A Literature Review of Equine Piroplasmosis

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Introduction

Equine piroplasmosis (EP), also referred to as babesiosis, is a disease of equids including horses, donkeys, mules, and zebras. EP is caused by the blood-borne protozoan parasites *Babesia caballi* or *Babesia equi*. These parasites are naturally transmitted from host to host via tick vectors, and dual infection with both organisms has been reported in equids.

In 1998, Mehlorn and Schein proposed a reclassification of *B. equi* to *Theileria equi.* A second group of researchers have proposed that *B. equi* be classified as *Babesia equi* (incertae sedis) because based on genomic analysis it belongs in a lineage between *Babesia* and *Theileria* (D. Knowles, pers. comm., 2010). For the remainder of this report the nomenclature of *B. equi* (incertae sedis) will be shortened to *B. equi*.[1]

EP is endemic in equids in many tropical and subtropical regions, including Africa, the Middle East, Asia, Central and South America, the Caribbean (including Puerto Rico), and Europe. The United States, Canada, Australia, Japan, England, Iceland, and Ireland are not considered endemic areas.

The United States was considered free of EP in 1988 as a result of a joint USDA-Animal and Plant Health Inspection Services (APHIS) and State of Florida eradication program for *B. caballi* that began in 1962 (USDA, 2008).

EP is reportable to the World Organization for Animal Health (OIE) [OIE, 2009a]. The OIE publishes EP status information submitted by member countries, but does not specifically recognize countries as free of EP (OIE, 2009b). Movement requirements for equids usually depend on the perceived risk of disease introduction from importation from a given country by the receiving country. Importing countries may impose their own requirements for pre-import isolation and/or testing of equids from other countries, regardless of the exporting country's self-reported disease status. Similarly, each State in the United States has the authority to impose requirements for equids entering its State. State import regulations are available through the USDA (2010) or by contacting the State Veterinarian for the State of destination (USAHA, 2010).

APHIS regulations pertaining to the importation of animals and animal products into the United States are set forth in 9 CFR Parts 92-99 (USDA, 2009a). Horses are defined in these regulations as horses, asses, mules, and zebras (9 CFR 93.300). Horses for importation are required to undergo quarantine at an APHIS-approved quarantine facility in the United States, or, in the case of Mexican horses, a Mexican facility (9 CFR 93.308 and 93.324). The minimum quarantine duration varies depending on the animal health status of the region of origin of the horse. Conditions for release from quarantine include the absence of evidence of communicable disease, as well as a negative official test for EP. The official test for EP (*B. caballi* or *B. equi*) for importing equids into the United States is the cELISA, as specified by VS Memorandum 291.58 (USDA, 2005). Horses entering the United States from Canada and Iceland are not required to be tested for EP.

In addition, horses for importation must be accompanied by a certificate of a salaried veterinary officer of the national government of the country of origin. The certificate must verify that no cases of EP occurred on the premises of origin or adjoining premises in the 60 days preceding export (9 CFR 93.314). In addition, except for horses from Canada, horses for importation must be certified as having been inspected and found to be free from ectoparasites (USDA, 2009b, 2009c, 2009d). In the United States, EP clinical cases have recently been detected in Florida (August 2008), Missouri/Kansas (June 2009), and Texas (October 2009). Epidemiological investigations of the Florida and Missouri/Kansas outbreaks

[1] Within the historical section the terminology used for EP pathogens and ticks was retained as referred to in the original cited reference.

suggest transmission was by iatrogenic means via needle sharing or blood exchange, not natural transmission via tick vectors.

As of November 2009, New Mexico has required that all horses entering New Mexico racetracks test negative for *B. equi,* and, as of March 8, 2010, a joint surveillance program conducted by the New Mexico Racing Commission and the New Mexico State Veterinarian has identified 16 infected horses. The Texas outbreak appears to be unrelated to those in Florida, Missouri/Kansas, and New Mexico. The source of the Texas outbreak was undetermined as of March 8, 2010, but transmission appears to have occurred on the index ranch over a period of at least 20 years via *Amblyomma cajennense* ticks and possibly *Dermacentor variabilis*. Updates for the investigations are available from OIE (2010).

As of February 2009, the Florida 2008 outbreak was resolved via a thorough investigation, including traceouts, testing, and removal of all infected horses from Florida premises. For the Missouri/Kansas outbreak, five horses infected with EP were euthanized; one infected horse was removed from a premises in Kansas prior to quarantine being initiated; and two infected horses were illegally removed from quarantine in Missouri. The 16 horses identified as infected through New Mexico surveillance have been euthanized or quarantined. The Texas investigation is ongoing, and the index ranch and any premises with horses traced to the index ranch have been quarantined.

The costs of responding to an EP outbreak vary and depend on the extent of response necessary, as well as movement and testing requirements. The joint APHIS–VS and Florida *B. caballi* eradication program initiated in 1962 took 25 years to be considered successful. This effort incurred direct costs of $12 million for tick inspections, testing, treatment of infected horses, and movement controls (USDA, 2008). When Florida experienced a less extensive EP outbreak due to *B. equi* in 2008,State personnel spent over 4,000 hours making 420 contacts during the investigation. This cost the State an estimated $150,000 (Short, pers. comm., 2009). The impact of the 2008 EP outbreak on the Florida horse industry is more difficult to estimate, but it may have cost the State's Thoroughbred breeding industry millions of dollars in lost sales and future earnings due to a Canadian ban on importation of horses from Florida during 2008 (Steffanus, 2008). Costs of subsequent outbreaks in other States have yet to be determined.

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Terms Used in This Report

Abiotic—Not associated with or derived from living things. Describes the physical and chemical aspects of an organism's environment such as light, temperature, moisture, and atmospheric gases.

Active stage for tick—Stage in which ticks are seeking hosts, feeding, and mating.

Biotic—Relating to, produced by, or caused by living organisms.

Dormant stage for tick—Inactive nymphal stage, from fall through following spring.

EC50—The concentration of half maximal effective concentration. Refers to concentration of a drug which induces a response halfway between the baseline and maximum after some specified exposure time. Commonly used as a measure of a drug's potency.

Generalist—Generalist species thrive in a wide variety of environmental conditions, making use of many different resources.

Generation time—Time taken for a tick to complete its life cycle from egg to adult.

Immature tick—Larval and nymphal stages of ticks. Ticks are unable to reproduce during these stages.

LD50—An index ot toxicity (lethal dose 50 percent). The amount of the substance that kills 50 percent of the test population of experimental animals when administered as a single dose.

Premunity—(a) Resistance to a disease due to the existence of its causative agent in a state of physiological equilibrium in the host; (b) immunity to a particular infection due to previous presence of the causative agent.

Questing—The act of a tick searching for a host. For example, when a tick climbs to the top of vegetation and slowly waves its forelegs while waiting for a passing host.

Current Situation—Prevalence and Geographic Distribution of EP

Review articles and studies can reveal general trends in EP prevalence and distribution, but regional differences must be cautiously interpreted with consideration of study design, sample size, and the tests used to determine infection status. No recent comprehensive data on the worldwide prevalence and geographic distribution of *B. caballi* and *B. equi* are available.

In general, the geographic distributions of *B. caballi* and *B. equi* are similar and include most of the world's tropical and subtropical regions (Brüning, 1996; Friedhoff et al., 1990). The distribution of *B. caballi* extends more often to northern latitudes than *B. equi* (Friedhoff et al., 1990; Friedhoff and Soulé, 1996). Both species of *Babesia* are endemic to many parts of Africa, Europe, the Middle East, and Asia (Brüning, 1996). In most regions of the world where EP is endemic, *B. equi* infections are more prevalent than *B. caballi* infections (Schein, 1988; Friedhoff et al., 1990; de Waal, 1992; Brüning, 1996; Friedhoff and Soulé, 1996; Heuchert et al., 1999; Kerber et al., 1999; Rothschild and Knowles, 2007).

Prior to the 2008 Florida outbreak (OIE, 2009b, 2009c, 2009d), the most recent case of EP on the United States mainland also occurred in Florida in 1978 (OIE, 2009b; USDA, 2009). Other equids infected with EP agents have been detected sporadically in the United States. Typically these cases have occurred in Florida in clinically normal horses with a history of importation into the United States. EP is present in Puerto Rico and the U.S. Virgin Islands (USDA, 2009). Canada is widely considered to be free of EP, although it has been detected sporadically, most recently in 1987 (AVMA, 2006; Center for Food Security and Public Health, 2008; CVO Australia, 2008; USDA, 2008; OIE, 2009a, 2009e). As of March 2009, the EP status of Mexico was listed by the OIE as confirmed infection but no clinical disease (OIE, 2009a).

Few data are available regarding the distribution of *B. caballi* and *B. equi* in Caribbean nations. In one recent study, 83 percent of 93 Thoroughbreds in Trinidad tested seropositive for *B. caballi, B. equi,* or both (Asgarali et al., 2007). The seroprevalence of *B. caballi* in this study was substantially higher than that of *B. equi* (69 and 33 percent, respectively).

EP is endemic to most of Central and South America, except in southern regions of Chile and Argentina (Friedhoff and Soulé, 1996). In several studies, large proportions of horses in one or more regions of Colombia, Brazil, and Chile tested positive for *B. caballi, B. equi,* or both, as determined by serological or nucleic acid detection methods. In several South American surveys, seroprevalence was higher for *B. equi* than for *B. caballi* (Schein, 1988; Tenter et al., 1988; Pfeifer Barbosa et al., 1995; Heuchert et al., 1999; Kerber et al., 1999; Xuan et al., 2001; Battsetseg et al., 2002; Heim et al., 2007).

EP is endemic to most regions of Africa (Schein, 1988; Friedhoff and Soulé, 1996). The majority of clinical EP cases in Africa are due to *B. equi* infection (Motloang et al., 2008). In one serological study, 80 percent of 6,350 serum samples from various regions of South Africa were positive for *B. equi,* and approximately 50 percent were positive for *B. caballi* (Zweygarth et al., 2002b). Both *B. caballi* and *B. equi* were detected in zebras from two national parks in South Africa by serological and culture methods (Zweygarth et al., 2002a).

B. caballi and *B. equi* are endemic to southern Europe, including Portugal, Spain, France, Belgium, Italy, and the Balkan Peninsula (Schein, 1988; de Waal, 1992; Rothschild and Knowles, 2007). The distribution of *B. caballi* and *B. equi* extends east to Hungary, Romania, and to the southern and western regions of the Commonwealth of Independent States (Schein, 1988; Friedhoff and Soulé, 1996). Most infections that have occurred in regions of Europe where *B. caballi* and *B. equi* are not endemic have been traced back to Spain, France, Italy, or the Commonwealth of Independent States (Rothschild and Knowles, 2007). Across the endemic regions of Europe, *B. caballi* and *B. equi* seroprevalence in equids is highly variable, ranging from about 8 to 35 percent for *B. caballi* and from 20 to 40 percent for *B. equi* (Camacho et al., 2005; Hornok et al., 2007; Acici et al., 2008; Sevinc et al., 2008).

Equine infections with *B. caballi* and *B. equi* are also prevalent in the Middle East and Asia (Donnelly et al., 1980; Schein, 1988; Friedhoff and Soulé, 1996; Rothschild and Knowles, 2007), and both *B. caballi* and *B. equi* are widespread in China and Mongolia (Friedhoff and Soulé, 1996; Xuan et al., 2002; Xu et al., 2003; Boldbaatar et al., 2005; Rothschild and Knowles, 2007). Japan has never reported an occurrence of EP to the OIE and is widely considered to be free of the disease (Friedhoff and Soulé, 1996; Ikadai et al., 2002; Rothschild and Knowles, 2007; OIE, 2009b). However, a recent seroepidemiologic study of more than 2,000 banked horse-serum samples collected for EIA surveillance from 1971 to 1973, found that 5 percent of the samples tested positive for *B. caballi,* and 2 percent were positive for *B. equi,* as determined by ELISA. This finding suggests that *B. caballi* and *B. equi* infections have occurred in Japan (Ikadai et al., 2002).

Neither *B. caballi* nor *B. equi* has become established in Australia or New Zealand (de Waal, 1992; Brüning, 1996; Friedhoff and Soulé, 1996; Martin, 1999; AVMA, 2006; Rothschild and Knowles, 2007; Center for Food Security and Public Health, 2008; CVO Australia, 2008; USDA, 2008a). Sporadic occurrences in Australia in the 1950s, 1960s, and 1970s were attributed to introduction by imported horses (CVO Australia, 2008). According to OIE data, EP occurred in Australia most recently in 1976. New Zealand has never reported the occurrence of EP to the OIE (OIE, 2009e, 2009f).

References—Prevalence and Geographic Distribution of EP

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Historical Perspective

Note: For this section, names of ticks and causative agents used in original reports are maintained.

History of EP organisms' discovery and early literature

Before 1901, EP was not recognized as a distinct disease and was often confused with other diseases. EP has been called anthrax fever, bilious fever or bilious form of horse sickness, biliary fever, and equine malaria (Roberts et al., 1962). EP was referred to as equine malaria because the clinical signs of the hemoparasitic infection observed in equids in Pretoria, South Africa, were similar to malaria infection (plasmodiidae) found in humans (Theiler, 1903). Based upon morphology, the hemoparasite was classified with other pear-shaped protozoa known as piroplasms, and was named *Piroplasma equi* (Laveran, 1901).

In the early 20th century, South African veterinarian Sir Arnold Theiler unsuccessfully attempted to transmit the disease agent from affected horses to unaffected horses via blood transfusion. Theiler speculated that he was unable to create the disease because native horses were already immune to it or, perhaps because the disease agent required a tick vector to be infective (Theiler, 1902). Theiler also reported that the disease did not appear to be directly contagious and that it was recognized most often in horses imported to South Africa from New Zealand, England, Australia, and the Argentine Republic. Although it did not appear that the disease was spread by direct contact, Theiler indicated that epizootics (epidemics) of equine malaria could occur when large numbers of susceptible animals were present. In addition, the disease was recognized more frequently in summer, especially during the rainy season. Theiler also indicated they experience more than one episode of clinical disease. In 1912, Nuttall and Strickland (1912) discovered that EP could be caused by two different agents, *B. equi* and *B. caballi*.

When Giemsa's, Leischemann's, or Wright's stains are used, *B. caballi* is characterized by two pear-shaped, basophilic-staining bodies approximately 4 µ in length in a single red blood cell (Retief, 1964). In a blood smear, *B. caballi* is much larger than *B. equi.* The intraerythrocytic form of *B. equi* may appear as a Maltese cross formation, ameboid, or signet-ring shaped with a clear halo and about 2 µ in length (Retief, 1964). During the Florida *B. caballi* outbreak in the early 1960s, blood samples obtained from jugular veins and from ear notches of 136 horses were compared because some investigators thought that blood obtained from a tissue sample (ear notch), and thus from capillaries, was more likely to contain the parasites. Of the 136 samples tested, 17 had *B. caballi* in red blood cells. In no instance did samples taken from ear notches yield a superior ability to detect the organism than samples taken from jugular veins (Sippel et al., 1962).

