POPULATION DYNAMICS OF ORIBATID MITES (ACARI: ORIBATIDA) ON HORSE PASTURES OF NORTH CENTRAL FLORIDA

By

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Abstract of Thesis Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Master of Science

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Population dynamics of oribatid mites on pastures were studied to evaluate the seasonal risk of infection of horses with Anoplocephaline cestodes in Florida. At monthly intervals during calendar years 1999 and 2000, core soil samples were collected to a depth of 10 cm with a bulb planter from multiple (10-30) sites on two horse farms in Alachua and Marion counties in north central Florida. Mites were extracted into 70% ethyl-alcohol by placing soil samples in Berlese Funnels for 7 days. Mites recovered included *Ceratozetes* n.sp., *Epilohmannia minuta*, *Eupelops* sp., *Galumna jacoti*, *Galumana minuta*, *Lamellobates* sp., *Lohmannia* sp.nr. *jornoti*, *Nesiacarus* sp., *Oribatella* sp., *Peloribates* sp. nr. *hirsutus*, *Protoribates capucinus*, *Rostrozetes ovulum*, *Rysotritia ardua*, *Scheloribates* sp., *Tectocepheus velatus*, *Tectoribates* sp., *Trichoribates* sp., *Zygoribatula* sp. nr. *floridana*, and a complex of members of the family Oppiidae consisting of four species: *Acroppia* sp. nr. *antillensis*, *Oppiella nova*, *Neoamerioppia* sp., *Striatoppia* sp. No obvious seasonal pattern in mite availability was observed.

Three-way analysis of variance found no effect on mite count due to date, month and year, or location. Seasonal mite counts were correlated with weather data such as monthly temperatures, rainfall, and water budget with no significance between any of the correlations except with total monthly rainfall, and in that case, the coefficient of determination was less than 10%. To study population distribution the annual dominance and frequency of each species were calculated. Population distributions were then studied using Taylor's power law. Fourteen of the mite species were found to have highly aggregated distributions on our study pastures.

LITERATURE REVIEW

This research project was initiated due to a lack of information about that portion of the horse tapeworm's lifecycle involving the intermediate host, the oribatid mite, in north central Florida. Before initiating the study, a complete literature review was done to gain knowledge about these mites and the tapeworm, *Anoplocephala perfoliata*.

Anoplocephala perfoliata

The equine cestode *Anoplocephala perfoliata* is taxonomically placed in the order Cyclophyllidea van Beneden in Braun, 1900. This order contains fifteen families of tapeworms that infect a broad range of definitive host species, including amphibians, reptiles, birds, and mammals (Schmidt, 1986; Jones et al., 1994). Three species within the family Anoplocephalidae Kholodkovskii, 1902 infect the gastrointestinal tract of horses and donkeys: *Anoplocephala perfoliata* (Goeze, 1782) Blanchard, 1848, *A. magna* (Abildgaard, 1789) Sprengel, 1905, and *Anoplocephaloides mamillana* (Mehlis, 1831) Rausch, 1976 (Schmidt, 1986).

All members of the family Anoplocephalidae are similar in that the scolex is devoid of rostellum, hooks, or hooklets during all stages of development and the suckers are unarmed (Skrjabin and Spasskii, 1951; Beveridge, 1994), thus any member of this subfamily has an unprotected scolex. Due to the strong possibility that this family is polyphyletic, four subfamilies have been described based on type of uterine development (Beveridge, 1994). All members of the subfamily Anoplocephalinae Blanchard, 1891, including the three equine tapeworm species, can be defined by the following

morphologic and biologic characteristics (Soulsby, 1982; Beveridge, 1994). The most defining morphological characteristic of members of the subfamily Anoplocephalinae is that the uterus persists in gravid proglottids. The uterus is initially a transverse tube or reticular network of tubes, but becomes saccate in the final stage of development. Additionally, members have a cysticercoid larval type that occurs within the hemocoele of oribatid mites. Oribatid mites are known to be natural intermediate hosts for all members of the subfamily. There is however experimental evidence that some genera of the subfamily (*Avitellina, Thysaniezia*, and *Thysanosoma*) may use other hosts (Denegri et al., 1998). In contrast, members of the subfamily Linstowiinae Fuhrmann, 1907 use coleopterans as their intermediate hosts, and members of the subfamily Thysanosomatinae Skryabin, 1933 use psocopterous insects as their intermediate hosts (Beveridge, 1994). At this time, life cycles of the members of a fourth subfamily, Inermicapsiferinae Lopez-Neyra, 1943, have not been adequately documented (Beveridge, 1994).

<u>Morphology</u>

Distinguishing characteristics of adult *Anoplocephala perfoliata* (Figure 1) are the following: (1) the length of gravid adult specimens is usually 25 to 40 mm, but may reach 80 mm, (2) the width of the body of *A. perfoliata* is generally between 8 and 14 mm, (3) the scolex or "hold-fast" organ is distinct and much smaller than the body, measuring only 2-3 mm, and (5) the strobila, which is directly caudal to the scolex and contains the first proglottids, has a width of the 1.0 to 1.2 mm. (Skrjabin and Spasskii, 1951).

The morphology of the scolex of *A. perfoliata* is distinct. Four ear-shaped lappets are situated posterior to the four apical muscular suckers. Two lappets project from the caudal edge of the scolex on both the dorsal and ventral surface of the scolex. The

lappets measure 0.5 to 1.0 mm (Lichtenfels, 1975) and cover the first several dozen immature proglottids of the strobila. The four apical muscular suckers are used for mucosal attachment within the definitive host's gastrointestinal tract.

The morphology of proglottids of A. perfoliata has been described extensively (Skrjabin and Spasskii, 1951). Individual proglottids are always much wider than long. Immature proglottids are one hundred times wider than long and gravid proglottids are forty times wider than long. Each proglottid is hermaphroditic, containing a single set of both male and female reproductive organs. Each proglottid has a single genital apparatus; the apertures are unilateral and are found in the cranial half of the lateral margin. The gravid uterus is transverse, large, sac-like, and lobed (Soulsby, 1982). The female reproductive system also consists of a poral ovary, a vagina posterior to the cirrussac, and a seminal receptacle. Both internal and external seminal vesicles are present as part of the male reproductive system and the testes are scattered throughout the medulla (Beveridge, 1994). Each proglottid also contains a muscular system, a tegument, and an excretory system. Cestodes do not have an alimentary system and must absorb their nutrition, which consists of intestinal content or chyme, through the tegument of their proglottids (Barker et al., 1993; Kassai, 1999). This surface is specially adapted for both absorptive and secretory functions.

The morphology of mature eggs of *A. perfoliata* is unique (Figure 2). The eggs are 65 to 80 micrometers in diameter. The eggs are round to "D" shaped with an outer vitelline membrane and a thick (eight to ten micrometers) dark albuminous middle shell. The innermost membrane is flame or pear-shaped and consists of a chitinous pyriform apparatus. The length of the pyriform apparatus is approximately equal to the radius of

the egg, measuring about 48 micrometers. This pyriform apparatus in turn contains the hexacanth embryo characteristic of Cyclophyllidean cestode eggs (Skrjabin and Spasskii, 1951; Soulsby, 1965). The diameter of the embryo measures approximately 16 micrometers. Eggs seen on fecal flotation often have an amber cast due to contact with excreta; however, eggs dissected from gravid proglottids of the adult tapeworm are colorless (Skrjabin and Spasskii, 1951).

The morphology of the larval stages of *Anoplocephala perfoliata* within the intermediate host has been studied and documented. Bashkirova (1941) documented the stages of larval development of *A. perfoliata*. Stunkard (1938) and Potemkina (1948, in Skrjabin and Spasskii, 1951) have extensively described the course of ontogenetic development of the tapeworm of sheep and goats, *Moniezia expansa*; thus the literature generally presents a detailed ontogenesis of *Moniezia expansa*. The morphology of anoplocephalid larval development is very similar among many species. The morphology of cysticercoids of anoplocephalids is so comparable that it is difficult to determine the generic membership from studying the cysticercoid (Skrjabin and Spasskii, 1951).

Detailed figures of the larval development of *M. expansa* and *A. perfoliata* were available in Skrjabin and Spasskii (1951) and Stunkard (1938). However in the text they described only the ontogenesis of *Moniezia expansa*. Drawing from both the figures and text, one can describe the ontogenesis of *A. perfoliata*. Once the tapeworm egg has been ingested, the oncosphere emerges and penetrates the intestinal wall of the oribatid mite. The oncosphere appears in the mite's body cavity within 48 hours, and is very motile and active for several days to weeks. The second stage of development is the "large sphere"



Figure 1. Adults of Anoplocephala perfoliata.



Figure 2. Egg of Anoplocephala perfoliata.

stage in which the oncosphere form is lost and the larva becomes immobile. During this period, the oncosphere increases in size and undergoes internal reorganization of the organs. The six hooks lose positioning and move to the tail of the larva while the body of the larva fills with round cells. The third stage of growth within the mite's hemocoele is the "extended larva" stage in which the body lengthens. The fourth stage is called the "segmented larva" stage. In this phase of development, the body continues to elongate but also divides into two parts that are separated by a constriction. The portion anterior to the constriction has four suckers, which will become the scolex of the tapeworm, and the portion posterior to the constriction is a spherical capsule. Embryonic hooks are positioned caudal to the capsule. During the fifth stage of larval development the anterior portion of the larva invaginates into the posterior capsule portion. The cysticercoid stage is the final stage of larval development. Morphologically the body is spherical with a dense cuticle. Within the cuticle is the scolex with four suckers. The wall of the cyst is stratified. At this phase of development, the larva is infective for the final host. In six experimentally infected *Scheloribates* sp. the average size of the *A. perfoliata* cysticercoids was 141 micrometers by 119 micrometers (Romero et al., 1989).

<u>Life Cycle</u>

The life cycle of *Anoplocephala perfoliata* is indirect, as it requires an intermediate host as well as a definitive host. Stunkard completed the developmental cycle of Anoplocephaline cestodes in 1937 when he discovered that certain members of one genus of oribatid mites (Acari: Oribatida), *Galumna* sp., could serve as the intermediate hosts of the sheep tapeworm, *Moniezia expansa*, a closely related cyclophyllidean that infects ruminants (Stunkard, 1937). A few years later, Bashkirova (1941) determined the complete developmental cycle of *Anoplocephala perfoliata*.

An indirect life cycle is typical among the cestodes. With this type of transmission, the prevalence of cestode infection in the intermediate host is low while it is high in the definitive host (Mackiewicz, 1988). Improved transmission efficiency within a two-host life cycle also allows for high definitive host specificity. Concurrently, the probability of transmission within an indirect life cycle is increased when there is increased diversity among the intermediate hosts (Mackiewicz, 1988). Intermediate host specificity is low in the various anoplocephaline cestode species since many species of oribatid mites may become infected (Kates and Runkle, 1948).

Horses with patent *Anoplocephala perfoliata* infections shed tapeworm eggs in their feces. The adult tapeworm attaches near the ileo-cecal valve and releases gravid proglottids into the horse's digestive tract. The process of shedding mature proglottids is known as apolysis. The proglottids are broken up via digestion during transit through the large intestines, thus only eggs are passed in the feces (Carmel, 1998; Proudman and Trees, 1999). After a gravid proglottid is shed, more than forty-eight hours may elapse before its eggs are passed in the feces (Proudman and Trees, 1999).

Survival time of an infective egg on pasture is important because it potentially allows for an improved chance of exposure to the intermediate host. Since little is known about the longevity of *Anoplocephala perfoliata* eggs in the environment, the more completely studied ruminant cestode, *Moniezia* spp., may be used as a guide. Ruminant cestode eggs in the soil have a survival time ranging from a few days to four months (Yannarella et al., 1981 as cited by Denegri, 1993). Mackiewicz (1988) reported that the egg stage of many cestodes generally survive for less than one year and Dunn (1978) reported that oncospheres can survive on the ground for about nine months as long as

there are no freezing weather conditions. This last report was contradicted by a 1986 report of eggs of the sheep tapeworm, *Moniezia expansa*, as well as cysticercoids in oribatid mites, overwintering on pastures in Germany (Barutzki and Sabzeh Parwar, 1986). Narsapur (1988) hypothesized that egg survival may be shortened in tropical climates. These studies do not differentiate between egg survival and its potential to develop within the intermediate host. Thus, the infectivity of cestode eggs over time in natural conditions has not been determined at this time.

Moniezia eggs can survive in feces for about nine days (Kuznetsov, 1959). The primary difference between eggs of *Anoplocephala perfoliata* and eggs of *Moniezia* is that the eggs of *Moniezia* are generally encased within the proglottid when passed in the feces. Stunkard (1938) believed that the eggs of most anoplocephaline tapeworms are not best suited for remaining within the definitive host's feces. He stated that a redistribution of the tapeworm eggs by rain into the upper layers of the soil would allow the eggs to remain viable for a longer period of time and enable the oribatid mite intermediate hosts to encounter the eggs, pick them up, and feed upon them.

The life cycle continues when oribatid mites ingest viable eggs of *A. perfoliata* on the pasture. Oribatid mites are free living mites found on herbage and in the soil of pastures. Since the cestode eggs are presumably too large to be accidentally eaten, the intermediate host may interpret them as prospective or preferred food (Denegri, 1993). Mackiewicz (1988) hypothesized that Anoplocephaline tapeworm eggs may use chemoattraction to increase the likelihood of being eaten by the oribatid mites. This would especially hold true if the tapeworm eggs were eaten as a food item rather than as a food contaminant. On the other hand, the oribatid intermediate hosts and *A. perfoliata*

eggs may be so abundant that chance encounters between the egg and mite may be the sole strategy of transmission (Kates and Runkle, 1948; Mackiewicz, 1988).

Whatever the transmission strategy, once ingested by the intermediate host, the larva or oncosphere is freed from within the tapeworm egg shell or embryophore, presumably via digestion. Activation factors that stimulate the oncosphere to tear through the intestinal wall using its hooks are unknown (Dunn, 1978). Once in the mite's body cavity, the development time to an infective cysticercoid within the invertebrate intermediate host is variable. Growth of the cysticercoid within the mite's hemocoele is dependent on environmental conditions, especially temperature. The infective cysticercoid stage is formed within 8 to 20 weeks under natural conditions. In experimental infections, fully infective cysticercoids may develop in as little as 28 days when mites are incubated at 28°C (Schuster et al., 2000). Once the cysticercoid is fully developed and infective within the oribatid mite, it is ingested by a grazing horse.

Cysticercoids of the porcupine tapeworm *Monoecocestus* (Cestoda: Anoplocephalidae) cannot penetrate the intact mite exoskeleton (Freeman, 1962). If intact cysticercoid containing mites were fed through a stomach tube, no tapeworm infection occurred, but, if the mites were first crushed so that their exoskeletons were cracked, the definitive host became infected. Therefore, in naturally acquired infections chewing must initiate the infection by breaking the mite's exoskeleton and allowing the larvae to excyst.

The infective cysticercoid has a scolex with four fully developed suckers. It is assumed that the excysted larvae of *Anoplocephala perfoliata* move along the gastrointestinal tract with ingesta until reaching the ileo-cecal valve area, at which point

the larvae attach to the gastrointestinal mucosa. Behind the scolex of the larvae there are germinal cells that will multiply via proglottidation or asexual reproduction to produce the proglottids of the adult tapeworm (Stunkard, 1938; Kennedy, 1983; Stunkard, 1983). The caudal end of the larvae contains the excretory pore that becomes the terminal segment of the adult tapeworm (Stunkard, 1938). The prepatent period following ingestion of an infected oribatid mite is between six and sixteen weeks (Soulsby, 1965; Bain and Kelly, 1977; Beroza et al., 1985; Drudge and Lyons, 1986).

