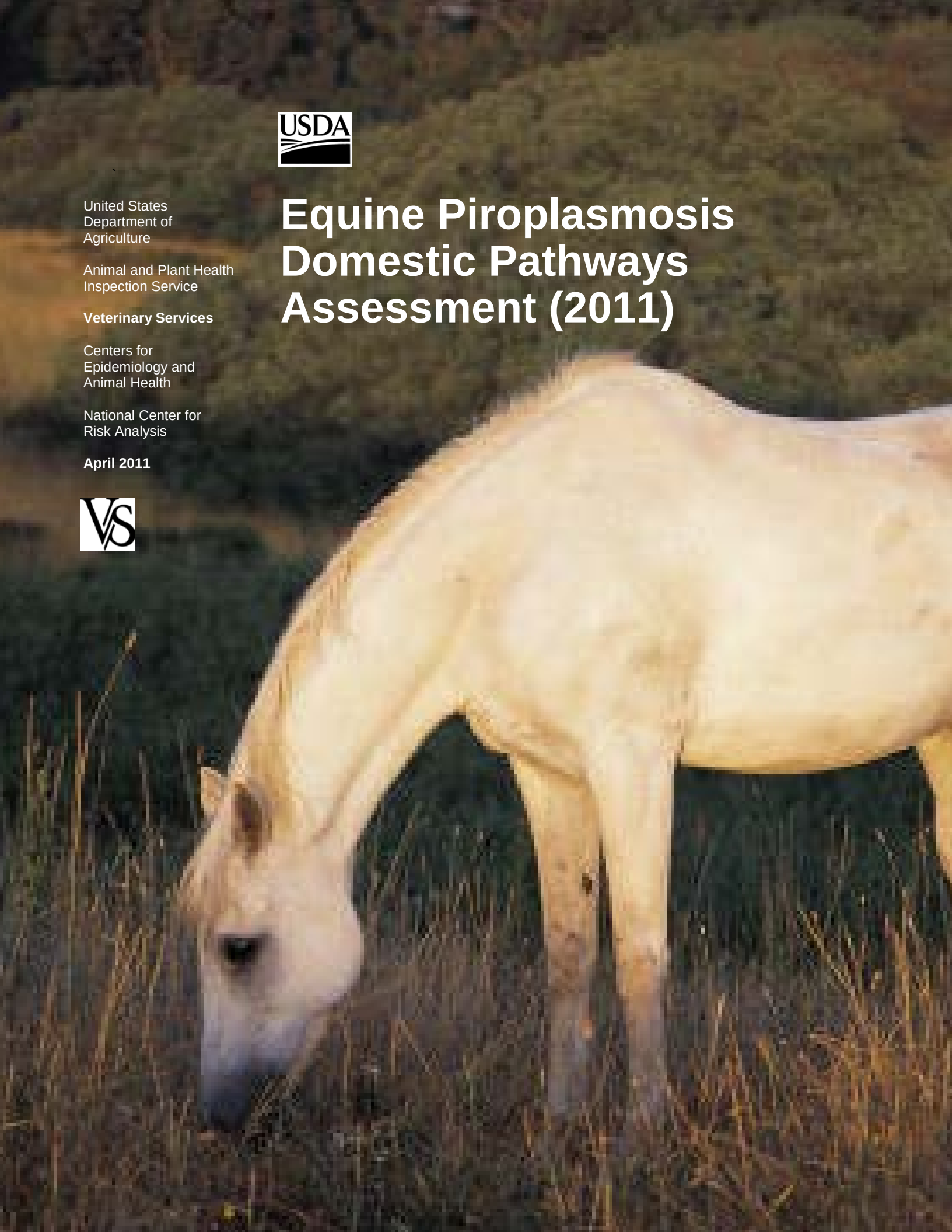

Equine Piroplasmosis Domestic Pathways Assessment (2011)

United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Centers for Epidemiology and Animal Health

National Center for Risk Analysis

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Abstract: Equine piroplasmosis (EP) is a tick borne disease of equids. It is considered a foreign animal disease in the United States. However, from January 2009 through November 2010, 542 confirmed positive cases have been identified in 16 different States. This domestic pathways assessment evaluates the risk of releasing an EP pathogen (*Theileria equi* or *Babesia caballi*) from a quarantined premises through movement of horses. In addition, this assessment evaluates the risk of disease transmission by ticks, vertical transmission, or iatrogenic transmission.

When an acaricide is applied correctly, the risk of EP transmission by ticks to a horse is low. In addition, infected reservoir hosts, environmental factors, and competent vectors must be present for the disease transmission cycle to occur. Vertical transmission of *T. equi* is considered a moderate risk pathway and the risk of vertical transmission of *B. caballi* is negligible.

Iatrogenic transmission via whole blood transfusion, blood doping, commercial serum/blood plasma, and contaminated equipment poses the highest risk of disease transmission. Blood is an efficient vehicle of transmission for EP pathogens and even a small volume of blood can be infectious. Exposure of an uninfected horse to any of these pathways is likely to result in EP transmission. Iatrogenic exposure may be difficult to regulate. Management practices such as testing blood donors would help mitigate this risk but these practices vary throughout the equine industry.

The overall risk of EP spread by the movement of a horse from a quarantined premises is moderate.

Keywords: Equine piroplasmosis, *Babesia caballi*, *Theileria equi*

Questions or comments on data analysis, contact: Dr. Katie Portacci (970) 494-7000: E-mail: CEAH@aphis.usda.gov

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Equine Piroplasmosis
Domestic Pathways Assessment (2011)

Pathways assessment for the spread of the causative agents of equine piroplasmosis from the movement of a horse from a quarantined premises within the contiguous United States.

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Authors:

Mr. Tim Clouse, USDA:APHIS:VS:CEAH:NCRA

Dr. Julie Gauthier, USDA:APHIS:VS:NCIE

Dr. Robert Harris, USDA:APHIS:VS:CEAH:NCRA

Dr. Angela James, USDA:APHIS:VS:CEAH:NCRA

Dr. Katie Portacci, USDA:APHIS:VS:CEAH:NCRA

Dr. Laurel Voelker, USDA:APHIS:VS:NCIE

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Acronyms

APHIS	Animal and Plant Health Inspection Service
cELISA	competitive inhibition enzyme-linked immunosorbent assay
CF	complement fixation test
EP	equine piroplasmosis
IFAT	indirect fluorescence antibody test
IPM	integrated pest management
LAMP	loop-mediated isothermal amplification
MAb	monoclonal antibody
NAHMS	National Animal Health Monitoring System
NVSL	National Veterinary Services Laboratories
OIE	World Organization for Animal Health
PCR	polymerase chain reaction
USDA	United States Department of Agriculture
VS	Veterinary Services

Definitions

confirmed positive horse	A horse that has tested positive for an EP pathogen with either a complement fixation test (CF) or a competitive enzyme-linked immunosorbent assay (cELISA) conducted by NVSL. A horse can be classified as a confirmed positive case without showing evidence of clinical disease of EP. (USDA 2009)
contiguous United States	The 48 United States that have common land borders with each other.
biocontainment	Prevention of disease spread within or between operations.
biosecurity	Prevention of disease introduction.
EP pathogen	*Babesia caballi* or *Theileria equi.*
exposed horse	A horse in the same herd as a confirmed positive horse or a horse that has had recent direct and sustained contact with a confirmed positive horse, as determined by State and Federal regulatory officials in consultation with epidemiologist. (USDA 2009)
exposure assessment	The exposure assessment estimates the likelihood of exposure and the risk of transmission if an uninfected horse is exposed to the EP pathogen by: ticks, vertical transmission, or iatrogenic transmission of blood or blood components.
exotic	Not known to be present in the contiguous United States.
gold standard	Gold standard test refers to a diagnostic test or benchmark that is regarded as definitive.
high risk	This event would be very likely to occur.
horse	*Equus caballus*

infected undetected horse	A horse that is infected but has been tested with either a complement fixation (CF) test or a competitive enzyme-linked immunosorbent assay (cELISA) conducted by NVSL and results indicate the animal is not infected.
intrastadial transmission	Transmission of *B. caballi* or *T. equi* to a horse by a tick that acquired the pathogen during the same lifecycle stage.
known donor	A horse used as a blood donor and whose identity is “known” by the teams performing the transfusion.
low risk	The event would be unlikely to occur.
moderate risk	This event would occur with an even probability.
negligible risk	The event would be unlikely to occur.
operation	An area of land managed as a unit by an individual, partnership, or hired manager.
potential tick vector	Any tick species considered competent to transmit *B. caballi* or *T. equi* to a horse.
additions	A horse purchased from outside an operation and transported to the purchasing operation for housing, training, breeding, riding, or racing.
quarantined premises	Premises with at least one confirmed positive case of EP and that is subject to the biosecurity measures outlined in VS Memorandum 555.20 low-risk premises. (USDA 2009)
race horse	A horse whose primary use is competition on sanctioned or unsanctioned race tracks.
release assessment	The release assessment estimates the likelihood that an EP pathogen will be released from a quarantined premises. The pathways for release examined were confirmed positive horse and a test negative exposed horse.
show/event horse	A horse used primarily in competition other than racing.
sanctioned racing	Horseracing conducted by the approval and regulation of State authority and/or racing commissions.
spread	New infection of at least one horse or tick vector with an EP pathogen.
trail and ranch horses	Horses used for farm, ranch or other noncompetitive uses.
transovarial transmission	Transmission of *B. caballi* or *T. equi* from a female tick to its offspring by infection of the eggs in the ovaries of the tick.
transstadial transmission	Transmission of *B. caballi* or *T. equi* to an equid by a tick in a lifecycle stage subsequent to the stage in which the tick acquired the pathogen.
unsanctioned racing	Horseracing conducted without the approval and regulation of State authority or official racing commission.

EXECUTIVE SUMMARY

Equine piroplasmosis (EP) is a tick borne disease of equids and is considered an exotic disease in the United States. From January 2009 through November 2010, 542 confirmed positive cases were identified in 16 different States (Figure 1). Of those, 412 cases were associated with one outbreak initiating in Texas. The National Equine Piroplasmosis Working Group, consisting of industry, State, and Federal representatives, developed long-term recommendations for the management and removal of EP in the United States. One recommendation was to conduct an assessment to estimate the risk of EP spread posed by the interstate movement of horses from a quarantined premises to events or other premises.

Release Pathways

This pathways assessment evaluates the risk of releasing an EP pathogen (*Theileria equi* or *Babesia caballi*) from a quarantined premises through movement of horses. The release pathways considered were:

1) movement of confirmed positive horses, and
2) movement of infected undetected horses.

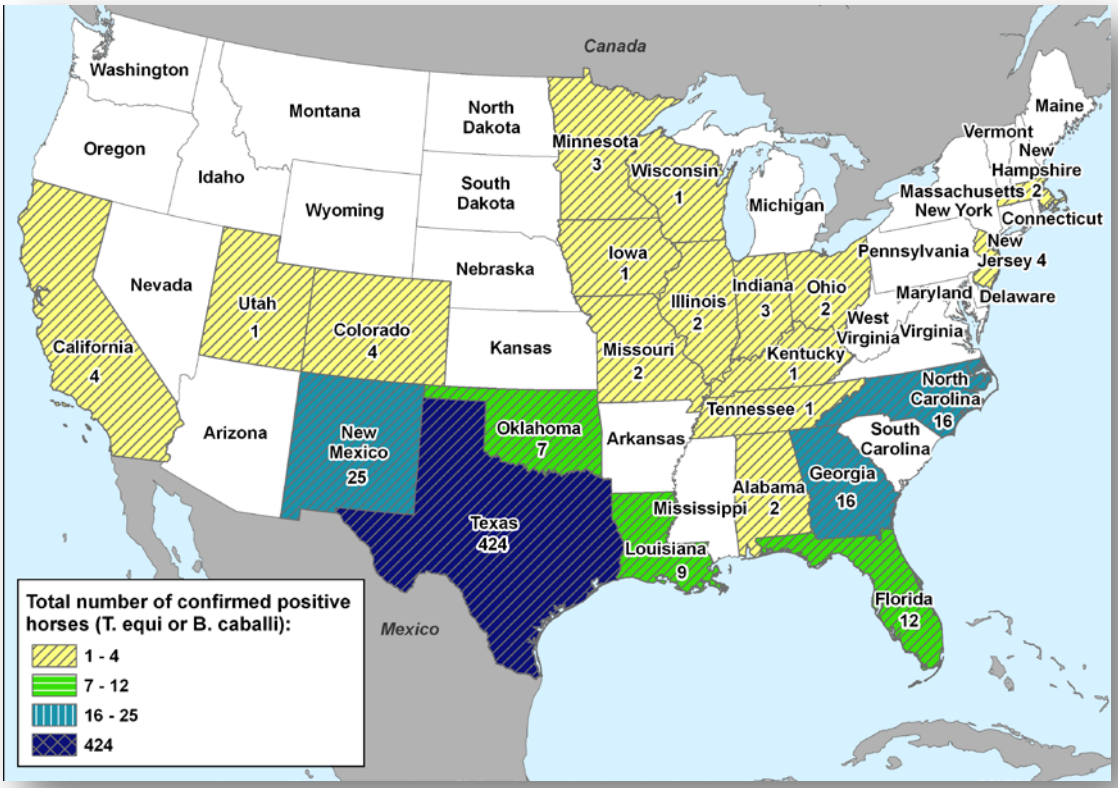

Figure 1. Total number of horses confirmed positive with equine piroplasmosis from January 2009 through November 2010.

The testing protocol outlined in VS Memorandum 555.20 results in a low risk that an infected horse will test negative for EP (i.e., infected undetected horse) when tested with both cELISA and CF. The risk is moderate if a horse is tested with cELISA alone. If a horse is confirmed positive, the likelihood that the horse is truly infected with an EP pathogen is high; therefore, the risk of releasing an EP pathogen via this pathway is high.

Exposure Pathways

The exposure pathways assessed for EP transmission to an uninfected horse were ticks, vertical transmission, and iatrogenic transmission via blood contamination. In this document, the risk associated with an exposure pathway is based on the ability of that pathway to result in transmission. Horse to horse contact alone is not sufficient for transmission of EP. If an EP pathogen is released onto a premises, the uninfected horse must be exposed to blood from an infected horse via mechanical or tick transmission.

The risk is low that an infected tick would remain attached to a horse moving off a quarantined premises when acaricide is applied correctly. Within a tick population, EP infections quickly die out without the presence of infected hosts because each generation of tick must be exposed to the organism. Therefore, EP is only maintained if at least one infected horse is present in the population. As a result, the risk of continued spread of an EP pathogen by ticks alone is low.

Photo by Angela M. James, USDA:APHIS:VS:CEAH:NCRA. Cumberland Island, GA. 1996.

Vertical transmission of *T. equi* from mares is a moderate risk pathway. Because transmission has not occurred between mares infected with *B. caballi* and their offspring, vertical transmission of *B. caballi* is a considered negligible.

Blood is an efficient vehicle of transmission for EP pathogens. Even a small volume of blood can be infectious. Of the iatrogenic exposure pathways assessed, whole blood transfusion, blood doping, commercial serum/blood plasma, and contaminated equipment are all considered high-risk pathways. Germplasm is the only iatrogenic exposure pathway considered in this assessment to pose a negligible risk.