Sippel and others (1962) described a technique for concentrating affected erythrocytes, which entailed mixing equal parts of 2-percent sodium citrate with the blood to be tested followed by centrifuging at 500 to 700 rpm for 3 to 5 min. The supernatant fluid was then decanted and recentrifuged at 1,500 to 2,000 rpm for 15 to 20 min. The supernatant fluid was discarded and smears were prepared from the sediment. The infected cells were lighter in weight than normal cells, enabling the method described above to concentrate the infected erythrocytes.

After the initial discovery of the causative agents for EP, it was confirmed that the disease was transmitted by ticks. Although many species of ticks reportedly transmitted the disease agent in South Africa, red ticks (*Rhipicephalus evertsi)* were the main vectors. Most horses in South Africa that were not stabled were carriers of the disease agent but seldom developed signs of disease because they were immune. In fact, only one clinical case of babesiosis was found during a 3-year period, in a horse on the veldt; however, 50 cases were found in stabled Thoroughbreds that had been de-ticked regularly (Retief, 1964). This report suggests that the de-ticked horses were susceptible to repeat infections as they did not develop a lasting immunity to the disease agent(s).

Premunity to EP in horses depends on the presence of the parasite in the body and lasts 6 months to 1 year. If, however, an immune horse is bitten by another infected tick during this period, a further period of premunity is conferred, which may explain why horses living in the veldt in South Africa become lifelong premune carriers. It has also been reported that foals in endemic areas are generally immune for the first year of life; presumably resistance is acquired through colostral transfer. If a foal is exposed to infected ticks while still immune from passive acquired antibodies, it acquires active immunity (Retief, 1964).

History of EP in the United States

The first reported infections of *B. caballi* and *B. equi* in U.S. equids occurred in 1961 and 1964, respectively. In August 1961, a private veterinary practitioner examined a horse in Dade County, Florida, with clinical signs consistent with equine infectious anemia (EIA) [Strickland and Gerrish, 1964]. Blood from the horse was submitted to a private laboratory, and intraerythrocytic organisms tentatively diagnosed as *B. caballi were* found. This diagnosis was confirmed at the USDA Animal Disease and Parasitological Research Laboratory in Beltsville, Maryland, and at the National Animal Disease Laboratory in Ames, Iowa. The mode and time of introduction of *B. caballi* into the United States was reported as unknown by one author (Knowles et al., 1966) and speculated by another to be the result of the importation in 1959 of 50 Cuban Walking Horses into Davie, Florida in 1959 (Sippel et al., 1962; Stiller and Coan, 1995). These Cuban Walking Horses were then sold to local individuals as privately owned mounts and were placed in various boarding facilities in the Miami-Fort Lauderdale area. Knowles speculated that EP might have gone undiagnosed for years because it was confused with EIA (Knowles et al., 1966). Initially, the only means for diagnosis were clinical signs consistent with the disease and the detection of the parasite in erythrocytes, using specific staining techniques and confirmation through animal inoculation studies (Roberts et al., 1962).

In Florida, from 1961 through September 1963, 141 cases of EP were detected. Infections in horses were diagnosed on 103 Florida premises, based on identification of *B. caballi* via blood smears. The majority of these cases were found in Broward and Dade Counties, which contained large populations of the tropical horse tick *Dermacentor nitens* (Strickland and Gerrish, 1964). These counties also had large equine populations, particularly pleasure horses kept at pasture where tick exposure was likely. Veterinarians in the counties had access to high-quality diagnostic facilities, and the system for reporting cases was very good (Strickland and Gerrish, 1964). However, it is likely that the number of cases was higher than reported because some infected animals were not tested due to lack of clinical signs following resolution of acute parasitemia, and some owners were reluctant to report sick horses due to fear of the regulatory response, which included quarantine. In 1964, a mixed infection of *B. caballi* and *B. equi* was found in the blood of a Florida horse (Ristic et al., 1964), and in 1965 a pure infection of *B. equi* was discovered in an equid in the United States in Florida (Knowles et al., 1966).

By 1969, four *B. equi* infections had been diagnosed in the United States—three in Florida and one in New Jersey. The New Jersey horse had developed signs of EP after importation from Europe. Experiments indicated that the ticks detected on horses in Florida as of 1969 were not transmitting *B. equi* (Taylor et al., 1969). By 1969, EP cases had been identified in five States (Taylor et al., 1969). All *B. caballi* cases outside Florida occurred in horses that had come from Florida, Puerto Rico, or the U.S. Virgin Islands (Taylor et al., 1969; Coffman, 1997). Anemia, fever, and edema were the most commonly reported clinical signs of EP due to *B. caballi* infection. Taylor and others (1969) suggested several reasons for the large number of cases of EP in 1968: the advent of the complement fixation test (CFT) to diagnose the infection; the fact that practicing veterinarians were becoming more aware of the disease and were pursuing a diagnosis; increased tracing by State and Federal animal health officials; and mild weather with greater than average spring rainfall in Florida, which likely contributed to the tick population. It should be noted that the number of cases reported by Taylor differ from the number reported by Coffman. Taylor's source for the number of cases was unpublished data from R.C. Knowles, Chief Staff Veterinarian, Equine Diseases, USDA–ARS, Animal Health Division, Hyattsville, Maryland. Coffman did not indicate the source of the data he reported.

EP cases from 1962 through 1971, as reported by Taylor et al. (1969) and Coffman (1997)			
Year	State	Number of Horses with *B. caballi* (Taylor et al., 1969)	Number of Horses with EP Infection* (Coffman, 1997)
1962	Florida	127	56
	Georgia	4	4
1963	Florida	14	94
1964	Florida	4	4
1965	Florida	4	31
1966	Florida	7	30
1967	Florida	4	66
1968	Florida	175	301
	New Jersey	2	2
	North Carolina	2	2
	Mississippi	1	1
1969	Arkansas	2	1
	Florida	37 (through May)	146
	New Jersey	4 (through May)	4
	Tennessee	26 (through May)	26
1970	Florida	Not reported	160
1971	Florida	Not reported	243

*Report agent not specified in original.

Dermacentor nitens, the tropical horse tick, was the only type of tick identified in *B. caballi* transmission in Florida in the 1960s (Retief, 1964; Knowles et al., 1966). This tick was first described by Neumann in 1897 from collections taken in Jamaica and Santo Domingo (Strickland and Gerrish, 1964). In 1901, the tick was identified in Guatemala, Venezuela, and Puerto Rico. Since then, *D. nitens* has been reported in most Caribbean countries, Central America, Mexico, part of South America, Florida, and several counties in south Texas. The tick was first reported in the United States around 1908 in Cameron County in southern Texas. *D. nitens* has specific temperature and humidity requirements which restricted its geographic distribution. *D.nitens* has been found in the ears of equids as well as the nasal diverticulum, mane, perineum, and along the ventral midline.

In one report, a case fatality rate of about 10 percent was reported among 52 horses in the United States diagnosed with *B. caballi* infection (Sippel et al., 1962). Taylor speculated that some of the fatalities attributed to EP in these reports may have been the result of a concurrent EIA infection. Of the four

horses diagnosed with *B. equi* infection in the United States in the 1960s, two showed signs of disease and none died from the infection (Taylor et al., 1969).

In 1962 a joint equine piroplasmosis outbreak control task force was formed, which operated until 1978 (Coffman, 1997). In 1962 Florida implemented a regulation that designated EP as a reportable disease and gave the Florida Department of State authority to carry out the objectives of the task force. The Florida EP Control Program involved horse testing, tick surveillance, quarantine of EP-positive horses and the premises on which positive horses or ticks were found, treatment of both clinical cases and inapparent carriers, and treatment of tick-infested horses and premises. EP-positive horses were identified with a lip tattoo or brand that included a *P* and an assigned number (Florida Department of State, 1962). The initial U.S. outbreak was reportedly brought under control in 1971 (Coffman, 1997). From 1962 to 1971, 1,150 EP-positive horses were identified in Florida, and 40 EP-positive horses were found in other States (Coffman, 1997). All EP cases found in States other than Florida were traced to horses originating in Florida, Puerto Rico, or the U.S. Virgin Islands.

The initial U.S. outbreak was reportedly brought under control in 1971 (Coffman, 1997). EP due to *B. caballi* was brought under control by eliminating *D. nitens* via pesticides and by administering babesiacidal drugs to known positive horses identified by CFT. The enzootic area included Florida's Dade, Broward, and Palm Beach Counties. Bryant and others (1969) reported that numbered hoof brands were used to identify EP-positive horses. Brands were renewed every 3 to 4 months.

In an effort to protect the race horse population from EP, the Florida Racing Commission required that: (1) horses arriving in Florida for racing season be inspected for ticks upon entry into the race tracks; (2) race horses be lodged at race tracks or approved stables (where vector control was enforced); and (3) hay and bedding used at the tracks in Florida could not originate from premises in the counties where EP had been diagnosed (Knowles et al., 1966).

Florida continued to operate a tick surveillance and treatment program until 1988 (Coffman, 1997). From 1974 to 1984, only 15 new cases of EP were found in Florida. In 1984, 293 Paso Fino horses at 19 different locations in the State were tested under court order. Of the 293, 35 (12 percent) tested EP positive.[2] All but one of the positive horses had been legally imported in 1983 or 1984 from Puerto Rico and South America. All of the EP-positive horses were either exported or treated until they tested negative (Coffman, 1997).

The 1994 Florida State Administrative Code required all horses from EP endemic areas to test negative for both *B. caballi* and *B. equi* prior to shipment to Florida and be quarantined and retested between 30 and 60 days after arrival in Florida. After the requirement to retest was implemented, EP-positive[2] horses were found, usually at low titers on the CFT. These horses were required to remain under quarantine and were treated at the owner's expense or exported (Coffman, 1997). In addition, horses residing on premises where an EP-positive horse had been identified were inspected for ticks by State personnel; if ticks were found, the owner was required to treat all animals and the premises for ticks in accordance with guidelines established by the Department of Agriculture in Florida. The estimated cost for controlling the EP outbreak that began in the 1960s in Florida was $7 to 8 million, although no details were provided as to how the estimate was made (Coffman, 1997).

History of U.S. import testing related to EP

Import testing of equids for the presence of antibodies to EP agents using the CFT began in October 1970. In 1979, an APHIS proposed rule (Federal Register, 1979) called for amending regulations to eliminate the requirement that horses must have a negative EP test before being imported into the United States. The rule change suggested that the procedure was no longer necessary and its elimination would reduce unnecessary time and expense associated with the importation of horses. The proposed rule also stated that based on a review: "(1) the tick vectors of this disease are severely limited in number and

[2] The parasite (*B. equi* or *B. caballi*) was not specified in the cited report.

distribution in this country; (2) the potential for disease dissemination without the availability of vectors is markedly lower than with other forms of communicable diseases; (3) many animals with positive test reactions do not show symptoms of the disease; (4) many horses that otherwise meet the import requirements are denied entry because they carry antibodies; (5) the presence of antibodies does not always mean there is infection in animals; and (6) the disease has not been shown to be a significant economic threat to the United States, nor has it been considered a significant economic threat to other countries."

Based on this proposed rule the Infectious Diseases of Horses Committee of the U.S. Animal Health Association stated in their 1979 proceedings, "The Committee wishes to iterate its strong urging that this import requirement be continued." The committee also addressed the USDA procedure for importation and quarantine of horses from Mexico that were consigned to slaughter; the Committee indicated, "it felt that the controls over these imported animals before going to the final destination was not adequate and the Committee opposes any changes in rules relating to the identification and final disposition of these animals."

A notice of withdrawal of the proposed rule from APHIS was posted and effective in September 1980 (Federal Register, 1980). This notice reported that an EP panel held public meetings to solicit public comment, especially from experts that had personal experience with EP. The panel recommended that, based on the evidence received, the EP test requirement should not be eliminated. Ten comments were received during a 60-day comment period. The majority of comments supported maintaining the testing requirement. Once completed, the review by the EP panel indicated that the CFR Part 92.11(d) and 92.34 (c) would not be amended at that time. The CFT was used as the official entry test for imported horses until August 2005 when the competitive enzyme-linked assay (cELISA) was implemented as the official test (USDA, 2009).

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Etiology of EP Agents: Life Cycle and Transmission

B. caballi and *B. equi* are protozoan organisms and are obligate intracellular parasites of blood cells (Schein, 1988; Friedhoff and Soulé, 1996). Equids are natural hosts of *B. caballi* and *B. equi*, and ixodid ticks are natural vectors (Uilenberg, 1995; Friedhoff and Soulé, 1996; Rothschild and Knowles, 2007). *B. caballi* and *B. equi* parasites have been detected in dogs; however, the epidemiologic significance of these detections is unknown (Criado-Fornelio et al., 2003; Beck et al., 2009). The lifecycles of *B. caballi* and *B. equi* are similar and include developmental stages in both the equine host and the vector tick. The life cycles of both parasites include sporozoites (an asexual infective stage), merozoites (an asexual blood stage), and gametocytes (a sexual blood stage).

Ticks are biologic vectors and become infected through ingestion of gametocyte-containing erythrocytes (red blood cells) while taking a blood meal from an infected host (Uilenberg, 2006). In the ticks' digestive tracts, the gametocytes develop into gametes, which fuse to form zygotes (Mehlhorn and Schein, 1998; Ueti et al., 2003; Uilenberg, 2006). *B. caballi* zygotes multiply and invade numerous tissues and organs of the ticks, including the ovaries, but not, initially, the salivary glands. *B. caballi* infection can be passed transovarially to the next tick generation, and development to an infective stage for equids occurs in the salivary glands of immature and adult ticks (Uilenberg, 2006; Rothschild and Knowles, 2007). In contrast, *B. equi* zygotes develop into kinetes, which invade the ticks' hemolymph and salivary gland cells (Mehlhorn and Schein, 1998; Ueti et al., 2003; Uilenberg, 2006; Rothschild and Knowles, 2007). Further development to a stage of *B. equi* infective for equids is generally complete within 6 to 24 days after the ticks are infected. The transovarial transmission of *B. caballi* within *Anocentor nitens* is epidemiologically significant in that such ticks are an additional reservoir for transmission, in addition to the persistently infected horse (Rothschild and Knowles, 2007).

An equid can be infected when tick saliva containing infective stages of the parasites is injected into the equid while a tick is feeding (Uilenberg, 2006; Rothschild and Knowles, 2007). *B. caballi* development in the equid host does not include a lymphocyte stage (Friedhoff and Soulé, 1996; Uilenberg, 2006; Rothschild and Knowles, 2007). Although two reports suggested that the initial development of *B. equi* in the equid host occurs in the lymphocytes (white blood cells) [Mehlhorn and Schein, 1998; Uilenberg, 2006] based on in vitro studies another research group has failed to find a lymphocyte phase of *B. equi* infection in vivo in the horse (Knowles, pers. comm., 2010). Importantly, lymphocyte transformation is not a component of *B. equi* infection as is the case in bovine theilerosis, thus suggesting *B. equi* does not meet one of the criteria for reclassification as *Theileria equi* (D. Knowles, pers. comm., 2010). Further development and asexual reproduction occurs in erythrocytes (Friedhoff and Soulé, 1996; Uilenberg, 2006).