No actual data are available on the life span of adult *Anoplocephala perfoliata*, but the life span of an adult cestode may vary from a few months to several years (Jubb and Kennedy, 1970; Kennedy, 1983). A basic cestode life cycle strategy, based on iteroparity (long-duration, non-seasonal terrestrial egg release), infers a long adult Cyclophyllidean life span that often lasts for years or as long as the definitive host lives (Mackiewicz, 1988; Schmidt and Roberts, 1989; Kennedy, 1983). Furthermore, there is selection for iteroparity and high fecundity when the pre-reproductive life span is long (Kennedy, 1983). In the case of *A. perfoliata*, this pre-reproductive life span may be one to one and one-half years while the cysticercoid is in the oribatid mite (Fritz, 1982; Norton, 1994).

Epidemiology

The distribution and prevalence of *A. perfoliata* are great enough to cause concern among both horse owners and veterinarians. *Anoplocephala perfoliata* is found worldwide and is currently accepted as the most common and the most pathogenic of the equine tapeworm species (Soulsby, 1982; Lyons et al., 1983; Drudge and Lyons, 1986; Beroza et al., 1987; Lyons et al., 1992a; Pearson et al., 1993; Lyons et al., 2000)

The prevalence of *A. perfoliata* in North America has been reported extensively. Due to the lack of sensitivity of antemortem diagnostic tests, most epidemiological studies report prevalence of infection at necropsy. In 1979 necropsy data collected from eight states, including Kentucky, found that 18% of foals and 26% of adult horses were infected with tapeworms (Hass, 1979). The most common tapeworm seen was *Anoplocephala magna*. Two other studies published in the same year reported the prevalence of *Anoplocephala perfoliata*, based upon fecal examination, at 13% in North Carolina (Bello, 1979) and 14% in Canada (Slocombe, 1979).

Later studies, conducted the early 1980s, reported the prevalence of *A. perfoliata* in Thoroughbred horses in Kentucky at necropsy to be 53-54% (Lyons et al., 1983; Lyons et al., 1984). Elsewhere in the United States, prevalences at necropsy are reported as similar or lower: Louisiana 47% (Torbert et al., 1986), New England 53% (Beroza et al., 1985), Ohio 18% (Reinemeyer et al., 1984). The most recent epidemiological data, collected in 1995-1999 in central Kentucky, are similar at 52% (Lyons et al, 2000).

In other countries the prevalence of *A. perfoliata* in horses also has been examined. New Zealand is considered a highly endemic area, with a reported prevalence of up to 81.5% (Bain and Kelly, 1977). Other countries have reported prevalence rates as follows: England 31% to 69% (Imrie and Jacobs, 1987; Owen et al., 1988; Lyon et al., 2000), Ireland 51% (Fogarty et al., 1994), The Netherlands 21% (Borgsteede and van Beek, 1996), Sweden 65% (Nilsson et al., 1995). Why different regions have such varying prevalence rates for this parasite is not known. It is possible that the variations could be due to differences in pasture type (thus creating a better or worse environment for the intermediate hosts), pasture stocking rates, climate, or other management or

environmental differences. Even with the variation in prevalence, there seems to be a trend toward higher prevalences of infection in countries with temperate climates (Proudman and Trees, 1999).

Many parasitologists have speculated as to what factors may have caused an increase in reports of Anoplocephala perfoliata infection. Edwards (1986) and Geering and Johnson (1990) proposed that extensive use of ivermectin, which was new to the market, removed the nematode parasites with greater efficacy than drugs previously available. This selective removal of other intestinal parasites allowed tapeworms to flourish due to lack of competition, hence the increase in prevalence of A. perfoliata in recent decades. This hypothesis was refuted by French et al. (1994), who reported that the use of ivermectin for five years did not promote increase of A. perfoliata. Others propose that antiparasitic drugs, such as pyrantel pamoate and fenbendazole, used before ivermectin was available may have had some cestocidal activity (Duwel et al., 1975; Owen et al., 1988; Lyons et al., 1989). Other hypotheses include changes in climactic factors which may in turn have a positive influence upon intermediate host numbers and changes in stocking rates and other pasture management practices (Geering and Johnson, 1990). However, there is no unequivocal data to suggest a true increase in the prevalence of A. perfoliata over time has occurred. Lyons et al. (1992a) reported that the prevalence of tapeworm remained essentially the same in their necropsies of Thoroughbred horses conducted from 1951-1990.

Equids of all ages can be infected with tapeworms, and unlike cattle and small ruminants, there is no acquired nor age resistance (Drudge and Lyons, 1986; Lyons et al., 1992a; Lyons et al., 2000). Living in the lumen of the gut usually does not trigger

pronounced immune responses. This allows the same host to be repeatedly infected over the course of its life (Mackiewicz, 1988).

Horses as young as weanlings can host patent infections since the prepatent period is between 6 and 16 weeks. Adult horses as old as forty years of age (age estimated by owner according to time in family) reportedly have been infected with *A. perfoliata* (Lyons et al., 2000). Prevalence is lower in foals less than one year of age (30-31%) than animals that are yearlings or older (52-60%) (Lyons et al., 1987; Lyons et al., 2000). No gender difference in the prevalence of *A. perfoliata* has been reported. In foals, prevalences were similar for colts and fillies at 33% and 24%, respectively. In adult horses, prevalences also were similar at 59-61% in mares, 43-57% in geldings, and 41-57% in stallions (Lyons et al., 1987; Lyons et al., 2000).

<u>Pathology</u>

Much has been published regarding the pathology associated with *A. perfoliata* infection. Although the mechanisms are not completely understood, presumably both mechanical damage and parasite antigens play a role in the process (Proudman and Trees, 1999). The four unarmed suckers on the scolex of *A. perfoliata* cause pathologic changes at the attachment site. The anatomy of the scolex can be directly related to the features of the lesion when viewed with a scanning electron microscope in that pegs of mucosa are pulled up into the four suckers (Williamson et al., 1997). The distance between the tissue pegs corresponds to the distance between the suckers. Long before scanning electron microscopy was available Skrjabin and Spasskii (1951) described ulceration caused by the scolex of *A. perfoliata* embedded in the intestinal wall. These ulcers were inflamed and contaminated with food and intestinal microflora. Case reports had been made of

perforation, peritonitis and death resulting from infection with *Anoplocephala perfoliata* (Skrjabin and Spasskii, 1951).

Adult *A. perfoliata* inhabit the intestinal tract, attaching in clusters primarily at the ileo-cecal junction, in the cecum near the ileo-cecal junction, and less commonly in the terminal ileum and ventral colon (Fogarty et al., 1994; Williamson et al., 1997). This clustering of the adult tapeworms can markedly exacerbate the lesions associated with tapeworm attachment. In areas of tapeworm clustering lesions were found to extend into the submucosa and therefore were more likely to disrupt intestinal blood supply and the nervous regulation (Fogarty et al., 1994). There is no scientific explanation for the clustering of the tapeworms at or near the ileo-cecal junction. There has been documentation and study of an aggregation pheromone, nippolure, secreted by the female of the nematode *Nippostrongylus brasiliensis* (Roberts and Thorson, 1977; Bone et al., 1980), although these parasites have separate sexes and this pheromone is believed to mediate pairing for mating.

Lesions caused by *A. perfoliata* account in part for the parasite's association with colic. Mild prolapse of the terminal ileum into the lumen of the ileo-cecal junction was noted in five of fifty horses examined at necropsy that had adult tapeworms attached to the mucosal surface of this area (Williamson et al., 1997). Extensive mucosal ulcerations near the ileo-cecal valve were found at necropsy in 63.1% of horses in a highly endemic area (Bain and Kelly, 1977).

Damage to the intestinal lining varies with the intensity of *A. perfoliata* infection (Pearson et al., 1993; Fogarty et al., 1994; Williamson et al., 1997). The primary feature of the gross pathological lesions found at the site of tapeworm attachment, either at the

ileo-cecal junction or on the cecal wall, is mucosal ulceration (Williamson et al., 1997). The depth and severity of the ulceration was increased as the numbers of tapeworms attached to the surrounding area increased. Pearson et al. (1993) associated numbers of tapeworms with severity of damage. Superficial congestion with slightly raised focal ulceration was seen in horses infected with up to 20 tapeworms, whereas, the mucosa was raised, thickened, and ulcerated, with nodular swellings at the area of worm attachment, in horses infected with more than 100 tapeworms. Reports have described other significant gross pathologic changes associated with attachment of *A. perfoliata* such as a yellow diptheritic membrane and gross edema of the mucosa (Bain and Kelly, 1977; Fogarty et al., 1994; Williamson et al., 1997). Additionally, a vertucose granulomatous lesion projecting from the mucosa of the ileo-cecal junction was reported in two of twenty horses examined by Pearson et al. (1993) and in 7.8% of the 65 horses examined by Bain and Kelly (1997).

Histopathologic sections from the areas of attachment revealed ulcerations of various depths from the superficial mucosa to the muscularis mucosa and submucosa (Pearson et al., 1993). In severe cases, the mucosal damage was so extensive that the glandular anatomy was distorted by fibrosis and infiltration of eosinophils into the lamina propria (Williamson et al., 1997). Inflammatory cells, eosinophils and lymphocytes infiltrated the areas of damage and in one case a submucosal abscess was reported (Pearson et al., 1993). Verrucose granulomatous lesions were made up of granulation tissue, primarily lymphocytes, and associated fibrinoid necrosis, primarily neutrophils (Bain and Kelly, 1977).

Clinical Syndrome

Clinical symptoms such as poor body condition (Fogarty et al., 1994), poor growth or chronic ill thrift, reoccurring diarrhea, progressive weight loss, and anemia have been associated with infections by *A. perfoliata* (Soulsby, 1965). These parasites do not cause blood loss that would result in anemia. The anemia could be an anemia of chronic disease, otherwise known as anemia of inflammatory disease, and is the most common form of anemia in horses (Rose and Hodgson, 1993). The clinical symptom of anemia is caused by a decrease in red blood cell manufacturing in the bone marrow, erythropoiesis, that is due to iron sequestration. Iron sequestration is a nonspecific reaction of the immune system to inflammation mediated by cytokines (Waner and Harrus, 2000).

The literature throughout the 1980s contains many clinical reports of intestinal crises in horses, such as intussusception, cecal perforation, and cecal torsion, that were circumstantially related to concurrent infection by *A. perfoliata* (Barclay et al., 1982; Beroza et al. 1983; Edwards, 1986). Barclay and coworkers associated intussusception with *A. perfoliata* in five cases admitted to the Illinois Equine Hospital, which accounted for 55 percent of the intussusceptions seen during a one-year period at that clinic. The intussusceptions all involved the ileum or the cecum (ileal-ileal, ileo-cecal, or cecal-cecal) (Barclay et al., 1982). The association between *A. perfoliata* and acute gastrointestinal crisis was described in three reported cases of peritonitis. The peritonitis was credited to perforation of the cecum, which in all cases was associated with infection by *A. perfoliata* (Beroza et al., 1983). Horses described in these three cases of peritonitis harbored high intensities of the parasite, up to 300 worms. In contrast, as few as two adult tapeworms were observed during laparotomy or necropsy of horses with

intussusceptions (Barclay et al., 1982). Since adult tapeworms were highly visible, and in some areas more than half of all horses were infected, finding adults of *A. perfoliata* during a surgery or necropsy was highly suggestive, but not necessarily incriminating, as to the cause of the abdominal crisis.

Even after an extensive review of the literature by Owen et al. (1989) no direct conclusions as to the role of tapeworms in association with equine intestinal disease could be made. This interpretation began to change in the early 1990s when Proudman and Edwards (1993) demonstrated an association between the presence of equine tapeworms and ileo-cecal colic. Although the association was not very strong, they found that the risk of ileo-cecal colic was increased by the presence of tapeworms. More recently *A. perfoliata* was found to be a significant risk factor for spasmodic colic and ileal impaction in the horse, and the risk of spasmodic colic increased as worm intensities rose (Proudman et al., 1998). Twenty-two percent of the spasmodic colics and 81% of the ileal impactions were tapeworm associated based on serological and coprological diagnoses. Their matched case-control studies suggested a dose-response link between infection intensity (as revealed by ELISA) and risk of clinical disease (Proudman et al., 1998).

It is possible to relate the gross and microscopic pathology caused by *A. perfoliata* to factors predisposing horses to intussusceptions involving the ileo-cecal area of the gastrointestinal tract, the most common site of intussusception in horses (Tennant et al., 1972). Two factors, segmental atony and hyperperistalsis, are thought to be necessary for intussusception to occur (Rooney, 1965), although other common denominators may predispose to intussusception. Lesions associated with tapeworm attachment may alter

the pattern of intestinal motility and thus be a potential cause of intussusception (Edwards, 1986). Three of these other factors that may predispose to intussusception occur with equine tapeworm infection (Reymond, 1972). First is a local inflammatory change in the bowel wall. Tapeworm infection causes inflammatory change when ulceration and granulation tissue form at the area of attachment. Second is a change in the bowel wall diameter. An abrupt change in bowel diameter occurs at the ileo-cecal junction- a common site of attachment for the equine tapeworms. The third factor is an intraluminal linkage between non-adjacent segments of bowel. Although *A. perfoliata* adults are only 25-80 mm long there is potential for intraluminal linkage between non-adjacent segments of the bowel as with any gastrointestinal nematode. Given these factors, it is reasonable that intussusception could be initiated by the presence of equine tapeworms.

<u>Diagnosis</u>

Antemortem diagnosis of infection by *A. perfoliata* can be made using serological antibody tests or by finding eggs on fecal examination. Diagnosis can also be made at necropsy. Location and appearance best identify adults of *A. perfoliata*. The tapeworms are found in clusters at or near the ileo-cecal junction.

Serological testing for *A. perfoliata* is a new diagnostic technology not yet available to veterinary practitioners, but tests that measure serum antibodies specific for antigens of *A. perfoliata* have been developed. The first group to develop an ELISA (indirect enzyme-linked immunosorbant assay) used a scolex antigen of *A. perfoliata* (Hoglund et al., 1995). This test did not show cross-reactivity to concurrent nematode infection (Hoglund et al., 1995). Serum antibody responses to scolex antigens of *A. perfoliata* in weanling horses were detected using an ELISA at two to four months prior

to fecal egg output detection (Hoglund et al., 1998). One month following treatment with a cestocidal drug, both fecal egg output and serum antibody levels declined. The authors were not able to demonstrate any correlation to numbers of tapeworms by measuring the humoral response to scolex antigens. One problem associated with the test was its inability to distinguish false positives from true positives thus, the specificity of the test is low (Hoglund et al., 1995).

Proudman and Trees (1996) developed and validated an ELISA using excretory/secretory antigens of *Anoplocephala perfoliata*. The diagnostic sensitivity of this test was 68% (n=38). The specificity was 95% (n=20) when helminth naive horses were used but fell to 71% when horses were used that were *A. perfoliata* negative at necropsy. These horses may have had prior exposure to the parasite and thus residual circulating antibodies were still present. Antibody levels were found to correlate with tapeworm infection intensity (Proudman and Trees, 1999). This immunodiagnostic test may improve our ability to diagnose and treat horses that have high numbers of *A. perfoliata*, monitor herd levels of infection, and as a diagnostic tool in epidemiological studies (Hoglund et al., 1995; Proudman and Trees, 1999).