In order for a horse to become infected by the exposure pathways described above, the horse must have the opportunity for sufficient contact with an infected population. Biosecurity practices vary throughout the equine industry. As a result, it is difficult to estimate the impact of mitigation measures other than regulatory requirements. Because of the continued monitoring, acaricide treatment, and identification of confirmed positive horses as outlined in this document, these

horses would have little opportunity to expose uninfected horses. In contrast, infected undetected horses have no requirements for identification. They also do not require acaricide treatment or additional testing after leaving the quarantined premises. This population poses a much greater risk of exposing uninfected horses to EP agents.

Summary

In summary, if an infected undetected horse releases EP from a quarantined premises, iatrogenic mechanisms pose the greatest risk for transmission to a new horse. This is due to the uncertainty about biosecurity practices, the large number of organisms present in a small volume of blood, and the small infectious dose. Iatrogenic transmission via blood or blood products has been the cause of several outbreaks worldwide. It is unknown how frequently practices such as blood doping or sharing of equipment occur throughout the industry. Currently, test negative horses leaving a quarantined premises have no requirements for identification or continued monitoring to ensure these horses are not infected undetected. The overall risk of this pathway is moderate.

If a confirmed positive horse releases EP from a premises, iatrogenic transmission may still occur. In addition, vertical transmission may occur if these horses are bred. The overall risk posed by the movement of confirmed positive horses is moderate.

1. Introduction

1.1. Background

The United States was considered free of equine piroplasmosis (EP) in 1988. Since 2009, 16 States have identified at least one EP case related to previous importation or trace investigations from other affected premises. Of those cases identified, 124 cases have been associated with *T. equi* (Figure 2) and 6 cases have been associated with *B. caballi* (Figure 3). In addition, as of November 1, 2010, 412 horses (out of a total population of 2,489 horses tested) were confirmed positive to *T. equi* (Figure 4) associated with an outbreak from one-affected premises in Texas.

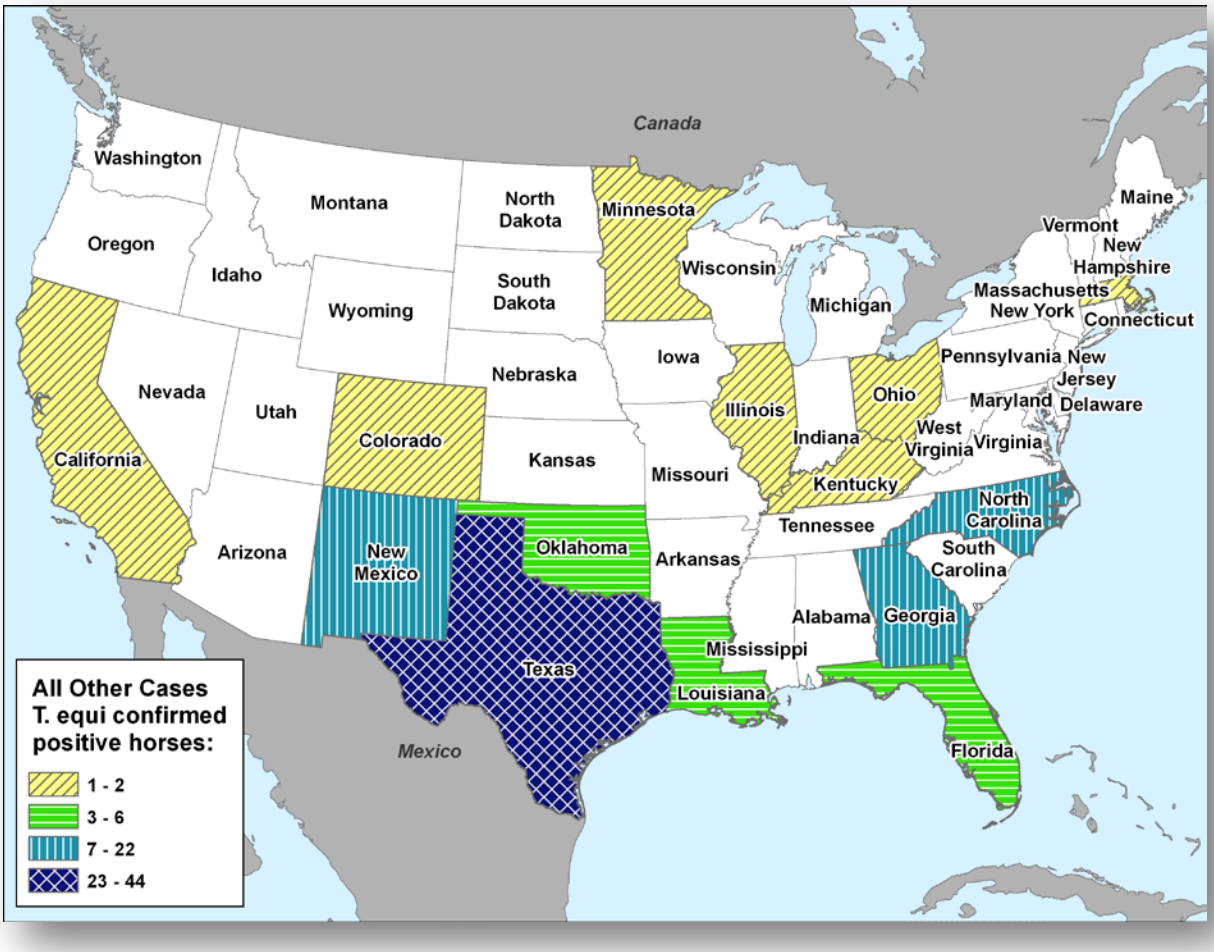

Figure 2. The number of horses confirmed positive with *T. equi* in the United States from November 1, 2009 through November 1, 2010, excluding those associated with the Texas outbreak.

Figure 3. *B. caballi* confirmed positive horses from November 1, 2009 through November 1, 2010.

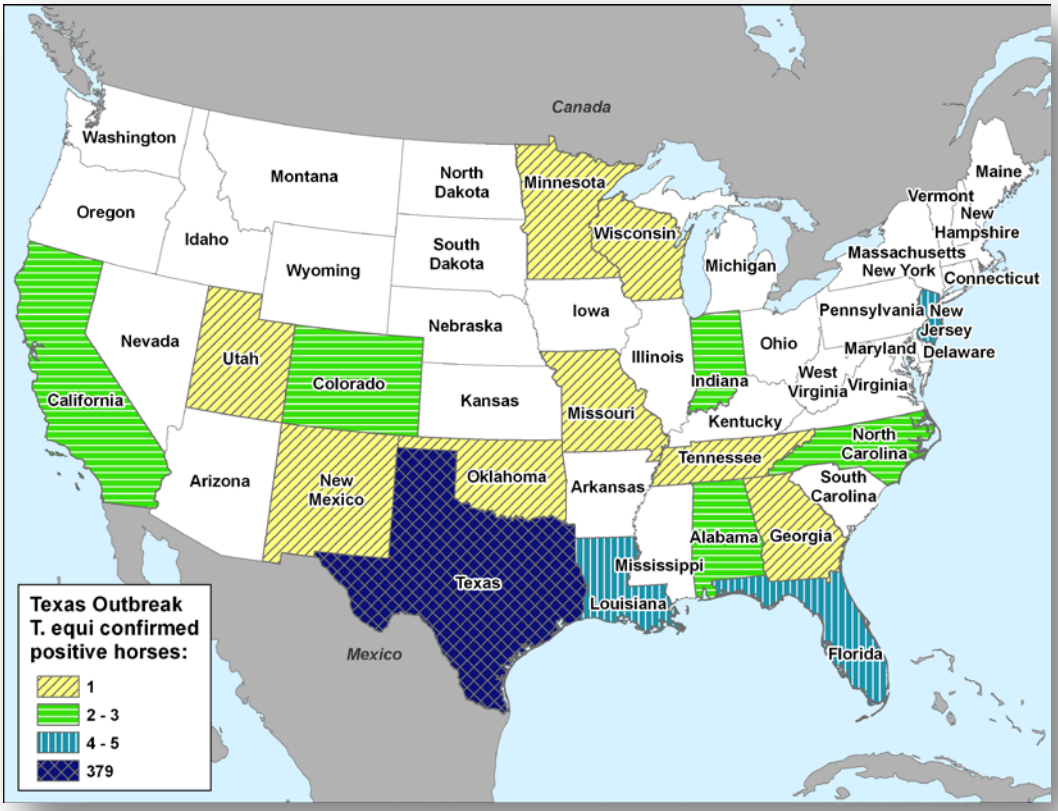

Figure 4. Number of horses confirmed positive with *T. equi* associated with an outbreak initiating in Texas from October 2009 through November 2010.

The National Equine Piroplasmosis Working Group, which consists of industry, State, and Federal representatives, was established to provide guidance to USDA:APHIS:VS and the States to control EP. In April 2010, the group requested a risk assessment be conducted to estimate the risk of EP spread posed by horses moving off a quarantined premises.

The objective of this assessment is to identify the likelihood that the interstate movement of a horse off a quarantined premises will result in at least one horse in a new location becoming infected with an EP pathogen. This risk assessment evaluates the efficacy of current management practices for quarantined premises, as well as an option to allow confirmed positive horses to attend shows, races, or other equid events. Results of this work will be provided to the National EP Working Group and VS to strengthen current management practices and inform policy.

1.2. Methods

The process used in this assessment is a modification of OIE's guidelines for import risk analysis (OIE 2008), which consists of a hazard identification, release assessment, exposure assessment, consequence assessment, and an overall risk estimation. Because the objective is to identify the likelihood of at least one horse becoming infected, this assessment does not consider the magnitude of the effect (i.e., biological or economic consequences) once a new horse becomes infected. Without this consequence assessment, this analysis is referred to as a "Pathways Assessment" rather than a "Risk Assessment." A pathways assessment describes the biological pathways necessary for the pathogen to spread from the infected population to the uninfected population of concern, and the likelihood of these events occurring. The hazards identified are *B. caballi* and *T. equi*, the causative agents of EP.

In this assessment, the infected population is defined as horses on quarantined premises that are infected with an EP pathogen. The infection in each of these horses might be confirmed by a positive EP test result, or it might be undetected because of a false negative EP test result. The uninfected population at risk is any uninfected horse exposed to the infected population through movement of an infected horse from a quarantined premises. The likelihood that a horse that tests negative on a quarantined premises is infected was estimated, based on USDA:APHIS:VS' approved testing protocol, through a stochastic simulation model using risk and decision analysis software @RISK by Palisade Corporation (Palisade 2009).

Published literature and reports from recent outbreaks were used to identify the potential release and exposure pathways. Appendix A. Literature Review of EP Release and Exposure summarizes the results of a literature review aimed at determining sources of introduction of EP to new areas and means of spread either following introduction or in an enzootic area. Worldwide, importation of infected horses is the only introduction pathway, which has been reported to lead to outbreaks of EP in regions in which the disease was not enzootic. As the table shows, EP has been shown to spread from infected to uninfected horses via ticks, vertical transmission, and iatrogenic transmission such as needle sharing or syringe reuse.

The *release assessment* estimates the likelihood that an EP pathogen will leave a quarantined premises. The release pathways considered were:

a. A confirmed positive horse temporarily leaving the premises

b. An infected, undetected horse permanently leaving the premises

Given that the pathogen successfully left a quarantined premises, the *exposure assessment* estimates the likelihood that exposure to a specific pathway would result in transmission to an uninfected horse. The exposure pathways considered include:

a. Ticks
   - An infected tick attached to a horse leaving a quarantined premises
   - Establishment of infection in the tick population on a new premises

b. Vertical transmission

c. Iatrogenic transmission via blood or blood components such as:
   - Whole blood transfusion (for medical purposes)
   - Blood doping (for non-medical purposes)
   - Commercial serum/plasma products
   - Equipment (e.g., needles and dental or tattoo equipment)
   - Germplasm

Infected ticks may attach to other hosts, which may move off a quarantined premises, however this assessment is specifically addressing the interstate movement of horses and did not consider other host movements off the premises.

While the risk of transmission associated with each of these exposure pathways can be described (Figure 5), the likelihood of exposure to an EP pathogen is more difficult to estimate due to uncertainty about management practices throughout the industry or the use of biosecurity or other mitigation measures. The baseline mitigation measures evaluated were current Federal regulations and recommendations. A general description of risk specific to certain sectors was provided.

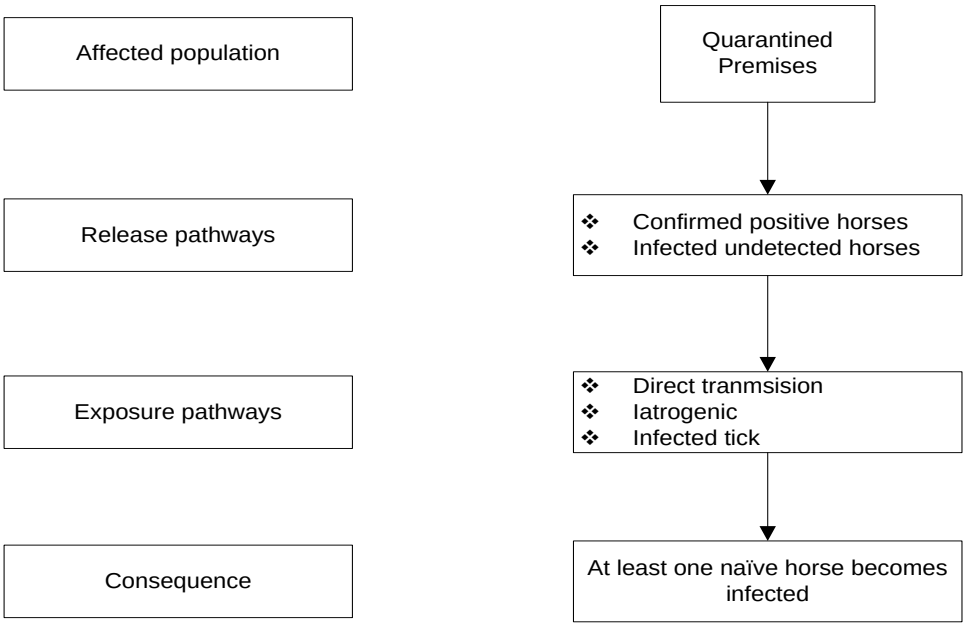

Figure 5. Pathway scenario for the general equine population.

The overall estimation of risk is based on the risk of release, risk of transmission from exposure, and likelihood of exposure.

1.3. Risk and Uncertainty Estimation

For each pathway, the likelihood that the pathway would result in the EP pathogen leaving the premises (release) or infection of a new horse (exposure) was estimated through qualitative or quantitative methods. Both results were communicated through a qualitative risk ranking system described in Table 1.

Table 1. Definitions for risk estimates.

Risk estimation	Descriptive definition of qualitative results	Probability outcome for quantitative results
High	This event would be very likely to occur	>40%
Moderate	This event would occur with an even probability	1-39.999999%
Low	The event would be unlikely to occur	.000001-1%
Negligible	The event would almost certainly never occur	<.000001%

In addition, the degree of uncertainty was captured based on the level of information available for qualitative estimates (Table 2).

Table 2. Level of uncertainty about the likelihood estimates.