Iatrogenic transmission of the parasites can occur via the reuse of needles, syringes, and through blood transfusions from untested, infected donor horses. Sharing equipment, such as dental or tattoo equipment, that may be contaminated with blood is also a potential pathway for transmission.

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Pathogenesis and Clinical Findings of EP

EP should be included in the differential diagnosis for equids with anemia, jaundice, fever, and signs of chronic inflammatory disease. However, clinical signs are variable and nonspecific, and EP cannot be diagnosed based on clinical signs alone (Rothschild and Knowles, 2007; Center for Food Security and Public Health, 2008). *B. caballi and B. equi* infections cannot be distinguished clinically, but differentiation between the two infections may be important for successful treatment and control (Rothschild and Knowles, 2007).

The incubation period, or time from infection to manifestation of clinical signs, ranges from 10 to 30 days for *B. caballi* and 12 to 19 days for *B. equi* (de Waal, 1992). Infection can result in a variety of clinical signs. The course of the disease may be peracute, acute, subacute, or chronic (Rothschild and Knowles, 2007). Peracute and acute signs may include fever, jaundice, anemia, hemoglobinuria, bilirubinuria, digestive or respiratory signs, and occasionally death. Equids with subacute piroplasmosis may display anorexia, lethargy, weight loss, anemia, limb edema (swelling), poor performance, increased heart and respiratory rates, and splenomegaly. Chronic piroplasmosis is clinically indistinguishable from other chronic inflammatory diseases and generally presents with nonspecific signs, such as inappetence, poor body condition, and poor performance. Anemia may be minimal or absent in equids with chronic or persistent infection; these animals are termed carriers and are reservoirs for tick and iatrogenic transmission (Rothschild and Knowles, 2007).

The majority of horses seropositive for *B. equi* or *B. caballi* are carriers which, by definition, are persistently infected with EP pathogens but show no clinical signs and serve as a source of infection for other equids throughout their lives (Rothschild and Knowles, 2007). Carrier mares can transmit *B. equi* to their offspring, resulting in abortions, stillbirths, neonatal disease or death, or carrier offspring (de Waal, 1992; Lewis et al., 1999; Phipps and Otter, 2004; Allsopp et al., 2007; Rothschild and Knowles, 2007). Persistent infection of mares by *B. equi* has been reported to be a very common cause of equine abortion in endemic regions; in a study of Thoroughbred mares in South Africa up to 11 percent of abortions were attributed to *B. equi* infections (de Waal, 1992; Lewis et al., 1999). In addition to the negative effects of *B. equi* infections on equine reproduction, persistent infection with EP pathogens can also adversely affect performance. Among equine athletes, seropositive horses may have decreased performance compared with seronegative horses and may be at risk for developing overt clinical disease or even sudden death (Hailat et al., 1997; Rhalem et al., 2001; Rothschild and Knowles, 2007).

Mortality due to EP pathogen infection ranges from 5 to 10 percent among horses native to endemic regions, depending on the pathogen strain, the health of the horse, and treatment (Rothschild and Knowles, 2007). Among naïve mature horses introduced into endemic regions, the case fatality rate commonly exceeds 50 percent (Maurer, 1962; Rothschild and Knowles, 2007). Importation of infected horses into regions where populations of naïve horses and vectors are present may also result in high mortality among indigenous horses. For example, importation of infected horses into southern France resulted in an EP outbreak characterized by a 69-percent case fatality rate among untreated indigenous horses (Maurer, 1962).

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Equine Immune Response to EP

Collective data indicate that the equine immune response is unable to eliminate *B. equi,* and infected horses are reported to remain infected for life (Schein, 1988; Rothschild and Knowles, 2007). Research for an effective treatment that eliminates or clears the horse of *B. equi* infection is underway. Although infections with *B. caballi* have been described as self-limiting, lasting up to 4 years after infection (de Waal and van Heerden, 2004), many horses that have apparently recovered from *B. caballi* infection relapse, suggesting lifelong infection and a period of time in which the pathogen was undetectable but still present. In addition, some scientists believe that the observations of *B. caballi* clearance were made before the development of more sensitive diagnostic methods, such as nested polymerase chain reaction (PCR) (Rothschild and Knowles, 2007). Additional research is warranted to determine whether *B. caballi* and *B. equi* infections can be eliminated and will rely on the development of a testing scheme that ensures the detection of any remaining pathogens in treated animals.

Resistance to clinical disease and re-infection in previously exposed animals has been described and is thought to be due to continued immune stimulation by the parasites, although the exact mechanisms of resistance have not been elucidated (Maurer, 1962; Rothschild and Knowles, 2007). Clinical relapses in carrier equids may be due to immune suppression, concurrent disease, or splenectomy (Maurer, 1962). There is no cross immunity between *B. caballi and B. equi* (Maurer, 1962; Taylor et al., 1969).

Persistent infection of equids is extremely important in the maintenance of EP in nature. Even though the number of EP pathogens in the erythrocytes of carrier equids is typically low, these animals act as reservoirs of EP pathogens and may serve as sources for dissemination of the pathogens wherever tick vectors or other transmission opportunities occur (Ueti et al., 2005; Rothschild and Knowles, 2007).

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Diagnostic Testing for EP Pathogens

Due to the variability of the presence of EP organisms and antibodies in different stages of infection, diagnosing infection in individual horses often requires a variable approach, which may include combinations of microscopy and various serologic tests (Short, pers. comm., 2008). In the United States, the proper State and Federal authorities should be contacted so that they can assist practitioners in appropriate sampling and submission protocols (Rothschild and Knowles, 2007). Careful and secure handling of samples is essential, and samples should be submitted only to laboratories authorized to do the official testing.

Microscopy

Diagnosis of clinical cases of *B. equi* and *B. caballi* can be made by identifying the parasites within erythrocytes via Giemsa's, Wright's, or Diff-Quick stains (Donnellan and Marais, 2009). Blood smears must be thoroughly examined, as the level of parasitemia, even in acute cases, can be low.

B. equi trophozoites are predominantly oval organisms up to 3 µm. Merozoites usually occur as four piriform parasites, 1.5 µm long, in the characteristic Maltese cross formation. The percentage of erythrocytes parasitized is usually 1 to 5 percent in clinically diseased animals, but may exceed 20 percent (Donnellan and Marais, 2009). Rothschild and Knowles (2007) suggested that the level of parasitemia with *B. equi* often ranges from 1 to 7 percent of erythrocytes, with a maximum of 95 percent.

B. caballi is larger than *B. equi. B. caballi* trophozoites are oval or elliptical with pear-shaped merozoites occurring in pairs. The percentage of detectable erythrocytes in peripheral blood parasitized by *B. caballi* typically does not exceed 1 percent and may be lower than 0.1 percent (Rothschild and Knowles, 2007; Donnellan and Marais, 2009). The sensitivity of detecting parasitized erythrocytes can be increased by using thick blood smears (Rothschild and Knowles, 2007). In addition, some literature has described additional methods for optimizing parasitemia detection, including maintaining blood in a cool environment until slides are made, and a centrifugation process that concentrates the parasitized cells (Theiler, 1902; Sippel et al., 1962). Because it is very difficult to detect the parasites in blood, serologic methods have been used for diagnosis (World Organisation for Animal Health [OIE], 2005).

Serology

Because it is very difficult to detect the parasites in blood, serologic methods have been used for diagnosis (OIE, 2005).

Serologic tests for detecting antibodies to either *B. equi or B. caballi* include the indirect fluorescent antibody test, the complement fixation test, and the competitive inhibition enzyme-linked immunoassay. Prior to July 2010, the USDA–APHIS–VS, National Veterinary Services Laboratories (NVSL) in Ames, Iowa, was the only official testing site in the United States; however, USDA–APHIS–VS Notice 10-06, related to the approval of additional National Animal Health Laboratory Network (NAHLN) laboratories to perform movement testing, was released in March 2010. As of July 2010 eight laboratories in six States (Colorado, Florida, Kentucky, New Mexico, Oklahoma, and Texas) have been approved to conduct serologic screening tests. Confirmation of positive screening tests is still performed at NVSL. [http://www.aphis.usda.gov/animal_health/lab_info_services/downloads/ApprovedLabs_piroplasmosis.pef].

<u>Complement fixation test (CFT)</u>

The CFT relies on fixation of complement during a reaction between the specific antigen and antibody (Brüning, 1996; Friedhoff and Soulé, 1996). Sera that react positively at a dilution of 1:5 are considered positive. The CFT detects antibodies as early as 8 days after infection; titers decline at 2 to 3 months after exposure. The CFT is a very specific test for acute infections; however, it has low sensitivity in chronic infection because some of the antibody types which develop in chronic infections do not fix complement.

This test cannot evaluate sera with anticomplement activity (Friedhoff and Soulé, 1996). The CFT was first implemented as an import test for equids entering the United States in 1970 (Federal Register, 1979) and remained the official test until 2005. When testing horses with acute infections, CFTs have been positive, while other serologic tests, such as the cELISA, have been negative (Short, pers. comm., 2010). Disadvantages associated with the CFT include the need to produce large quantities of antigens and the occurrence of false-negative results (Brüning, 1996).

Indirect Immunofluorescent Antibody Test (IFAT)

The IFAT is a more sensitive test than the CFT and has been used as a supplemental test when CFT results are inconclusive (Rothschild and Knowles, 2007). In this assay, parasite antigens are bound to a glass slide and allowed to react with test sera. Bound antibodies are visible under ultraviolet light after binding of the fluorescein-labeled antiequine sera. Sera are considered positive if they show strong fluorescence of the parasites at a dilution of 1:80 or higher. In one study, the earliest antibody responses detected with the IFAT in horses experimentally infected with *B. caballi* and *B. equi* occurred 3 to 20 days after infection, with titers still detected in the chronic stages of infection. To increase specificity with the IFAT, the serum must be diluted, resulting in a concurrent loss of sensitivity (Rothschild and Knowles, 2007). The IFAT is time consuming, requires large amounts of antigen and, because of the subjectivity in interpreting fluorescence, is difficult to standardize. Per the OIE manual, the IFAT is one of the prescribed tests for purposes of equine international trade (OIE, 2005).

Enzyme-linked Immunosorbent Assay (ELISA) and Competitive Inhibition ELISA

Knowles and others (1991), using *B. equi* equine merozoite antigen (EMA)-1 and specific monoclonal antibodies, developed a competitive inhibition ELISA (cELISA) to detect *B. equi* infection. This cELISA was later improved by using recombinant protein instead of culture-derived whole parasites (Knowles et al., 1992). The cELISA has detected chronic infection in experimentally infected horses not detected by the CFT (Brüning, 1996). The use of the recombinant protein facilitates standardization of the assay and overcomes the need for in vitro cultivation of the parasite or the artificial infection of horses for antigen production, making the cELISA an ideal screening test for *Babesia* infection (Rothschild and Knowles, 2007).

In 1999, a cELISA using recombinant *B. caballi* rhoptry-associated protein-1 (RAP-1) was developed by Kappmeyer and others (1999). In a field survey, this test identified 25 percent more sera as positive for *B. caballi* than did the CFT.

In 2004, the OIE approved the cELISA—for both *B. equi* and *B. caballi* detection—as a prescribed test for international horse movement (OIE, 2005; Rothschild and Knowles, 2007). The NVSL's "Competitive ELISA for Serodiagnosis of Equine Piroplasmosis (*Babesia equi* and *B. caballi*) and Production of Recombinant *B. equi* and *Babesia caballi* cELISA Antigens" is the cited reference for cELISA EP test methods in the OIE's "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals" (OIE, 2005). Subsequently, two test kits manufactured by Veterinary Medical Research and Development (VMRD) were developed—one for detecting *B. equi* antibodies and one for detecting *B. caballi* antibodies. These kits are licensed by the USDA–APHIS–VS Centers for Veterinary Biologics (CVB) based on performance requirements for the kits and each batch of reagents. The sale of these licensed cELISA test kits in the United States is restricted to specific USDA-approved laboratories.

Approval of test kits for use in the United States

The VMRD cELISA test for *B. caballi* and *B. equi* are listed as licensed kits by CVB on its Web site (USDA, 2010). In another section of the Web site there is a remark that diagnostic test kits for diseases with U.S. State and/or Federal eradication/control programs will be provided to the NVSL by CVB for evaluation for program use. In some cases, APHIS may provide the core samples for a reference serum panel. The license and/or permit for such kits may restrict distribution to APHIS-approved laboratories.

Western blot

The Western blot test is also called the immmunoblot assay or Iblot. The materials and methods for this test have been described by Schwint and others (2009), and thus far the test has been used primarily in research. When the Iblot was compared to CFT and cELISA in two horses treated for *B. caballi* infection with imidocarb dipropionate, the Iblot test remained positive longer than either the CFT or cELISA. However, in a serosurvey conducted by USDA–APHIS–VS the confirmatory testing of VMRD cELISA-positive samples via Western blot did not lead to usable results due to several factors, including inadequate remaining sera from some of the samples, poor sera quality, and unexpected results (banding patterns) when Western blot testing these field samples. These results were in contrast to those from limited experimental use of the Western blot in which clear indication for positive and negative horses was provided (USDA, 2009).

Polymerase chain reaction (PCR)

The PCR test relies on amplification of DNA from the EP pathogens and the detection of specific, unique components of that DNA. There are four different types of PCR test used for detecting *Babesia* parasites: conventional or primary PCR (one set of primers); real-time PCR (quantifies level of parasite in peripheral blood); nested PCR (two sets of primers used to increase sensitivity); and nested PCR with hybridization (probe specific for gene target results in enhanced sensitivity and specificity) [D. Knowles, pers. comm., 2010]. It is important to note that specificity in the PCR can be defined by several methods. These include molecular mass of the band, restriction endonuclease treatment of the band, sequencing of the band, and hybridization with specific probes (D. Knowles, pers. comm., 2010). Therefore, under experimental conditions false positives can be avoided. A real-time PCR test that allows for quantitative detection of *B. caballi* and *B. equi* has been reported (Bhoora, 2010). Quantification of the parasite in blood would allow for studies related to factors that may alter the level of parasitemia.

A nested PCR for *B. equi* based on the sequence of the EMA-1 gene has detected the equivalent calculated parasitemia of 0.000006 percent (Nicolaiewsky et al., 2001). In a field study, this nested PCR detected 3.6 times more infections than did microscopy and 2.2 times more infections than did conventional PCR (Rothschild and Knowles, 2007).

To assess the diagnostic sensitivity and specificity of the PCR, studies that include testing horses in endemic areas are necessary. In addition, there is a need to define the transmission risk posed by a seropositive, but PCR-negative, horse in order to define the horse's status from a transmission and regulatory perspective. Currently, the PCR is used only for research purposes in the United States; use of the test for regulatory purposes to determine a horse's infection status will not be considered until further validation of the tests is completed.

Animal inoculation

The OIE Terrestrial Animal Health Manual indicates that when equivocal results are encountered in serological tests for EP, the inoculation of large quantities of whole blood transfused from the suspect horse into a susceptible splenectomized horse will assist in diagnosis (OIE, 2008). The recipient horse is observed for clinical signs of disease, and its red blood cells are examined for parasites. Alternatively, a specific tick vector is fed on the suspect animal and the parasite may then be identified either in the vector or through transmission by the vector to another susceptible animal. Transmissions by direct blood inoculation and by tick feeding were used to demonstrate the clearance of *B. caballi* based on treatment with imidocarb dipropionate (Schwint et al., 2009).