Coprological examination for parasite eggs is the most commonly used diagnostic technique to detect any gastrointestinal parasite. The prevalence of equine cestodiasis often may have been underestimated because veterinary practitioners base their diagnosis upon finding eggs using the microscopic fecal examination technique with a standard flotation solution (Beroza et al., 1987). Slocombe (1979) stated that the reason that diagnosis has proven difficult is because the eggs levitate poorly with common fecal flotation solutions. One study found 3% of horses to be positive for eggs of *A. perfoliata*

using flotation with sodium chloride solution (specific gravity 1.18) (Lyons et al, 1983) and 7% of horses to be positive using zinc sulfate solution (Lyons et al., 1984). At necropsy, 54% of these horses were proven infected with *A. perfoliata*. Another study found that the eggs, when present, appear to float with all of the standard flotation solutions (French et al., 1994). Although saturated sugar solution is the most sensitive of the standard flotation media (Beroza et al., 1985), this levitation medium is not commonly used by veterinary practitioners. The use of zinc sulfate is less time consuming and difficult to prepare than the saturated sugar solution. Zinc sulfate solution (specific gravity = 1.22) was shown to yield the greatest egg counts of eight different solutions, including saturated sugar solution, in a comparison of coprological tests for the detection of *A. perfoliata* (Duncan, P. and Jordan, M., unpublished data).

The primary reason that all flotation techniques are inconsistent is that the proglottids containing the eggs are shed sporadically (Beroza et al., 1987). It is believed by many that the shed proglottids disintegrate prior to the feces passing from the horses gastrointestinal tract (Drudge and Lyons, 1986; Fogarty et al., 1994). An alternative hypothesis is that tapeworm eggs are retained in the small, easily overlooked proglottids making few eggs available in the feces for diagnosis (Soulsby, 1965; French and Chapman, 1992). Treating suspect animals with ivermectin is reported to increase the visualization of *A. perfoliata* eggs (French et al., 1994). Perhaps due to the massive reduction of strongyle eggs passed in the feces, making the eggs of *Anoplocephala* spp. easier to detect microscopically.

Alternatives to simple flotation for fecal diagnosis of equine tapeworms are double centrifugation techniques that use sedimentation followed by flotation. Although

more time consuming, these techniques improve the diagnostic accuracy. Beroza et al. (1987), found that the use of double centrifugation first in water then in sucrose solution improved the recovery of known numbers of formalin-fixed equine tapeworm eggs by ten times over a technique using only gravitational flotation in sucrose solution. Centrifugal techniques improve the accuracy, but these techniques often lack sensitivity due to the sporadic shedding egg that is an inherent feature of equine tapeworms.

The Wisconsin Sugar Flotation Method (Sloss, 1970) is arguably the best technique for recovery of tapeworm eggs. A variation of this technique, the Cornell-Wisconsin centrifugal fecal flotation technique (Egwang and Slocombe, 1982), was 32% sensitive and 98% specific when used prior to and 24 hours after de-worming with pyrantel pamoate (Hearn and Hearn, 1995). This double centrifugation technique uses sequential sedimentation and flotation of a five-gram fecal sample to increase the chances of finding the tapeworm eggs.

Because egg counts may be low, repeated sampling may be necessary to demonstrate tapeworm eggs in a horse. Fecal diagnosis of *A. perfoliata* has been attempted using a method similar to the Wisconsin Sugar flotation Method, but with larger amounts of feces, 40 grams in 500 ml of tap water. Using large quantities of feces plus a concentration technique involving centrifugation to first sediment and then levitate with a sucrose solution, improved the diagnostic sensitivity of the test to 54% (Meana et al., 1998). Proudman and Edwards (1992) validated a technique involving triple centrifugation and an initial 40 gram sample of feces. The sediment was concentrated by double centrifugation and then centrifuged once with sucrose to levitate the eggs. This technique had a sensitivity of 61% and a specificity of 98%. Regardless of the fecal

diagnostic method used, no correlation has been recognized between numbers of adult tapeworms and egg detection using these techniques (Proudman and Edwards, 1992; Nilsson et al., 1995; Meana et al., 1998).

Treatment and Prevention

No drugs are approved by the FDA for the treatment of *A. perfoliata* in the United States, but several products are effective when used in an extra label fashion. However, the most popular class of equine anthelmintics in use today, the macrocyclic lactone endectocides are ineffective against equine tapeworms (Costa et al., 1998; Torbert et al., 1982).

Pyrantel salts are the most economical and effective treatment, but the activity of pyrantel pamoate is rather variable against *Anoplocephala perfoliata*. Administration of pyrantel pamoate orally at the FDA approved therapeutic dose for strongylate nematodes (6.6 mg base/kg) reportedly removes 15% (Slocombe, 1979) to 87% (Lyons et al., 1989) of *A. perfoliata*. Twice the label recommended dosage (13.2mg base/kg) reportedly removes 93% (Lyons et al., 1986) to 97.8% of adult tapeworms (Slocombe, 1979). Even three times the label recommended dosage administered every eight weeks was not 100 percent effective (Kivipelto et al., 1998).

Pyrantel pamoate also may not have good activity against the immature *A*. *perfoliata* (Lyons et al., 1986). Therefore the optimal time to treat with this drug against adult *A. perfoliata* infections would be during the winter or spring seasons (Hoglund et al., 1998), to insure that the tapeworms acquired during the previous grazing season will be fully mature and susceptible to the treatment. One weakness of this approach is that maturity is predicted on the basis of detection of eggs in the feces, which may be highly inconsistent due to factors such as sporadic proglottid release and seasonality of egg shedding (Kivipelto et al., 1996). A second weakness of this approach is that it is designed for temperate regions where horses are only allowed to graze pastures during the late spring, summer, and early fall. Treatment regimens have not been adapted for milder climates where grazing is continuous, such as north central Florida.

Administration of 2.64 mg per kg pyrantel tartrate on a daily basis removes adult worms, stops tapeworm egg shedding, and prevents reinfection (Greiner and Lane, 1994; Lyons et al., 1997b; Kivipelto et al., 1998). The daily use of this drug is arguably one of the best choices at this time for tapeworm prophylaxis on properties infested with this parasite, but it is both expensive and inconvenient.

Praziquantel is a cestocide widely used in small animal medicine, but treatment with praziquantel is much more expensive than with pyrantel salts. Administration of praziquantel orally or via nasogastric tube at a dose of 1.0 mg/kg removed 89 to 100% of *A. perfoliata* present, and a dose of 0.75 mg/kg removed 82 to 100% (Lyons et al., 1992b). Although it is not approved for use in the United States at this time, closantel at doses of 20 or 40 mg/kg reportedly has high activity against *Anoplocephala perfoliata* adults (Guerrero et al., 1983).

Fenbendazole is effective at stopping shedding of *Moniezia* spp. segments in sheep when dosed with 10 mg/kg, and a 95% reduction of tapeworm burdens was observed when the dose was increased to 15 mg/kg (Duwel et al., 1975). Surprisingly, both granular and paste forms of fenbendazole administered at various dosages failed to eliminate the tapeworm infections in approximately one-half of the horses tested (Tausend, 1989). Neither oxfendazole administered at the dose of 10 mg/kg nor oxibendazole administered at doses of 5, 10, or 15 mg/kg had any effect against *A*. *perfoliata* (Lyons et al., 1981; Kates et al., 1975; Kingsbury and Reid, 1981).

Good management will help prevent and control tapeworm infection by reducing both the numbers of eggs passed into the environment in horse feces and the exposure of horses to cysticercoid-containing mites. One should maintain reasonable stocking rates on pastures. Overcrowding of pastures with infected animals in the season when oribatid mites may have peak populations would likely produce heavy parasite burdens in the definitive host (Narsapur, 1988). Rotation of pastures also has been recommended as a means of prevention. While this could reduce egg numbers on the pasture, given the longevity of the adult mites on pastures (one to one and one-half years) transmission probably would not cease unless the horses were kept off of the pasture for at least that time (Fritz, 1982; Norton, 1994).

A most important control method is to isolate new animals on arrival to the farm. Since fecal testing may produce false negative results, all new horses should be treated with an appropriate cestocidal drug prior to entering the grazing area (Urquhart et al., 1996). If this guideline is not followed, the potential for initiating the life cycle in a previously uninfested pasture is great. Resident horses should be routinely tested and dewormed as well (Bain and Kelly, 1977). Plowing and reseeding pastures has been suggested to reduce the numbers of oribatid mites (Soulsby, 1965; Bain and Kelly, 1977). In reality, control of *A. perfoliata* infection by reduction of mite numbers is impractical for most horse owners due to the ubiquitous nature of these mites, thus the only viable alternative is prophylactic cestocidal treatment. Many parasites of livestock can be controlled by a limited number of strategically timed treatments (Herd, 1988; Barger,

1999), but at present not enough is known about the transmission of tapeworms to allow complete recommendations. If there were a seasonal fluctuation in mite numbers on pastures, strategically-timed treatment of horses to reduce shedding of tapeworm eggs in feces just prior to the seasonal increase in mite populations would be advantageous (Narsapur, 1988).

Oribatid Mites

Oribatid mites are members of the subclass Acari, order Acariformes, suborder Oribatida (= Cryptostigmata, Oribatei). This cosmopolitan suborder includes over 150 families and about 1,000 genera with approximately 7,000 species that are found in North America (Norton, 1990; Norton, 1994).

Within the suborder Oribatida there are two large groupings or supercohorts, the Macropylina or "lower" oribatid mites and the Brachypylina or "higher" oribatid mites. Distinguishing characteristics of mites in the group Macropylina are (1) shallow acetabula where the legs articulate with the body, (2) anal and genital plates that are not distinctly separate, and (3) a notogaster that is sometimes divided by transverse furrows (Norton, 1999b). The mites in the supercohort Brachypylina are grouped together because they all have a circumdehiscent nymphal molting pattern (Norton, 1994; Norton, 1999a). Other distinctive features of members of this group are (1) deep acetabula where the legs articulate with the body, (2) distinctly separate anal and genital plates, and (3) a holoid body type, which is the most rigid of body types (Norton, 1999b).

<u>Morphology</u>

Oribatid mites have incredible morphologic diversity yet some biological aspects are highly conserved (Norton, 1994). Oribatid mites are often covered with large sclerotized protective plates and have been said to look like minute beetles (Walker, 1994). Another distinguishing characteristic of mites in the suborder Cryptostigmata, including the oribatids, is concealed stigmata (Walker, 1994). In general, oribatid mites may be distinguished from other mite suborders by having chelate-dentate chelicera, simple palpi, bothridial sensilla, and they are usually well sclerotized (Krantz, 1978). The internal organ systems consist of the digestive system, the reproductive system, the glandular system, and the nervous system. The internal organs are bathed in hemolymph that moves throughout the hemocoele due to muscular movements of the mites' body (Krantz, 1978).

Many mite families have setal (hair-like) structures that are sensory, primarily tactile and chemoreception. These often have taxonomic significance since they often have different patterns and structure. One key characteristic of oribatid mites is that they have one pair of highly specialized propodosomal trichobothria, commonly called bothridial sensilla, that may be used for vibroreception and anemoreception (Krantz, 1978). The base of a trichobothrial structure sits in a cavity called a bothridia. Trichobothria have a solid core of actinopiline, an optically active substance that is birefringent in polarized light. Oribatid mites may be taxonomically distinguished by observing the distinctive shapes of their bothridial sensilla.

The morphology of oribatid mites makes it difficult for them to spread tapeworm infections from one pasture location to another (Fritz, 1982). Oribatid mite dispersal primarily occurs when adults, which are very are slow moving and do not travel more than a few centimeters horizontally, seek food or favorable oviposition sites (Norton, 1994). Oribatid mites are rarely adapted to phoresis (or attachment) as their legs are

generally not adapted for grasping and if disturbed, they retract their legs and fall to the ground. The legs may end in one or more large claws but never in suckers (Walker, 1994).

Basic Biology

The majority of oribatid mites are members of the soil fauna. All oribatid mites are free-living and none are parasitic (Johnston, 1982). Oribatida are the most diverse and numerically dominant arthropods in the organic layers of temperate forest soils (Norton, 1994). Oribatid mite densities within the organic layers of soil can easily range from twenty thousand to several hundred thousand individuals per square meter (Jacobs, 1986; Norton, 1990; Norton, 1994). Surveys of oribatid mite populations of grassland soils in temperate regions will yield at least thirty to fifty species (Norton, 1990).

Oribatid mites promote soil fertility by breaking up and digesting organic matter (Hartenstein, 1962). Because oribatid mites are a major component of the soil fauna they are believed to be key contributors to the breakdown of organic debris (Crossley, 1977). This contribution may be due to indirect effects that the mites have within the ecosystem such as fragmentation of litter, inoculation with microbial spores, and stimulation of microflora by grazing (Crossley, 1977). Schuster (1956) closely examined the role that oribatid mites play in processing the soil litter into humus substrate. He reported that no actual decomposition of soil litter occurs during passage through oribatid mites intestinal tracts. However, this process does prepare the material for subsequent decomposition of the fecal pellets in the soil.

Factors that influence oribatid mite distribution

Oribatid mites are ubiquitous but flourish especially well in certain climates and microclimates due to both biotic and abiotic influences. Microclimatic preference

probably plays a large role in the clumping distribution seen in open pasture environments (Ibarra et al., 1965). Mitchell (1979) reviewed environmental influences on oribatid mite distribution. The two most critical abiotic environmental factors affecting oribatid mite distribution are temperature and moisture, but one must take into account the soil depth. The primary biotic factor affecting the oribatid mites is food availability.

Temperature greatly affects oribatid mites because they, like other invertebrates, are exothermic. The environmental temperature must directly affect many of the physiological processes, such as respiration, ingestion, growth and survival of oribatid mites. Oribatid mites can survive in temperatures up to forty-five degrees Celsius (Mitchell, 1979).

Moisture was the second abiotic factor that affects oribatid mites (Mitchell, 1979). Different species and different life stages within a species have variable tolerance to moisture. A direct effect of both the environmental moisture and temperature upon an oribatid mite is its transpiration. It would seem logical that oribatid mites that are more frequently found to inhabit the upper layers or the soil should be more tolerant to desiccation.

The final abiotic factor that affects the habitat of an oribatid mite is the soil. The soil depth influences the temperature, moisture, organic matter quality, and pore space. Smaller sized oribatid mite species are more likely to predominate in deeper soils (Mitchell, 1979). Also, the type of soil may affect mites. Schalk (1968 referenced by Van Nieuwenhuizen et al., 1994), recorded that sandy soils containing humus and vegetation are ideal for oribatid mites. These well-ventilated soils may make deep
vertical migration of the mites more likely (Usher, 1975). However, the typically dry, sandy pastures of the coastal plain of the southeastern United States may be inhospitable to oribatid mites, causing the prevalence of equine tapeworms to be lower in this region (Proudman et al. 1998).

Feeding habits

The most important biotic factor that influences oribatid mites is food availability. The availability of food also is influenced indirectly by all of the abiotic factors that influence oribatid mite distribution. Food preference among an oribatid mite population would greatly depend upon the species present. However, species in the same family often have similar food preferences (Denegri, 1993). Since oribatid mites have many different feeding habits, the possibility of tapeworm egg ingestion varies greatly among potential intermediate hosts (Pinto et al., 1998).