Uncertainty category	Definition
Low	The data available are solid and complete. Multiple published references or reliable databases and records are available. Different sources are generally in agreement.
Medium	Some, but not complete data are available. A small number of published references or reliable databases and records are available. If personal communication or anecdotal evidence is used in combination with published information, then it is from multiple reliable sources that are generally in agreement.
High	No published data are available. The only evidence is in the form of personal communications, anecdotal reports, or unpublished data.

1.4. Assumptions

The baseline mitigation measures that were considered for the release assessment were based on the VS Memorandum 555.20. It is assumed that horses on quarantined premises will be treated as test negative horses on Low-Risk premises (as defined in the VS Memorandum), which initial intervention includes:

- All exposed horses are tested for EP and negatives are retested 30 days later
- A 10-foot separation is maintained between negative and positive horses
- The facility is inspected by State animal health authority
- Vegetation is minimized and an acaricide is applied
- Treating horses with acaricide twice
- Acaricide application is consistent with VS Memorandum 556.1.

In addition, horses with a negative test within 30 days and with acaricide application within 14 days can move off the premises permanently. No additional testing or treatment is required.

VS Memorandum 555.20 does have provisions for the interstate movement of test-positive horses. Therefore, test-positive horses were assumed to move temporarily and consistent with a combination of draft measures outlined by the Texas Animal Health Commission (2009) and USDA:APHIS:VS (unpublished). Management of grounds on the premises was not considered as a standard mitigation because it was not a standard recommendation.

The mitigations applied to the movement of test positive horses in this assessment include:

- A permit is issued by the State or Federal Animal Health Agency prior to movement.
- Horses are spray treated with a pyrethroid not less than 24 hours or more than 14 days prior to any movement.
- Horses are sprayed with acaricide every 14-18 days while off the premises.
- Horses have a unique identifier and their location and transport monitored.
- Horses return within one day of finishing the event.
- Horses will not be accepted as blood donors.
- Foals born to positive mares will be maintained under hold order until they are weaned /separated from the mare and have negative cELISA, CF, and PCR tests at a minimum of 6 months of age, and met the requirements listed above.

2. Hazard Identification

Epidemiology describes the distribution and determinants of a disease in the population. The epidemiology of a disease is an important component in estimating risk because it describes how a pathogen is transmitted as well as host susceptibility. In order for a disease to occur the appropriate agent, host, and environmental conditions must be present. Equine piroplasmosis is a tick-borne parasitic infection of horses, mules, donkeys and zebras (Friedhoff and Soulé 1996).

2.1. Agents

Equine piroplasmosis is caused by the protozoan parasites *Babesia caballi* or *Theileria equi*. The etiologic agents of EP, *B. caballi* and *T. equi*, have complex life cycles that include obligate sexual stages in the guts of their tick vectors. Consequently, only ticks that are competent vectors (ticks that are capable of supporting the development of the parasite) biologically transmit these parasites.

The nomenclature of *T. equi* (versus *B. equi*) has been debated due to the developmental phase of *T. equi* in the lymphocyte (Ali, Sugimoto et al. 1996). OIE guidelines state that: "*Theileria equi* was previously designated as *Babesia equi* but compelling evolutionary, morphologic, biochemical, and genetic evidence supports its reclassification as a *Theileria*" (OIE 2009a). This document will remain consistent with OIE terminology.

The following table demonstrates some key characteristics and differences between the two organisms.

Table 3. Characteristics and key differences between *B. caballi* and *T. equi.*

	B. caballi	*T. equi*
Distribution in the United States; Nov 2009-Nov 2010 (USDA Sit Rep, Nov 2010, unpublished)	6 positive horses in 5 States	124 positive horses in 16 States plus an ongoing outbreak investigation originating from Texas that includes 412 horses
Tick Transmission	Zygotes can be found in various organs of tick vectors and must transmit transovarially from egg to larva to be found in the salivary glands (Uilenberg 2006)	Zygotes develop in salivary glands of tick vector and not found in other tick organs; not transmitted transovarially from egg to larva (Uilenberg 2006)
Vertical Transmission	Intrauterine infections have been reported but are rare and usually lead to abortion (de Waal 1992)	Mares can transmit the infection throughout their lives to their offspring, resulting in abortions, stillbirths or carrier offspring (de Waal 1992)
Infection in horses	Development does not include a lymphocyte stage (Uilenberg 2006) and does not cause adhesions of infected erythrocytes to vascular endothelium (Ali, Sugimoto et al. 1996).	Initial development in the lymphocyte with further development and asexual reproduction in erythrocytes (Uilenberg 2006)
Incubation period	10 to 30 days (de Waal 1992)	12 to 19 days (de Waal 1992)
Parasitemia	0.1-10% (Ali, Sugimoto et al. 1996)	May be >20%, but 1-5% most common (Ali, Sugimoto et al. 1996)

	B. caballi	*T. equi*
Clinical Signs	Infections are more likely to be unapparent or mild (Ali, Sugimoto et al. 1996). However, kinin releases may lead to cerebral babesiosos (Ali, Sugimoto et al. 1996)	Variable
Immune Response	Once described as self-limiting but lasting up to 4 years after initial infection, however, may be lifelong but undetected by current tests (Rothschild and Knowles 2007)	Cannot be eliminated from the body by the immune response; even with treatment the horses remain infected for life (Rothschild and Knowles 2007)
Diagnosis: Blood Smear	Merozoites are seen exclusively in erythrocytes and typically as pairs joined at the posterior end (de Waal 1992)	In macrophages early in infection. Visualized in erythrocytes as merozoites in a Maltese-cross formation of 4 pyriform parasites (de Waal 1992)
Diagnosis: serology	Higher Se/Sp on Complement Fixation compared to *T. equi* on CF	Higher sensitive (Se) and specific (Sp) on cELISA compared to *B. caballi* on cELISA

2.2. Host

2.1.1. Susceptibility

Horses in endemic regions may have a low case fatality rate (5-10 percent) (Rothschild and Knowles 2007). However, the case fatality rate may increase significantly among naïve mature horses (Maurer 1962; Rothschild and Knowles 2007). Persistent infection of *T. equi* is thought to be a common cause of abortion in endemic regions (de Waal 1992; Lewis, Penzhorn et al. 1999). Susceptibility does not appear to vary with age (Acici, Umur et al. 2008) or sex (Asgarali, Coombs et al. 2007).

Clinical signs vary from acute death in the peracute form (rare); fever, anorexia, anemia, and lethargy in the acute form (most common): and reduced performance and weight loss in the chronic form. Transmission occurs through ticks as biological vectors, or through mechanical transmission by iatrogenic inoculation with infected blood (Ali, Sugimoto et al. 1996). Vertical transmission of *T. equi* also occurs in horses. Dual infections with both organisms have been reported and cross-immunity does not occur.

2.2.2. Description of the Equine Population

Currently no accurate estimate of the current total number of horses in the United States exists. According to the 2007 Census of Agriculture, the total inventory of horses in the United States is 4,028,827 on 575,942 farms (Figure 6). However, this census only counts horses that are on a farm, or places that sells $1,000 of agriculture products or has 5 or more equids (other than commercial enterprises such as racetracks) (VS 2007).

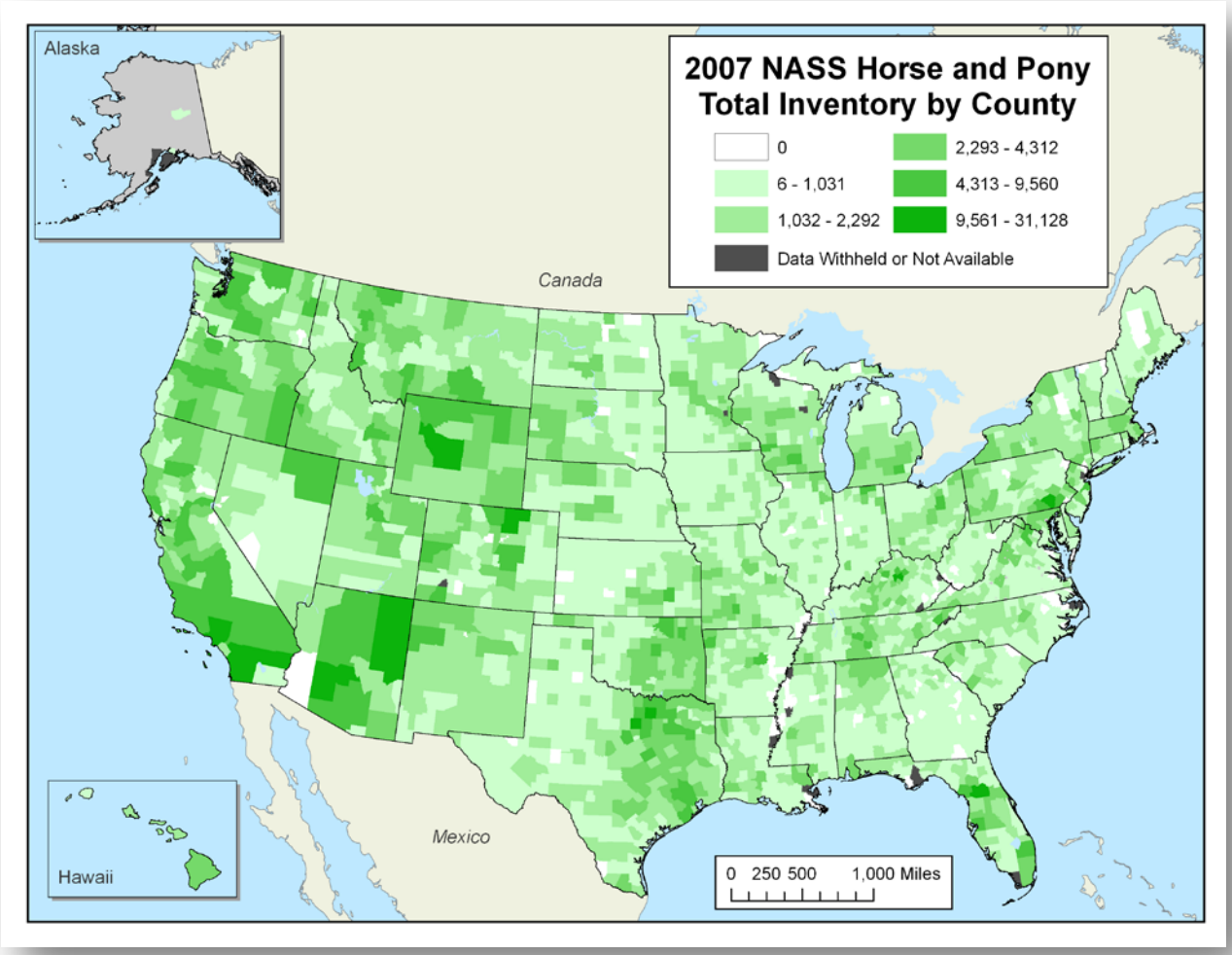

Figure 6. Horse and Pony Inventory, NASS 2007

The United States Department of Agriculture also described U.S. equine populations in a survey of operations with 5 or more horses in 28 States and 4 regions representing 78 percent of all horses and 78.6 percent of all premises (VS 2007). The highest rate of household horse ownership was in the Mountain Region (3.2 percent of households), followed by the Central (2.6 percent) and South Central (2.8 percent) Regions. The Pacific region was intermediate (2.1 percent), followed by the North Central (1.4 percent) and Atlantic regions at about 1 percent. Small operations (5-9 animals) contained about 66 percent of the horse population, medium operations (10-19 horses) contained about 26 percent, and large operations (20 or more horses) contained about 8 percent of the total population. The horse population in the United States is distributed between private users such as ranches, farms, and hobby owners and commercial operations engaged in breeding, boarding, training, racing, and showing. By function, about 40 percent of premises were farms or ranches, 37 percent were personal use, and the remaining 23 percent of operations were primarily boarding and breeding facilities (NAHMS 2006) . The pet horse population was estimated at about 7.3 million horses housed on about 2.1 million facilities and households (AVMA 2007).

Equine piroplasmosis has been identified in Quarter Horse racehorses, Thoroughbred racehorses, and ranch horses during 2009 through November 2010.

2.3. Vector

Equine piroplasmosis is primarily a tick-borne disease. There are three main modes of tick-borne transmission of *B. caballi* parasites: transstadial, intrastadial, and transovarial. Tick borne-transmission of *T. equi* can occur through transstadial or intrastadial mechanisms; transovarial transmission of *T. equi* in ticks is uncertain or absent. Transstadial transmission occurs when a tick (larval or nymphal) acquires the pathogen from an infected host in one lifecycle stage and then transmits it to another host in the next lifecycle stage. 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 and transmits to a naïve host without development or molting to another life stage. Transovarial transmission occurs when an infected female tick passes the pathogen to eggs, resulting in infected offspring (Ueti, Palmer et al. 2008).

Historically, *Anocentor (Dermacentor) nitens*, the tropical horse tick, was thought to be the only known natural vector of EP (*Babesia caballi*) in the United States (Roby and Anthony 1963). Recent evidence suggests that *D. variabilis*, American dog tick and *Amblyomma cajennense*, the cayenne tick, may be natural vectors of *T. equi* as demonstrated with the field collection of adults and transmission of *T. equi* to naïve horses (Scoles 2010).

Rhipicephalus microplus is an experimental vector of *T. equi*, and evidence is growing that *R. microplus* is likely a natural vector of *T. equi* in subtropical and tropical regions of the Americas (Knowles, Kappmeyer et al. 1992; Guimarães, Lima et al. 1998; Heuchert, de Giulli Jr. et al. 1999; Battsetseg, Lucero et al. 2002). Transstadial transmission of *T. 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 (Stiller, Goff et al. 2002; Ueti, Palmer et al. 2005). Additionally, *R. microplus* males can acquire *T. equi* parasites from chronically infected horses and transmit the parasites to naïve horses through intrastadial transmission (Ueti, Palmer et al. 2008).

In addition to the four species of ticks that are proven or suspected to be natural EP vectors, *Dermacentor albipictus*, the winter tick, has been shown to transmit *B. caballi* and *T. equi* under laboratory conditions. *Rhipicephalus sanguineus*, brown dog tick, has been reported as a vector of *B. caballi* and *T. equi*, but there is no evidence that this tick species is an EP vector in the United States (Kouam, Kantzoura et al. 2010). A list of competent vectors with evidence for vector competence is located in Table 4. A map of potential vector distribution can be found in Figure 7.

2.4. Environment

In addition to tick borne transmission, EP pathogens may be transmitted mechanically through fomites contaminated with blood. Blood infected with *Babesia microti*, the causative agent of human babesiosis, may remain infective for up to 3 days at room temperature and up to 17 days with refrigeration (Eberhard, Walker et al. 1995). Survival of other *Babesia species* in blood is presumed to be similar to *B. microti*.

Table 4. Known natural and experimental EP tick vectors in the United States.