Reporting criteria for National Animal Health Reporting System (NAHRS)

Equine piroplasmosis is considered a foreign animal disease in the United States. Accredited veterinarians are required to report all animal disease conditions to regulatory veterinary authorities if the differential diagnosis could include a foreign animal disease. A USDA-certified Foreign Animal Disease Diagnostician is assigned to investigate using standardized protocol and submits diagnostic materials to

the NVSL. Reporting through NAHRS requires the concurrence of the USDA and the State Chief Animal Health Official. Diagnosis requires serologic confirmation (CF) at the NVSL, an epidemiologic investigation, and concurrence of the State Chief Animal Health Official and the Federal Area Veterinarian in Charge (NAHRS, 2008).

Laboratory Criteria: OIE Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals 2008, Chapter 2.5.8 (OIE, 2005)

1.1. *Agent identification:* Geimsa stained blood or organ smears taken during the acute phase of the disease can be used to identify the parasites, but this method may be quite difficult in samples from chronically infected carrier animals. Thick blood film samples may help in chronic infections. Polymerase chain reaction (PCR) tests have been developed.

1.2. *Serological tests:* Serum is tested for the presence of antibodies by complement fixation (CF), or indirect fluorescent antibody (IFA), or competitive enzyme-linked immunosorbent assays (c-ELISA). Positive CF and IFA results indicate a more acute infection and start to wane by 180 days. cELISA positive results develop at approximately three weeks and indicate a more chronic infection.

2. Case Definition*:*

2.1. *Suspect case:* Any equids with:

2.1.1. Clinical signs consistent with equine piroplasmosis; **OR**

2.1.2. History of exposure; **OR**

2.1.3. An inconclusive or positive test (Geimsa-stained blood film, or CF, or IFA, or c-ELISA, or PCR) performed on sample taken during routine screening or surveillance for equine piroplasmosis.

2.2. *Presumptive positive case:* Any suspect case with:

2.2.1. Epidemiological information consistent with equine piroplasmosis; ***AND***

2.2.2. A positive test for equine piroplasmosis (Geimsa-stained blood film, or CF, or IFA, or c-ELISA, or PCR).

2.3. *Confirmed positive case:* Any equids tested confirmed positive for equine piroplasmosis by **NVSL or a laboratory designated by the Secretary of Agriculture** by at least one of the following methods: CF ***OR*** c-ELISA.

3. Reporting Criteria*:* Equine piroplasmosis is a U.S. foreign animal disease (FAD) outside Puerto Rico and the U.S. Virgin Islands, and is an OIE notifiable disease.

3.1. Follow standard FAD procedures according to Veterinary Services Memorandum No. 580.4.

3.2. Any suspect cases must, by law, be reported immediately to State Veterinary Officer or Federal authorities (AVIC).

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Treatment of Equids with EP

Treatment regimens for equids with EP often vary and may depend on whether the equids are in endemic or free countries, and whether the desired goal of treatment is parasite elimination or resolution of clinical disease.

Treatment of EP in endemic countries is usually aimed at eliminating clinical signs of disease without eliminating the parasite from the body. It is detrimental to eliminate the parasite from equids in endemic countries because premunition depends on continued presence of the parasite at low levels (Donnellan and Marais, 2009).

In contrast, in countries where EP is not considered endemic, treatment of infected equids is aimed at chemosterilization. Clearing the parasites by treating infected equids with antiprotozoal drugs is challenging and treated horses may continue to be reservoirs. These pathogens may persist in immunocompetent hosts at levels below the limits of routine microscopic detection and without overt signs of disease, yet serve as efficient reservoirs of arthropod vector-borne transmission (Ueti et al., 2008; Schwint et al., 2009). Defining the criteria to determine if parasite clearance has occurred is also a challenge, as the goal is to be confident that the treatment has eliminated the transmission risk. At this time, treating infected equids in the United States is not authorized, unless the horse is enrolled in a USDA research trial.

Antiparasitic drugs

A variety of drugs have been used in attempts to treat EP. In general, *B. equi* has been reported to be more refractory to babesiacidal drugs than *B. caballi*. The drugs commonly used to treat EP in equids are imidocarb and diminazene.

Historically, amicarbalide isothionate and eufalvine were used to treat EP, but these drugs are no longer readily available (Donellan and Marais, 2009). Tetracycline has been reported as a treatment for *B. equi*; however, in these cases the drug was given prior to definitive identification of the causative agent, so treatment response in suspect cases might have been the result of resolution of a different type of infection (Zobba, 2008). Antitheilerial compounds, including parvaquone and buparvaquone, have been shown to reduce parasitemia of initial infection but failed to sterilize *B. equi* infections. Imidocarb appears to be the most effective of the drugs explored to date (Donnellan and Marais, 2009).

<u>Imidocarb</u>

Imidocarb is a caranilide derivative usually administered as the dipropionate salt by intramuscular injection. Although imidocarb has been used for more than 20 years to treat babesiosis, minimal information was available about the pharmacokinetic behavior of the drug in equids until 2002, when Belloli and others (2002) published an article on the topic. Belloli's study showed that: (1) imidocarb was effectively distributed to tissues; (2) a prolonged period was required for complete elimination of the drug from tissues; and (3) there was evidence of possible sequestration of imidocarb in vascular and extravascular compartments.

The short-lasting persistence of imidocarb in blood reported by Belloli was not considered a favorable kinetic behavior for a drug to affect hemoprotozoan parasites. Reports of the plasma concentration of imidocarb necessary for effective therapy are lacking for equine *Babesia* spp. Based on the effective diffusion of imidocarb across cell membranes, the short-lasting persistence of the drug in blood may be enough to kill *B. caballi* (Belloli et al., 2002).

The liver was reported to be a storage tissue for imidocarb. In Belloli's study, imidocarb was detected in the milk of mares, but mammary elimination did not appear to be important. The concentration of imidocarb in milk reflected the state of the plasma drug levels (Belloli et al., 2002). Given the rapid clearance of imidocarb from plasma, repeating multiple courses of treatment with several days in between

may ensure that high drug levels are maintained in storage tissues, thereby supplying the body with delivery reservoirs from continuous release of imidocarb. The storage tissues (liver and kidney) may also be sites in which circulating infected cells are exposed repeatedly to elevated drug concentrations (Belloli et al., 2002).

Research results regarding the efficacy of imidocarb for *B. caballi* and *B. equi* clearance vary. Schwint and others (2009) experimentally inoculated horses with a Puerto Rican strain of *B. caballi* then treated them with imidocarb dipropionate (Imizol, Schering Plough Animal Health) via intramuscular injection. Treated horses failed to transmit infection to naïve horses by transfer of whole blood or by tick feeding. The treated horses converted from seropositive to seronegative status on the cELISA test. The authors concluded that the aggressive imidocarb treatment cleared the strain of *B. caballi* inoculated in this study from persistently infected horses. Treated horses met the regulatory standard for international movement (seronegative on cELISA test) and eliminated the transmission risk to ticks.

Paso Fino horses imported to the island of Curacao that were seropositive to *B. caballi* and *B. equi*—based on testing with the indirect fluorescent antibody test (IFAT)—were treated with five consecutive doses of imidocarb dipropionate. This treatment protocol temporarily resulted in negative serologic tests; however, samples collected 6 and 18 weeks after treatment tested positive for both *B. caballi* and *B. equi* antibodies (Butler et al., 2008).

Ongoing research at the NVSL is evaluating the efficacy of imidocarb dipropionate in the clearance of *B. equi* from experimentally infected horses. Dr. Tom Bunn, Director of Diagnostic Bacteriology Laboratory at the NVSL, reported that the goal of this research is to attempt to demonstrate the ability of imidocarb to clear horses infected with *B. equi* (USAHA, 2009). Twelve ponies were inoculated with *B. equi*, 8 of which were subsequently treated with imidocarb. Clearance was defined by a negative PCR test, the failure to establish infection by direct inoculation of blood to susceptible recipient horses, and the failure of ticks to acquire parasites after feeding from treated ponies. At the time of this report, three naïve horses had been inoculated with red blood cells (RBCs) from treated, PCR-negative ponies and showed no seroconversions after 90 days (USAHA, 2009). Two naïve intact horses inoculated with RBCs from untreated ponies seroconverted by both CFT and cELISA after 90 days. The next steps in the project are to continue subinoculation of seronegative horses (USAHA, 2009). If blood inoculation of horses fails to result in seroconversion, ticks that have fed on treated horses will be fed on susceptible horses to determine if *B. equi* is transmitted (USAHA, 2009). As a final test of clearance, splenectomies will be performed.

Imidocarb causes a dose-dependent hepatotoxicity and nephrotoxicity (Donnellan and Marais, 2009). In clinical cases with dehydration and anemia drug disposition kinetics may potentiate the negative effects of imidocarb. The LD50 for an imidocarb injection given intramuscularly was 15.99$\pm$1.49 mg/kg, with mortalities occurring within 6 days following the first injection. Increasing levels of imidocarb was associated with increasing mortality and morbidity (local and systemic reactions). Mortalities were attributed to acute renal cortical tubular necrosis and acute periportal hepatic necrosis induced by two injections of 16 or 32 mg/kg of imidocarb (Adams, 1981).

Treatment of donkeys with EP

Donkeys are reported to be more susceptible than horses to the toxic effects of imidocarb (Donnellan and Marais, 2009). The therapeutic efficacy of imidocarb, artesunate, arteether, buparvaquone, and a combination of arteether and buparvaquone against *B. equi* of Indian origin was evaluated in splenectomized donkeys with experimentally induced acute infection (Kumar et al., 2003). Individually, arteether and buparvaquone had no efficacy for clearing parasitemia; treated donkeys died within 5 to 6 days after showing high parasitemia and clinical signs of disease. Parasite multiplication was restricted in donkeys treated with artesunate, but only while drugs were given. Donkeys treated with imidocarb and a combination of arteether and buparvaquone cleared *B. equi* from blood circulation 2 to 5 days post treatment; however, reoccurrence of the parasite was detected in both groups 55 to 58 days post treatment.

In vitro testing[3]

The literature contains information about in vitro effects of compounds on EP agents and their relation to the survival of *B. caballi* and *B. equi*. Chemicals or drugs that kill the parasites in the laboratory may not have the same effect when given to equids and may in fact be toxic to equids. Before a drug is used in equids, stringent evaluation of its efficacy and safety is usually undertaken by the manufacturer. However, the costs associated with the research, development, and licensing of a new drug can be substantial, and a manufacturer will not make such an investment unless a market exists for the drug.

Evaluating the effect of antimicrobial drugs in an in vitro model has utility in screening antiparasitic drugs for potential in vivo use. As an example of a model, the effect of oxytetracycline, imidocarb, and enrofloxacin on *A. marginale,* a hemoparasite of cattle, was recently evaluated using short-term erythrocyte cultures (Coetzee et al., 2009). In this evaluation, a significant association existed between ultrastructural changes in the parasite and infectivity.

In vitro growth inhibition of *B. equi* and *B. caballi* was reportedly induced by exposure to triclosan, with no adverse effects on host cells (Bork et al., 2003). The multiplication of *B. equi* and *B. caballi* in an in vitro cell culture model was significantly inhibited by heparin, resulting in complete clearance of the intracellular parasites (Bork et al., 2004). Growth inhibition of *B. equi* and *B. caballi* in vitro was reported due to exposure to three antimalarial drugs, including artesunate, pyrimethamine, and pamaquine (Nagai et al., 2003). *B. equi* was more resistant to pyrimethamine than *B. caballi* and more sensitive to artesunate and pamaquine than *B. caballi*.

A *B. caballi* culture system has been used to evaluate the in vitro antibabesial efficacy of four ethnoveterinary plants: *Rhoiscissus tridentate*, *Elephantorrhiza elephantine*, *Aloe marlothii,* and *Urginea sanguindea* in vitro *(*Naidoo et al., 2005*).* To validate the model, the plants were compared with imidocarb diproprionate and diminazene aceturate. Effectivity was established as the degree of inhibition of color change, the percentage of parasitized cells on thin culture smears, and calculation of the degree of residual infectivity. The model demonstrated the in vitro efficacy of imidocarb and diminazene indicating EC50 values of 0.08 and 0.3 µg/ml, respectively. Of the ethnoveterinary plants, only the *E. elephantine* rhizomes acetone extracts at a concentration of 100 µg/ml were effective. This study showed that the color change method was not very sensitive in determining the activity of crude plant extracts (Naidoo et al, 2005).

Additional treatment and management

Antibabesial treatment should be accompanied by supportive care of horses with clinical disease. Supportive care would involve management of anemia through blood transfusion and fluid treatment in animals with pigmenturia or dehydration.

Equine piroplasmosis is a reportable disease in the United States, and veterinarians should contact State or Federal regulatory officials rather than treat suspect cases.

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Distribution, Biology, and Ecology of U.S. EP Tick Vectors (revised 11/10/10)

Ticks are commonly thought of as insects but are actually arachnids like scorpions, spiders, and mites. All members of this group have four pairs of legs as adults and have no antennae. Adult insects have three pairs of legs and one pair of antennae. Ticks are among the most efficient carriers of disease agents because they attach firmly to a host when sucking blood, feed slowly, and may go unnoticed for a considerable time while feeding. There are at least 850 tick species worldwide and approximately 85 tick species within the United States (Horak et al., 2002).

Life stages/life cycle

Ticks are divided into two groups: hard ticks and soft ticks. Hard ticks such as the American dog tick have a hard shield just behind the mouth parts (sometimes incorrectly called the “head”). Unfed hard ticks are shaped like a flat seed. Soft ticks do not have the hard shield and are shaped like a large raisin. Soft ticks prefer to feed on birds or bats and are seldom encountered by people or other types of animals unless their host animals are nesting or roosting in an occupied building.

Ticks have four life stages: egg, six-legged larva, eight-legged nymph and adult. After the egg hatches, the tiny larva feeds on an appropriate host. The larva then develops (molts) into the larger nymph. The nymph feeds on a host and then molts into an even larger adult. Both male and female adults find and feed on a host, the females lay eggs after feeding (Sonenshine, 1991). Female adult ticks die after laying eggs. Male adult ticks can feed and mate multiple times before dying, and their life span varies, depending on species. When seeking a female for mating, male ticks might move from one animal host to another.

Ticks are generally classified as one-, two-, or three-host ticks.

<u>One-host tick</u>

A one-host tick attaches to a single host and feeds on that same host throughout all of its life stages, from larva through adult. A one-host tick attaches to a host at the larval stage, feeds, molts to the nymphal stage on the host, feeds again on the same host, molts to an adult stage on the host, and feeds on the same host. The female will drop from the host after feeding and lay her eggs in a suitable habitat.

<u>Two-host tick</u>

A two-host tick attaches and feeds on at least two different hosts during its life stages. Generally, the immature stages feed on one host and the adult feeds on another.