Feeding habits of oribatid mites have been categorized and summarized by Krantz (1978). Few oribatid mites are phyophagous or feed on live plant tissues. Most oribatid mites are classified as mycophagous (Mitchell, 1979). Mites within this group feed on fungal hyphae in woody plant tissues, in soil, or in humus. Oribatid mites may or may not prefer various foods at different life stages (Schuster, 1956; Sengbusch, 1954). Due to the differences in preference for and availability of fungi, different soil inhabiting stages of the same oribatid mite species may be vertically distributed at different soil depths. The juveniles of a species may live in a habitat other than soil, such as wood. This biotype change may initiate a dietary change (Schuster, 1956). The third feeding category is the saprophagous oribatids. As with the mycophagous feeders, many oribatid mites fit within this category. Members of the saprophages are vital in the decomposition of organic materials and nutrient recycling. Mites in this group feed on dead insects and

other dead invertebrates such as earthworms within the soil in addition to organic debris such as leaves. The ingestion of animal debris by oribatid mites is rare and may be accidental (Hartenstein, 1962; Schuster, 1956). In Schuster's studies of oribatid mite feeding habits, none were found to be predatory or obligatory carrion feeders. The final feeding group is the coprophages. *Galumna formicarus* and at least four *Oppia* spp. have been found to ingest fecal material (Krantz, 1978). However, Schuster (1956) reported that coprophagy generally does not occur in the natural habitat when abundant food is available.

A second, simpler scheme has been used. Within this second classification scheme are both macrophytophages and microphytophages. The macrophytophages eat decaying higher plant material such as wood, fallen leaves, coniferous needles, foliar hairs, and root particles (Schuster, 1956). The microphytophages eat fungal hyphae, fungal spores, yeast, bacteria, pollen grains, moss and lichen debris, and algae (Schuster, 1956). *Oppia* spp. are typical microphytophages (Schuster, 1956). If an oribatid species ingests foods from both categories, as do *Scheloribates* spp. (Hubert et al., 1999) and *Galumna* spp. (Schuster, 1956), it is referred to as panphytophagous or a non-specialist. The strategy of a panphytophagous mite is to survive and have population growth while using a wide spectrum of food sources (Hubert et al., 1999). Some oribatid mites classified as microphytophagous are capable of ingesting macrophytophagous food particles, but they cannot complete normal life cycles while eating this unnatural food source (Schuster, 1956).

Other factors influence oribatid mite feeding habits. As mites decrease in size, the larger the proportion of fungal matter ingested (Denegri, 1993). This relationship

explains why the majority of oribatid mites are microphytophagous. Food moisture levels are also important (Schuster, 1956). In laboratory experiments if moisture levels in the food substrates were too low the mites stopped feeding.

Reproduction

Classic fertilization occurs within the suborder Oribatida, and both male and female progeny are produced (Krantz, 1978). Reproduction begins when the male places a freestanding stalked spermatophore, with a sperm packet at the tip, on the substrate on which the mites live. The female then envelops the spermatophore with her genital vestibule and the sperm is transferred. Fertilization using spermatophores is thought to be an ancestral trait within the order Acariformes. This system of reproduction, used by most oribatid mite species, does not require any direct male-female contact (Norton, 1990; Norton, 1994). Sex determination is diplo-diploid, or having four times the haploid number of chromosomes in the nucleus (Johnston, 1982).

A second form of reproduction not requiring fertilization occurs in nearly 10% of oribatid mites (Norton and Palmer, 1991; Norton, 1994). Thelytoky, a form of parthenogenesis, involves the production of females by parthenogenic females with unfertilized eggs (Krantz, 1978). This phenomenon is common within certain oribatid mite families and its occurrence is suspected in others due to the absence or rarity of males in collections (Norton, 1990). Thelytoky is considered adaptive and may be important in this group of mites due to their generally low fecundity (Norton, 1994). Small body size and deep soil habitat seem to correlate with thelytoky in certain genera (Norton, 1994).

Though most female oribatid mites are thought to be iteroparous i.e. capable of repeated reproduction, the lifetime fecundity of oribatid mites is low in relation to other

mite groups (Norton, 1990; Norton, 1994). Factors reported to influence fecundity under laboratory conditions are crowding, food quality, temperature and humidity (Norton, 1994). Although oribatid mites are believed to have moderately stable populations with generational overlap, Mitchell (1977a) proposed that oribatids inhabiting the fermentation or humus layer of the soil tend to oviposit seasonally, primarily in the spring and summer. He reported that the egg stage is not sensitive to desiccation and that larvae appear when moisture conditions and food resources are more favorable. Oviposition generally occurred in *Galumna* spp. from spring through the fall under field conditions and was correlated with temperature in the laboratory (Sengbusch, 1954). These same species, in field studies, deposited eggs twice yearly, once in the spring and again in the fall. This process seemed to be controlled in part by temperature (Sengbusch, 1954). Other species do not have an egg laying period and have eggs present in females throughout the year (Mitchell, 1977a).

Life Cycle

The life cycle begins as eggs are passed out of the female through the genital valves. Oviposition through this small opening is only possible due to the elasticity of the eggs. Oviposition is also believed to be an ancestral trait within the order Acariformes and most oribatid species do lay eggs (Norton, 1994). Oribatid mites as a group have a specialized ovipositor that holds the eggs while at the same time probing the substrate for a suitable niche in which to deposit the egg (Krantz, 1978). Some oribatid mites do consistently larvaposit, but all of these species are aquatic (Norton, 1994).

Postembryonic developmental stages include prelarva, larva, protonymph, deutonymph, tritonymph, and adult (Krantz, 1978; Norton, 1994). The first two life stages, egg and prelarva, may occur within the female (Norton, 1990; Norton, 1994).

Once oviposition occurs, the egg chorion partially splits. The exposed prelarva, lacking legs and mouthparts, remains within the egg since it is immobile. In all known oribatid mites this prelarval stage is said to be in "calyptostasis" because it is essentially a featureless sac (Krantz, 1978; Norton, 1994). The six-legged larva is the first of five mobile feeding instars (Norton, 1990). Larvae have little or no sclerotization and lack external genitalia. Identification of the larval stages is almost impossible below the level of suborder or superfamily due to a lack of identifying structures (Krantz, 1978).

As development continues through the three nymphal stages and on to the adult, body segments are added to the terminal end of the body and setae are added to the body and appendages. This process of interstadial change is called anamorphic development (Norton, 1990). Progression through the life stages may involve slight or striking changes in appearance. The latter is more common in the higher oribatids, Brachypylina (Norton, 1990). Immature-adult heteromorphy is the trend within the suborder Oribatida, although sexual dimorphism among the adults is rare (Johnston, 1982). All nymphal and adult stages have eight legs.

The majority of mortality occurs in the immature instars (Norton, 1994). Once an oribatid mite has reached the adult stage it may live for one to one and one-half years in pastures or up to 2 years in temperate forests (Fritz, 1982; Norton, 1994). The expected longevity within the class Acari as a whole ranges from one month to several years (Krantz, 1978). There seems to be an evolutionary emphasis on adult survival seen in morphological adaptations for predator defense (Norton, 1994). Examples of adult defense adaptations are hardening of the cuticle, by sclerotization or calcification, and

ptychoidy, in which the legs are retracted and the prodorsum folds ventrally to cover them (Norton, 1994).

Oribatid mites are generally thought to have long life cycles, extended development, adult longevity, and iteroparity. This combination of factors produces a stable population structure (Mitchell, 1979; Norton, 1994). The time for completion of an oribatid mite's life cycle is dependent on temperature, moisture, and the availability of food (Krantz, 1978; Mitchell, 1979). Development from egg to adult generally requires from five months to one year (Krantz, 1978). An oribatid mite in a temperate zone may require several months to one year to complete its life cycle (Norton, 1994). Even while under constant laboratory conditions, the time required for different life stages to develop is highly variable, however, in general, the smaller the mite and the warmer the climate, the less time it takes to complete a life cycle (Cleat, 1952; Mitchell, 1977a; Narsapur, 1988). Field studies report a generational time of most oribatid mites of one or two years in temperate climates (Norton, 1994).

Oribatid Mites as Anoplocephaline Intermediate Hosts

From a fairly simple approach, there are two basic prerequisites for completion of this life cycle. The first is the size of the intermediate host must be large enough to ingest the egg and maintain it during larval development. The second condition required for transmission is that sufficient numbers of infected intermediate hosts are present in the environment to guarantee maintenance of the life cycle (Sengbusch, 1977). In general, cysticercoid development within the body cavities of oribatid mites is influenced by the species of the mite, the intensity of infection, and the environmental temperature (Narsapur, 1988).

In total 127 species of oribatid mites, representing 27 families, have been reported to serve as the intermediate hosts of anoplocephaline tapeworms (Balogh, 1972). Only 42 species were found to naturally carry anoplocephaline cysticercoids, thus the majority of the intermediate hosts were infected under laboratory conditions (Denegri, 1993). Demonstration of cysticercoid development within a species under laboratory conditions does not justify the conclusion that this species is a natural intermediate host. A natural intermediate host must be present in the environment where the definitive host eats (Kates and Runkle, 1948).

Species of oribatid mites may differ in their efficiency as intermediate hosts (Kates and Runkle, 1948). It is believed that on any given pasture there may be only one or at best, a few species that serve as principal intermediate hosts (Fritz, 1982; Kates and Runkle, 1948). Likewise, in experimental infections, different species of mites have different infection rates, infection intensities, and cysticercoid maturation times (Xiao and Herd, 1992). This difference in efficiency among intermediate hosts may also depend a great deal upon the tapeworm species being studied (Narsapur, 1976).

Since only 3-6% of mites on pasture are naturally infected with cysticercoids (Fritz, 1982; Kates and Runkle, 1948; Schuster, 1988 in Schuster et al., 2000), it is likely that large tapeworm infections in horses result from the prolonged, continuous ingestion of small numbers of cysticercoid containing mites (Narsapur, 1988). Since the adult oribatid mite is generally believed to live for 1- 1 ½ years and estimates have been made at up to 1-3 years (Kassai, 1999), infective cysticercoid stages could persist in the environment even in the absence of definitive hosts (Stoll, 1935a; Mackiewicz, 1988). Infected adult mites are capable of over-wintering, even in harsh climates (Stoll, 1935a).

Oribatid mites infected with cysticercoids of *Moniezia expansa* survived for up to 24 months (Potemkina, 1959 cited by Worley et al., 1974). Infective oribatid mites were shown to persist on a pasture for up to 17 months when sheep were not present (Stoll, 1935a). This study period included two winters and took place in a temperate climate. Determining Factors for an Effective Intermediate Host

Location within the soil, morphology, and feeding habits are important factors in determining which oribatid mites are likely to be intermediate hosts (Denegri, 1993). Oribatid mites ingest tapeworm eggs accidentally while feeding on other organic matter in the upper levels of the soil and herbage. Temperature, moisture, and preferred food availability influence a mite's location within the soil (Mitchell, 1979; Denegri, 1993). Most researchers agree the mites only need be large enough to ingest a tapeworm egg and sustain it throughout larval growth (Sengbusch, 1977), however most research has primarily involved the adult oribatid mite as intermediate hosts. Nymphal stages of oribatids can also be infected with *Moniezia* spp. (Narsapur, 1988; Schuster et al., 2000) in which the tapeworm larvae survives consecutive molts within the mite's body cavity (Narsapur, 1988).

In addition to body size, the design and dimension of the mouthparts might influence the fitness of an intermediate host. Feeding habits of oribatid mites have been classified and discussed previously. Although specific reasons were not provided, some authors believe that of the feeding groups it was unlikely that phyophagous or coprophagous oribatid mites would serve as intermediate hosts (Krantz, 1978; Denegri, 1993). It does not seem unlikely that if an oribatid mite is coprophagous, it would ingest the infective eggs shed in the feces. Most reported intermediate hosts for anoplocephaline tapeworms were panphytophages, unspecialized plant feeders, or zoophages, feed on living animal material (Denegri, 1993).

Known Intermediate Hosts of A. perfoliata

Eighteen oribatid species are known to transmit *A. perfoliata*. The probability of transmission within any indirect life cycle is increased when there is increased diversity among the intermediate hosts (Mackiewicz, 1988).

A list of species of oribatid mites known to act as intermediate hosts of *Anoplocephala perfoliata* includes:

Achipteria spp. Berlese, 1885; Carabodes spp. Koch, 1835; Ceratozetes
bulanovae Kuliev, 1962; Eremaeus oblongus Koch, 1883; Galumna
dimorpha Krivolutski, 1952; G. nervosus Berlese, 1914; G. obvius Jacot,
1929; Hermanniella granulata Nicolet, 1855; Liacarus spp. Michael,
1898; Liebstadia simils Michael, 1888; Parachipteria punctata Nicolet,
1855; Platynothrus peltifer Koch, 1839; Scheloribates laevitus Koch,
1835; S. latipes Koch, 1844; Scheloribates spp. Berlese, 1908;
Trichorbates incisellus Kramer 1897; Urubambates schachtachtinskoi
Kuliev, 1961; and Zygoribatula microporosa Bulanova-Zachvatkina, 1967
(Marshall et al, 1987; Denegri, 1993).

The species most frequently reported as an intermediate host of *Anoplocephala perfoliata* is *Scheloribates laevitus* (Denegri, 1993), body size 500-600 µm in length (Xiao and Herd, 1992). Two genera, *Scheloribates* and *Galumna*, are considered highly specific intermediate hosts for common Anoplocephalines (Narsapur, 1988).

Effects on the Intermediate Host

The hypothesis that infection or hyper-infection of oribatid mites with anoplocephaline larvae may cause increased mite mortality was proposed shortly after the intermediate host of *Moniezia* was discovered (Kates and Runkle, 1948; Stunkard, 1938). Experimental infections of *Scheloribates* spp. with *Moniezia expansa* eggs decreased mite survival rates from 90% in control mites to 62-82% in infected groups (Schuster, 1995). High infection doses (20 proglottids per culture jar) caused a high mortality rate of mites in culture (Xiao and Herd, 1992). Similar, observations regarding larval development within the intermediate host and the effects of parasite burden on intermediate host mortality hold true for a number of other cestodes (Stunkard, 1938; Fritz, 1982; Narsapur, 1988).

In addition to reducing survival rates, cysticercoid infection of oribatid mites also reduces reproductive capacity. Oribatid mites carrying cysticercoids usually do not carry mite eggs or at least have fewer eggs than uninfected mites (Kates and Runkle, 1948; Schuster, 1995; Schuster et al., 2000). Since infection with cysticercoids may adversely affect a mite longevity or reproductive capacity (Fritz, 1982), it was hypothesized that tapeworm infection in grazing animals could paradoxically limit the oribatid mite population on pastures (Narsapur, 1988). However, this has little to no effect on the overall mite population due to the low infection rate (<4%) under natural conditions (Kates and Runkle, 1948; Schuster, 1995; Schuster et al., 2000). Fritz (1982) proposed that if concurrent infection with tapeworm larvae reduces female reproductive fitness, then selection would benefit mites that did not ingest the tapeworm eggs, but this is yet to be proven.

