wild Mammals*, 2nd ed. Iowa State University Press. U.S.A.

Sellnow, Les. July 1998. Parasite Update.
http://www.thehorse.com/0798/parasite_update0798.html. Accessed March 1, 2001.

Turner, Allison. 2001. Assateague Island National Seashore Feral Horse Fertility Control-2000. Assateague Island National Seashore, Division of Resource Management 2000 Annual Reports. Pp 1-8.

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

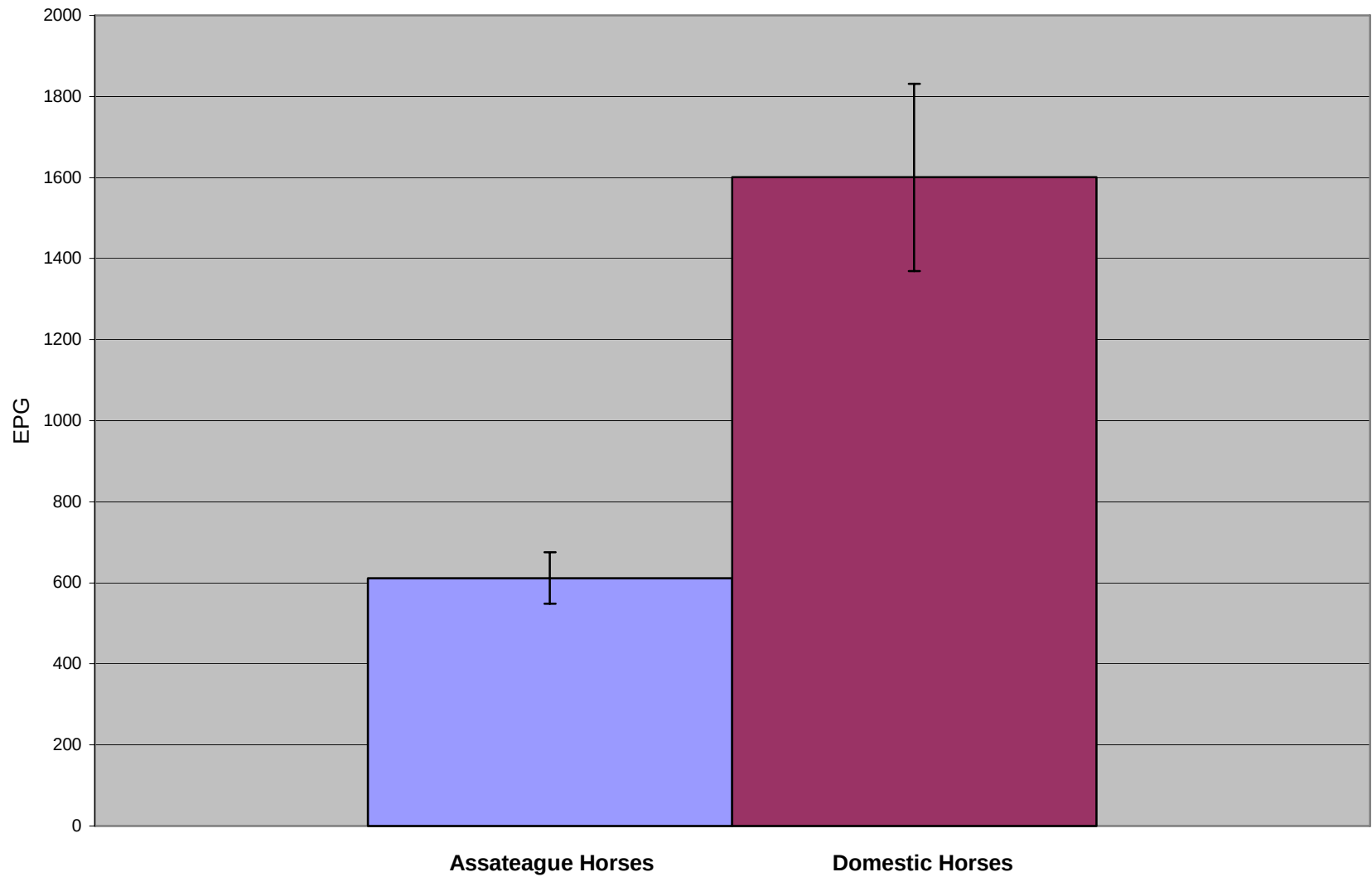


Figure1: Mean EPG levels of Assateague horses and domestic horses shown with one standard error of the mean. Domestic horse EPG levels are significantly higher than Assateague horse EPG levels.