	Babesia caballi		*Theileria equi*		
	Evidence of competence	**Mode of transmission**	**Evidence of competence**	**Mode of transmission**	**Distribution**
R. microplus			Experimental	Transstadial and Intrastadial	Limited to southern Texas
A. nitens	Natural	Transovarial Transstadial			Limited to southern Texas
D. variabilis	Experimental	Transovarial	Natural and Experimental*	Intrastadial	Wide distribution across the United States
A. cajennense			Natural and Experimental*	Intrastadial	Limited to southern Texas
D. albipictus	Experimental	Transovarial			Wide distribution across the United States

Adapted from (Stiller and Coan 1995; Stiller, Goff et al. 2002; Ueti, Palmer et al. 2008)
**(Scoles 2010)*

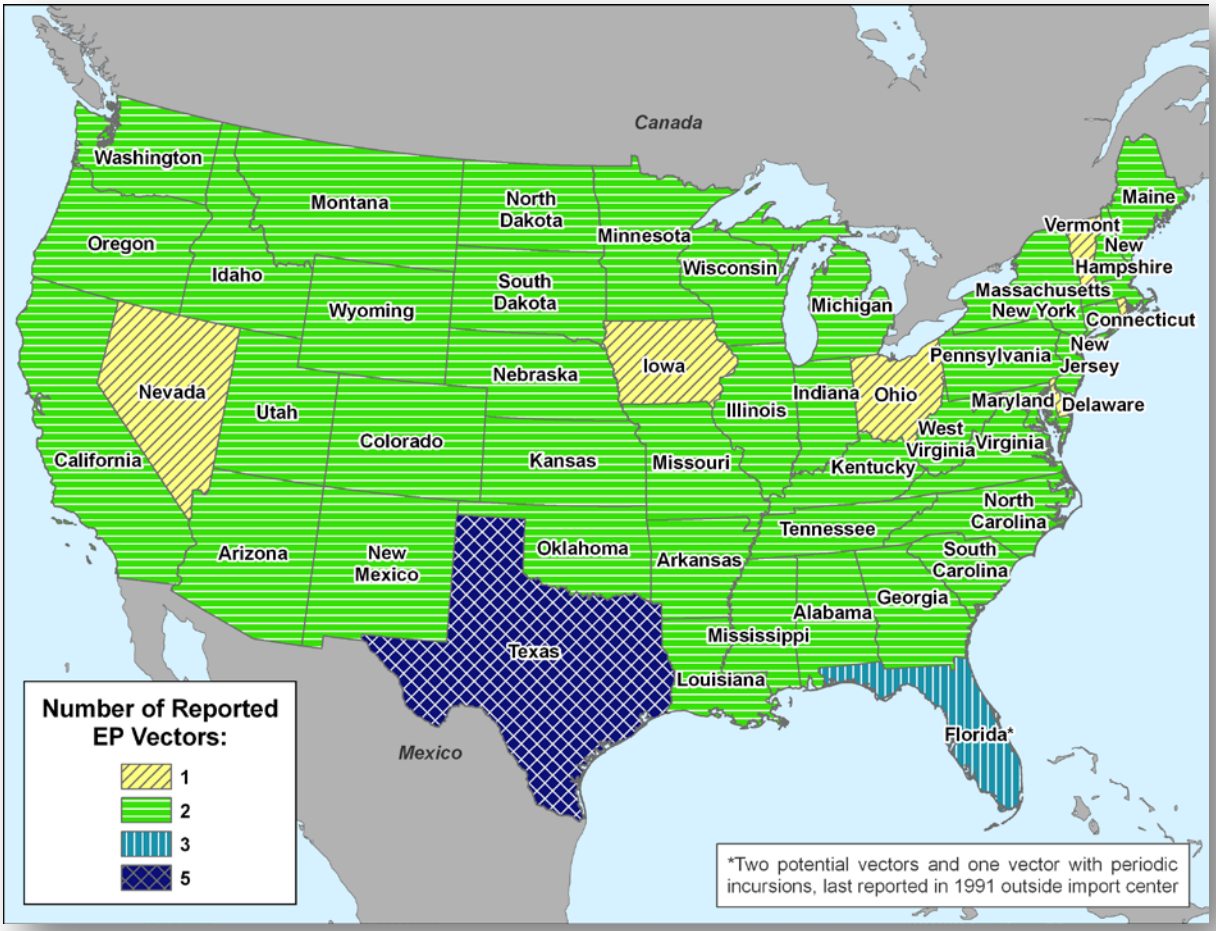

Figure 7. Number of natural or experimental equine piroplasmosis vectors identified by NVSL in each State between 1989 and 2006. (Source: NVSL National Tick Surveillance program.) *[Map revised 08/09/11]*

3. Release Assessment

The release assessment estimates the likelihood that an EP pathogen will be released from a quarantined premises. The pathways for release examined were the movement of a confirmed positive horse and a infected undetected horse.

3.1. Diagnosis

To understand the likelihood of release, the accuracy of diagnostic tests must first be discussed. Microscopic identification of parasites in stained blood is possible; however, identification in chronically infected animals may be difficult due to low parasitemia. Other techniques for identifying the organism, such as polymerase chain reaction (PCR) and the loop-mediated isothermal amplification (LAMP) can be used to detect DNA of the EP pathogens (Alhassan, Govind et al. 2007). These tests are not currently approved for regulatory diagnostic purposes in the United States.

Several serologic tests are available for the detection of *T. equi* or *B. caballi* infections. OIE currently recommends the indirect florescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA) for international trading purposes. However, the tests currently approved to classify horses in the United States include the CF and cELISA tests. These are the only tests evaluated in this assessment. The cELISA uses a recombinant protein and a specific monoclonal antibody (MAb) to identify antibody response to surface proteins on a specific lifecycle stage of the EP pathogens (merozoite). Antibody detection with the CF test disappears 2-3 months after infection in *B. caballi*- infected horses (Weiland 1986). The cELISA is more sensitive than CF at detecting chronically infected horses (Knowles, Kappmeyer et al. 1992; Kappmeyer, Perryman et al. 1999a).

A description of test performance reported in the literature can be found in Appendix C. Performance of Diagnostic Tests Over Time.

3.1.1. Sensitivity/specificity

No diagnostic test is 100 percent accurate at detecting the presence or absence of disease in a population. The ability of a diagnostic test to correctly identify an infected animal as positive on a diagnostic test is referred to as sensitivity. Animals that are infected but test results indicate they are not infected (test negative) are referred to as false negatives or infected undetected. The ability of a diagnostic test to correctly identify an uninfected individual as negative is referred to as specificity. Animals which are not infected but tests results are positive are referred to as false positives.

Reported sensitivities and specificities for serologic tests for EP are difficult to interpret due to a lack of a gold standard to compare with the serologic tests. For this assessment, the diagnostic characteristics of cELISA and CF tests for detection of *T. equi* and *B. caballi* were estimated using Bayesian analysis using WINBUGS version 3.0.3 software (MRC Biostatistics Unit 2007). Specifically, the uncertainty distributions (posterior) for the test characteristics were estimated from cross-testing data where the target populations were tested using both cELISA and CF. This

analysis only included the cELISA currently approved and licensed in the United States (VMRD cELISA).

The benefits of Bayesian analysis for this application include:

- Data from different sources can be combined to estimate the test characteristics.
- Data with unknown characteristics, such as prevalence in the target population, can be utilized.

3.1.1.1. Assumptions

The assumptions used in this analysis included:

- The sensitivity and specificity of the cELISA and CF tests are the same in data from various sources considered in the analysis.
- The results of the cELISA and CF tests are conditionally independent, depending only on whether the sample is a true positive or true negative.
- The impact of time since exposure to the parasite on cELISA and CF test characteristics are not considered in the current analysis.
- The sensitivity and specificity of cELISA for detecting *T. equi* and *B. caballi* are greater than 50 percent.

3.1.1.2. Model

The distributions for the sensitivity and specificity were estimated separately for *T. equi* and *B. caballi*. The uncertainty distributions (posterior) for the test characteristics were estimated through Markov Chain Monte-Carlo sampling using WINBUGS v 3.03 software. Uniform prior distribution was used for most parameters. Four chains were simulated for 50,000 iterations each. Convergence was monitored by comparing the results across different chains.

3.1.1.3. Data

The data used for this analysis included:

For *T. equi*

- A comparison of CF and cELISA results with sera sequentially obtained from 4 horses for 60 days after experimental exposure to *T. equi* and to *B. caballi* (Katz, Dewald et al. 2000).
- 154 samples from 19 countries were tested for *T. equi* using CF and cELISA (Knowles, Perryman et al. 1991).
- CFT and cELISA test results for 292 samples classified as true positives (VS reported outbreak data, unpublished).

For *B. caballi*

- 289 samples submitted to NVSL and tested for *B. caballi* using CF and cELISA (Kappmeyer, Perryman et al. 1999b).

3.1.1.4. Results

The sensitivity of cELISA for detecting *T. equi* was estimated to be 96 percent (90 percent C.I. 94-98) as shown in Table 5. A caveat in the application of these results is that sensitivity of cELISA could be considerably lower if there are a higher proportion of recently exposed horses in the target population. In addition, test variation may occur depending on the cELISA test kit used. According to VS Memorandum 555.20, all exposed equids must be retested at least 30 days from the last exposure to a positive equid. This analysis did not consider retesting of these animals. The sensitivity of cELISA for detecting *B. caballi* may be lower than for detecting *T. equi* based on the preliminary results (Table 6). The specificity of the cELISA is greater for *B. caballi* (98 percent) than *T. equi* (95 percent).

There is greater uncertainty regarding the sensitivity of CF, which is 47 percent for the detection of *T. equi* and 88 percent for the detection of *B. caballi.* The sensitivity estimates from different data sources had a greater variance for this parameter.

Table 5. Test Characteristics of CF and cELISA for detecting *T. equi.*

Test	Test Characteristic	Mean	95 Min	95 Max
C-ELISA	Sensitivity	96%	94%	98%
	Specificity	95%	83%	99%
CF	Sensitivity	47%	42%	51%
	Specificity	94%	83%	99%

Table 6 .Test characteristics of CF and cELISA for detecting *B. caballi.*

Test	Test Characteristic	Mean	95 Min	95 Max
C-ELISA	Sensitivity	91%	85%	96%
	Specificity	70%	60%	85%
CF	Sensitivity	88%	63%	99%
	Specificity	98%	95%	99%

3.2. Release Pathway 1: Confirmed Positive Cases

Currently, under VS Memorandum 555.20 a confirmed positive case is any horse that has tested positive by the National Veterinary Services Laboratories (NVSL) with either a CF or cEISA test. A horse can be classified as a confirmed-positive case without showing evidence of clinical disease. (USDA 2009) Animals with positive test results on one test may not be positive on another test (Donnelly, Joyner et al. 1980; Tenter and Friedhoff 1986a; Heuchert, de Giulli Jr et al. 1999). There is no cross immunity between the two organisms (Maurer 1962; Taylor, Bryant et al. 1969), so infection with only one organism would not likely result in a positive test for the other organism.

When an animal tests positive for EP, two possibilities exist: 1) the horse is truly infected, or 2) the horse is uninfected and the test result (for any number of reasons) is incorrect. In this section, the likelihood that a horse with a positive test result is truly infected with EP will be determined.

The positive predictive value of a test is the likelihood that an animal is truly infected, given a positive test result. In order for a confirmed positive case to release an EP pathogen off the premises, the horse must test positive and be infected with the organism at the time of movement.

In order to estimate the number of confirmed positive animals that are truly infected, the positive predictive value was estimated based on the sensitivity and specificity described above using the following formula:

Positive predictive value =

$1-[(1-Sp_{Test1})*(1-Sp_{Test2})*(\ldots) / ((1-Sp_{Test1})*(1-Sp_{Test2})*(\ldots)+Se_{Test1}*Se_{Test2}*\ldots)]$

The positive predictive value was estimated for horses that underwent both the CF and cELISA tests, and horses tested with only cELISA. It is not common practice to test with CF only. From 100 test positive animals for *T equi*, the mean number of animals which may be truly infected is 76 using cELISA, and 98 when tested with both CF and cELISA. This is similar for *B caballi* (Table 7). Therefore, the likelihood that a horse with a positive test result is infected with EP is high. It then follows that if a confirmed positive case leaves the quarantined premises, the risk that it will carry the EP pathogen off the premises (release) is high (>40 percent).

Table 7. Positive predictive value. Probability that a test positive animal is infected.

		Mean	Min (5% confidence)	Max (95% confidence)
cELISA only	*T equi*	76.23%	64.05%	88.21%
	B. caballi	77.70%	64.70%	91.02%
cELISA and CF	*T equi*	98.31%	93.53%	99.92%
	B caballi	99.14%	96.51%	99.87%

Title 9 of the Code of Federal Regulations, section 71.3 does not currently allow for interstate movement of confirmed positive cases, therefore it is assumed that these horses only move temporarily to a race, show or other equid event as described in section 1.4.

Some treatments for *B. caballi* have been considered effective at eliminating the parasites (Weiland 1986); however, treatment is not part of routine management of EP cases. Therefore, movement of confirmed positive animals after treatment was not assessed.

The risk that a confirmed positive case is infected and releasing EP agent is high.

3.3. Release Pathway 2: Infected Undetected Horses

Horses are allowed to move off a quarantined premises after negative tests and acaricide application. When an animal tests negative for EP, one of two possibilities exist: 1) the animal is truly uninfected or 2) the animal is infected but the test fails to detect the infection. It is this later population, referred to as infected undetected horses, which can move EP pathogens off the premises.

The likelihood that a test will fail to detect a truly infected animal is the complement of the test sensitivity (1-Se). If multiple tests are used, then a failure to detect occurs only if all tests fail to detect the pathogen ($(1-Se_{TestA})*(1-Se_{TestB})*\ldots$)). The sensitivity and specificity of the CF and cELISA tests were determined by the analysis described in section 3.1.1.

Diagnostic test performance can vary by animal and situation, therefore a single probability of detection cannot be applied to a test. Instead, a probability distribution is used to address these potential situational variations. These results were then fit into beta distributions using a risk analysis software program @RISK (Palisade 2009) in order to reflect the uncertainty about the true, but unknown values of these tests as applied in the field. The beta distributions for this analysis can be found in Appendix B. Beta Distributions.

According to VS Memorandum 555.20, a horse is considered to be infected with EP if it tests positive on either the CF or cELISA tests. A commonly used protocol is to test with CF and cELISA in parallel; therefore, a horse would be considered uninfected only if it tested negative on both tests. The probability of nondetection using this parallel testing approach was evaluated. The other common scenario is testing with cELISA alone and considering a test negative animal not infected. Thus, the probability of nondetection describes the likelihood that an infected horse will test negative in either scenario. As noted above, there is uncertainty about the test sensitivity, so there will be uncertainty about the nondetection probabilities as well. For example, the mean probability of not detecting an infected horse using both tests for *B. caballi* is 0.88 percent. The probability of an infected horse testing negative is shown in Table 8.

Table 8. Probability of nondetection, CF and cELISA.

Percentiles	*B. caballi*	*T. equi*
5th	0.12%	0.03%
50th	0.88%	0.09%
95th	3.06%	0.26%

If a single test is used (cELISA) the nondetection probabilities increase, shown in Table 9 (must test positive to both tests).

Table 9. Probability of nondetection, cELISA only.

Percentiles	*B. caballi*	*T. equi*
5th	4.57%	1.26%
50th	8.43%	3.40%
95th	13.80%	7.10%

The probabilities calculated above can also be used to estimate the number of infected horses that test negative for EP, given that 1,000 infected horses are tested. This is calculated by using a binomial distribution. A binomial distribution estimates the number of successes (infected nondetected horses) in a population (1,000 infected horses) given a probability (the probability on nondetection).