The space in a mite's hemocoele is limited, thus whether oribatid mites are infected with one or more cestode larvae may affect the development of those larvae. Developing parasite larvae may compete with other larvae or with mite eggs for both space and nutrients (Fritz, 1982; Schuster, 1995). The carrying capacity of cysticercoids within the body cavity of an oribatid mite is limited due to volume, and is directly proportional to the oribatid's size (Kates and Runkle, 1948; Schuster et al., 2000). Both Stunkard (1938) and Kates and Runkle (1948) observed that a single cysticercoid within a mite's hemocoele was larger than when multiple larvae were present. Also, the development of the larvae was slower in cases of multiple larvae than in cases of single larva infection, and when multiple larvae are developing within a mite's body cavity, the cysticercoids tend to be smaller in size at maturity (Stunkard, 1938). However, larval development within the oribatid mite was not related to mite host species, to crowding within the hemocoele of the host, or to the temperature (Gleason and Buckner, 1979). Variations in the Oribatid Mite Populations

The prevalence of cestode infection is linked to the abundance of oribatid mite intermediate hosts and this in turn is dependent on environmental factors (Van Nieuwenhuizen et al., 1994). The many environmental factors that influence oribatid mite distribution patterns have both seasonal and diurnal fluctuation patterns. Due to variations in seasonal mite populations within different strata of the soil and daily vertical migrations into vegetation, it has been suggested that there is a greater risk of infection in months with greater rainfall and in the early mornings (Van Nieuwenhuizen et al., 1994; Schuster et al., 2000). Climactic factors such as temperature, rainfall, relative humidity, soil moisture, and solar radiation most often showed a correlation with mite abundance (Van Nieuwenhuizen et al., 1994).

One must keep in mind when studying the population dynamics of soil arthropods that they are seldom dispersed randomly. Many species tend to form communities in discrete aggregations or clumps. The success of a given species of oribatid mite as an intermediate host of anoplocephalid cestodes is determined by the abundance and distribution of the mites in the pasture (Wallwork and Rodriguez, 1961).

Seasonal Variations in Oribatid Mite Populations

There have been investigations into the seasonal variation of oribatid mite populations and the consequence this may have on the epidemiology of cestode infections. However, the reports differ in their findings. Dunn (1978) suggests that A. *perfoliata* infections are seasonal, being most abundant in summer and autumn, thus reflecting the seasonality of the mite vectors. Ibarra et al. (1965) reports that there are generally two peaks per year in the adult oribatid population in pastures of temperate regions. The first peak is in the spring due to the emergence of overwintering adults and nymphs that mature to adults. The adult is the chief overwintering life stage in most instances (Stamou and Sgardelis, 1989). These mites produce eggs that will give rise to the second peak in the adult mite population in the autumn. Another hypothesis for these two peaks is that there is a population recruitment during moist conditions (Mitchell, 1977a). Lebrun (1984) reported seasonal variation in total numbers of the oribatid species studied that peaked only once in the summer, primarily due to the large variation in larval numbers. These variations in population density decreased with increasing ontogenetic level and adults mite numbers remained constant throughout the three-year study in a temperate region.

Denegri and Alzuet (1992) investigated the seasonal variation of oribatid mite population and the consequence it had upon sheep infection by *Moniezia* spp in

Argentina. They showed that factors which positively influenced cestode transmission include: increased temperatures, increased numbers of oribatid mites, increased numbers of available male and female oribatid mites, rapid maturation of cysticercoids (45-60 days at 23-25° C) and co-causative factors associated with livestock management. Another influencing factor may be that the diversity of species within an oribatid community in a habitat may change at different times of the year (Fritz, 1982).

Based on previous reports, it seems that the stage most climate dependant is the larval oribatid. The larvae die when humidity is low and appear in large numbers when the amount of moisture in the environment increases (Mitchell, 1977a). This environmental influence may be a key to controlling the intermediate host (Denegri and Alzuet, 1992). Dry seasons and drought has been shown to negatively influence the number of definitive hosts contracting tapeworms (Stoll, 1935b). This may be due to the environmental influence upon the oribatid community. Proudman et al. (1998) suggested that regional variations, such as dry sandy pastures of the southern United States are inhospitable to oribatid mites, this may limit horse contact with the intermediate host and decrease *A. perfoliata* prevalence in this geographical area.

A seasonal fluctuation in mite numbers may not occur in all climates. A study of *A. perfoliata* prevalence and pathogenicity in South Auckland, New Zealand, states that the mites are found in the pastures year round with little seasonal variation (Bain and Kelly, 1977). Similarly, Kates and Runkle (1948) found no relationship between numbers of mites collected and seasonal and temperature conditions within a given sampling locality near Beltsville, Maryland. However, in Greece, a region that is intermediate between temperate and mediterranean climates, both the population density

and reproductive activity of the forest dwelling oribatid mite species studied exhibited seasonality that seemed to be governed by fluctuations in temperature (Stamou and Sgardelis, 1989). In China, seasonal variations of oribatid mite numbers have been reported from different regions. In Xinjiang, during one year the oribatid mites had a period of peak activity, July to September, and a dormant period, December to March (Lizhen et al., 1988). During the dormant period mites moved to deeper layers of the soil as the temperature dropped. A study in Foochow reported two peaks in oribatid numbers the first in October and the second from April to June (Yu-kwang et al., 1975). Climate seemed to be a deciding factor in numbers of adult mites found in the upper layers of the soil and herbage, which is necessary for completion the tapeworm life cycle. Both cold and dry weather and hot and dry weather greatly reduced the number of mites collected. Daily Variations in Oribatid Mite Distribution

The frequency of oribatid mite migration onto the vegetation when the definitive host is grazing is very important in maintaining the life cycle. It has been hypothesized that the diurnal fluctuations in temperatures may be responsible for the vertical migrations of the surface dwelling mites as described by many researchers. These mites would be subject to the largest extremes in temperatures both daily and seasonally. Another important factor influencing vertical migration of oribatid mites may be soil moisture (Metz, 1971). High soil moisture and temperature combine to elevate the relative humidity in the herbage. Mites have been found to be most active on pastures at dawn, dusk, and midnight and most idle at noon (Yu-kwang et al., 1975; Lizhen et al., 1988). Van Nieuwenhuizen et al. (1994) reported no difference in the numbers of mites recovered from the total sample (herbage, surface litter, and soil below the surface liter) during various times of the day from a pasture in South Africa. However, there was a

decrease in mite numbers found on the herbage at midday and throughout the afternoon perhaps because the mites migrate back into the surface litter and soil to escape the midday unfavorable conditions (Van Nieuwenhuizen et al., 1994). Not all mites have daily migrations up and down herbage. *Galumna virginiensis*, a known intermediate host of *Moniezia expansa* was found to stay at a comparatively high density in pasture vegetation throughout the day (Wallwork and Rodriguez, 1961). Oribatid mites prefer to be shielded from direct solar radiation. Fewer mites were collected from pasture grasses on sunny days, more on cloudy days, and the most were collected on rainy days (Yu-kwang et al., 1975).

SEASONAL AVAILABLITY OF ORIBATID MITES

Objective

The perceived pathogenicity of *Anoplocephala perfoliata* has changed enormously over the last few decades. At first the parasite was regarded as nonpathogenic, but it is currently believed to be associated with colic. Because colic is the single most widespread cause of death in horses, and causes significant emotional and economic losses to the horse owners when not fatal (Tinker et al., 1997), this parasite needs to be properly diagnosed and treatment regimes prescribed based on its epidemiology.

This observational study was designed to study the relationship between weather data such as monthly average temperature, monthly total rainfall and monthly oribatid mite numbers on horse pastures of north central Florida. The purpose of this study is to gain knowledge of the epidemiology of *Anoplocephala perfoliata* by studying its intermediate host availability, thus facilitating development of better treatment and prevention strategies. This project also allowed study of the species of oribatid mites present on the two study site pastures.

Hypothesis

It is my hypothesis that there is no seasonal variation in the numbers of oribatid mites on horse pastures of north central Florida.

Materials and Methods

The seasonal abundance of oribatid mites on horse pastures of north central Florida was determined by the monthly collection of soil samples, the extraction of oribatid mites from these soil samples, and the identification and enumeration of the oribatid species present. Samples were collected from January 1999 through December 2000.

Study Sites

Two pastures were used throughout the two-year sample collection period. Both pastures are a part of The University of Florida Department of Animal Sciences facilities. These pastures have housed horses for at least ten years prior to our study and continued to do so throughout the study.

Pasture P-6 is located at the Horse Teaching Unit (Figure 3). This facility is a part of The University of Florida's campus in the Gainesville, Florida area. Pasture P-6 is a five-acre fenced area with herbage consisting of sparse Bahiagrass (*Paspalum notatum*) and Bermudagrass (*Cynodon* spp.), probably due to a history of overgrazing. The stocking rate throughout the study period was approximately 1.2 horses per acre.

Pasture 21-23 is located at the Horse Research Center (Figure 4), near Ocala, Florida. Pasture 21-23 is a twenty-acre fenced area, and the primary forage is Bahiagrass (*Paspalum notatum*). During the study period the stocking rate was less than one adult horse per acre. Additionally, there were nursing foals with the mares for three months each spring.

The soil at both the Horse Teaching Unit and the Horse Research Center is sand overlaying a thin layer of clay on a lime rock base (University of Florida, 1982).



Figure 3. Layout of Horse Teaching Unit. Asterisk indicates location of the study pasture.



Figure 4. Layout of Horse Research Center. Asterisk indicates the location of the study pasture.

The two study sites were selected based on our goal to study seasonal variation in oribatid mite populations on horse pastures in north central Florida. North central Florida includes Ocala, Florida located in Marion County and Gainesville, Florida located in Alachua County. This area has the second largest concentration of thoroughbred horses in the United States and is the heartland of Florida's thoroughbred industry, which generates billions of dollars annually. We chose to sample two pastures in different locations in an attempt to prevent the study from observing seasonal differences in oribatid numbers driven by unique local conditions.

Soil Sample Collection

Thirty soil samples were collected from each pasture during odd numbered months (January, March, May, July, September, November) and ten soil samples were collected from each pasture on even numbered months (February, April, June, August, October, December). The pastures were divided into a grid pattern and sections were marked by brightly colored tape placed around the fence posts. Systemic sampling insured that the entire pasture was sampled evenly. Sampling was consistent within the grid pattern during the entire study. No attempt was made to mark exact locations of monthly sample collections.

Sampling was done between 0800 and 0900 to decrease differences in numbers of mites collected due to vertical migration (Van Nieuwenhuizen et al, 1994). Prior to soil sampling, the two temperature recordings were made in the study log. First, the ambient air temperature was recorded then the soil temperature approximately ten centimeters below the soil surface was recorded.

Soil core samples were gathered using a standard tulip bulb planter. The objective of soil sampling was to deliver non-compacted samples of standard size. The

depth of a standard tulip bulb planter is ten centimeters. At this depth, all organic horizons are included as are the vast majority of soil arthropods (Mitchell, 1977b). The volume of a standard tulip bulb planter is 359 cc minimum and 442 cc maximum. Thus, the volume of each soil sample collected was presumed to be within this range. Grass above the soil sample collected was extended through the hole in the top of the tulip planter and taken with the soil. Individual soil samples with corresponding herbage were placed in plastic bags and sealed. Each bag was labeled numerically according to the pasture grid pattern to identify the region from which the sample had been collected and transported to the laboratory.

Each soil sample was weighed upon arrival at the laboratory and again after seven days in the mite extraction apparatus.

Mite Extraction

The classic technique for small arthropod extraction from soil is the Berlese funnel (Borror et al., 1989; Krantz, 1978). The mite extraction process introduced a nonsystemic bias to data, which cannot be eliminated with statistical methods (Stamou and Sgardelis, 1989). The limitation of this method of sampling is that the extraction efficiency may decrease the accuracy of population estimates (Mitchell, 1977b). It would seem that this sampling bias would only be a limiting factor in a study that is attempting to estimate the oribatid mite population using absolute numbers and not pose a problem in a study that is simply estimating variations in the population over time.

Our laboratory apparatus was made of wooden support structures that support funnels holding the soil (Figure 5). The funnels used in this experiment had top diameter of approximately seventeen centimeters. A single layer of cheesecloth was placed within the funnels under the soil to prevent the soil from falling into the collection cups. Collection cups containing 70% ethyl alcohol were placed under the funnels.

Light sources were hung approximately 30 centimeters above the soil samples. There were two light bulbs (40 watts each) for each bank of six soil samples. The light sources provided heat to the top of the sample and gradually reduced the moisture through the layers of soil. Both heat and drying drive the mites, via forced migration, to burrow deeper into the funnels and eventually cause them to fall out the bottom into the collection cups. Soil arthropods are triggered to burrow when the substrate humidity reaches less than 20 to 25% (Krantz, 1978). These bulbs also provided light for the downward migration of the negatively phototropic mites (Kates and Runkle, 1948).

Soil samples were kept in the extraction apparatus for seven days. After this time, the soil samples were weighed and discarded, and the collection jars were capped and stored in the laboratory after adding a sufficient amount of 70% ethyl alcohol to insure preservation until identification.

Taxonomic Identification

The techniques involved in manipulating oribatid mites are specialized due to their large size and heavy sclerotization (Krantz, 1978). Prior to examining most adult higher oribatids, the mites must be cleared. Proper clearing technique involves placing the mites into lactic acid and for one to three weeks. Although applying heat may speed up the process, this method was not used. Temporary mounts are generally used to identify mites. This entails the use of a cavity slide, coverslip, and lactic acid as the



Figure 5. Berlese funnel apparatus.

mounting medium (Krantz, 1978). Once mites are properly mounted they can be rolled to any position by moving the coverslip that covers the cavity.

Mite identification to genus was accomplished using unpublished Keys to the Genera of "Lower" Oribatid Mites of the USA and Canada prepared by Roy Norton and Keys to the Genera of the Brachypyline Oribatid Mites of North America as extracted and slightly modified from Balogh and Balogh (1992) by Roy Norton. Both keys were distributed at The 49th Annual Acarology Summer Program August 9 through August 14, 1999.

Tentative generic identifications were sent to Dr. Roy Norton, SUNY- College of Environmental Science and Forestry, Syracuse, NY for confirmation of identification. These specimens were permanently mounted with on glass slides with Hoyer's medium (Krantz, 1978). Dr. Norton confirmed the generic and specific identities of all but one mite that is believed to be a new species of *Ceratozetes*. Dr. Valerie Behan-Pelletier, research scientist at the research branch of Agriculture and Agrifood, Ottawa, Canada, will describe and report *Ceratozetes* new species in the future.

Tapeworm Infection in Horses

Confirmation of the presence of *A. perfoliata* infected animals on the study pastures was done periodically throughout the study. Fecal samples were taken from the horses residing in both pastures in January 1999, April 1999, January 2000, and final collections were done in November 2000 from the horses in P-6 at the HTU and in December 2000 from the horses in P21-23 at the HRC. Fecal material was obtained per rectum or from the ground if we observed the horse defecating, placed in an arm-length sleeve, labeled with the animals identification number, name, or a brief description of the animal. The samples were then returned to the laboratory and examined the same day. The Wisconsin Sugar Flotation Method (Sloss, 1970) was used to determine the presence or absence of equine tapeworm eggs in addition to strongyle type eggs. The presence of *Anoplocephala perfoliata* eggs was confirmed by measuring the eggs diameter of 65 to 80 microns (Soulsby, 1982) and outermost shell thickness of 8-10 micrometers (Skrjabin, 1951). The other two equine cestodes, *Anoplocephala magna* and *Anoplocephaloides mamillana* measure 50-60 micrometers in diameter and 51 by 37 micrometers respectively (Soulsby, 1982). Both of the latter cestodes have thinner outer shell membranes of only 4 micrometers (Skrjabin, 1951).

The managers of the two horse facilities were asked to not administer pyrantel salts to the horses pastured in the study sites during the two-year study period. The seasonal de-worming program of the horses in pasture P21-23 at the Horse Research Unit consisted of use of ivermectin at the recommended dose of 200 mcg/kg on the following dates: June 1998, January 1999, May 1999, September 1999, January 2000, April 2000, and August 2000. The horses pastured in P-6 at the Horse Teaching Unit are normally dewormed according to a standard protocol in which random fecal samples are run on two horses in the pasture. Treatment is done if needed during the months of September (pyrantel pamoate), November (ivermectin), January (ivermectin), and March (pyrantel pamoate).