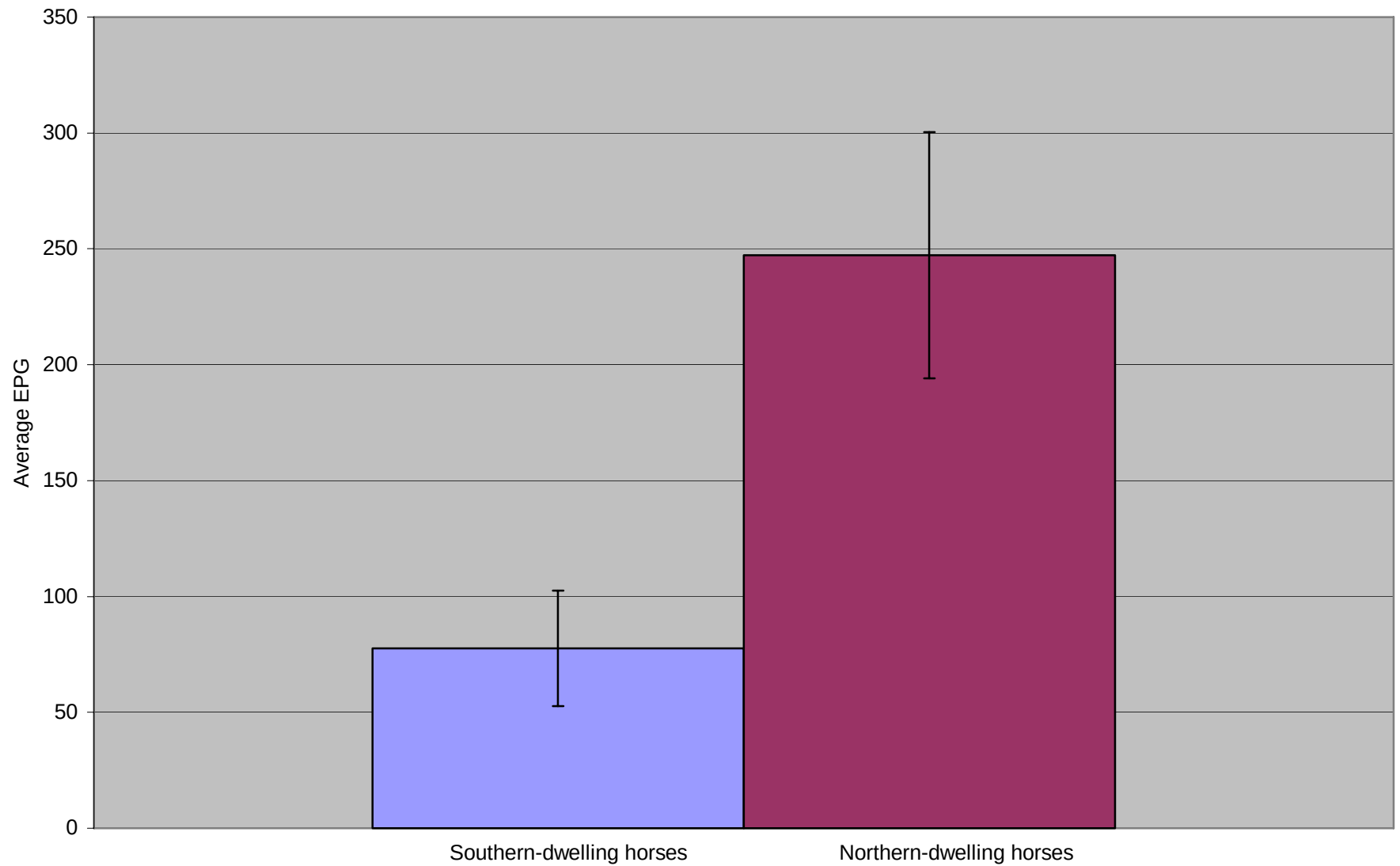


Figure 2: Mean EPG levels of feral horses dwelling in southern and northern Assateague Island shown with one standard error of the mean. Northern-dwelling horses have significantly higher EPG levels than southern-dwelling