If both the cELISA and CF tests are used the horses must test negative to both. Therefore, the number of truly infected horses that would test negative would be:

- *B. caballi*: 9 (median), 32 (upper 95 percent), 1 (lower 5 percent)
- *T. equi*: 1 (median), 4 (upper 95 percent), 0 (lower 5 percent)

If only the cELISA test is used:

- *B. caballi*: 84 (median), 140 (upper 95 percent), 44 (lower 5 percent)
- *T. equi*: 34 (median), 73 (upper 95 percent), 11 (lower 5 percent)

The risk of a negative exposed horse being infected undetected and allowing the release of the pathogen off a quarantined premises is low (< 1 percent) when testing with cELISA and CF, and moderate (1.4 percent) for *B. caballi* with cELISA only.

3.4. Overall Likelihood of Release

The risk of release is the likelihood that the movement of the pathway off a quarantined premises will result in the successful release of *B. caballi* or *T. equi* from the premises. Horses that test positive for either EP pathogen are a high-risk pathway for release, while infected undetected horses pose a low risk of being infected and therefore moving an EP pathogen off a quarantined premises (exception in *B. caballi* infected horses that are tested with cELISA only and pose a moderate risk).

Table 10. Risk of release pathway.

	B. caballi		*T. equi*	
Release pathways	Risk	Uncertainty	Risk	Uncertainty
Test positive case- cELISA only	High	Low	High	Low
Test positive case- cELISA and CF	High	Low	High	Low
Infected undetected- cELISA only	Moderate	Low	Low	Low
Infected undetected- cELISA and CF	Low	Low	Low	Low

4. Exposure Assessment

The exposure assessment estimates the likelihood of exposure and the risk of transmission if an uninfected horse is exposed to the EP pathogen by: ticks, vertical transmission, or iatrogenic transmission of blood or blood components.

4.1. Likelihood of Exposure to Release Pathway

The likelihood an uninfected horse exposed to an EP pathogen by the interstate movement of a horse off a quarantined premises is based on the generalized practices in the equine industry and the mitigation measures discussed in section 1.4. The practices of specific sector and State requirements will also be discussed.

4.1.1. General Biosecurity Practices

Biosecurity practices are variable throughout the equine industry. A recent study conducted by USDA:APHIS:VS asked producers to report on common practices. General equine industry biosecurity and biocontainment practices reported include isolation, infection control, and equipment disinfection (USDA 2007). Management practices also included limiting contact between animals, insect control, manure management, and preventing feed contamination.

Insect control was reported on 88.9 percent of operations. Control measures included repellents, reducing vegetation, emptying and refilling water containers, using facemasks on the horses, and applying insecticides in housing areas. Some insect control measures could also be effective against ticks.

Overall, about 65 percent of operations isolated equids for infection control. The most common requirements were Coggins tests (45.3 percent), vaccination (36.3 percent), and worming (33.6 percent). Larger operations (20 or more horses) were most likely to require isolation followed by medium (10-19 horses) and small operations(5-9 horses). Health evaluation and quarantine were often practiced when new nonresident animals entered existing facilities. Quarantine prior to contact was not often required and where resident horses departed and returned to a facility, 60.6 percent of operations did not conduct reentry quarantine. Only 2.8 percent of operations routinely quarantined returning resident horses.

The American Association of Equine Practitioners (AAEP 2006) has published biosecurity information, recommendations, and guidelines for equine practitioners (AAEP 2006). The AAEP recommends multi-language instructions and that a specific individual care for an affected horse. They also recommend restricted facility access and segregation of sick animals. The guidelines further recommend that tack be horse-specific and shared equipment be thoroughly sanitized. Facilities should be constructed of nonporous material, which should be periodically and thoroughly disinfected. While many of these biosecurity practices may help reduce the risk of tick exposure between horses, they have little impact on the iatrogenic routes of EP transmission.

4.1.2. Industry and State Specific Requirements

While biosecurity and management practices are not well described for the equine industry, it is recognized that some practices may be more common in various sectors. In addition, management, testing, and movement requirements vary by State, which could impact risk. The management of an infected horse by status (known positive or infected undetected) also has an impact.

The current Veterinary Services memorandum restates that 9 CFR 71.3 prohibits *interstate*, but does not restrict *intrastate*, movement of EP positive horses. Many individual States have not formulated or universally applied EP specific quarantine and movement regulations. Examination of a list of State movement requirements (U.S.Rider 2010) shows that only 6 of the 50 States have equine movement regulations which specifically address EP. Some States deny entry to horses from Texas, some deny entry of horses from "infected premises" or state that "no horse that has ever tested positive" for EP may enter the State. Constantly changing State requirements indicate that Web sites may not always reflect the latest movement regulations; therefore, State specific mitigations were not considered in this assessment.

An exhaustive review of equine health management at events was last performed in 2005 (NAHMS 2006), before the current outbreaks of EP in Texas, Florida, and Missouri. The study presented in 2005 remains the best available data and contains information, which can be used to estimate relative risk of transmission of EP at certain events or under known conditions. In a few cases, it can be determined that horses must be CF or cELISA negative for EP to participate in an event, but there remains substantial possibility that events can and will occur, in which the EP status of the participating horses is unknown.

In 2005, 57.1 percent of all events examined did not require a CVI for horses attending an event. National events, in general, were more likely than Regional or State events to require a CVI or Health Certificate. However, the presence of a CVI does not indicate the animals were tested for EP; therefore, has little impact on the potential for EP spread but does indicate physical examination by a veterinarian. A site veterinarian was present at 22.8 percent of all events but an apparently healthy animal would remain undetected on clinical exam.

Insect control (ticks not addressed) was not performed at 49.5 percent of the events studied.

The following table estimates relative risk of EP transmission for the five industry sectors using the likelihood of tick presence, most likely release pathway, length of direct contact with other horses, most likely exposure pathway, and likelihood of exposure (based on biosecurity).

Table 11. Summary of risk by sector.

	Purchased additions to small operations	Show horses	Trail and ranch horses	Sanctioned racetrack	Unsanctioned racetrack
Likelihood of tick present in environment	Moderate- climate dependant	Low	High	Low	Moderate
Length of direct contact with other horses	Long	Short	Long	Short	Short to unknown
Most likely release pathway	Infected undetected	Confirmed positive horses or infected undetected	Infected undetected	Confirmed positive horses or infected undetected	Infected undetected
Most likely exposure pathway	Vectors, iatrogenic	Iatrogenic	Vectors, iatrogenic	All unlikely	Iatrogenic
Likelihood of exposure (based on biosecurity)	High	Low	High	Low	High

4.1.3. Exposure to Confirmed Positive Horses

Based on characteristics of tests for EP, a confirmed positive horse that was allowed to leave a quarantined premises has a high likelihood of actually being infected and thus causing release of the EP pathogen. However, the limitations on movement and use of these horses assumed in this assessment serve to limit exposure of uninfected horses to EP pathogens via any of the iatrogenic exposure pathways examined. Acaricides applied prior to movement off the quarantined premises would reduce the risk of an infected tick attached and the continued application of acaricide would reduce the risk of ticks feeding on these horses. These horses are allowed to breed which puts foals at an increased risk of exposure to *T. equi*. Because these horses are only allowed to move temporarily under a permit issued by the State and identified, it is unlikely these horses would be lost in the interstate movement process. Restricted movement of horses decreases the likelihood that an infected horse will be a source of infection for ticks in other locations. However, unpublished reports indicate that confirmed positive horses have bypassed monitoring requirements when owners are unwilling to cooperate.

4.1.4. Exposure to Infected Undetected Horses

Based on the characteristics of EP tests when used in accordance with VS Memorandum 555.20, a test-negative horse has a low likelihood of being infected undetected with EP and thus allowing an EP pathogens to be released from the quarantined premises. However, if an animal is infected undetected, it could leave a quarantined premises with unrestricted movement, no identification, and no additional testing. These horses are likely to have a great deal of contact with naïve horses in other locations and it is unknown how frequently blood doping, equipment sharing, or other high-risk practices occur. In addition, lack of acaricide treatment, once this population has permanently left the quarantined premises, would allow for exposure to ticks in the environment.

The acaricide application prior to movement would reduce the risk of a tick attached at the time of movement. No breeding restrictions are identified therefore any foal born to infected undetected mares are at high risk of exposure to EP pathogens.

4.2. Exposure Pathway 1: Ticks

Ticks may be responsible for EP transmission to an uninfected horse through two scenarios:

1) An infected tick introduced by horse movement off a quarantined premises would have to feed directly on an uninfected horse on the new premises while infected.
2) A tick on the new premises would need to feed on an infected horse introduced and feed on an uninfected horse while still maintaining infection.

4.2.1. Infected Tick Attached to Horse Moving Off a Quarantined Premises

In order for an infected tick to move off a quarantined premises, the tick must first become infected with the EP pathogen on that premises and survive acaricide treatment prior to its arrival onto the new premises. VS Memorandum 555.20 contains several mitigations designed to prevent ticks from moving off a quarantined premises. Ticks may move off a premises by other mechanism, but only horse movement was considered in this assessment.

Horses leaving a quarantined facility must be treated with an acaricide not less than 24 hours or more than 14 days prior to movement. 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 the ticks. 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, spraying is acceptable when dipping is not practical (VS Memorandum 556.1).

A laboratory study compared 17 formulations of 15 acaricides on 7 species of engorged female ticks (Drummond and Ossorio 1988). 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*. With the exceptions of permethrin and coumaphos, these compounds demonstrated toxicity to ticks; however, most of these compounds do not meet EPA requirements for low human health risk, rapid environmental degradation, and low ecological toxicity to nontarget organisms which means they cannot be registered for use in the United States (Document 2007; EPA 2008).

Problems of acaricide resistance in ticks (as a result of prolonged use), animal product contamination, and environmental residues may render acaricide treatments ineffective over time as a control method. 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 (Jongejan and Uilenberg 1994). An integrated pest management (IPM) approach, as

discussed in VS Memorandum 555.20 should be considered for tick control and to minimize exposure to ticks in view of the increased acaricide resistance in ticks on global basis.

The likelihood of a tick carrying an EP pathogen off a premises and infecting a new horse is low in most cases, however, the efficacy of acaricides should be monitored during prolonged usage. The uncertainty around this is high due to the lack of understanding of resistance of some tick species to acaricides over time, conflicting information on the topic, and possibility of incorrect application.

4.2.2. Ticks in the Environment

In order for a tick in the environment to transmit EP, an infected horse would need to be introduced to the premises, the tick would need to feed on the infected horse, and be capable of transmitting the organism during subsequent feeding. For many tick borne diseases including EP, the presence of the tick is not sufficient for an infection to occur. The major components 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 (Kitron and Kazmierczak 1997). Transmission of most tick-borne diseases is seasonal because many tick species seek hosts during a well-defined period of the year. These time periods or seasonal activities are when ticks can transmit diseases and when vertebrate hosts are most likely at risk (Estrada-Peña 2008).

Wildlife and pet animals such as cats and dogs can serve as hosts for multiple stages of ticks, potentially increasing equine exposure to ticks. Contact with nonequine animals was reported on many operations. Dogs (76.9 percent of operations) and cats (66.4 percent of operations) were the most common contact, but cattle (43.2 percent of operations), poultry (18.6 percent of operations) and skunks-raccoons-opossums-bats (25-50 percent) were also present (NAHMS 2006). However, these species are not considered to be reservoir hosts for EP.

The prevalence of infection in host-seeking ticks depends directly on the frequency of encounters between ticks and reservoir hosts, which, in this case, are horses infected with EP pathogens. Moreover, the risk of infection for hosts depends on the number of infected questing ticks and of the number of hosts in an area. As the number of tick bites per host increases, the probability of transmission increases, resulting in higher prevalence of infection in the host population. The frequency of tick encounters with horses is affected by the behavior of individual ticks and horses.

The longer the host is infected with a pathogen the greater the opportunity for transmission to take place and less likely that the tick's seasonal activities will influence the maintenance of enzootic cycles. *B. caballi* has a relative shorter infective period in horses than *T. equi*, where the horse maintains lifelong infections (Ueti, Palmer et al. 2005; Ueti, Palmer et al. 2008). The lifelong nature of *T. equi* infection in horses provides a continual source for pathogen acquisition by ticks, which is required by each tick generation as the *T. equi* infection cannot be maintained by the tick alone. Ueti et al. (Ueti, Palmer et al. 2005) indicated that the threshold level for nymphal *R. microplus* to acquire *T. equi* and have development progression of the pathogen to the

salivary glands after molting to an adult was less than $10^{5.8}$ *B. equi* parasites per milliliter of horse blood. There was no difference in the percentage of adult ticks that developed infection in the salivary glands whether they fed on a horse in the acute phase ($10^{9.4}$/ml) versus the chronic phase ($10^{5.8}$ to 10^{6} /ml). This is consistent with other blood-borne pathogens. Once the threshold level is reached a further increase does not result in a larger percentage of ticks being infected. The exact number of ticks needed to transmit *T. equi* to horses is unknown. There is evidence to suggest that fewer than 10 ticks are needed to transmit the pathogen to a naïve horse. A study examined the salivary glands of fed ticks with *T. equi* in their salivary glands at the time of transmission. The minimal number of ticks with detectable *T. equi* in the salivary gland at the time of successful transmission feeding varied from 4- to 10-ticks per horse (Ueti, Palmer et al. 2005). Therefore, it appears that only a few infected ticks are required for the successful transmission of *T. equi* to horses in a laboratory setting. Other possible influences on successful tick transmission are the number of sporozoites in the tick salivary gland, pathogen, and tick strain differences, and the duration of the tick feeding in the field and those that require further investigation.

Although *Anocentor nitens*, the tropical horse tick, is a natural vector of EP caused by *B. caballi,* it may not be an important vector in the United States because of its limited distributional range. *Anocentor nitens* is unable to maintain EP infections by transovarial transmission for more than a few generations and may only be successful as a short-term reservoir (Schwint, Knowles et al. 2008a). On the other hand, *D. albipictus* and *D. variabilis* may be important vectors because they experimentally transovarially transmit *B. caballi*, naturally infest horses, and are widely distributed throughout the United States. *Rhipicepalus microplus* transtadially transmits *T. equi* and can be found on horses particularly if horses are kept with cattle. *Rhipicepalus microplus* is distributed throughout southern Texas and is being controlled through USDA's cattle fever tick program. Despite the wide distributions of *D. albipictus* and *D. variabilis* in the United States and the presence of EP infected horses, it is unclear why EP has not become well established in these tick populations. It may be that the frequency of contact between these tick species and horses are below the threshold to maintain or spread EP. The lack of contact may be related to the host preferences of the immature stages of these tick species (i.e., the American dog tick is frequently found on rodents), varying seasonal activities of the each of the tick life stages, tick density, and animal husbandry management practices, and low prevalence of EP in the United States. Moreover, both the level of tick susceptibility to EP infection and the likelihood of tick-mediated transmission to a horse will vary with tick and EP pathogen strain (Stiller and Coan, 1995).