<u>Three-host tick</u>

As a larva, a three-host tick will stay on the ground or climb up a plant to wait for a host to pass by. The seed tick (a larval form of a tick, which is very small) waits with its front claws outstretched to grab the first animal that comes by, usually a mouse, vole, squirrel, chipmunk, mole, shrew, muskrat, or rabbit (first host). The larva engorges with blood for about 4 days then drops to the ground, molting (shedding its skin) in leaf litter and becoming an eight-legged nymph. The nymph climbs up a plant and waits for a new host (second), which may be another small mammal or a moderate-sized animal like an opossum or raccoon. Once the nymph has attached to a new host, it engorges with blood for approximately 6 days then drops from its second host and molts on the ground. At this stage, it may take weeks before the nymph molts and becomes an adult tick.

As adults, both the male and female ticks look for a host (third in life cycle), most likely a larger mammal than its first or second host such as a raccoon, opossum, fox, skunk, woodchuck, deer, dog, or human. Once attached to a host, the female will feed to produce eggs. The male mates with the female while she completes her blood meal. A female will not complete her feeding period without mating. Rather, she will

remain on the host with a partial blood meal until she mates. After mating, the male dies and the female drops to the ground and lays her eggs, which begins the cycle again.

During any of its stages, a three-host tick can survive for several years without finding a host. In such cases, a tick may enter a diapause or a period of inactivity or rest, during which development is suspended (Oliver, 1989). Diapause is an adaption by ticks used to survive unsuitable conditions. Ticks wait for host animals from the tips of grasses and shrubs (not from trees). When brushed by a moving animal or person, they quickly let go of the vegetation and climb onto the host. Ticks can only crawl; they cannot fly or jump. Some species of ticks will crawl several feet toward a host (Sonenshine, 1991).

Transmission and vector competency for EP pathogens

There are three main modes of tick-borne transmission of *Babesia* parasites: transstadial, intrastadial, and transovarial. Transstadial transmission occurs when a tick stage (larval or nymphal) acquires the pathogen from an infected host and the subsequent tick stage within the same generation transmits the pathogen to an uninfected host. The pathogen is retained by the tick through the molting process (i.e., nymph to adult). Intrastadial transmission occurs when a tick acquires the pathogen (i.e., male) transmits to a naïve host with no development or molting to another life stage prior to transmission. Transovarial transmission is the passage of the pathogen from one tick generation to the next through the ovaries (Ueti et al., 2008).

EP pathogens are enzootic on several islands in the Caribbean, areas in Central and South America, and in Mexico, and are transmitted by three genera of ticks, including *Hyalomma, Dermacentor*, and *Rhipicephalus* species (de Waal, 1992; Strickland and Gerrish, 1964). *B. caballi* is transmitted by seven *Dermacentor* species, six *Hyalomma* species, and two *Rhipicephalus* species. *B. equi* is transmitted by four *Dermacentor* species, four *Hyalomma* species, and five *Rhipicephalus* species*. B. caballi* is transmitted transstadially (nymph to adult) by 10 tick species and transovarially (female to eggs) by 11 tick species. *B. equi* is transmitted transstadially by 13 tick species and only transovarially by *H. anatolicum* (Neitz, 1956; de Waal, 1992; Stiller and Coan, 1995). *Anocentor nitens*, the tropical horse tick, is currently the only known natural vector of EP in the United States (Roby and Anthony, 1963). *B. caballi* and *B. equi* have been shown to be experimentally transmitted by three additional U.S. tick species: *D. albipictus*, the winter tick; *D. variabilis*, the American dog tick; and *B. microplus,* the southern or tropical cattle tick. *B. caballi* can be transmitted by *A. nitens*, *D. albipictus*, and *D. variabilis,* whereas *B. equi* is transmitted only by *D. variabilis* and *Rhipicephalus (Boophilus) microplus* (Stiller et al., 2002). Although *R. microplus* has been considered an experimental vector of *B. equi*, evidence is growing in Brazil that *R. microplus* is likely a natural vector of *B. equi* in Brazil (Guimaraes et al., 1998, Heuchert et al., 1999; Battsetseg et al., 2002). *B. equi* infects horses and is transmitted by *R. microplus* in subtropical and tropical regions of the Americas (Knowles et al., 1992; Guimaraes et al., 1998). Transstadial transmission of *B. equi* by *R. microplus* has been confirmed with the acquisition of parasites by the nymphal stage from chronically infected horses and transmitting as a newly molted adult to a naïve host (Ueti et al., 2005). Additionally, *R. microplus* males can acquire *B. equi* parasites from a chronically infected horse and transmit the parasites to a naïve horse. Therefore, intrastadial transmission has been demonstrated with this tick vector, and targeted control methods should consider this mechanism of transmission (Ueti et al., 2008). Additionally, there is some recent evidence that *D. variabilis* and *Amblyomma cajennense*, the cayenne tick, may be natural vectors of *B. equi,* as demonstrated with the field collection of adults and transmission of *B. equi* to naïve horses (Scoles, pers. comm., 2010). It is likely that there is some intrastadial transmission of *B. equi* (same tick stage, i.e., males) by American dog tick and cayenne tick populations to naïve hosts (Stiller and Coan, 1995; Scoles, pers. comm., 2010).

Distribution, host associations, and seasonal activity

The major factors involved in the occurrence of a vector-borne disease include: the abundance of vectors and reservoir hosts; prevalence of pathogens within vectors and vertebrate hosts; local environment conditions, particularly temperature and moisture for tick vectors; and host resistance in the targeted host population (Sonenshine, 1991).

Natural vectors are arthropods capable of transmitting pathogens under essential environmental conditions whereby the pathogens survive and multiply within the vector and the pathogen is transmitted to a naïve host. The vector is capable of obtaining the pathogen via intake of a large blood meal from an infective host and maintaining the pathogen through multiple life stages. An experimental vector is defined as an arthropod that is infected via an inoculated host in a laboratory setting and that can maintain and transmit the pathogen to naïve host under artificial conditions (Sonenshine, 1991). A tick species is considered established when it is capable of reproducing successfully (i.e., completes its life cycle) under favorable abiotic and biotic conditions in a particular region. If the tick species is considered "reported" from an area, it is likely that the success of reproductive status of that particular tick species has not been confirmed and the tick(s) might simply have been imported on a host (Dennis et al., 1998).

Natural and experimental EP tick vectors in the United States

Natural vectors

Tropical horse tick (Anocentor nitens)

A natural vector of *B. caballi* in the Americas is the tropical horse tick *Anocentor nitens* (also known as *Dermacentor nitens*) (Horak et al., 2002; Barker and Murrell, 2004); its distribution in the United States is limited to the southernmost parts of Florida and Texas. The tick is widespread in the tropical and subtropical regions of northern South America, Central America, Mexico, and the West Indies. In 1901, *A. nitens* distribution extended from Jamaica and Santo Domingo to include Puerto Rico, Guatemala, and Venezuela. By 1964, *A. nitens* distribution extended to the Caribbean, Central America, Mexico, and South America. The first report of *A. nitens* in the United States was in 1908 in Cameron County, Texas, with additional reports coming from Hidalgo, Nueces, San Patricio, Webb, and Willacy Counties by 1964. *A. nitens* was known to be established in the southernmost tip of Texas until 1960, when it was found in Palm Beach, Florida (Friedhoff et al., 1990). From 1960 through September 1963, there were 705 collections of *A. nitens* from 24 counties in Florida (Strickland and Gerrish, 1964). In 1962, *A. nitens* was collected from Coffee County, Georgia, on four separate occasions, but it did not become established in that State. From 1962 to 1963, collections of *A. nitens* were only made in Texas, Florida, and Georgia (Knowles et al., 1966; Bryant et al., 1969; Taylor et al., 1969). *A. nitens* submissions to NVSL from 1999 to 2008 are currently reported only from southern Texas (figure 1).

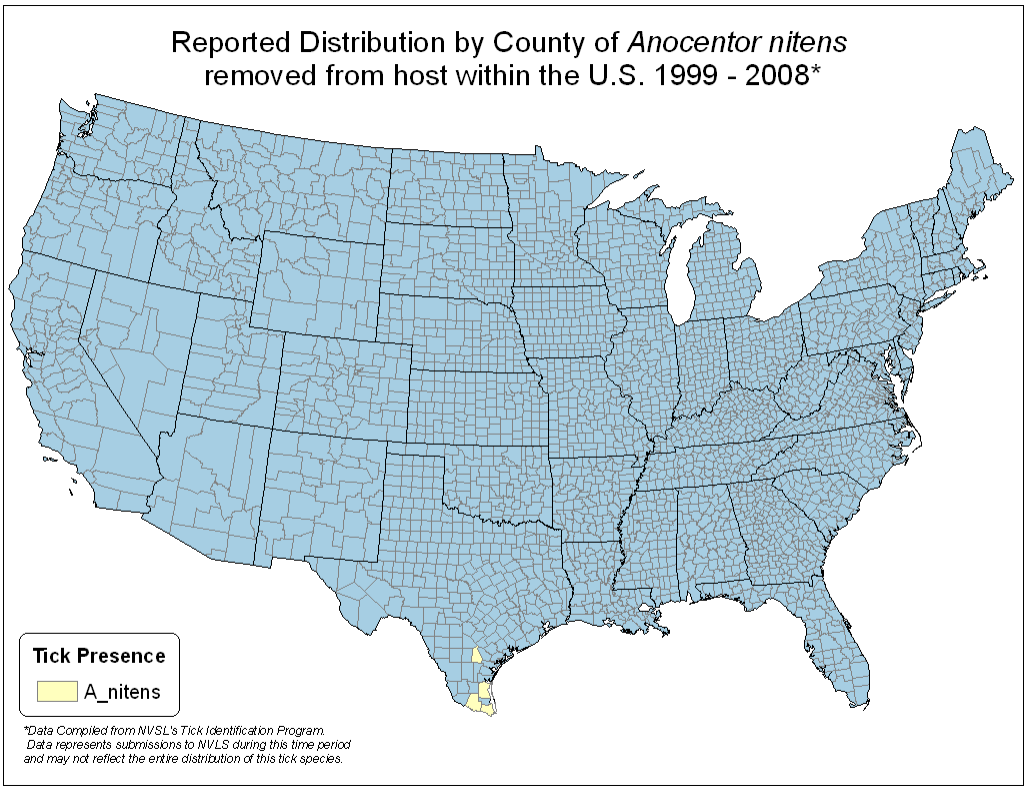

Figure 1

Horses, donkeys, mules, and other equids are the preferred hosts of *A. nitens*; however, this tick is also found on cattle, goats, sheep, and deer (Thompson, 1969). *A. nitens* is widespread in Brazil (Borges et al., 1999). It is typically found in the nasal diverticulum, the mane, perineal and perianal regions, and particularly the ears of the host. Heavy infestations of this tick can cause severe lesions and predispose a host to secondary infections.

As a one-host tick, *A. nitens* remains on its host through two molts and requires approximately 26 days to complete its development; it produces new generations at 75- to 90-day intervals. This extended development on the host provides opportunity for the tick to travel long distances (Bishopp and Trembley, 1945). This tick is found in all stages on hosts throughout the year (Holbrook et al., 1968; Borges et al., 2002).

Cattle tick (Rhipicephalus [Boophilus] microplus)

Although *B. microplus*, the southern or tropical cattle tick, was recently reclassified into the genus *Rhipicephalus*, for purposes of clarity, we will maintain the previous genus assignment of *Boophilus* (Keirans and Durden, 2001; Murrell and Barker, 2003). *B. microplus* is considered a natural vector of EP in South America. Neither *B. equi* nor *B. caballi* has been detected in field collections of *B. microplus* in the United States.

B. microplus is found worldwide in subtropical and tropical regions. This tick is endemic in the Indian subcontinent, much of tropical and subtropical Asia, northeastern Australia, Madagascar, southeastern Africa, the Caribbean, and many countries in South and Central America and Mexico. In 1906, *B. microplus* was well established within 14 southern U.S. States and southern California. By 1943, *B. microplus* was eradicated from all southern States except Florida, which successfully eradicated the tick by 1961. Although the tick has been eradicated from most of the United States, it can sometimes still be

found in Texas in a buffer quarantine zone along the Mexican border (Estrada-Pena, 2006) (figure 2). *B. microplus* is considered the most important tick of livestock in the world, based on economic impact and animal health. Heavy tick burdens on animals can decrease production and damage hides (Utech et al., 1983; Estrada-Pena, 2006). This hard tick can be found on many hosts including cattle, buffalo, horses, donkeys, goats, sheep, deer, pigs, dogs, and some wild animals.

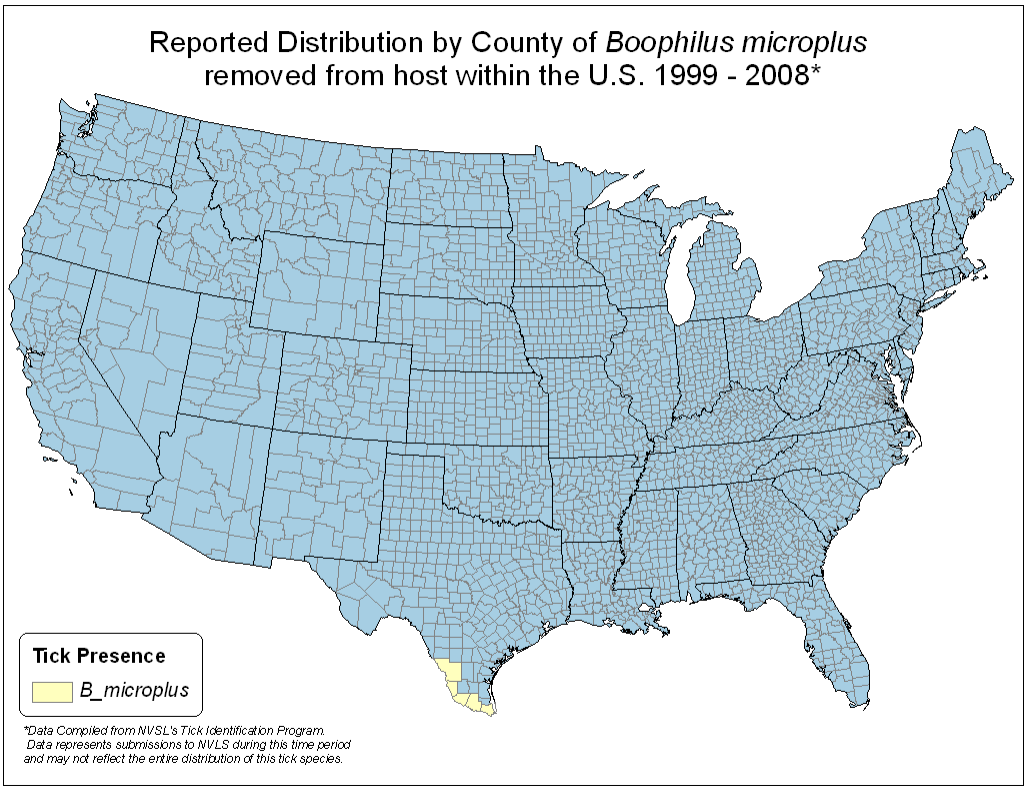

Figure 2

B. microplus is a one-host tick; all stages are spent on one animal. Its eggs hatch in the environment and the larvae crawl up grass or other plants to find a host. They may also be blown by the wind. In the summer, the *B. microplus* can survive for as long as 3 to 4 months without feeding, and under optimal conditions it completes its life cycle in 3 to 4 weeks. In cooler temperatures, the tick may live without food for up to 6 months. Newly attached tick larvae are usually found on the softer skin of the inner thigh, flanks, and forelegs of the host. They may also be found on the abdomen and brisket. After feeding, the larvae molt twice to become nymphs and then adults. Each developmental stage (larva, nymph, and adult) feeds only once, but the feeding takes place over several days. An adult male tick becomes sexually mature after feeding and mates with feeding females. An adult female tick that has fed and mated detaches from the host and deposits a single batch of many eggs in the environment (Sutherst et al., 1988; Guglielmone et al., 1990; Estrada-Pena, 2006).