Weather Data and Soil-Water Budgets

Monthly summaries of weather data from January 1999 through December 2000 were obtained from the two National Oceanic and Atmospheric Administration (NOAA) weather stations operating throughout the study and located nearest the two study sites. The weather stations fitting these criteria were Gainesville Regional Airport (co-op ID 083326) located in Alachua County at latitude 29° 42' N and longitude 82° 17' W and

Ocala (co-op ID 086414) located in Marion County at latitude 29° 12' N and longitude 82° 05' W. Weather data was obtained to determine whether monthly differences in oribatid mite counts were related to monthly mean temperature, total monthly precipitation, or the departure from normal monthly precipitation and departure from normal monthly temperatures.

To determine the effect that farm location, month, and year of collection had on mite numbers we ran a 3-way analysis of variance using Sigma Stat statistical software version 2.0. Then to determine if any of the weather data (total monthly rainfall, monthly temperature, ambient temperature on day of collection, soil temperature on day of collection) had any significant relationships to monthly mite counts, Pearson product moment correlations were done, also using Sigma Stat statistical software version 2.0.

The monthly water budget of estimated soil moisture storage was calculated to determine whether monthly oribatid mite counts were related to the available soil moisture by using a computer program for estimating evapotranspiration by the Thornthwaite method (Sellinger, 1996). This method was chosen because it is simple to calculate using a computer program available from the U.S. Department of Commerce, and the data required for calculation is available from NOAA weather stations. To run the program the following data for both Ocala and Gainesville was required:

(1) mean monthly air temperature;

(2) total monthly precipitation:

(3) information on the water holding capacity of the depth if soil for which the balance is to be computed.

(4) the latitude and hemisphere of the watershed.

The mean monthly temperature, total monthly precipitation, and latitude data were obtained from the NOAA weather stations databases. The soil moisture storage was estimated to be 150 mm (Kaplan, 1995). After the computer program was run, the output data included potential evapotranspiration (PET) and actual evapotranspiration. Weather data for the previous year, 1998, was used to determine the initial soil moisture storage for January 1999 and we began January 2000 with the remaining water budget from December 1999. The water budget, or change in storage of moisture in the soil, was calculated by subtracting monthly actual evaporation from monthly precipitation.

Each soil core sample was kept separate from others and the mites were enumerated and entered into the database separately. Since every other month there were either 10 or 30 soil cores, the total numbers of mites collected from these cores were adjusted by either multiplying by three or one as needed. Adjusted monthly mite counts were correlated using Sigma Stat statistical software version 2.0 with the following weather data: monthly water budget, monthly mean temperature, ambient air temperature at the time of sampling, soil temperature at the time of sampling, and the monthly total precipitation.

Calculation of Population Distribution in Study Pastures

The characteristics of the oribatid mite communities in the pastures were determined by calculating the dominance and frequency of each species (Wallwork and Rodriguez, 1961). The monthly dominance (D_m) of a species relates the size of the population of a given species to the sum of the population sizes of the remaining species in the community and is affected by mite clumping.

 $D_{m} = \frac{\text{Number of individuals from a given species}}{\text{Number of individuals from all species}} \times 100$

The frequency number of a given species (F) is the percentage occurrence in a series of samples. This statistic is, to some extent, independent of the amount of clumping of mites.

$$F = \frac{\text{Number of samples in which the species occurs}}{\text{Total number of samples in a series}} \times 100$$

Finally, an aggregation index of each oribatid mite species was calculated by determining the slope (*b*) of a linear regression of plot of \log_{10} of variance (s^2) vs. \log_{10} of the arithmetic mean (*m*) for each species. This was done with both study sites and all sample dates pooled to have sufficient data to run the analysis. The variance can be related to the mean by Taylor's power law: $s^2 = am^b$ (Taylor, 1961). Parameters *a* and *b* are both population parameters (a = b = 1 when dispersion is random). Parameter *a* is a constant that is characteristic of the population in question and depends chiefly on the size of the sampling unit (Elliott, 1977). Parameter *b* is a true population statistic, an "index of aggregation". When this index of aggregated. Taylor's power law was used rather than estimation of a common $k(k_c)$, where *k* is an index of clumping in a population, for a series of samples because the power law covers a wider range of distributions than the negative binomial and the transformations derived from *b* are often easier to apply than those derived from the negative binomial (Elliott, 1977).

Culturing Oribatid Mites

Oribatid mites were cultured in the laboratory to determine which species of oribatid mites that commonly occur in north central Florida horse pastures are suitable intermediate hosts for *Anoplocephala perfoliata*. Oribatid mites were collected, extracted, and cultured in humidity controlled chambers in the laboratory.

The study sites used were the same as those for the monthly collections throughout the study of seasonal availability of oribatid mites. The mites collected from these pastures could have been naturally infected with *A. perfoliata* since at least one horse per pasture was shown to be shedding *A. perfoliata* via fecal diagnostic techniques for most of the study period.

Techniques for soil sample collection were the same as those described above except the collection was not done at regular monthly intervals or at a particular time of day. Collection was done as necessary to keep oribatid mites available for experimentation in the laboratory.

Extraction techniques were the same as described for the study of seasonal availability of oribatid mites except the mites were not collected into 70% ETOH. Instead, the collection jars contained filter paper moistened with tap water and they were covered with Parafilm-K to insure that moisture would not be lost. The collection jars were monitored via dissecting microscope daily or every two days for the presence of oribatid mites. Oribatid mites found were transferred with a fine tipped paintbrush to a culture jar.

Culture jars were glass with tight fitting plastic caps in which round holes were cut and a fine mesh cloth was placed underneath to allow air to circulate through the jars while keeping the small oribatid mites from escaping. The bottoms of the culture jars were made of plaster and charcoal (ratio 5:1). This substrate has been highly successful due to the ease of humidity control and reduction of contamination (Krantz, 1978). Many of the culture jars could be kept inside a large humidity chamber. The humidity chambers had a supersaturated solution in the bottom to keep a constant humidity within the chamber. The small culture jars were kept above the solution in the bottom by placing them on large plastic petri dishes that were upside down. The humidity chambers were kept at room temperature (22° Celsius). There were three humidity chambers (A, B, and C) used for culturing. The relative humidity and the saturated solution used in the chambers were 76% Ammonium Monophosphate, 79% Ammonium Sulfate, and 91% Sodium Chloride respectively (Winston and Bates, 1960).

Yeast extract and baker's yeast were used for food. These substrates proved least likely to grow mold and they seemed palatable to the mites. The yeast extract or baker's yeast was placed on moistened strips of filter paper and was added to the cultures as needed.

Gravid *A. perfoliata* adults for mite infection trials were obtained from horses necropsied by the University of Florida's VMTH Anatomic Pathology Service. If a positive horse had a greater than 12 hour lapse between time of death or euthanasia and necropsy, it was likely that the gravid proglottids had already been lost. Whether this was due to the host gastrointestinal enzymes or a mechanism of the tapeworm is unknown. Tapeworm eggs have been previously collected and used to infect colonies of oribatid mites. Xiao and Herd (1992) stored *Moniezia benedeni* eggs at 4° C from one to five weeks before exposure to oribatid mites and reported decreased infectivity, decreased infection intensity, and slower cysticercoid maturation time with even short term exposure to low temperatures. In the current trial, the gravid proglottids were dissected and stored in physiologic saline and then stored overnight at 4° C.

Exposure time of the oribatid mites in culture to *A. perfoliata* eggs was set at a minimum of 48 hours. This time period was chosen because when Freeman (1952)

exposed oribatid mites to porcupine tapeworm eggs, exposure time of less than 24 hours resulted in greatly decreased numbers of infected mites and with longer exposure time the numbers of infected mites did not increase dramatically. Other important factors for infection is that the oncospheres be fully mature and contain active larvae (Stunkard (1938). We did not observe the *A. perfoliata* eggs for active larvae.

The cultures were experimentally exposed to gravid *A. perfoliata* proglottids twice. The first exposure was on October 28, 1999. The proglottids were teased apart and placed on moistened pieces of filter paper then placed into all culture chambers containing live mites in the laboratory. The second exposure was on March 23, 1999. At this time gravid proglottids were crushed in physiologic saline solution. The number of eggs contained in the saline was approximately 95,000 eggs per ml. This measurement was obtained with a hemocytometer. Either 0.05ml of the solution (4,750 eggs) or 0.1 ml of the solution (9,500 eggs) was added to each of the culture chambers containing live oribatid mites.

The culture jars were examined at weekly or bi-weekly intervals for dead mites until 20 weeks post-exposure. Dead mites were placed in 70% ETOH solution for storage. The dead mites were identified under a dissecting microscope and then six to ten mites of the same species were placed in one to two drops of saline on a clean microscope slide and covered with a cover glass to which gentle pressure was applied to crack their exoskeletons. These slides were examined at 100x for evidence of egg ingestion or cysticercoid development.

Results

Oribatid Mites

A total of 28,752 oribatid mites was collected from the two pastures and identified during the two-year study. The oribatid taxa identified are presented in Table 1 and the predominant oribatid mite genera are pictured in Figure 6. Twenty-two different taxa were recovered. Thirteen of the 22 taxa collected have supported tapeworm development (Fritz, 1982; Denegri, 1993). Within each soil sample an average of 3.6 taxa were found, with a standard deviation of 3.3 taxa per soil sample.

The four species identified in the superfamily Oppiodea, *Acropia* sp. nr. *antillensis* (Manunka), *Neoamerioppia* sp., *Oppiella nova* (Oudemans), and *Striatoppia* sp., are nearly indistinguishable and thus have been grouped into to the Complex Oppiidae *sensu* Norton 2000 throughout the study.

Considerable variation was found in the number of mites extracted from each soil sample. The mite number per soil sample varied from a low of zero to a high of 1882. The greatest number of mites in one soil sample was found in May 2000 from the Horse Research Center in Ocala. The average number of mites per soil sample was 8.4 with a standard deviation of 35.8.

Prior to any further calculations, monthly mite counts were corrected for the months in which only 10 soil samples were collected by multiplying the raw data by three. A three-way analysis of variance was performed using Sigma Stat statistical software version 2.0. No statistically significant effect of farm location, month of collection, or year of collection on the monthly mite counts were detected. These results are in agreement with the null hypothesis, which states that there is no seasonal variation in the numbers of oribatid mites on horse pastures of north central Florida.

Table 1. The mite taxa.

Drachymylina			Genus Known IH
/ Macropylina	Superfamily	Species	01 Anoplocephalidae*
Macropylina	Epilohmannoidea	Epilohmannia minuta Berlese	Yes
Macropylina	Lohmannioidea	<i>Lohmannia</i> sp. nr. <i>jornoti</i> Manunka	No
Macropylina	Lohmannioidea	Nesiacarus sp.	No
Macropylina	Euphthiracaroidea	Rhysotritia ardua Koch	No
Brachypylina	Ceratozetoidea	Ceratozetes n. sp.	Yes ¹
Brachypylina	Ceratozetoidea	Trichoribates sp.	Yes ¹
Brachypylina	Galumnoidea	Galumna jacoti Marshall, Reeves,	Yes ¹
Brachypylina	Galumnoidea	Galumna minuta Ewing	Yes ¹
Brachypylina	Oppioidea	Acroppia sp. nr. antillensis Manunka	No
Brachypylina	Oppioidea	Neoamerioppia sp.	No
Brachypylina	Oppioidea	Oppiella nova Oudmans	Yes
Brachypylina	Oppioidea	Striatoppia sp.	No
Brachypylina	Oribatelloidea	Oribatella sp.	Yes
Brachypylina	Oribatuloidea	Peloribates sp. nr. hirsutus Banks	Yes
Brachypylina	Oribatuloidea	Protoribates capucinus Berlese	Yes
Brachypylina	Oribatuloidea	Rostrozetes ovulum Berlese	No
Brachypylina	Oribatuloidea	Scheloribates sp.	Yes ¹
Brachypylina	Oribatuloidea	Zygoribatula sp. nr. floridana Fritz	Yes ¹
Brachypylina	Phenopelopoidea	Eupelops sp.	Yes
Brachypylina	Tectocepheoidea	Tectocepheus velatus Michael	Yes
Brachypylina	Unplaced	Lamellobates sp.	No
Brachypylina	Unplaced	Tectoribates sp.	No

* Source: Fritz (1982); Denegri (1993) ¹ Members of this genus are known IH for *A. perfoliata*



Figure 6. Selected oribatid mite genera (collected during the study). a) *Rostrozetes ovulum* (Berlese); b) *Ceratozetes* n sp.; c) *Epilohmannia minuta* (Ewing); d) *Zygoribatula* sp. nr *floridana* (Fritz); e) *Galumna minuta* (Ewing).

Fecal Analysis

All fecal samplings during the study confirmed that at least one horse in each of the study pastures was shedding *A. perfoliata* eggs with the exception of the November 2000 sampling at HTU. The negative results obtained in November from the HTU can be explained by the fact the management at the HTU farm dewormed the horses in the study pasture P-6 in October 2000 with pyrantel pamoate paste at the label directed dosage.

Weather Data and Soil-Water Budgets

Figure 7 and Figure 8 show the monthly total precipitation and its deviation from average in both Gainesville and Ocala. The monthly mean temperatures and their deviation from the 30-year average in Gainesville and Ocala are shown in Figure 9 and Figure 10.

The Pearson product moment correlation coefficient (r), when monthly mite count and total monthly rainfall were entered as the variables, was -0.314 (P value 0.0297). The data and regression line are shown in Figure 11. The negative correlation implies a negative relationship between monthly mite count and total monthly rainfall: that is, as monthly total rainfall decreases, total monthly mite counts increases. The coefficient of determination (r^2) measures the amount of variation in the response variable, in this case the monthly mite counts explained by monthly total rainfall. Thus, with a coefficient of determination (r^2) of 0.099, the fitted regression model explains only 9.9% of the variation in the monthly mite counts. Therefore, total monthly rainfall is obviously not a variable that has much impact on oribatid mite population size. When the other response variables, monthly mean temperature, ambient air temperature, soil temperature, and monthly water-budget, were correlated with monthly mite counts using the same analysis, no significant relationships were found.

Population Distribution in Study Pastures

The values of annual mean, minimum, and maximum dominance of each mite species at each study site may be seen in Table 2 (Horse Research Center in 1999), Table 3 (Horse Research Center in 2000), Table 4 (Horse Teaching Unit in 1999), and Table 5 (Horse Teaching Unit in 2000). The four species with the highest mean percent monthly dominance at the Horse Research Center were *Rostrozetes ovulum* (Berlese), *Zygoribatula* sp. nr. *floridana* (Fritz), *Scheloribates* sp. (Berlese), and *Epilohmannia minuta* (Ewing). In 1999 these four species comprised 72.0% of the oribatid mites found on the pasture and in 2000 they comprised 77.7% of the oribatids found on the pasture. The two co- dominant species at the HRC were *Rostrozetes ovulum* and *Zygoribatula* sp. nr. *floridana*, and *Scheloribates* sp., are known to be intermediate hosts for *A. perfoliata* (Denegri 1993). Members of the genus *Epilohmannia* are known to be intermediate hosts of Anoplocephalidae (Denegri, 1993). *Rotrozetes ovulum* has not been implicated to be an intermediate host.