The outbreak in Texas involved transmission by ticks, but tick mediated spread has not been confirmed for other outbreaks in the United States. The ranch appears to be a suitable habitat for the maintenance of large numbers of competent tick vectors (high vector density) and large numbers of horses and cattle (high host density). In addition, the common animal management practice of mixing horses with cattle and working horse activities (find strays in a tick habitat) create an environment of high frequency of contact between the tick vectors and hosts. It is possible that this situation could occur if *T. equi* were introduced onto another premises with similar environment conditions and management practices. The tick populations alone cannot sustain the EP pathogen as they are not reservoirs of the pathogen. *Anocentor nitens*, can only

maintain *B. caballi* through one generation with no reservoir host present therefore the risk of *B. caballi* establishment may be lower than *T. equi*.

Based on this scenario the risk of transmission from ticks in the environment, given an infected horse enters the premises, is low assuming acaricides still maintain efficacy. However, the risk may vary by environmental factors and vector competency. The uncertainty around this is high due to the limited information available on EP transmission and density of vectors in the United States. The appropriate density of infected horses, competent vectors, and environmental conditions for the tick to survive and move through its lifecycle would be needed for EP to persist, or the tick would need to feed directly on an uninfected horse during the same life stage. Therefore, a tick can only become infected, and the infection maintained in a tick population, with the presence of an infected host.

4.3. Exposure Pathway 2: Vertical Transmission

In order for vertical transmission to cause spread of *T .equi* on a new premises as defined in this assessment, the introduced horse would have to be a *T. equi* infected, test-negative mare that subsequently gives birth on a new premises.

Transmission of *T. equi* from infected mares to their offspring in utero has been described (Phipps and Otter 2004; Allsopp, Lewis et al. 2007). A short report from 2004 described two horses in the United Kingdom born to a carrier mare imported from Portugal, where *T. equi* is endemic. The horses were 2- and 5- years old when infection was detected on CF and IFAT. Organisms were also detected on blood smears. An epidemiological investigation led to the conclusion that the most likely route of transmission was transplacental (Phipps and Otter 2004). Piroplasmosis due to *T. equi* is a common cause of equine abortion. In addition, in utero infection of a fetus with *T. equi* can also result in the birth of foals with neonatal piroplasmosis or clinically normal carrier foals (Erbsloh 1975; de Waal and van Heerden 2004; Phipps and Otter 2004; Allsopp, Lewis et al. 2007; Rothschild and Knowles 2007).

In 2007, a study on transplacental transmission of *T. equi* in a group of 17 chronically infected mares concluded that *T. equi* transmission from mother to fetus occurs across the normal placenta and can occur as early as the first trimester of pregnancy (Allsopp, Lewis et al. 2007). The authors were able to detect *T. equi* organisms via DNA probe in artificially aborted fetus as early as 130 days gestation. In addition, the study results indicated that congenital infection of foals born to *T. equi* carrier mares is common: In the study, six *T. equi* carrier mares were allowed to carry foals to full term, and all foals were born clinically normal but *T. equi* probe positive. The samples were collected 12 hours after birth, and the foals were kept in a tick-free experimental barn, making tick-mediated transmission unlikely. The authors concluded that if a *T. equi* carrier mare gives birth, the resultant foal would likely be infected with *T. equi*.

A recent case report described a foal in Trinidad that was born weak and severely icteric with hematuria. A blood smear at 10 hours postpartum revealed that 63 percent of the foal's red blood cells were parasitized, and reverse line blot and nested PCR identified *T. equi*. The mare was clinically healthy and a blood smear and reverse line blot were both *T. equi* negative. (These are different from the standard tests approved by VS; therefore, Se/Sp for these tests has not been

described in this document.) Nested PCR performed on the mare's blood was *T. equi* positive. The authors concluded that this case demonstrated that carrier mares could transmit *T. equi* to their foals. However, based on the data in the case report, it is impossible to determine at which point during pregnancy or parturition *T. equi* transmission occurred (Georges, Ezeokoli et al. 2010).

In contrast, another recent study failed to amplify *T. equi* DNA in 6 neonatal foals born to chronically infected dams (3 horses, 3 donkeys) and concluded that transplacental transmission did not occur in this group of animals (Kumar, Kumar et al. 2008). The authors argued that the normal equine placenta should serve as a barrier to molecules as large as *T. equi*. The conflicting results could be due to *T. equi* strain behavior, differences in host immunity, and differences in test performance, or study design.

In the recent *T. equi* outbreak in Texas, there is evidence that vertical transmission, at least of that specific strain of *T. equi*, is inefficient (Knowles 2010). Of the 24 mares that foaled, all 24 foals were PCR negative at birth (USDA unpublished).

There is sufficient evidence of transplacental transmission to conclude that there is moderate risk that the foal of a *T. equi* infected mare will be born infected. However, due to conflicting evidence, the uncertainty surrounding this estimate is medium.

No evidence of vertical transmission of *B. caballi* resulting in infected foals was found, thus the risk that a foal born to a mare infected with *B. caballi* will be infected is negligible, and the uncertainty surrounding this estimate is medium.

4.4. Exposure Pathway 3: Iatrogenic via Blood or Blood Components

Horse blood is a documented source of *B. caballi* and *T. equi* infection (Sippel, Cooperrider et al. 1962; Ristic, Oppermann et al. 1964; Sibinovic, Ristic et al. 1965; Holbrook and Frerichs 1968; Holbrook 1969; Hurcombe, Mudge et al. 2007; CFSPH 2008). Purely mechanical transmission of EP was confirmed in a herd of British horses (Gerstenberg, Allen et al. 1999). Experimentally, both organisms have been transmitted by direct blood inoculation (Tenter and Friedhoff 1986b; Ueti, Palmer et al. 2005; Ueti, Palmer et al. 2008; Schwint, Ueti et al. 2009).

Transfer of blood and products could occur via direct, purposeful administration of blood (or blood product) to a horse, or via blood contaminated fomites. The likelihood that blood, a blood product, or a contaminated fomite will transmit EP is affected by the number of organisms in the blood, the survivability of the pathogens, and the infectious dose.

4.4.1. Infection risk from blood

4.4.1.1. Number of Organism in Blood of an Infected Horse

In naturally infected horses, *B. caballi* parasitemia is frequently as low as 0.1 percent, even in acute cases (de Waal 1992; Heim, Passos et al. 2007). The highest reported parasitemia is 10 percent (Holbrook 1969; Rothschild and Knowles 2007). Horses in the chronic, subclinical phase of infection have parasite concentrations of less than 10^5 parasites per milliliter of blood (Holman, Frerichs et al. 1993; Schwint, Knowles et al. 2008b; Schwint, Ueti et al. 2009).

Parasitemias in natural *T. equi* infections typically range from 1 to 10 percent (de Waal 1992; Friedhoff and Soulé 1996). Chronically infected, subclinical horses may have extremely low circulating parasitemias (Maurer 1962). Parasitemias as low as 0.1 percent have been reported in naturally infected, subclinical horses (Heim, Passos et al. 2007). However, in some cases parasitemias can exceed 20 percent, and the highest reported parasitemia is 95 percent (Holbrook 1969; de Waal 1992; Rothschild and Knowles 2007). Experimentally, horses with chronic infection (15- to 40-months duration) showed parasite levels which fluctuated from 10^3 to 10^6 parasites per milliliter of blood (Ueti, Palmer et al. 2005; Ueti, Palmer et al. 2008). Parasite levels within an individual horse fluctuated over time. With both *B. caballi* and *T. equi* infection, animals with the higher levels of parasitemia were clinically ill or moribund.

Equine piroplasmosis pathogens exist in the merozoite life stage in red blood cells. Cells infected with *B. caballi* and *T. equi* typically have 2 or 4 merozoites per infected red cell, respectively. Additionally, in *T. equi* infections, the sporozoite life stage transmitted from a tick bite initially infects lymphocytes, which can contain up to 200 merozoites prior to rupturing and infecting new red blood cells (Rothschild and Knowles 2007). No data is available on the percentage of lymphocytes that would potentially be infected, or the length of time that a horse would be expected to have infected lymphocytes following tick-mediated transmission.

The reference range for red blood cells in hot- and cold-blooded breeds is 8.2-12.2x1012 cells/L, and 5.5-9.5x10^{12} cells/liter, respectively (Lording 2008). Table 12 shows the potential number of infected red cells per milliliter of blood across horses with a variety of red blood cell concentrations and parasitemias As the table shows, even a horse with an extreme anemia (2x10^9 red blood cells/ml), and a very low parasitemia (0.05 percent), can have 5x10^5 infected cells per milliliter.

Table 12. Infected red blood cells per milliliter.

	Number infected RBC's per ml				
	Parasitemia (%)				
RBC's/ml (x10^9)	0.05	0.1	1	5	10
1	5.0x10^5	1.0x10^6	1.0x10^7	5.0x10^7	1.0x10^8
2	1.0x10^6	2.0x10^6	2.0x10^7	1.0x10^8	2.0x10^8
3	1.5x10^6	3.0x10^6	3.0x10^7	1.5x10^8	3.0x10^8
4	2.0x10^6	4.0x10^6	4.0x10^7	2.0x10^8	4.0x10^8
5	2.5x10^6	5.0x10^6	5.0x10^7	2.5x10^8	5.0x10^8
6	3.0x10^6	6.0x10^6	6.0x10^7	3.0x10^8	6.0x10^8
7	3.5x106	7.0x10^6	7.0x10^7	3.5x10^8	7.0x10^8
8	4.0x10^6	8.0x10^6	8.0x10^7	4.0x10^8	8.0x10^8
9	4.5x10^6	9.0x10^6	9.0x10^7	4.5x10^8	9.0x10^8
10	5.0x10^6	1.0x10^7	1.0x10^8	5.0x10^8	1.0x10^9
11	5.5x10^6	1.1x10^7	1.1x10^8	5.5x10^8	1.1x10^9
12	6.0x10^6	1.2x10^7	1.2x10^8	6.0x10^8	1.2x10^9
13	6.5x10^6	1.3x10^7	1.3x10^8	6.5x10^8	1.3x10^9

4.4.1.2. Ability of EP Pathogens to Survive

Little conclusive information is available regarding the duration and levels of *B. caballi* or *T. equi* infectivity in horse blood or blood products outside of the host. *Babesia caballi* has been reported to remain infective in horse blood during cryopreservation (Holman, Frerichs et al. 1993). Little information regarding the ability of the EP pathogens to survive in blood ex vivo is available. However, a body of published work exists concerning *Babesia microti*, a related organism with a similar life history and characteristics (Homer, Aguilar-Delfin et al. 2000; Uilenberg 2006). Blood from hamsters experimentally infected with *B. microti* was able to infect naïve hamsters for up to 3 days if stored at room temperature, and for up to 21 days if refrigerated under conditions similar to those in a human blood bank. The blood used in this experiment was treated with EDTA to prevent coagulation (Eberhard, Walker et al. 1995). Transfusions of red blood cells, deglycerolized red blood cells, and platelets have transmitted *B. microti* to humans. In order for this transmission to occur, the pathogens survived common blood bank procedures including refrigeration, removal of white blood cells, filtration, and freezing. It is thought that *Babesia sp.* can survive freezing because the glycerol added during the process prevents red blood cell lysis (Gubernot, Lucey et al. 2009). EP pathogen spread via blood contaminated fomites has been documented, indicating that the EP pathogens can survive in blood ex vivo for at least some period of time.

4.4.1.3. Infectious Dose

A definitive infectious dose of either EP pathogen was not found. Experimentally, inoculation of $10^{5.2}$ organisms into naïve horses resulted in *B. caballi* infection, but the authors were not specifically attempting to determine infectious dose (Schwint, Ueti et al. 2009). Infectious dose of *B. bovis* for calves is less than 100 organisms (Goff, Johnson et al. 1998). Schwint et al., used those findings to conclude that the infectious dose for babesial organisms in general is less than 100 organisms (Schwint, Ueti et al. 2009).

4.4.2. Administration of Blood or Blood Components from a Known Donor

In this portion of the assessment, the term “known donor” is used because it is assumed that the team performing a blood transfusion also removes the blood from the donor horse, rather than purchasing the blood from a licensed source (covered in section 4.4.4). Thus, the medical team knows the identity of the horse being used as a blood donor.

This section discusses situations during which blood or blood components are collected from a known donor and are administered to a horse under the direction of a veterinarian for the purposes of correcting a medical problem. For the purposes of this risk assessment, this process will be referred to as transfusion. In order for an EP infected horse to spread EP under this scenario, the infected horse would need to be used as a donor for a transfusion (use of contaminated needles or transfusion equipment is covered in section 4.4.5).

The most common substance used in equine transfusions is whole blood, which consists of blood from a donor without components removed or separated. Stored whole blood is still not widely or readily available in equine practice, and its use is still relatively expensive (David 2009). Within

the context of a veterinary client patient relationship, veterinarians can legally administer unlicensed blood and blood products, including keeping a donor horse at their practice (Kaler, Curry-Galvin et al. 2006; Evans 2010). Thus, the majority of whole blood transfused into horses in veterinary practice is collected by the veterinarian performing the transfusion from a known donor horse (Slovis and Murray 2001; David 2009) It is recommended that whole blood be used within 24 hours of collection. Many equine blood donors are pre-identified as donors and are typically housed at a veterinary practice or privately owned location by a client of the practice (David 2009). In emergency situations, however, veterinary tests and the AAEP transfusion guidelines describe the appropriate use of a horse that has not been pre-screened as a donor. Quarterhorses, standardbreds, and Morgans have a low prevalence of genes coding for highly immunogenic equine alloantigens, and thus are preferred blood donor horses (Slovis and Murray 2001; David 2009).

Standard guidelines for infectious disease screening of equine blood donors were not found. Published articles, including the American Association of Equine Practitioners' conference proceedings, recommend that the donor should be generally healthy and free of infectious disease. In the literature published in the United States, a test for Equine Infectious Anemia (EIA) is the only infectious disease screening specifically mentioned (Gonzales 2001). Several available equine medicine textbooks were reviewed, and piroplasmosis testing for blood donor horses was not specifically suggested (Corley and Stephen 2008; Muir and Hubbell 2008; David 2009; Reed, Bayley et al. 2009). More recently, APHIS published a factsheet that recommended that all horses being considered for use as blood donors be tested for EP (USDA APHIS 2010b). Heightened awareness of EP will likely lead to increased testing, but it is unclear at this time the proportion of equine blood donors that are being tested for piroplasmosis.

Blood from a known donor in a medical setting could potentially be separated into its component parts, for example plasma, or concentrated red cells. The level of contamination of plasma with red or white blood cells depends on the method used to separate the plasma, though plasma produced in a hospital setting is likely to have cell contamination (see section 4.4.1).

Blood donation is a relatively uncommon procedure and any one individual horse has a low likelihood of being chosen as a donor. For this assessment, it is assumed that a confirmed positive case would be moving off the quarantined premises temporarily, would be identified, would have location and transportation monitored, and would not be allowed to donate blood. A horse with a history of residency on an EP affected premises but tested negative would be unlikely to be chosen if its history is known. However, negative horses are not required to be tracked or retested. Testing for EP is becoming more common but not standard across the industry, therefore an apparently healthy EP infected donor would be as likely as any other horse to be used as a donor.