Cayenne tick (Amblyomma cajennense)

A. cajennense, the cayenne tick, is a native of the Americas. *A. cajennense* ranges from the southern part of the United States to northern Argentina, through Central America and some of the Caribbean (Cooley and Kohls, 1944). In the United States, the reported distribution is confined to the southern tip of Texas (Bishopp and Trembley, 1945) (figure 3).

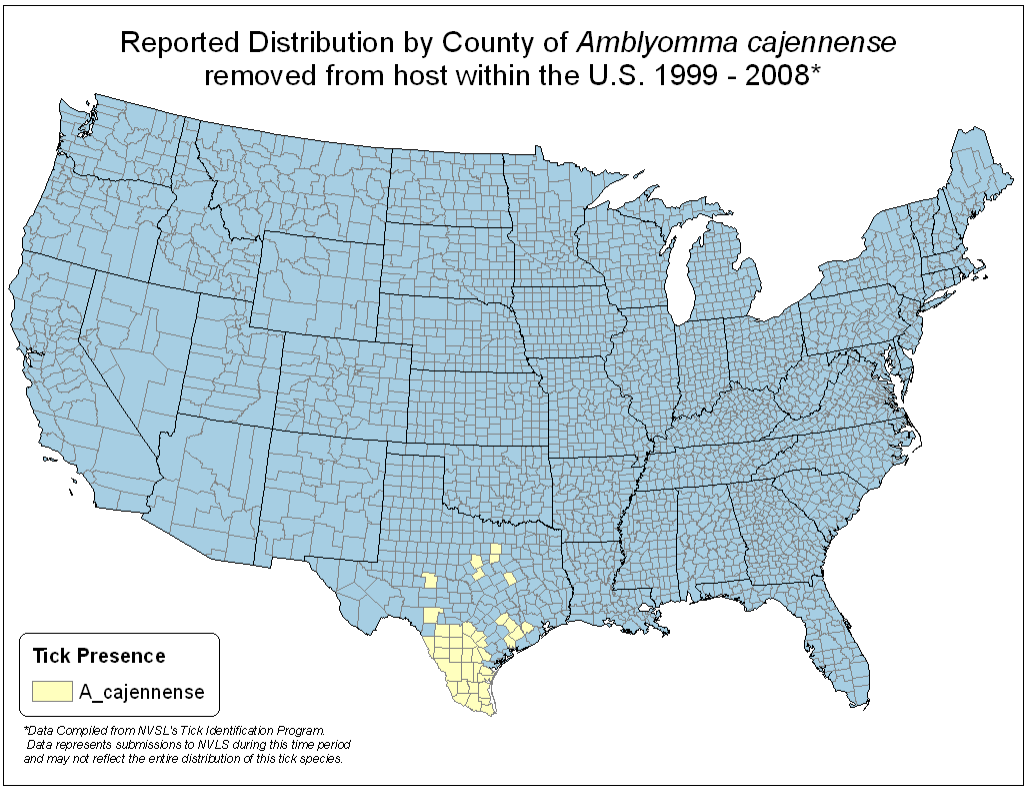

Figure 3

A three-host tick, *A. cajennense* (Fabricius) feeds on equids, but it may infest other mammals such as bovids, cervids, wild and domestic canids, birds, and even humans (Dias and Martins, 1939; Lemos et al., 1997; Rojas et al., 1999; Horta et al., 2004; Guedes et al., 2005). The active immature stages of this tick are indiscriminate in host choice; livestock and a large variety of avian and mammalian wildlife serve as hosts. Although *A. cajennense* is found mainly on horses, Smith (1974) suggested that determining the role of secondary hosts of this tick, maintenance of its biological cycle in the wildlife populations, and the possibility of the dispersion of immature stages by birds was vital for the establishment of appropriate control programs.

A. cajennense is a species that can have economic consequences and is generally quite abundant and active throughout the year. People and livestock can be severely irritated by clusters of *A. cajennense* larvae in wooded and high-grass areas. Most adult ticks attach on the lower body surface, especially between the legs; some feed elsewhere on the body (Evans et al., 2000; Oliveira et al., 2000).

A higher number of males than females were observed on hosts with an increasing ratio of males to females from August and onward. *A. cajennense* males produce pheromones 5 to 6 days after feeding that attract nymphs, females, and males of the same species. Laboratory studies have indicated that *A. cajennense* males can survive for more than 80 days on hosts. Females typically complete their feeding within 2 weeks (Pinter et al., 2002). In some months, certain stages of *A. cajennense* were not present on horses, suggesting that a diapause may occur during some phases of the *A. cajennense* life cycle (Oliveira et al., 2003). Labruna and others (2003) observed 1-year generation times (egg to adult) in Brazil that are primarily controlled by larval behavioral diapauses, as indicated by larval hatching in the spring/summer months. Oliver (1989) suggested that the behavioral diapauses observed in tick populations are adaptive behaviors used to survive unfavorable conditions in the environment (i.e., dry, cold temperatures). Male feeding behavior and larval diapauses should be considered when developing seasonally targeted control programs for tick-borne diseases for equids. Adult cayenne ticks, collected

from EP-positive horses during an outbreak of equine piroplasmosis on a ranch in south Texas, were allowed to re-attach and feed on a naïve horse, resulting in successful transmission of *Babesia equi* (Scoles, pers. comm., 2010).

Experimental vectors

American dog tick (Dermacentor variabilis)

D. variabilis (Say), the American dog tick, is widely distributed in the United States east of a line drawn from eastern Montana to western South Texas. The tick species is also found in California, west of the Cascade and the Sierra Nevada Mountain ranges and in Canada, east of Saskatchewan (figure 4). This species is most abundant in the eastern United States from Massachusetts south to Florida but is also common in central areas of the United States, including Iowa and Minnesota (Bishopp and Trembley, 1945; Matheson, 1950). The tick also occurs in certain areas of Canada, Mexico, and the U.S. Pacific Northwest (Mcnemee et al., 2003).

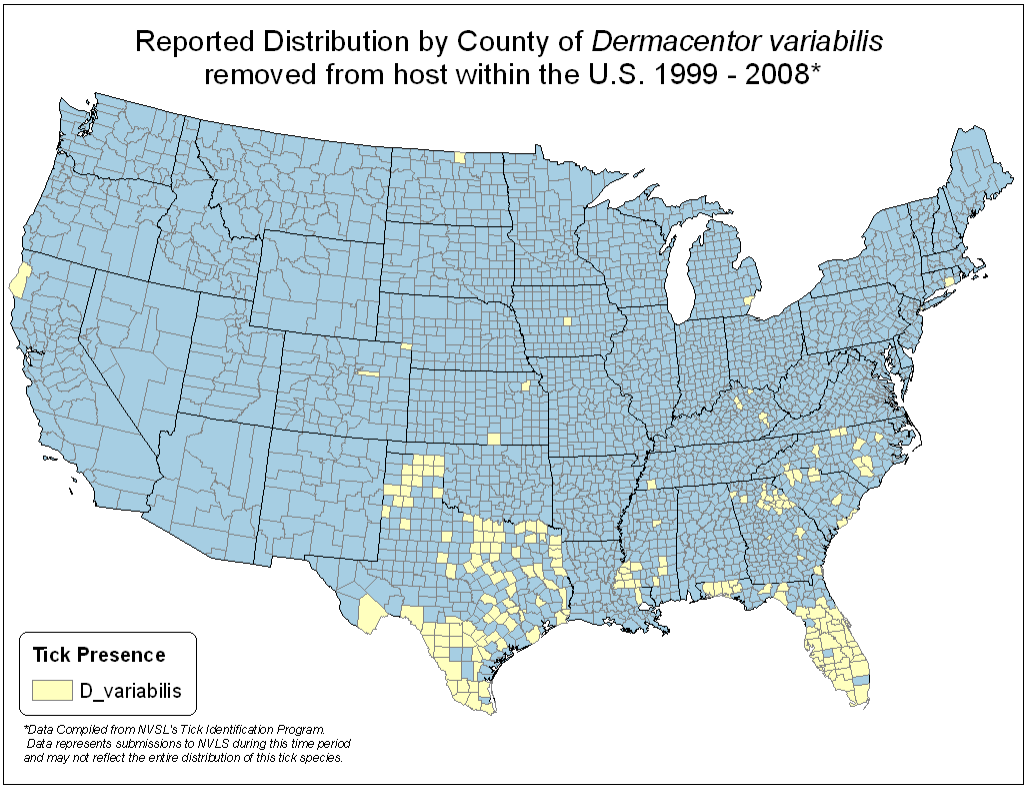

Figure 4

D. variabilis is one of the most frequently encountered ticks by livestock, equids, and humans. *D. variabilis* is a three-host tick with larval and nymphal stages found on a variety of hosts, including mice, birds, chipmunks, voles, squirrels, rats, opossums, and raccoons. Adult ticks are usually found on more medium- to large-sized mammals such as horses, cattle, white-tailed deer, opossums, raccoons, dogs, and humans. Immature ticks prefer rodents, so the abundance of the hosts can be an influencing factor on its distribution and numbers. Meadow voles (*Microtus* sp.) and white-footed mice (*Peromyscus* sp.) are common hosts for immature *D. variabilis*, although they will feed on other species of small mammals. Arthur (1961) indicated that immature stages were found on pine mice, jumping mice, meadow mice, white-footed mice, cotton rats, Norway rats, rabbits, squirrels, and house mice. Adult ticks were found on canids, horses, raccoons, and opossum in Ohio. Immature ticks are typically distributed on the host

around the head region, neck, and shoulders as immatures, while the adults attach around the neck, dewlap, brisket, axillae, groin, genitalia, and abdomen; some feed elsewhere on the body and around the head region (Conlon and Rockett, 1982).

Environmental conditions and terrain can affect tick survival and distribution. High humidity and temperature seem to be important factors for tick survival, as does the abundance of grassy or brush-covered surroundings.

Adult *D. variabilis* winter in the soil and are most active from around mid-April to early September. Larvae are active from about March through July and nymphs are usually found from June to early September (Goddard 1996). In northern areas, such as Massachusetts and Nova Scotia, adults appear from April to August with a peak in May and June (Campbell and MacKay, 1979; Campbell. 1979; McEnroe, 1979a). In central latitudes of the United States such as Virginia, adults are active from April to September/October with peaks in May and July (Sonenshine and Stout, 1971; Carroll and Nichols, 1986). In Ohio, adult activity occurs between April and September with a peak in May and June and a second smaller peak in August and September (Conlon and Rockett, 1982). In Georgia, adult ticks are active from late March to August with peaks from early May to late June (Newhouse, 1983). In Florida, adult *D. variabilis* activity occurs from April to July (McEnroe, 1979b). A study done in Lexington, Kentucky, found the duration of *D. variabilis*' spring activity was related to its overwintering success. This study also concluded that overwintering adult *D. variabilis* ticks remained active throughout the entire season (Burg, 2001). Lastly, the seasonal activity distribution for *D. variabilis* can be bimodal, with peaks of activity occurring twice in a year. In an Ohio study, bimodal peaks occurred in mid-spring and late summer, with a large peak in May and a smaller one in early September. Many of the larvae overwinter, become active in the spring, enter nymphal activity, and molt to adults in late summer with a resulting second peak of adults in early September. Atwood and Sonenshine (1967) found that the questing behavior of *D. variabilis* is positively correlated with the amount of solar radiation received at ground level.

The life cycle of *D. variabilis* requires at least 54 days to complete, but can take up to 2 years depending on host availability, host location, and temperature. In the southern States, the life cycle can be completed in 1 year, with 2 years typical in the northern States (Burg, 2001). The American dog tick occurs primarily in wooded, shrubby, and long-grass areas. However, it is possible for residential areas to support populations of this tick. Shrubs, weeds, tall grass, clutter, and debris attract rodents that are hosts for immature ticks. It has been suggested that adult ticks crawl to the edge of the roads and trails in an attempt to find a host. Some have hypothesized that the scents left by animals following trails attracts these ticks, to the trails (Mcnemee et al., 2003).

Winter tick (Dermacentor albipictus)

D. albipictus, the winter tick, is widely distributed in the northern and western United States, and in Canada and Mexico. The species is abundant in Maine, Washington, and Oregon. In addition, the dark form of this tick, known previously as *D. nigrolineatus*, can be found in arid parts of Texas, New Mexico, and northern Mexico (figure 5). This tick also is found occasionally on horses and cattle in the southern U.S. States. *D. albipictus* feeds on large mammals like deer, cattle, and horses. This tick species attaches to the host as a larva and remains attached throughout its life (Bishopp and Trembley, 1945).

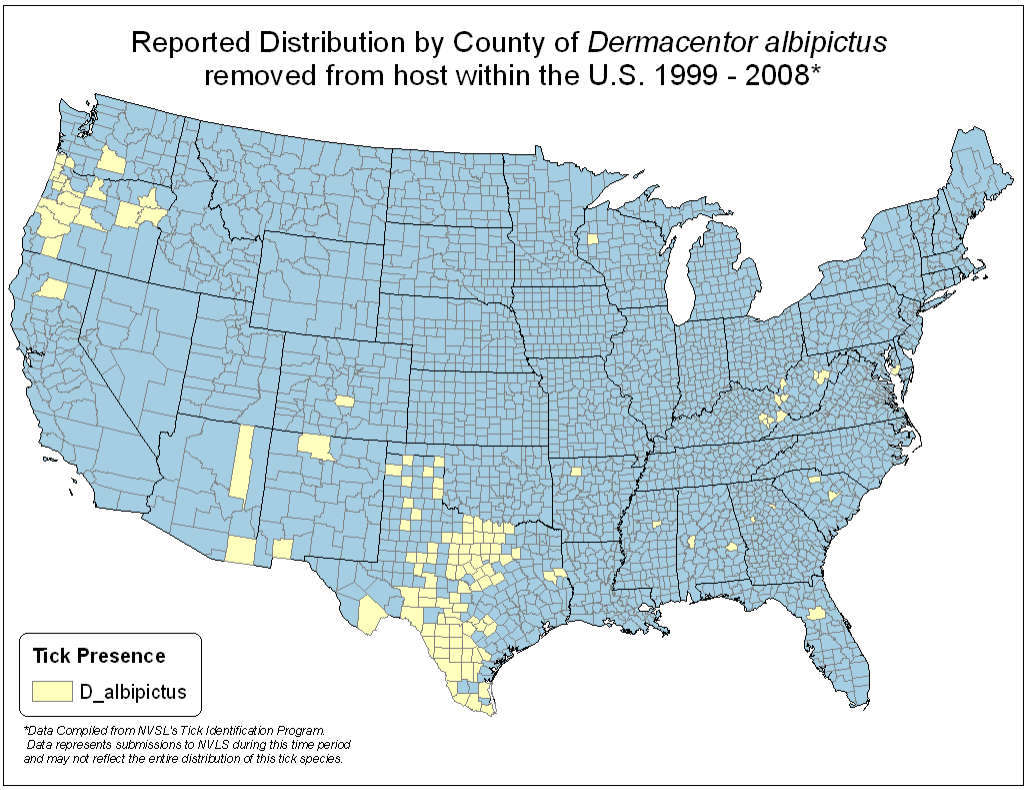

Figure 5

D. albipictus appears to prefer larger hosts such as horses, elk, and moose, but is also abundant on cattle and deer (Bishopp and Trembley, 1945; Gregson, 1956, 1973; Addison and McLaughlin, 1988). Moose and elk can die as a result of heavy infestations of this tick, particularly when the animals' food is in short supply during late winter and early spring. This tick prefers to attach on the dewlap, brisket, abdomen, groin area, and head region, but may be found all over the body in cases of heavy infestation.