There were three species, Oppidae, *Zygoribatula* sp. nr. *floridana*, and *Rostrozetes ovulum* that made up the majority of mites counted at the Horse Teaching Unit in 1999 (55.63%) and 2000 (56.81%). The four species making up the Complex Oppidea *sensu* Norton 2000 (*Acroppia* sp. nr. *antillensis*, *Oppiella nova*, *Neoamerioppia* sp., and *Striatoppia* sp.) are dominant at the HTU. Of the four species in this complex, only *Oppiella nova* is known to be an intermediate host of Anoplocephalidae.

The annual percent dominance and mean monthly frequency for each species at each study site are shown in Table 6 (HRC in 1999), Table 7 (HRC in 2000), Table 8 (HTU in 1999), and Table 9 (HTU in 2000). There are many species that are dominant
and that also have high frequencies during the two years of sampling. At the HRC the top three most frequently collected oribatid mites are also in the top four dominant species in both 1999 and 2000. However at the HTU in 1999 the most frequently sampled species, *Ceratozetes* n. sp., was only the fourth highest in dominance and in 2000 this species was the second most frequently samples species, but was only the fifth most dominant species. The other three species of the four most frequently sampled species at the HTU in 1999 and 2000 were also the three most dominant species during the two years of study.

To test the hypothesis that when a high frequency value is associated with a high dominance value or when a low frequency value is associated with a low dominance value, there is an absence of a high degree of aggregation (Wallwork and Rodriguez, 1961), we calculated the index of aggregation for each species found during the study (Table 10). The calculations were based on Taylor's power law: $s^2 = am^b$ (Taylor, 1961). Parameter *a* is a constant that is characteristic of the population in question and depends chiefly on the size of the sampling unit, parameter *b* is a true population statistic, an "index of aggregation", and *m* is the arithmetic mean for each species (Taylor, 1961; Elliott, 1977). A Sigma Stat statistical software version 2.0 linear regression model successfully calculated an index of aggregation (*b*) for 14 of 19 of the species found. The parameter *b* was found to be greater than two for all of the 14 species and all are considered to have highly aggregated populations (Taylor, 1961).

The 5 oribatid mite species that had the highest dominance values on the study pastures, *Rostrozetes ovulum*, *Zygoribatula* sp. nr. *floridana*, *Scheloribates* sp., *Epilohmannia minuta*, and the Complex Oppidea *sensu* Norton 2000, were evaluated for

any seasonal or monthly variation. Of these, only *Rostrozetes ovulum* is not known to be an intermediate host of Anoplocephalidae. We first ran two-way ANOVAs with both date and study site as the variables and found that there was no significant effect due to farm. Then we ran Kruskal-Wallis One Way Analysis of Variance on Ranks to compare differences among the individual species and the month and year collected. The result for all of the tested species was that there were differences in the median values among the groups that were greater than expected by chance (P < 0.001). When pairwise multiple comparison procedures (Dunn's Method) were done to isolate the groups that differ from the others, no month could be isolated as being different from the other months for Scheloribates sp., Rostrozetes ovulum, or the Complex Oppidea sensu Norton 2000. The same proceedures were performed to compare the numbers of mites recovered in the different months for Zygoribatula sp.nr. floridana and Epilohmannia minuta and some months were found to be statistically different (P<0.05). However, when these statistically different months were compared with graphs showing the total monthly mite counts for the species, no monthly or seasonal pattern was observed.

Oribatid Mite Cultures

Throughout the infection study only one mite was found to have ingested *Anoplocephala perfoliata* eggs. The mite belonged to the species *Epilohmannia minuta* Berlese (*=Epilohmannia pallida* Wallwork).

The oribatid mites that were exposed to *A. perfoliata* eggs from October 28, 1999 through November 2, 1999 were examined weekly. The dead mites were pooled together for the first four weeks post-exposure and dissected (Table 11). One *Epilohmannia minuta* Berlese (= *Epilohmannia pallida* Wallwork) ingested four *Anoplocephala perfoliata* eggs. There was evidence of four oncospheres in body cavity (Figure 12).

Another member of this genus, *Epilohmannia pallida*, has been found to act as an intermediate host for *Moniezia* spp (Denegri, 1993).

No other mites were found to have ingested any *A. perfoliata* eggs or have developmental stages of *A. perfoliata* larvae within their hemocoele. The identification and enumeration of the mites removed from the cultures between 5 weeks and 20 weeks post-exposure are found in Table 12.

The remaining live mites in culture 20 weeks after the first exposure to *A*. *perfoliata* eggs were re-exposed on 3/23/00. From this date other culture chambers that had previously been exposed to different food stuffs, such as potato, brewer's yeast, and yeast extract, to see if this affected their longevity or reproduction were used as unexposed controls as these mites were unexposed to *A*. *perfoliata* eggs in our laboratory. All dead mites were removed and fresh feed supply was added to all chambers on the following dates : 3/27/00, 4/3/00, 4/10/00, 4/18/00, 4/27/00, 5/3/00, 5/8/00, 5/18/00, 5/24/00, 6/1/00, 7/13/00, 7/20/00, 8/3/00, 8/17/00, 9/15/00, 10/3/00, 10/25/00, 11/6/00, 1/31/01. None of the dissected mites were found to contain *A*. *perfoliata* eggs or larvae (Table 13). Since no mites were found to have *A*. *perfoliata* larvae, and all mites observed were either exposed to *A*. *perfoliata* eggs on the study pastures or both on the study pastures and in the laboratory, I would conclude that the prevalence of mites infected with *A*. *perfoliata* larvae is low.

Discussion

The results of monthly-adjusted mite counts when correlated with monthly total rainfall, monthly mean temperature, ambient air temperature on the day of sampling, soil temperature on the day of sampling, and water budget failed to reveal any significant

relationships thus proving our null hypothesis correct. This finding is disappointing because it means that it appears unlikely that an effective strategic treatment program can be developed for control of *A. perfoliata* in north central Florida based upon seasonal variation in the availability of its intermediate host.

The weather data immediately prior to and during the study period was investigated. It is obvious that both localities in north central Florida experienced a drought in the months prior to and during 1999 and 2000. The temperatures were milder than normal during the autumn and winter of 1998 and into the early months of 1999 at both locations. The end of 1999 and majority of 2000 were warmer than usual in Ocala. The final months of 2000 were cooler than average in both Gainesville and Ocala.

Because the focus of this research was to determine if there is a seasonal pattern in availability of the intermediate host, we must also examine other seasonal influences that have been found regarding the life cycle of *A. perfoliata*. Seasonal variations in the number of horses positive for tapeworms at necropsy, the number of adult tapeworms in the gastrointestinal tract, and in the number of horses that display patent infections have been reported (Hass, 1979).

Generally, research has found adult tapeworm infection intensity to be lowest in the spring with a gradual increase through the seasons until winter (Bain and Kelly, 1977; Benton and Lyons, 1994; Hass, 1979; Lyons et al. 1983; Lyons et al., 1984; Lyons et al. 1990; Lyons et al, 1997; Urquart et al., 1996). Bain and Kelly (1977) hypothesized that with colder weather a longer prepatent period and natural parasite loss occur, due to physiological aging or immunity. Thus, the numbers of adults seen are lowest in the spring and then there is an accumulation through the seasons until the winter. Presumably this increased numbers of adult tapeworms seen at necropsy in the fall and winter is due to exposure of the horses to the intermediate hosts throughout the grazing season. It has also been proposed that the development time of the larvae within the intermediate host and the prepatent period once the infective larvae is ingested by the horse, influences the seasonal differences in prevalence in a temperate climate where the grazing season is limited (Owen et al., 1988). Though no actual data is available on the life span of adult *Anoplocephala perfoliata*, but the life span of an adult cestode may vary from a few months to several years (Jubb and Kennedy, 1970; Kennedy, 1983). A basic cestode life cycle strategy, based on iteroparity (long-duration, non-seasonal terrestrial egg release), infers a long adult Cyclophyllidean life span that often lasts for years or as long as the definitive host lives (Mackiewicz, 1988; Schmidt and Roberts, 1989; Kennedy, 1983). These two theories do not agree.

Season may play a role in the epidemiology of *Anoplocephala perfoliata* with regards to egg shedding in the feces of the infected horse. Seasonal variations have been found in egg shedding in horses with patent tapeworm infections in north central Florida. Kivipelto et al. (1996) found that the percentage of horses displaying patent tapeworm infections was greater from July through January and lower from February through June.

Although no seasonal fluctuations in oribatid mite numbers available in pastures were found in this study, seasonal factors other that those controlling mite numbers actually ascending onto herbage where they can be eaten by horses may very well still play a key role in the epidemiology of *A. perfoliata* transmission. This study did not distinguish between those mites in the soil and those on the herbage. Since the mites that migrate up onto the herbage are more likely to be eaten by a horse, the next logical step

in this line of research would be to enumerate the mites on the herbage through the seasons.

Taylor's power law was used to determine the value of the index of aggregation (b). This was done to provide more information to support that general belief that oribatid mites populations exist in clumped or clustered patterns. It has been hypothesized that when a high frequency value is associated with a high dominance value and a low frequency value is associated with a low dominance value, there is an absence of a high degree of aggregation (Wallwork and Rodriguez, 1961). For the linear regression model to calculate a valid index of aggregation the following assumptions were required: normal distribution of the statistic, the mean of the distribution or the statistic must be zero, and the variance of the statistic must be the same for all factor levels. There were five species for which the index of aggregation could not be calculated. *Peloribates* sp. nr *hirsutus* did not pass the normality test, *Protoribates* capucinus was not of significant value, and Lamellobates sp., Rhysotritia ardua and Trichoribates sp. did not have sufficient numbers of specimens to run a valid test. The index of aggregation values that were obtained for the remaining 14 oribatid species ranged from 2.136 to 2.728 and therefore they are considered to be highly aggregated species (Taylor, 1961).

The coefficient of determination (\mathbb{R}^2) is an important statistic that is included in Table 4. The coefficient of determination measures the percentage of variability in the response variable accounted for by the model. \mathbb{R}^2 values ranging from 0.652 to 0.979 with mean of 0.874 explains that the predictive model used was reasonably accurate.

Laboratory controlled mite feeding experiments are believed to be more accurate to determine the predominant species of oribatid mite utilized in completing a tapeworm life cycle. When examining mites collected from grazed pastures and counting the percentage of mites infected with cysticercoids there is not uniform contamination of a pasture and it is impossible to distinguish species of tapeworm simply by observing the cysticercoid (Narsapur, 1976). Therefore, use of this study's collection of mites to determine the prevalence of cysticercoids in the different taxa may not be a worthwhile endeavor unless a technique employing genus or species specific nucleic acid probes are used to confirm that any cysticercoids belong to equine tapeworm species.

We found only one mite with evidence of *A. perfoliata* egg ingestion and no mites with cysticercoid development despite having examined 709 oribatid mites. All of the mites examined had been potentially exposed prior to collection from the study pastures and 240 of the mites were also exposed to high numbers of *A. perfoliata* eggs in the laboratory. Throughout the infection study only one mite was found to have ingested *Anoplocephala perfoliata* eggs. The mite belonged to the species *Epilohmannia minuta* Berlese (*=Epilohmannia pallida* Wallwork). This mite was exposed to high numbers of A. perfoliata eggs in the laboratory while in culture. Another member of this genus, *Epilohmannia pallida*, has been found to act as an intermediate host for *Moniezia* spp (Denegri, 1993). Our finding implies that the species *Epilohmannia minuta* should be further studied to resolve it's role as an intermediate host of *Anoplocephala perfoliata* in north central Florida horse pastures. This mite species exists on our study pastures at an overall 9.98% monthly dominance. The viability of this mite as an experimental or natural intermediate host needs to be examined closely.

One topic that must be addressed is the difference between natural intermediate hosts and mites that are capable of developing cysticercoids in laboratory cultures. Should all mites that are capable of developing cysticercoids be considered natural intermediate hosts? Most authors think not. Including only the arthropods that have the capacity to produce cysticercoids, and those that are constituents of the habitual diet have the probability of being natural intermediate hosts is one author's theory of trophism (Denegri, 1998). For example, Epilohmannia sp. and members of the family Oppidea live below the soil surface and thus would not be as likely to be ingested by an animal while grazing. However, one can not reject the possibility of a horse pulling up plants in the pasture and ingesting some of the roots and soil. Both groups are listed as being capable of transmitting Anoplocephaline tapeworms (Denegri, 1993). However, Fritz (1982) did not include the *Epilohmannia* sp. in his list of probable intermediate hosts for the goat tapeworm, *Moniezia expansa*. However the two most commonly found species, Zygoribatula floridana and Ceratozetes sp., are surface dwelling species and therefore were included as probable intermediate hosts (Fritz, 1982). During his study Fritz found 0.2 to 3.4% of oribatid mites to be naturally infected with cysticercoids on goat pastures in north central Florida (Fritz, 1982). One must know the susceptibility to tapeworm egg ingestion and larval development and the natural occurrence on pastures to know the epidemiological role of a particular oribatid mite species (Schuster et al., 2000).

The oribatid mite families most frequently found to be harboring cysticercoids of Anoplocephaline species were Oribatulidae, Galumnaidae, and Ceratozetidae while the genus that most frequently acted as an intermediate host, and that demonstrated the greatest natural and experimental susceptibility, was *Scheloribates* (Scheloribatidae)

(Denegri, 1993). Oribatulidae includies the family described by Norton (1999a) as Scheloribatidae. Oribatid mites in the genera *Galumna* (Galumnaidae) and *Scheloribates* (Scheloribatidae) have wide ranges of distribution (Kates and Runkle, 1948). The mites in these families are moderate to large in size and highly sclerotized and therefore would be more likely to inhabit the upper layers of soil where pore size is larger and they are more resistant to dessication (Mitchell, 1979). Also, mites within the two genera, *Galumna* and *Scheloribates*, have been found on herbage and in the top 5 cm of soil (van Niewenhuizen, 1994). Oribatid mites from all of the mentioned families were collected during this study, and it is assumed that one or more are natural intermediate hosts on horse pastures in central Florida.

There are very few reports of natural or experimental infection of oribatid mites with *Anoplocephala perfoliata*. Bashkirova (1941) was the first to complete the life cycle in a laboratory setting. Romero et al. (1989) experimentally infected six of 598 *Scheloribates* sp., four with one cysticercoid each and two with two cysticercoids each. The mites in the successful experiment were collected from the same depth and extracted using the same method and temperature as were used for this project, but different culture conditions were described. They used steel cuvettes as holding chambers and the substrate was sterilized soil mixed with parasite free bovine manure. Another difference was that they just added gravid proglottids to the substrate without prior dissection. Perhaps future attempts at infecting cultured oribatid mites should include this previously successful method, since *A. perfoliata* eggs are typically contained within proglottids rather than free in the feces as with *Moneizia* spp. However, it seems that even under the best of circumstances, artificial *A. perfoliata* infections may be difficult to produce.

In another study with oribatid mite exposure to cestode eggs of the species *A*. *magna*, *A. perfoliata*, and *Anoplocephaloides mamillana*, few mites developed larval stages within their body cavities (Pinto et al., 1998). The following factors were given as possible contributors to poor success: (1) gravid proglottids with non-infective eggs; (2) mites with low survivability, thus not allowing for larval development; (3) mites in the study had low susceptibility to cestode infection; (4) difficulty in maintaining *Galumna* spp. in artificial culture chambers. Other laboratory feeding trials have shown oribatid mite survivability to be low, especially in members of *Galumna* spp. (Gleason and Buckner, 1979). Although low numbers of oribatid mites are naturally infected with other tapeworm species cysticercoids, up to 90% may become infected under some experimental conditions (Schuster, 1988 in Schuster et al., 2000).