As described in the hazard identification, *T. equi* and *B. caballi* are intraerthrocytic parasites. A critically ill horse may receive up to 10 liters of blood in a single transfusion (Slovis and Murray 2001; David 2009). Thus, even a donor with low parasitemia would transmit a large number of organisms to the recipient. In addition, horses receiving blood in a medical situation are generally severely compromised. Therefore, the likelihood of transmission of either EP pathogen, when an

infected donor is used, is high. Because the transmissibility of the organism through small amounts of blood is well documented, the uncertainty surrounding this conclusion is low.

4.4.3. Blood Doping

Blood doping, the administration of large volumes of blood or concentrated red blood cells from a donor horse prior to racing, was reported as one of the practices used by those involved in bush track Quarter Horse racing in Florida (Holt 2008). Other names for this practice are blood boosting or blood packing.

The frequency of "blood doping" in the equine industry is unknown. It is unlikely that the donors would be screened for EP or other diseases. Anecdotally, this practice is prevalent in the unsanctioned racing segment of the industry. Unpublished epidemiology reports from Florida and Missouri indicate that unsanitary management practices such blood doping and sharing needles or syringes between horses are the most likely cause of transmission of EP on premises where competent natural vectors do not exist.

APHIS published an information sheet targeted at horse owners and trainers in 2010 (USDA APHIS 2010b) This sheet recommended testing all blood donor horses for EP. However, it is unlikely that this practice has become commonplace among individuals who perform blood doping.

As with medical transfusion (above) a large volume of blood or blood product is given to the recipient. Therefore, if a donor is infected, the risk of transmission is high. The uncertainty surrounding this estimate is low.

4.4.4. Commercial Plasma and Serum Products

Equine plasma and serum products are used to treat a variety of equine diseases, such as failure of passive transfer, septicemia, clotting disorders, and certain infectious diseases. Plasma is also administered in some cases of acute blood loss to expand blood volume and replace lost proteins and clotting factors. Under the conditions of this assessment, the introduced positive horse would need to be the donor of this plasma or serum in order for risk to occur. A literature review revealed no cases of commercially available horse blood or blood products serving as sources of *B. caballi* or *T. equi* infection.

Plasma and serum are produced by various methods of removing red and white blood cells from whole blood. Plasma contains clotting factors, while serum does not. Blood cell contamination (red or white) can lead to adverse reactions in the recipient, and cell degradation during storage decreases plasma quality (Feige, Ehrat et al. 2003). Thus, it is beneficial to create serum/plasma products with as few cells as possible. Techniques to separate plasma from blood cells include gravity sedimentation, centrifugation, plasmapheresis (applicable to plasma only) (Feige, Ehrat et al. 2003; Kaler, Curry-Galvin et al. 2006). Plasma prepared by plasmapheresis has fewer red and white blood cells than plasma prepared by gravity sedimentation or centrifugation (Feige, Ehrat et al. 2003). The results are summarized in Table 13.

The differences between the three speeds of plasmapheresis were not statistically significant, while the differences between the three methods were statistically significant.

Table 13. Absolute counts of erythrocytes and leukocytes per milliliter, by plasma preparation method.

	Median cells per microliter (range)	
Production Method	**Erythrocytes**	**Leucocytes**
Plasmapheresis (70 ml/min)	0.0 (0.0-2.5)	0.0 (0.0-0.0)
Plasmapheresis (85ml/min)	1.3 (0.0-5.0)	0.0(0.0-5.0)
Plasmapheresis (100 ml/min)	2.5 (0.0-22.5)	0.0 (0.0-7.5)
Blood bag centrifugation	615 (520-800)	10 (10-700)
Gravity Sedimentation	935(700-1200)	2900 (700-3800)

Adapted from (Feige, Ehrat et al. 2003)

Plasma and serums are given in large quantities (measured in liters rather than milliliters). A liter of plasma or serum containing only 2.5 red cells per microliter from an infected donor with a low parasitemia (0.05 percent) could contain 1,250 infected red cells. As the red cell contamination of the product increases, so does the number of infected red cells per liter. Note that *T. equi* replicates in equine white blood cells following a bite by infected tick. It is unknown how this relates to iatrogenic horse-to-horse transmission.

A wide variety of commercial plasma and serum products are available. However, under the Virus, Serum, and Toxins Act, only products that make specific "disease prevention or treatment claims" are required to have a license by the USDA. In order to obtain a license, these products must meet various conditions for safety and efficacy. Most relevant to this assessment, USDA licensed products are required to take certain steps to ensure that donor horses are free of infectious diseases. Donor horses must be clinically healthy and maintained at a licensed facility in a closed herd. New donors must be quarantined. Donors also are tested for piroplasmosis prior to entering the herd, however, no specific test is recommended, and yearly testing is not required. Therefore, some infected animals may be undetected (see section 3.3).

Products may be exempted from federal licensing requirements if licensed under a State program for intrastate movement if State requirements meet Federal standards (Kaler, Curry-Galvin et al. 2006; Evans 2010; USDA APHIS 2010a). As of 2009, the only program that met Federal requirements was California. The use of a licensed product is recommended whenever possible (AAEP 2009). There are no red or white blood cell contamination limits for USDA licensed products (Evans 2010).

Currently, there are only three USDA licensed producers of equine plasma; all of these use plasmapheresis. Some commercial plasma products produced by plasmapheresis are guaranteed by the manufacturer to be free of all blood cells (Hardefeldt, Keuler et al.). Plasmapheresis requires a special machine, and there are no commercially available systems designed for use in horses. Therefore, firms that use plasmapheresis to produce plasma must take the time and expense to modify a human machine. The licensed serum producers currently use sedimentation or centrifugation (Evans 2010).

Equine blood and serum products that do not make specific disease prevention or treatment claims fall under the regulatory authority of the Food and Drug Administration's Center for Veterinary Medicine (CVM). However, these products are low regulatory priority for the CVM, and there is no official approval process in place (Kaler, Curry-Galvin et al. 2006; AAEP 2009). Administration of unlicensed products within the context of a veterinary client patient relationship is legal (Kaler, Curry-Galvin et al. 2006; Evans 2010). In a 2009 white paper, the American Association of Equine Practitioners concluded that "equine plasma and serum products which make no disease or treatment claims are manufactured and sold without regulatory oversight." Therefore, veterinarians cannot be assured of the disease status of donor horses, or the quality of the process used to create the product. These products are most likely produced using centrifugation or sedimentation methods.

The likelihood that the donor will be EP infected is determined by management practices and testing of donors. The testing process for licensed products reduces the likelihood of using an infected donor, though infected donors may not be detected on all tests. Under the assumptions in this document, confirmed cases would not be allowed to enter into commercial plasma or serum production. In addition, horses with a known history of residence on a premises with an EP infected horse would be unlikely to be used as a donor, but, as described previously, these horses may not be identified. Horses used as donors in production of unlicensed products are less likely to be tested for EP, though no specific information is available on the practices of this industry. No information was available on the amount of plasma and serum products used in the equine industry.

In summary, the majority of plasma and serum products will have blood cell contamination. Administration of large volumes increases recipient exposure to potentially parasitized cells. Even at very low levels of contamination and an EP infected donor with very low parasitemia, a liter of plasma or serum could contain over 1,000 infected red cells. Therefore, the risk of transmission if the donor is infected with either EP pathogen is high. The uncertainty surrounding this estimate is low.

4.4.5. Contaminated Equipment

Contaminated hypodermic needles, syringes, and surgical instruments have been implicated as sources or potential sources of EP pathogen transmission (Callow 1984; Hermann, Baumann et al. 1987; Friedhoff 1988; Gerstenberg, Allen et al. 1999; Rothschild and Knowles 2007; CFSPH 2008; DAFF 2008; OIE 2009d; OIE 2009c). Reuse of syringes (but not needles) in a group of horses that had been bled regularly over several years resulted in *T. equi* infection of 61/66 horses. This scenario led to exposure to very small to miniscule amounts of blood, but resulted in very high transmission rates (Gerstenberg, Allen et al. 1999). When blood smears of 8 CF and IFAT positive mares were examined, parasites were identified in 4 mares, and parasitemia was less than 0.1 percent in all mares.

A small number of studies have examined residual blood volume in needles and syringes in human needlestick injuries and needle/syringe sharing. Needlesticks with 21 or 22 gauge needles transferred less than 1 microliter of blood (Hoffman, Larkin et al. 1989; Gaughwin, Gowans et al.

1991). These studies were designed to model accidental needle injuries sustained by health care professionals, in which the needle is in contact with the stick victim for a very short amount of time and penetrates skin or muscle. Subcutaneous or intramuscular injections in horses may be given with larger needles, and the needle remains inside the animal for enough time to inject a volume of fluid.

In needle sharing studies designed to simulate shared use of a needle and syringe for intravenous use, blood is drawn back into the syringe until just visible to check placement in the vein. Using a 2ml syringe and 25 gauge needle, one author demonstrated that a mean volume of 34 microliters of blood was transferred to the next user, with a range of 18-67 microliters over 20 trials (Hoffman, Larkin et al. 1989). Another research team estimated that the amount of blood transferred to the first subsequent user with a re-used or shared 22 gauge needle/1 ml syringe, and a re-used or shared 20 gauge needle/2ml syringe was approximately 0.5 and 5 microliters, respectively. When 0.5ml of blood was drawn into the syringe and ejected twice, the amount of blood transferred was up to 5 times greater. Washing with tap water decreased, but did not eliminate, transfer of blood. The authors concluded that regardless of the initial amount of blood contamination or washing, 2ml syringes transfer significantly more blood to a subsequent user than 1ml syringes (Gaughwin, Gowans et al. 1991). Another author hypothesized that the reason for this difference is the presence of a hub for a detachable needle on 2ml syringes. The hub creates a space between the plunger and the needle, which holds additional liquid, even when the plunger is fully depressed (Grund and Stern 1991).

In equine practice, the needles and syringes used for blood collection or intravenous administration of substances are generally larger than the equipment used in humans. This creates additional space for residual blood to collect. In addition, equipment such as intravenous administration sets and dental equipment can also transfer blood between horses.

As discussed above, previous incidents have demonstrated that the relatively small amounts of blood on contaminated needles and equipment can transmit the EP pathogens. As shown in Table 13 from 4.3, even a horse with an unrealistically low total number of red cells ($2x10^9$/ml), and a very low parasitemia (0.05 percent) will have 10^6 infected cells per milliliter, or 1,000 infected cells in one microliter of blood. Based on the studies above, contaminated needles and syringes that have been used for venipuncture in horses could realistically have at least 5 microliters of residual blood, for 20,000 infected cells given an infected horse with mid-range red cell numbers ($8x10^9$/ml) a low parasitemia (0.05 percent).

Washing with water only is likely to remove some, but not all blood (Gaughwin, Gowans et al. 1991). The addition of a detergent will increase the amount of organic material, including blood, that is removed (Rutala, Weber et al. 2008). The efficacy of disinfectants against intraerythrocytic pathogens like the EP pathogens is not well described, as these pathogens are assumed to have limited survival time outside the host (OIE 2009f).

The likelihood of exposure to contaminated veterinary equipment is determined by management practices. It is assumed that equipment contaminated with blood from a known infected horse would be cleaned prior to use, but cleaning may not be sufficient to eliminate EP infectivity. The

American Association of Equine Practioners has published biosecurity information, recommendations and guidelines for equine practitioners (AAEP 2006). These guidelines do not mention use or reuse of needles or other equipment. The recent APHIS information sheet clearly recommends against needle resuse and other risky practices, such as reusing uncleaned dental and surgical equipment (USDA APHIS 2010b).

No information is available regarding the duration of *B. caballi* or *T. equi* infectivity on contaminated equipment. Based on documented instances of transmission and the relatively high number of organisms present in even very small amounts of blood, the risk of transmission of either EP pathogen resulting from exposure to contaminated veterinary equipment is high. The uncertainty surrounding this estimate is medium due to the lack of information regarding the survival of the organisms.

4.4.6. Germplasm

Horse germplasm is considered here as a commodity already harvested from the source horse. Theoretically, pathogen-contaminated blood associated with germplasm from an infected horse could be a pathogen source (Metcalf 2001). Under the assumptions in this risk assessment, the germplasm would be collected from the infected horse after it had been allowed to move from a piroplasmosis quarantined premises. We found no reports of EP pathogen detection in equid germplasm, and no reports of pathogen transmission from contaminated germplasm. We found no reports that EP is transmitted venereally or through assisted reproduction.

Because of the lack of evidence that equid germplasm is associated with transmission of EP and handling that limits blood contamination, germplasm from an infected horse moved off quarantined premises poses negligible risk for EP spread.

4.5. Overall Risk of Exposure

The overall risk of exposure is based on the likelihood of successful transmission to an uninfected horse via the exposure pathway.

Table 14. Risk of exposure pathways.

		B. caballi		*T. equi*	
	Exposure Pathway	**Risk**	**Uncertainty**	**Risk**	**Uncertainty**
Ticks	Infected tick moving off quarantined premises	Low	Medium	Low	Medium
	Ticks on premises	Low	High	Low	High
Vertical	Vertical transmission	Negligible	Low	Moderate	Medium
Iatrogenic	Blood transfusion (medical purposes)	High	Low	High	Low
	Blood doping	High	Low	High	Low
	Commercial serum/blood plasma	High	Low	High	Low
	Contaminated equipment	High	Medium	High	Medium
	Germplasm	Negligible	Low	Negligible	Low

5. Overall Risk Estimation

The overall estimation of risk is based on the risk of release, the likelihood of exposure, and the likelihood that exposure leads to transmission. As discussed, simple horse-to-horse contact is not sufficient to transmit EP. Several exposure pathways, including tick transmission, vertical transmission, and iatrogenic mechanisms were examined.

This assessment examined the likelihood that the EP pathogen would leave a quarantined premises (release) and subsequently cause infection of a naïve horse (exposure). The exposure pathways examined are shown in Table 15.

Ticks pose a low risk of introducing or spreading infection onto a new premises. Iatrogenic transmission of blood from transfusions, blood doping, contaminated equipment, and commercial serum/blood plasma products is the most likely mechanism for new horses to acquire infection and mitigation efforts should focus on measures to ensure that infected horses, including those that are test negative, are not used for these purposes.

The movement of confirmed positive horses poses a high risk of EP release from a quarantined premises. It is assumed these horses undergo stringent biosecurity measures to minimize contact with uninfected horses. However, if iatrogenic exposure does occur, the risk of transmission is high. Therefore, the overall risk of this pathway is moderate. Additional enforcement to ensure iatrogenic exposure does not occur would minimize this risk.

The movement of infected undetected horses poses a low risk of EP release, however after released from a quarantined premises, these horse are presumed to move freely in the population. No additional testing, acaricide treatment, or identification is required of this population. It is unknown how frequently iatrogenic exposure occurs and any iatrogenic exposure is likely to result in infection, therefore the overall risk of this pathway is moderate.

Table 15. Overall risk estimation.