The seasonal occurrence of *D. albipictus* on hosts varies with latitude and altitude. During summer, *D. albipictus* exist only as unhatched eggs in the soil. Larvae of this species quest on vegetation in September and October and are quite resistant to cold temperatures. Adults appear by March–April and lay eggs in late June. *D. albipictus* remains on its host from 1 to 2 months, so it can be easily transported to other regions or countries. Development times are sensitive to decreasing photoperiod (i.e., decreasing day length) and freezing soil temperatures. *D. albipictus* is abundant in forested, upland, and mountain habitats, and all stages can occur on large mammals between fall and spring (Wright, 1971; Samuel and Barker, 1979; McPherson et al., 2000).

Ecological implications

For many tick-borne diseases, the presence of the tick alone does not present a sufficient risk for infection. A certain number of host relationships and seasonal dynamics of ticks are required to maintain a robust enzootic cycle. There must be established relationships between environmental conditions and tick-host associations, as both vertebrate host populations and tick questing behavior are influenced by abiotic factors (distributional limits, abundance, and seasonal dynamics) (Sonenshine and Clifford, 1973).

Distribution

A tick's geographical distribution, life history, hosts, and its ability to transmit diseases are determined by intrinsic and extrinsic factors. Intrinsic factors include biochemical and physiological aspects of the tick that determine its reaction to its environment (e.g., the number of offspring produced per reproductive cycle). Extrinsic factors include abiotic and biotic components of the environment that influence the biology of the tick. Climate—temperature, wind, precipitation, humidity, and other meteorological elements—is a main abiotic factor, as it influences horizontal and vertical relationships, life cycle, seasonal activities, population dynamics, and behavior. In addition to climatic factors, the spatial structure of the landscape and its connectivity and vegetation types also have an effect on tick populations and their distributions (Estrada-Pena et al., 2008). The most important biotic factor for a tick is a suitable host or a host that provides a blood source for development and reproduction.

In general, most tick species are less widely distributed than their principal hosts on local and continental scales, with climate appearing to limit a tick's range (Cumming, 1999). Ticks are intermittent parasites, spending part of their lives off their hosts in habitats where they are influenced by abiotic factors. Temperature and rainfall are the main factors affecting the ecology and population dynamics of tick species, and these operate at critical levels on selection of tick populations (Estrada-Pena et al., 2009). For example, the distribution of *Ixodes ricinus* in Britain was associated with several environmental factors, such as substrata composed of less permeable soil types and less permeable superficial/bedrock geology, which would support moist microhabitats. Their distribution was also associated with calcareous/neutral grassland and heathland habitats, particularly those grazed by livestock (Medlock et al., 2008). McEnroe (1977) reported that there were relative differences in the distribution of the American dog tick from region to region, likely due to moisture and temperature differences. Campbell (1979) reported shifts in tick distribution and abundance related to vegetative types and attributed environmental determinants to tick survival.

A tick's distribution or geographic range can be influenced by the physical, immunological, and behavior characteristics of its hosts. Many tick-borne diseases exist in an enzootic state or at a low, stable rate of infection; however, this cycle may change with the advent of a different and opportunistic tick species with a wide variety of acceptable hosts. In contrast, if the host range is narrowed, then transmission may become more focused, resulting in high infection rates in the host (competent reservoir host) and tick vector.

Host specificity may be defined as some form of restriction to a particular class, order, or genus of vertebrate. All ticks do not feed equally on all vertebrate hosts and show some degree of host specificity. As immatures, American dog ticks show a certain amount of host specificity, preferring rodent hosts. For example, in Virginia over 90 percent of the immature American dog ticks were collected from meadow voles and white-footed mice in old field habitats (Sonenshine, 1991). Host specificity may extend from one end of the spectrum (high degree of specificity) to the other (low degree of specificity). The cattle tick, *Boophilus microplus*, preferentially feeds on ruminants, especially cattle, but may also be found on horses when there are horses and cattle in the same pasture (Labruna et al., 2003).

In addition to host preferences, a tick's distribution may be influenced by its host's seasonal behavior. For example, the one-host tick *Dermacentor albipictus*is is found on certain wild animals, particularly moose and elk. All life stages of *D. albipictus*is occur on these large mammals between fall and spring. *D. albipictus*is larvae increase their questing activity in September, concurrent with the period of moose ruts in mid-September to early October (Wright, 1971). During the rut, moose increase their geographical range in search of mates and, with the ticks' increased larval activity during this same period, the contact rate between ticks and hosts increases, as does the ticks' distribution (Bishopp and Trembley, 1945; Gregson, 1956). Moreover, male moose travel farther than females during the rut, which may explain why males average twice as many ticks as females and may have greater influence on the expansion of the ticks' range (Kollars et al., 1997; Cortinas and Kitron, 2006).

Seasonal activity

Many tick species seek hosts during a specific time of year, making transmission of tick-borne diseases seasonal. Understanding the dynamics of these seasonal changes among certain tick populations can help determine the periods when vertebrate hosts are most likely at risk.

Ticks begin to quest for a host after emerging from a diapause. Questing ticks climb vegetation and wait for a host or actively seek a host by walking on the ground. Questing ticks are highly responsive to host CO_2 and body heat. Questing periods vary among each tick's geographic range and by life stage. The seasonal activity of a tick population is controlled by its response to a changing environment, such as photoperiods and ambient temperature. Questing activities can last days or even weeks, depending on the tick's body water loss tolerance. Ticks will return to a moist micro-environment when water loss reaches a certain threshold, and a certain cycle of dehydration-rehydration is not unusual. If environmental conditions become too unfavorable, ticks will enter a diapause rather than continue to seek hosts until the environmental conditions improve (Belozerov, 1982).

The initiation of diapause and the increased seasonal activity of ticks are influenced by a variety of climatic factors. For example, the eggs of the winter tick remain unhatched in the soil during summer and hatch as the temperature and photoperiod decrease in the fall. *Dermacentor albipictus* larvae climb vegetation in autumn and form clumps of more than 100 individuals that attach to passing ungulate hosts (Wright, 1971). The larvae are capable of entering a resting phase or diapause until favorable conditions are present. Larval *D. albipictus* hatch in mid-August to early September in Canada's Elk Island National Park and begin questing in mid-September in response to climatic factors such as photoperiod and above freezing temperatures (Wright, 1971). During the high ungulate host movement activity in the fall, the larvae form clumps around 50 to 190 cm above ground, which coincides with the torso heights of some moose, elk, and deer (McPherson et al., 2000). Larvae rapidly feed and molt to the nymphal stage from 10 to 22 days after attachment. The nymphal stage lasts about 3 months (until January) and thus the nymphal stage enters a diapause on the host during this time, which is likely an adaption by the ticks to survive colder temperatures. Adults appear on the host from January through March and April (Addison and McLaughlin, 1988). Additionally, Labruna and others (2003) observed larval behavioral diapauses in *A. cajennense* in Brazil, as indicated by larval hatching in the spring and summer as temperatures change. The cayenne tick is quite tolerant of desiccation and can quest for long periods of time in open grazed environment (Needham and Teel, 1991), but their questing activity can vary diurnally and seasonally with climate (Belozerov, 1982).

Diapause is significant not only for survival of the tick vector, but also because it influences the epidemiology of any associated pathogens. Norval and others (1991) indicated that the absence of *Theileria parva* infections in southern Africa may be due to the diapauses in *Rhipicephalus appendiculatus*. *Rhipicephalus appendiculatus* in not continuous throughout the year in southern Africa, with diapauses confined to the unfed adult. Many adults who emerge after July will not quest until November. In contrast, the same tick species in equatorial Africa does not have a diapause. All stages appear throughout the year (Madder et al., 1999). Photoperiods and temperature changes are likely factors that influence diapause activity in ticks (Belozerov, 1982; Fourie et al., 2001).

Abundance

In addition to climatic and landscape changes, changes in biotic factors such as host availability, host abundance, or quality of the blood meal have been proposed as possible determinants for changes in tick abundance (Ogden et al., 2007). Therefore, the population dynamics of ticks are influenced by the seasonality of the host dynamics. Immature American dog ticks can be quite abundant where large numbers of meadow voles exist or in oak/hickory and oak/hickory/pine forests in the southern United States. This increase in the abundance of American dog tick larvae and nymphs in relation to the abundance of the meadow vole in a region can increase the potential for infection of this host (Atwood and Sonenshine, 1967; Sonenshine, 1973). Additionally, tick abundance has been positively correlated with the abundance of larger mammals such as deer in Scotland and the United States (Wilson et al.,

1985; Gilbert, 2010). An increase in the number of individuals within a tick population as an effect of the abundant hosts within a region can effectively develop a zoonotic intensification of tick-borne diseases.

Tick abundance is also limited by abiotic factors that influence the ability to actively quest for a host. Questing ticks are influenced by a variety of abiotic factors such as increasing or decreasing day length or fluctuations in temperature. Additionally, temperatures can change the questing behavior in ticks; for example, decreasing temperature associated with increasing altitude will negatively affect the number of questing numbers of nymphs and adults (Randolph, 2004). Therefore, the opportunity to acquire a pathogen from a reservoir host can be positively or negatively impacted by abiotic factors. It has been observed with *I. ricinus* nymphs that drier conditions impacted the questing height, with more nymphal ticks questing lower and thus having increased exposure to rodents. In contrast, in wetter conditions, an increased number of larvae attached to rodents and fewer nymphs. These climatic factors influenced the questing behavior and thus the potential for a pathogen to be transmitted by nymphs or larvae feeding on rodents. The risk of infection to hosts depends on the number of infected questing ticks. In addition, the larger the number of hosts in an area the greater the probability of ticks attaching and progressing through their life cycle and transmitting a pathogen (Kitron and Kazmieerczak, 1997; Randolph, 2001).

Summary

Ecological information on tick species within in the United States is vital for developing and implementing a systematic approach to tick control. The assessment of the developmental times of the free-living tick stages on vegetation will be helpful in determining the major tick species' seasonal activities. With the additional analysis of environmental temperature, rainfall, and tick abundance, this information will aid in the prediction of peak abundance of ticks and the timing of appropriate tick control on a seasonal basis. Moreover, data on life cycles, habitat requirements, and factors influencing distributions and abundance of EP vector ticks would be helpful in the development of predictive models (Petney et al., 1987). This critical information has already been used as a parameter in population models (Sutherst and Maywald, 1985; Floyd et al., 1987) spatial models (Lessard et al., 1990), and climate-driven computerized systems (Sutherst and Maywald, 1985). Through the integration of field, laboratory, and modeling studies, we can gather new insights into the mechanisms of evolutionary ecology regarding the diverse spectrum of tick-borne pathogens, tick species, and their vertebrate hosts.

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Control of Tick Vectors of Equine Piroplasmosis

As ectoparasites of equids, ticks are difficult to control because they are small—especially during early developmental stages—which makes them a challenge to find on or off a host, and because they can be attached to a host for varying time periods. An overview of tick species commonly found on companion animals is provided by Dryden and Payne (2004).

In the case of multihost ticks, one or more larval, nymphal, or adult stages may each involve a different host, depending on feeding preferences and host availability. When multihost ticks are not blood feeding, they are normally free living in the environment, further complicating detection and effective control. Since ticks have seasonal patterns of abundance, it is important to select control strategies that are most effective against a specific developmental stage before pathogen transmission might occur. In the case of tick species that seek different hosts, awareness of the common questing practices can help in the development of management practices that minimize host exposure to ticks. For one-host ticks, each life stage may remain on the same host after feeding, and early detection followed by preventive treatment is critical. Varying host preferences among one- and three-host tick vectors make it unlikely that any single control strategy will succeed. Rather, a range of control methods is needed to prevent equine tick exposure and to avoid pathogen transmission.

Examining horses for ticks

USDA guidance on the inspection of horses for ticks recommends a thorough and systematic examination. The following examination procedure, known as “scratching” for ticks, has been recommended (Rowe, personal communication, 2010):

- Beginning at the horse’s head, examine the both ears and palpate inside of each ear, examine the false nostrils visually and palpate with the forefinger.
- Move to the forelock and, with thumb opposed to fingers, examine the forelock, continuing down the mane to the withers.
- Examine the submandibular/intermandibular space using flattened hand and fingers, feeling for any unevenness of the skin.
- Examine the axilla of one side, visually and through palpation.
- Examine the posterior fetlock to the coronet of the front foot, visually and through palpation.
- Visually examine the udder/scrotum area on one side.
- Examine the tail and perineum, visually and through palpation.
- Examine the posterior fetlock to the coronet of the back foot, visually and through palpation
- Examine the udder/scrotum of the other side.
- Examine the posterior fetlock to the coronet of the other back foot, visually and through palpation.
- Examine the posterior fetlock to the coronet of the other front foot, visually and through palpation.
- Examine the axilla of the other side, visually and through palpation.
- Perform hand hygiene after examining each animal.

Removing attached ticks

Many of the methods used to remove attached ticks from a host are based on folklore and might cause additional harm to the animal. Some of the common folk methods include applying petroleum jelly, fingernail polish, 70 percent isopropyl alcohol, or a hot kitchen match to the attached tick (Needham, 1985). While these methods are thought to induce a tick to “back out” of an attachment site, they actually stimulate a tick to secrete more saliva, cause regurgitation, or introduce other tick-body secretions or excretions into the wound. These procedures increase the risk of secondary infection around the bite

location. The best method, as described by Needham (1985), is to implement the following procedures in sequence:

1. Using blunt curved forceps, grasp the tick as close to the skin surface as possible and pull upward with a steady even pressure.
2. Avoid squeezing, crushing, or puncturing the tick's body.
3. Do not handle the tick with bare hands.
4. Cleanse the bite site with soap and water.

As a possible alternative to forceps, Stewart and others (1998) compared three commercial tick-extraction tools (Ticked-Off, Tick Nipper, and Pro-Tick Remedy) and found that all three were an improvement over forceps. These tools worked well for removing nymphs and adults by taking advantage of the tick's mouthpart morphology and body size to minimize mistakes that can be made when using forceps. One or more of these tools are available at outdoor and camping retail stores. Another method for removing ticks is spraying the ticks with pyrethrins or pyrethroids containing aerosol repellent, then spraying again within 1 minute. Ticks will fall off after treatment.