During this study we hoped to have reproduction of oribatids in culture and perhaps produce self-maintaining oribatid colonies. Development time of oribatid mites in culture depends on temperature, light conditions, food source, and density of culture (Mitchell, 1977a). Due to one of more of the above conditions the mites did not produce self-maintaining colonies while in our laboratory. Optimal mite densities are not published. During this experiment mites were kept at various densities and none seemed to change the outcome. We did however find evidence of reproduction such as eggs and nymphal stages in the culture jars. Due to the difficulty in identifying nymphal stages and since the cultures contained multiple adult species, the author is unsure which species were reproducing.



Figure 7. Monthly precipitation Gainesville.



Figure 8. Monthly precipitation Ocala.



Figure 9. Monthly temperatures Gainesville.



Month

Figure 10. Monthly temperatures Ocala.



Figure 11. Total rainfall vs. mite counts.

	Percent Monthly Dominance		
Mite Species	Mean	Minimum	Maximum
Rostrozetes ovulum	31.57	15.67	55.95
Zygoribatula sp. nr. floridana	16.54	4.46	41.46
Scheloribates sp.	14.96	1.67	52.52
Epilohmannia minuta	8.92	0.92	16.88
Complex Oppiidae sensu Norton 2000*	5.76	0.97	18.85
Galumna minuta	5.30	0.72	16.18
Lamellobates sp.	4.01	0.41	7.14
Unknown	3.64	0.73	8.35
Lohmannia sp. nr. jornoti	3.62	0.48	11.33
Tectocepheus velatus	2.77	0.79	5.15
Eupelops sp.	2.71	1.00	7.23
Protoribates capucinus	1.94	0.16	3.86
<i>Tectoribates</i> sp.	1.73	0.16	4.07
Ceratozetes n. sp.	1.56	0.17	4.36
Nesiacarus sp.	1.43	0.16	4.23
<i>Oribatella</i> sp.	0.73	0.16	1.63
Peloribates sp. nr. hirsutus	0.40	0.32	0.48
Galumna jacoti	0.24	0.24	0.24
Rhysotritia ardua	0.17	0.16	0.18

Table 2. Percent dominance by mite species at the Horse Research Center in 1999.

*= Acroppia sp. nr. antillensis, Oppiella nova, Neoamerioppia sp., Striatoppia sp.

	Percent monthly dominance		
Mite Species	Mean	Minimum	Maximum
Zygoribatula sp. nr. floridana	30.91	3.16	82.09
Rostrozetes ovulum	29.60	1.49	52.18
Scheloribates sp.	9.09	1.38	30.22
Epilohmannia minuta	8.11	0.54	20.91
Galumna minuta	6.45	1.00	17.56
Complex Oppiidae sensu Norton 2000	4.73	0.80	22.45
Tectocepheus velatus	3.59	0.89	9.32
Unknown	2.63	0.69	6.06
Eupelops sp.	2.31	1.20	3.51
Peloribates sp. nr. hirsutus	1.68	1.68	1.68
Tectoribates sp.	1.60	0.24	4.63
Ceratozetes n. sp.	1.55	0.30	2.99
Nesiacarus sp.	1.49	0.11	7.07
Lamellobates sp.	1.45	0.11	4.00
Lohmannia sp. nr. jornoti	1.39	0.24	5.56
Protoribates capucinus	0.57	0.12	1.49
Galumna jacoti	0.48	0.12	0.85
<i>Oribatella</i> sp.	0.12	0.12	0.12
Rhysotritia ardua	0.05	0.05	0.05

Table 3. Percent dominance by mite species at the Horse Research Center in 2000.

• • •	Percent Monthly Dominance		
Mite Species	Mean	Minimum	Maximum
Complex Oppiidae sensu Norton 2000	24.21	0.82	43.65
Zygoribatula sp.nr. floridana	16.09	2.18	58.33
Rostrozetes ovulum	15.32	4.17	30.03
Ceratozetes n. sp.	10.76	4.17	18.01
Epilohmannia minuta	7.52	0.83	25.00
Scheloribates sp.	7.35	0.94	18.00
Tectoribates sp.	4.44	1.10	18.80
Lamellobates sp.	4.22	0.21	21.95
Galumna minuta	3.88	0.64	10.32
Unknown	2.96	0.43	12.27
Eupelops sp.	2.38	0.67	4.92
Tectocepheus velatus	2.28	1.18	3.64
<i>Oribatella</i> sp.	1.65	0.48	4.92
Nesiacarus sp.	1.34	0.55	2.12
Galumna jacoti	1.16	0.46	2.46
Rhysotritia ardua	0.45	0.07	0.69
Peloribates sp. nr. hirsutus	0.29	0.29	0.29
Trichoribates sp.	0.14	0.14	0.14
Lohmannia sp. nr. jornoti	0.11	0.04	0.17

Table 4. Percent dominance by mite species at the Horse Teaching Unit in 1999.

• * *	Percent Monthly Dominance		
Mite Species	Mean	Minimum	Maximum
Complex Oppiidae sensu Norton 2000	21.19	3.23	37.08
Zygoribatula sp.nr. floridana	19.40	2.85	40.00
Rostrozetes ovulum	16.22	0.63	26.57
Epilohmannia minuta	15.40	0.40	69.62
Ceratozetes n. sp.	10.63	3.93	24.90
Scheloribates sp.	8.40	0.50	18.73
Tectoribates sp.	6.21	2.13	13.29
Unknown	6.05	0.63	40.00
Galumna minuta	2.57	1.00	5.59
Eupelops sp.	2.44	0.70	4.58
<i>Oribatella</i> sp.	2.23	0.11	6.59
Lamellobates sp.	1.76	0.50	4.78
Galumna jacoti	1.50	0.10	3.23
Tectocepheus velatus	1.05	0.48	2.19
Lohmannia sp. nr. jornoti	0.62	0.62	0.62
Rhysotritia ardua	0.54	0.11	0.97
Nesiacarus sp.	0.42	0.15	0.63
Trichoribates sp.	0.14	0.14	0.14
Protoribates capucinus	0.14	0.14	0.14

Table 5. Percent dominance by mite species at the Horse Teaching Unit in 2000.

Table 6. Annual dominance and frequency for mites from the HRC in 1999.

Mite Genus	Percent Dominance	Mean Monthly Frequency
Zygoribatula sp.nr. floridana	16.54	53.06
Rostrozetes ovulum	31.57	52.22
Epilohmannia minuta	8.92	34.72
Eupelops sp.	2.71	25.83
Galumna minuta	5.30	25.56
Tectocepheus velatus	2.77	25.28
Scheloribates sp.	14.96	24.72
Lamellobates sp.	4.01	21.94
Complex Oppiidae sensu Norton 2000	5.76	17.50
Unknown	3.64	16.67
Lohmannia sp. nr. jornoti	3.62	12.22
Tectoribates sp.	1.73	10.28
Protoribates capucinus	1.94	7.50
Ceratozetes n. sp.	1.56	5.83
Nesiacarus sp.	1.43	5.83
<i>Oribatella</i> sp.	0.73	1.11
Galumna jacoti	0.24	0.83
Peloribates sp. nr. hirsutus	0.40	0.56
Rhysotritia ardua	0.17	0.56

Table 7. Annual dominance and frequency for mites from the HRC in 2000.

Mite Genus	Percent Dominance	Mean Monthly Frequency
Zygoribatula sp.nr. floridana	30.91	53.61
Rostrozetes ovulum	29.60	51.67
Epilohmannia minuta	8.11	32.50
Galumna minuta	6.45	29.44
Unknown	2.63	26.39
Tectocepheus velatus	3.59	25.28
Complex Oppiidae sensu Norton 2000	4.73	25.00
Eupelops sp.	2.31	25.00
Scheloribates sp.	9.09	24.72
Tectoribates sp.	1.60	12.78
Lamellobates sp.	1.45	11.39
Lohmannia sp. nr. jornoti	1.39	8.89
Ceratozetes n. sp.	1.55	6.67
Nesiacarus sp.	1.49	5.28
Protoribates capucinus	0.57	3.61
Galumna jacoti	0.48	1.11
Peloribates sp. nr. hirsutus	1.68	0.83
<i>Oribatella</i> sp.	0.12	0.83
Rhysotritia ardua	0.05	0.28

Table 8. Annual dominance and frequency for mites from the HTU in 1999.

Mite Genus	Percent Dominance	Mean Monthly Frequency
Ceratozetes n. sp.	10.76	56.94
Zygoribatula sp.nr. floridana	16.09	54.72
Rostrozetes ovulum	15.32	43.89
Complex Oppiidae sensu Norton 2000	24.21	41.11
Scheloribates sp.	7.35	36.39
Epilohmannia minuta	7.52	31.67
Unknown	2.96	29.72
Tectocepheus velatus	2.28	22.78
Eupelops sp.	2.38	21.11
Tectoribates sp.	4.44	20.28
Galumna minuta	3.88	20.00
Lamellobates sp.	4.22	18.89
Galumna jacoti	1.16	10.56
Oribatella sp.	1.65	4.44
Rhysotritia ardua	0.45	2.22
Nesiacarus sp.	1.34	1.39
Lohmannia sp. nr. jornoti	0.11	1.11
Peloribates sp. nr. hirsutus	0.29	0.28
Trichoribates sp.	0.14	0.28

Table 9. Annual dominance and frequency for mites from the HTU in 2000.

Mite Genus	Percent Dominance	Mean Monthly Frequency
Zygoribatula sp.nr. floridana	19.40	45.28
Ceratozetes n. sp.	10.63	45.28
Rostrozetes ovulum	16.22	34.44
Complex Oppiidae sensu Norton 2000	21.19	29.72
Scheloribates sp.	8.40	28.61
Epilohmannia minuta	15.40	22.78
Unknown	6.05	20.83
Eupelops sp.	2.44	19.17
Tectoribates sp.	6.21	14.72
Galumna minuta	2.57	13.89
Tectocepheus velatus	1.05	12.78
Galumna jacoti	1.50	10.28
Lamellobates sp.	1.76	10.00
<i>Oribatella</i> sp.	2.23	5.00
Nesiacarus sp.	0.42	1.67
Rhysotritia ardua	0.54	1.39
Trichoribates sp.	0.14	0.28
Protoribates capucinus	0.14	0.28
Lohmannia sp. nr. jornoti	0.62	0.28

Species	Index of Aggregation	\mathbf{R}^2
Nesiacarus sp.	2.728	0.976
Galumna jacoti	2.584	0.835
Galumna minuta	2.461	0.839
Tectoribates sp.	2.453	0.923
Lohmannia sp. nr. jornoti	2.443	0.828
Complex Oppiidae sensu Norton 2000	2.410	0.929
Tectocepheus velatus	2.391	0.817
Oribatella sp.	2.352	0.652
Eupelops sp.	2.324	0.884
Epilohmannia minuta	2.277	0.884
Zygoribatula sp. nr. floridana	2.258	0.866
Scheloribates sp.	2.227	0.934
<i>Ceratozetes</i> n. sp.	2.203	0.925
Rostrozetes ovulum	2.136	0.949
Protoribates capucinus	*	
Lamellobates sp.	***	
Peloribates sp. nr. hirsutus	**	
Rhysotritia ardua	***	
Trichoribates sp.	***	

Table 10. Index of Aggregation and R^2 for each mite species.

Note: * indicates non significant regression (P>0.05); ** indicates invalid results due to lack of normality; *** indicates inadequate sample size

		A. perfoliata ingestion or
Mite Taxa	Number	development
Eupelops sp.	3	No
Galumna spp.	5	No
Epilohmannia minuta	9	Yes
Lohmannia sp. nr. jornoti	1	No
<i>Oribatella</i> sp.	2	No
Rostrozetes ovulum	11	No
Scheloribates spp.	8	No
Zygoribatula sp. nr. floridana	6	No
Unidentified	4	No
Total	49	

Table 11. Results of mite dissections 4 weeks post-exposure to A. perfoliata eggs.

Table 12. Results of mite dissections 5 to 20 weeks post-exposure to *A. perfoliata* eggs.

		A. perfoliata ingestion or
Mite Taxa	Number	development
Epilohmannia minuta	8	No
Galumna spp.	3	No
Lohmannia sp. nr. jornoti	1	No
Complex Oppiidae sensu Norton 2000	13	No
Rostrozetes ovulum	20	No
Scheloribates spp.	13	No
Zygoribatula sp. nr. floridana	3	No
Total	61	



Figure 12. *Epilohmannia minuta* Berlese (= *Epilohmannia pallida* Wallwork) oncospheres recovered from body cavity (photos). A) Two oncospheres next to remains of the mite; b) Higher magnification of one of the oncospheres.

Таха	Exposed	Not Exposed
Galumna jacoti	6	10
Galumna minuta	2	30
Epilohmannia minuta	0	10
Eupelops sp.	1	2
<i>Lamellobates</i> sp.	2	13
Oppiidae	28	98
Oribatella sp.	1	0
Rostrozetes ovulum	11	119
Rhysotritia ardua	0	2
Scheloribates spp. + Ceratozetes n. sp.	62	113
Tectocepheus velatus	2	4
Tectoribates sp.	0	24
Unknown	11	11
Zygoribatula sp. nr. floridana	4	13
Total	130	469

Table 13. Oribatid Mites Collected from Study Pastures (exposed mites offered A. perfoliata eggs in laboratory on 3/23/00).

CONCLUSIONS

From the information gathered during this study one can conclude that the transmission of *A. perfoliata* is theoretically continuous because there are oribatid mites available as intermediate hosts of *A. perfoliata* on horse pastures of north central Florida during all months of the year. Due to this conclusion it is recommended that treatment with an appropriate cestocidal drug would be prudent at regular intervals throughout the year. Taking into account the parasite's prepatent period, which is between 6 and 16 weeks, the intervals should be no greater than every 2 to 4 months.

Many of the genera identified in this study are known intermediate hosts for taxa within Anoplocephalidae or more specifically for *A. perfoliata*. The number of oribatid mites available was not affected by the farm location within north central Florida, the month of sampling, or the year of sampling during this two-year study. Mite numbers did not correlate significantly with weather data such as water budget, mean monthly temperature, ambient air temperature on the day of collection, or soil temperature on the day of collection. Although a negative linear correlation of statistical significance was found between monthly mite counts and total monthly rainfall, the fitted regression model could explain only 9.9% of the variation in the mite counts.

We were able to conclude that the majority of mites found during this study did show a high degree of clumping on the pastures studied by calculating the index of aggregation using Taylor's power law. This reinforces the knowledge that this clumping is characteristic of most oribatid mite populations.

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BIOGRAPHICAL SKETCH

Merijo Jordan was born and raised in Paris, Illinois, a small farming community. There she found pleasure in rescuing various cats, bringing them home to raise kittens, and riding her horse Missy along the grass water ways of the corn fields.

Merijo attended the University of Illinois for seven years. During this time, she studied animal sciences as an undergraduate and then veterinary medicine. With her doctor of veterinary medicine degree in hand, she and her Welsh Corgi moved to Dunlap, Illinois, to begin practicing veterinary medicine. Although practice was very fulfilling a decision was made to move south to attend the University of Florida. While completing her master's degree in parasitology she met her husband, Patrick Meeus. Now living in Micanopy, Florida, they have a small farm with three dogs, a cat, eight chickens, two goats, and a thoroughbred horse. She is currently employed as a veterinary professional services manager by Merial.