To submit the sample for identification, place the tick in a container of alcohol (optimally 70 percent ethanol or 70 percent isopropyl) and send the specimen to a State or Federal (National Veterinary Services Laboratories) laboratory responsible for tick identification.

Preventing tick infestations

Preventing equine tick exposure is complicated by the specific biological requirements of a tick species and whether a tick's life cycle is dependent on one host or multiple hosts. If a tick species utilizes multiple hosts, then host availability, access to suitable questing sites, and seasonal changes in tick abundance play a role in the likelihood of an equid becoming infested with ticks. A study by Labruna and others (2001) identified risk factors related to tick infestation of horses in São Paulo, Brazil. *A. nitens, A. cajennense, and B. microplus* were the only species feeding on horses, with *A. nitens* being the most abundant of the three species. *A. cajennense* ticks were most abundant in pastures with a mixed overgrowth of grasses and shrubs, and tick abundance on pasture vegetation was correlated with levels of tick infestation on horses. The authors found that mowing pastures once a year was an effective means of avoiding high infestations of *A. cajennense*. *B*. *microplus* infestations on horses were correlated with the presence of cattle on a shared pasture. The abundance of *A. nitens* ticks did not correlate with any of the risk factors evaluated in this study. It was also observed that *A. cajennense* infestations on horses were higher on farms that sprayed acaricide on horses. Similarly, applying a topical acaricide did not control *A. nitens* infestations; however, the authors could not confirm whether the topical treatments were applied as directed by the manufacturer's label.

A study by Lopes and others (1998) emphasized the important role of secondary hosts in dispersing the three-host tick *A. cajennense* to new areas. In areas where *A. cajennense* was present, one or more stages of this tick species were commonly found attached to equids, small mammals, birds, and domestic animals. The duration of attachment, or drop-off periodicity, varied by host species and a tick's life stage. On average, larvae and nymphs remained attached for 3 to 4 days. Results for the attachment times of adult ticks were inconclusive.

Nonchemical control of ticks

Information on a wide variety of tick control strategies is summarized in the "Tick Management Handbook" (Stafford, 2007) developed by the Connecticut Agricultural Experiment Station. For the purposes of this report, control methods directed primarily at populations of *Ixodes scapularis, Dermacentor variabilis, and Ambylomma americanum* ticks relative to Lyme disease and other zoonotic diseases will be emphasized. This report recommends viewing an area of possible tick exposure as a "tickscape." Using this approach, the potential of grasses, shrubs, and other vegetation to serve as tick habitat is considered in a landscape management plan. Modifications to desirable tick habitats serve to reduce the abundance of questing ticks and lessen host exposure.

Tickscape management plans should include habitat edges (i.e., ecotones) where a greater diversity of vegetation types is likely to occur. In addition to habitat management, other nonchemical methods of tick control focus on reducing populations or limiting movements of free-ranging wildlife hosts, such as deer. Examples of host-limiting habitat modifications include deer fencing, deer repellents, and substituting less palatable plants in a landscape. Although this approach works well in suburban residential landscapes, it might be impractical in rural areas.

Controlling ticks by using plants that ticks avoid has been tried in South Africa. Moyo and Masika (2009) reviewed various tick control methods used by resource-limited farmers in Eastern Cape Province, South Africa. Farmers there reported that the leaves of *Aloe ferox* (commonly known as Cape Aloe), a medicinal plant of the Asphodelaceae family, has insect and tick repellent properties. In addition, the bark of *Ptaeroxylon obliquum*, a member of the Rutaceae family, has repellent and acaricidal properties. Although no reports could be found on the incorporation of repellent and acaricidal plants in a landscape in the United States, this approach is worthy of consideration and further investigation.

Chemical control of ticks

Chemical control methods are effective in reducing host exposure to ticks, especially when appropriate chemicals are applied at times and locations that will have the greatest impact on the developmental stages of a tick, either on or off a host. To control multihost ticks that spend various amounts of time off a host, area-wide acaricides may be applied to tick habitats, such as woodland edges and grassy patches located near riparian areas. Area-wide acaricide treatments should correspond to times when larval and nymphal tick stages are most active. All chemicals used as insecticides or acaricides must be registered with the U.S. Environmental Protection Agency (EPA) and must be approved for areawide use or for use on a specific host species.

The most common compounds used in areawide control are carbamate insecticides (e.g., Sevin® with the carbaryl, 1-naphthyl methylcarbamate, as the active ingredient); natural pyrethrins (derived from seed cases of the *Chrysanthemum cinerariaefolium* plant and consisting of a combination of two stereoisomers, pyrethrin I and pyrethrin II, along with a cyclopropane core); and synthetic pyrethrins (often called pyrethroids). Pyrethrins have been recognized as neurotoxins of insects for nearly a century, following the report by Staudinger and Ružička (1924) of the insecticidal property of this natural compound. Staudinger and Ružička also reported that in low or nonlethal doses, pyrethrins had repellent properties. Pyrethrins are nonpersistent because they break down quickly when exposed to light and oxygen. Although pyrethrins do not persist in the environment, these compounds are toxic to many aquatic organisms, waterfowl, and bees (Aldridge, 1990; Anonymous, 1994). While human toxicity to pyrethrins is considered low, fatal asthma has been reported after the use of an animal shampoo containing pryethrin (Wagner, 2000).

A recent search of the National Pesticide Information Retrieval System was used to identify which EPA-registered pesticides are approved for the control of ticks on horses (Messenger, pers. comm., 2010). Active chemical ingredients in compounds registered with the EPA for the control of ticks on horses include: natural pyrethrins, pyrethroids (synthetic pyrethrins), coumaphos (an organophosphate insecticide), sulfur (a wettable sulfur powder), and tetrachlorvinphos (an organophosphate in a dust or wettable powder). These active ingredients are included in various formulations and marketed under various brand names. The acaricides on this list are also registered for use on cattle; however, more chemical compounds are registered for use on cattle than on horses. An important acaricide approved for use on cattle is amitraz (a triazapentadiene compound) which cannot be used on horses because it can cause irreversible gut stasis. The most common acaricide formulations for use on horses are natural and synthetic pyrethrins. Often these formulations include piperonyl butoxide as a synergist that inhibits rapid metabolism of the active compound by arthropods, thus prolonging the neurotoxic effects. Other synergists that may be added are: chloropyriphos, dichlorvos, or thiazolyn (Graf et al., 2004). Pyrethroid formulations frequently used for tick control on horses are: cypermethrin, deltamethrin, alphamethrin, tetramethrin, and prallethrin.

There are no systemic acaricides registered for internal use on cattle or horses. The high cost and the long development time needed to identify and synthesize potential acaricides, along with efficacy, safety, and environmental studies, have hindered the development of new products that control ticks on cattle or horses (Graf et al., 2005). Limiting factors in the development of new acaricidal products include improving product efficacy, reducing chemical resistance, and ensuring the safety of horses, humans, and the environment. When spraying a horse with an acaricide, all skin surfaces should be wetted, including the undercarriage. Acaricide should be wiped onto the surfaces of the pinna and false nostril. Dipping is the optimal method for applying acaricides, as this method ensures that all skin surfaces are wetted; however, this method is seldom used on horses.

A laboratory study by Drummond (1988) compared 17 formulations of 15 acaricides on 7 species of engorged female ticks. Effectiveness was measured as a reduction in the number of eggs produced per engorged female after exposure to a candidate acaricide. The most effective acaricides were chlorfenvinphos, lindane, chlorphyrifos, coumaphos, diazinon, permethrin, phosmet, amitraz, dioxathion, arsenic trioxide, malathion, tetrachlorvinphos, carbaryl, toxaphen, and ronnel. Tick species tested included *A. cajennense, A. nitens, B. annulatus,* and *B. microplus*. All compounds tested demonstrated toxicity to ticks; however, except for permethrin and coumaphos, these compounds do not meet EPA requirements for low human health risk, rapid environmental degradation, and low ecological toxicity to nontarget organisms (EPA, docket number: EPA-HQ-OPP-2008-0023; EPA 738-R-06-017, 2007). Studies by Jongejan and Uilenberg (1994) demonstrated that levels of tick infestation are usually decreased through the alternate use of chemical acaricides on animals and in the environment, while considering the seasonal dynamics of ticks. Nevertheless, these authors found the use of acaricides to be unsatisfactory due to problems of acaricide resistance in ticks, animal product contamination, and environmental residues.

Antiparasitic drugs such as ivermectin are approved for internal use in horses for controlling gastrointestinal parasites. The insecticidal and acaricidal properties of this macrocyclic lactone compound (22,23-dihydroavermectin B 1a + 22,23-dihydroavermectin B 1b) have been reported to be effective in the control of ticks in cattle and horses. Experimental studies on cattle conducted by Drummond (1985) demonstrated ivermectin's effectiveness against cattle grubs, mites, and eight species of ticks. Subcutaneous injections of ivermectin produced 95- to 100-percent control at lower doses than when this drug was administered by the oral route. Campbell and Benz (1984) reviewed the efficacy and safety of ivermectin in cattle, horses, sheep, swine, and dogs. The antiparasitic efficacy of ivermectin in the control of nematodes, mites, ticks, and parasitic flies was summarized for cattle. However, in horses the authors only reported efficacy studies to parasitic helminths, mostly intestinal nematodes. Efficacy of ivermectin in the control of ticks on horses was not reported.

USDA guidelines (USDA, 2008) for treating cattle, horses, and sheep with acaricides recommend dipping animals in vats containing an approved acaricide and strictly following the concentration amounts recommended on the label. When horses are imported to the United States for special events, APHIS–VS (USDA, 2003) requires all horses to be free of ticks or other external parasites. To ensure that this requirement is met, horses must be sprayed with a solution of dioxathion (p-dioxane-2,3 diyl ethyl phosphorodithioate) [trade name Delnav®], an organophosphate acaricide, which is also used to control insects and mites on citrus fruits, deciduous fruits, and nuts. An acceptable alternative to dioxathion is the use of 32.5-percent emulsifiable concentrate of Atroban®, a broad spectrum pesticide composed of permethrin (0.05 percent). In addition, veterinary examiners inspecting horses imported for special events must check the false nostril and external ear canal (inside pinna) for ticks and then treat these areas with one of the approved acaricides mentioned previously.

Use of vaccines in tick control and disease prevention

Another approach to controlling tick populations is inducing host-acquired immunity against ticks by active immunization with either crude, purified native, or recombinant antigens derived from ticks (Mulenga et al., 2000). Consequently, studies have focused on potent antigens for the development of efficacious vaccines (Willadsen, 2004). Two vaccines are commercially available for cattle: TickGARD Plus, (Rodriguez et al., 1995) and Gavac Plus (Willadsen et al., 1995), developed and tested in Cuba and

Australia, respectively. Both vaccines are constituted of the Bm86 antigen, which is a membrane-bound glycoprotein of 89kDa on the microvilli of tick-gut digestive cells (Rand et al., 1989; Willadsen et al., 1989; Gough and Kemp, 1993). Use of Bm86 antigen vaccine reduces the numbers and weights of engorged ticks, decreases egg laying capacity, and lowers egg viability leading to a decline of the *B. microplus* population (Kemp et al., 1989; Willadsen et al., 1995). An efficacy rate of 99 percent has been reported by the majority of studies testing the Bm86 antigen-based vaccine (Fragoso et al., 1998; de la Fuente et al., 2000a; de Vos et al., 2001; Canales et al., 2009).

The Bm86 antigen was originally identified in the cattle tick *B. microplus* and was successfully used against this same species; however, vaccine efficacy is inconsistent and usually much lower when using this antigen, or homologues of the antigen, in immunizing animals against other tick species, particularly in the most important genera of *Amblyomma, Hyalomma,* and *Rhipicephalus.* Vaccination has had variable efficacy, depending on the tick species used when testing vaccine efficacy (de Vos et al., 2001). This observation suggests that tick genetic and/or physiological differences may affect the efficacy of tick vaccines in different geographic regions (Fragoso et al., 1998; García-García et al., 1999, 2000; de la Fuente et al., 1999, 2000a, 2000b; de Vos et al., 2001; de la Fuente and Kocan 2006; Sossai et al., 2005; Canales et al., 2009). Several tick-antigen targets considered to date are from a limited range of functional classes. Such antigens consist of structural proteins, predominantly from salivary glands; hydrolytic enzymes and their inhibitors, mainly those implicated in hemostatic processes; and a group of membrane-associated proteins of indefinite function (Willadsen, 2006). Moreover, the use of an antigen mix to increase vaccine efficacy has been the subject of numerous experimental trials, with results that have been ambiguous and inconclusive (Willadsen, 2008).

Despite having new technologies available to identify antigens capable of eliciting a protective immune response against a tick infestation, the number of well-characterized antigens remains small and constitutes a significant challenge (Hope et al., 2010). Improving the existing Bm86 antigen vaccine is likely to rely on the discovery of other antigens that in combination might offer improved and multispecies efficacy. The choice of target antigens depends on whether an immunizing molecule can protect the host against both tick infestation and pathogen transmission. An ideal vaccine would be capable of reducing tick populations and protecting against infection by tick-borne pathogens and/or affecting tick vectorial capacity (de la Fuente et al., 1998, 2007; Rodríguez Valle et al., 2004). The high cost and technical problems associated with screening a sizeable number of tick-protective antigen candidates along with vaccination trials constitutes a major obstacle to obtaining approval for new vaccines. In recent research by Almazan and others (2010), antitick vaccination was shown to be a cost-effective alternative for the control of tick infestations, reducing the use of chemical acaricides and preventing selection of drug resistant ticks (de la Fuente et al., 2007).

Summary and recommendations

Tick vectors of EP pathogens are difficult to detect on equids and in their environment, and control is a challenge. The best approach is to use the principles of integrated pest management and incorporate multiple strategies aimed at various life stages, multiple hosts, and a variety of landscapes. Limiting tick access to equine hosts by developing landscapes poorly suited to tick-host interaction and use of tick repellents or repelling acaricides are initial approaches to avoiding exposure. Ivermectin shows promise in the systemic control of blood-feeding ticks. Although ivermectin has potential as a method of tick control, the impact of this compound on the epidemiology of EP is uncertain, because an infected tick may still transmit sporozoites to a host before being affected by ivermectin. In addition, ivermectin is not known to have antiparasitic properties against *B. caballi* or *B. equi.* Meanwhile, efforts should be made to obtain formal approval of ivermectin as a systemic acaricide. For long-term prevention and control, it is important to have one or more vaccines directed against blood-feeding ticks and against *B. caballi* and *T. equi* sporozoites, or other stages of the hemoprotozoans that can be used to elicit a protective immune response. More research is needed in the development of effective multispecies tick vaccines that protect horses from both tick vectors and the infectious organisms that cause piroplasmosis. Having preventive tickscapes, effective repellents, external acaricides, systemic acaricides, and specific vaccines will allow for the use of integrated pest management strategies that will limit host resistance to chemicals and provide long-term control options.

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