		Release Pathways			
		Confirmed Positive Horse		Infected Undetected Horse	
Exposure Pathways		***T. equi***	***B. caballi***	***T. equi***	***B. caballi***
CF and cELISA	Risk of release	High	High	Low	Low
cELISA only	Risk of release	High	High	Low	Moderate
Ticks attached to horse leaving quarantined premises	Risk of Transmission	Low	Low	Low*	Low
	Likelihood of Exposure	Low	Low	Low	Low
Ticks in environment	Risk of Transmission	Low	Low	Low	Low
	Likelihood of Exposure	Low	Low	High	High
Vertical Transmission	Risk of Transmission	Moderate	Negligible	Moderate	Negligible
	Likelihood of Exposure	High	High	High	High
Blood transfusion-medical	Risk of Transmission	High	High	High	High
	Likelihood of Exposure	Low	Low	High	high
Blood doping	Risk of Transmission	High	High	High	High
	Likelihood of Exposure	Low	Low	High	High
Commercial Serum/Plasma	Risk of Transmission	High	High	High	High
	Likelihood of Exposure	Low	Low	High	High
Contaminated Equipment	Risk of Transmission	High	High	High	High
	Likelihood of Exposure	Low	Low	High	High
Germplasm	Risk of Transmission	Negligible	Negligible	Negligible	Negligible
	Likelihood of Exposure	Low	Low	High	High
Overall Risk		Moderate	Moderate	Moderate	Moderate

**Moderate when male ticks of the following species are infected D.variabilis, A. cajennense, and R. microplus*

6. Data Limitations

6.1. Ticks

The high degree of uncertainty about these risk estimations is based on lack of available data. To gain a better understanding of the role of U.S. tick species in EP transmission, additional work is needed to understand the ecology of these vectors, density, and distribution. Transmission studies with U.S. tick species are also needed to better understand the unique cycle of transmission. In addition, studies are needed to better understand acaricide efficacy on cayenne tick or how effective it will remain over time.

6.2. Biosecurity

Additional information is also needed about biosecurity practices in various industries so that mitigation efforts can focus on the sectors of highest risk. USDA is currently unable to measure how well these mitigation measures are being implemented or enforced.

6.3. Test Performance

Due to the differences in cELISA tests available for EP, additional information is needed to compare NVSL versus licensed kit cELISA performance on *B. caballi* organisms.

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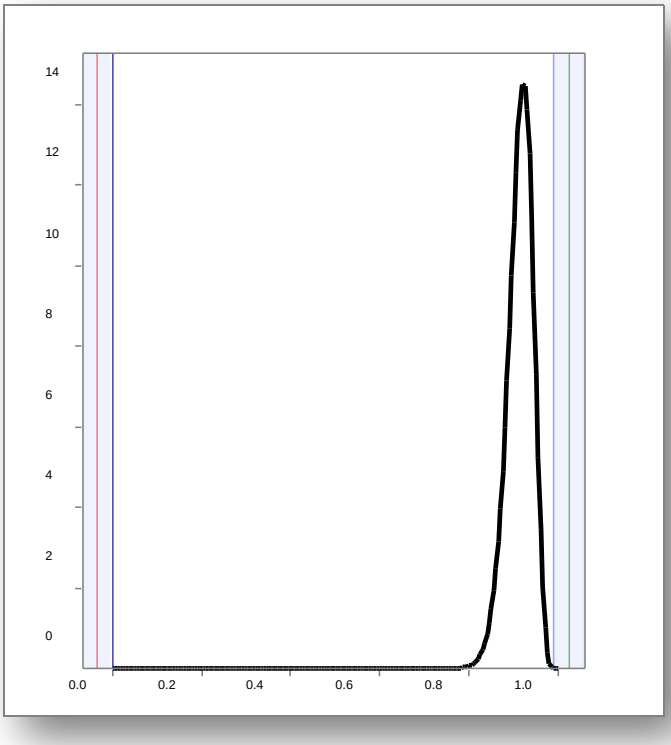
APPENDIX A. LITERATURE REVIEW OF EP RELEASE AND EXPOSURE

Location of introduction or spread	Estimated Introduction (year)	Index case detected (year)	Pathogen	Source of introduction	Mechanism of spread	Summary of outbreak	References
Release and Exposure							
United States (Florida)	1959 or 1960	1961	*B. caballi*	Walking Horses from Cuba	*D. nitens*	372 cases of *B. caballi* infection in horses in Florida until 1969	(Sippel, Cooperrider et al. 1962; Cooperrider 1963; Taylor, Bryant et al. 1969; Knowles 1988)
Australia	1950s and 1960s	1976	*T. equi*	Quarter horses from Texas	Needle sharing	Total number infected not reported; includes more than 50 locally bred horses.	(Callow 1984)
Switzerland	Unknown	1985	*T. equi*	Horse of unknown origin	Needle sharing	Fourteen racehorses on one premises.	(Hermann, Baumann et al. 1987)
United Kingdom	1981	1996	*T. equi*	Horse from North Africa	Syringe reuse	61/66 mares in an experimental herd of horses and donkeys	(Gerstenberg, Allen et al. 1999)
United Kingdom	≥8 years prior to detection	Not reported	*T. equi*	Horses from Portugal	Vertical	At least four infected horses were imported (three mares and one stallion). Vertical transmission from one mare to at least two offspring.	(Phipps and Otter 2004)
United States (Florida)	*Not reported*	2008	*T. equi*	Two horses from Mexico	Needle sharing	Twenty horses on seven premises.	(OIE 2009d)
Ireland	Not reported	2009	*T. equi*	Reported as "an animal" returning from an EP endemic region	Iatrogenic	Fifty horses on six premises	(OIE 2009e)
Release only							
Australia	1970s	1976	*T. equi*	Andalusian horses from Spain	No spread reported	Thirty horses in three geographic regions of Australia.	(Callow, McGregor et al. 1979; Callow 1984)
United States (California)	1988	1993	*T. equi*	Horse from France, or exposure in Florida	No spread reported	One Selle Français warmblood gelding.	(Holman, Hietala et al. 1997)
Germany	Within one year prior to detection	1997-1999	*B.* caballi & *T. equi*	Horses from various countries in Europe, Russia, and Ukraine	No spread reported	Eighteen horses.	(Zahler and Gothe 2000)
Hong Kong	1999	2000	*T. equi*	Horse from South Africa	No spread reported	One Thoroughbred gelding.	(Sippel, Cooperrider et al. 1962)

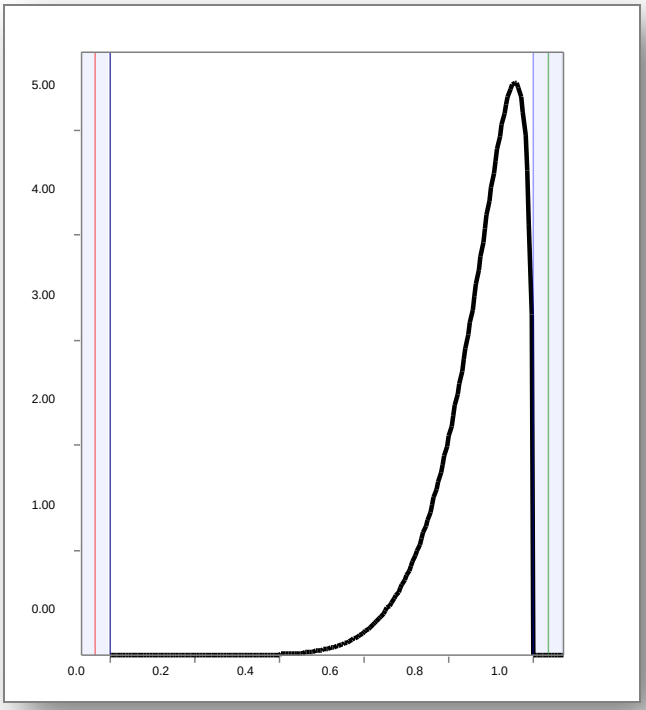
Location of introduction or spread	Estimated Introduction (year)	Index case detected (year)	Pathogen	Presumed Source of introduction	Mechanism of spread	Summary of outbreak	References
Australia	2000	2000	*T. equi*	Horse from Hong Kong	No spread reported	One Thoroughbred gelding.	(listed] 2000)
United Kingdom	Not reported	Not reported	*B. caballi*	Horse from Portugal	No spread reported	One mare. Seropositive for both *T. equi* and *B. caballi*.	(Phipps and Otter 2004)
Exposure only, or insufficient information to categorize							
Panama	Not reported	1913	*B. caballi*	Horses in Panama	*D. nitens* or *A. cajennense*	One “American driving horse”, shipped from the United States to the Panama Canal Zone; developed clinical signs 4 days after exposure to “native ponies” and ticks. Reported as "the first record of the parasite (*B. caballi*) in America".	(Darling 1913)
Panama	Not reported	1922	Not reported	Horses in Panama	*D. nitens*	Seventeen Army horses in the Panama Canal Zone	(Kelser 1922)
United States (multiple States)	Not reported	1962-1969	*B. caballi*	Exposure to horses from Florida or Puerto Rico	Not reported	Number of infected horses: Arkansas, 2; Georgia, 4; Mississippi, 1; New Jersey, 6; North Carolina, 2; Tennessee, 26	(Taylor, Bryant et al. 1969)
United States (Florida)	Not reported	1964	*B. caballi &T. equi*	Not reported	Not reported	One horse	(Riek 1964)
United States (Florida)	Not reported	1965	*T. equi*	Not reported	Not reported	One Thoroughbred horse in southern Florida	(Knowles, Mathis et al. 1966; Holbrook and Frerichs 1968)
United States (New Jersey)	1967	1967	*T. equi*	Not reported	Not reported	One horse on U.S. Olympic jumping team, returning from France	(Holbrook and Frerichs 1968; Taylor, Bryant et al. 1969)
Not reported	Not reported	1977	*T. equi*	Horses from Jordan	Needle sharing	Number of horses not reported.	(Knowles 1988)
Switzerland	Not reported	1994	*T. equi*	Not reported	Tick? (weak evidence).	First documented autochthonous case of EP in Switzerland.	(Sigg, Gerber et al. ; Gottstein, Pauli et al. 1995)
United States (Missouri)	Not reported	2009	*T. equi*	Not reported	Needle sharing	Eight quarter horses	(OIE 2009b)

Location of introduction or spread	Estimated Introduction (year)	Index case detected (year)	Pathogen	Source of introduction	Mechanism of spread	Summary of outbreak	References
United States (multiple States)	Not reported	2009	*T. equi*	Not reported	*A. cajennense*	Event not yet resolved; 408 horses in seven States (TX, AL, CO, LA, IN, NC, TN)	(USDA Sit Rep, Nov 2010, unpublished)
United States (multiple States)	Not reported	2009	*T. equi*	Not reported	Not reported	Event not yet resolved; 79 cases in six States (NM, TX, CO, OK, GA, NC).	(OIE 2010b)
United States-New Mexico	Not reported	2010	*B. caballi*	Not reported	Management practices	One Quarter horse race pony (euthanized)	(OIE 2010c)
United States (multiple States)	Not reported	2010	*B. caballi*			Event not yet resolved. Horse in NM: "under quarantine" per July 19, 2010 OIE immediate notification report; implies that this is not the same horse (euthanized) as listed in row above. As of OIE follow-up report 1: one horse in each of NM, TX, and IA.	(OIE 2010a)
United Kingdom	Not reported	2010	Not reported	Not reported	Not reported	In an OIE immediate notification report on equine infectious anemia: "The horse was also found to be positive for piroplasmosis."	(OIE 2010d)
Cuba		1951 (report date)	*B. caballi and T. equi*		*D. nitens*	"A group of horses that the Cuban army purchased from the United States"	(Roby, Anthony et al. 1964) secondary ref.; primary requested (de la Fuente, Naranjo et al. 2004)

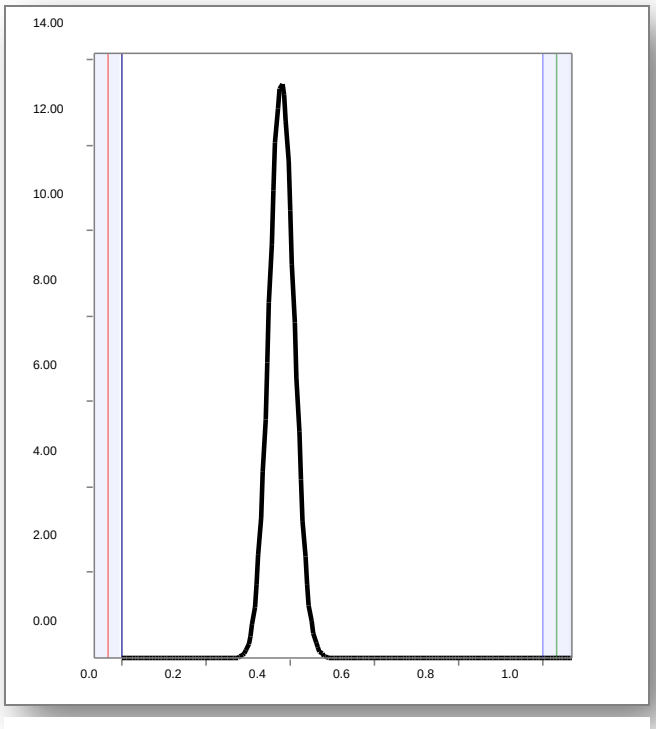
APPENDIX B. BETA DISTRIBUTIONS



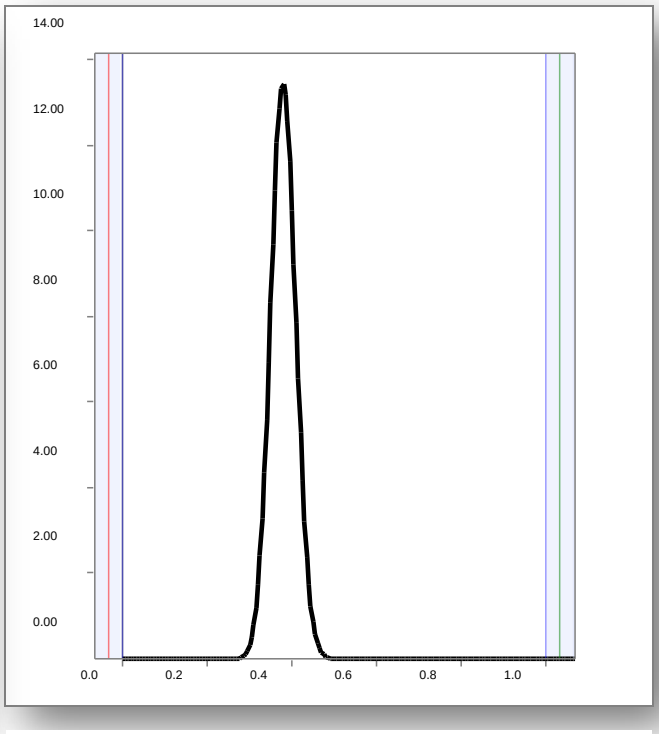
***A. B. caballi* cElisa Sensitivity**



***B. B. caballi* CF Sensitivity**



C. *T. equi* cELISA Sensitivity



D. *T Equi* CF Sensitivity

Appendix C. Performance of Diagnostic Tests Over Time

Organism: *T. equi*									
	Time to Detection			Time to Negative Test					
Blood Smear	CF	IFAT	cELISA	Blood Smear	CF	IFAT	cELISA	Infection Method	Reference
	11-20 days	7-14 days		364-455 days	63-174 days	>476 days		Inoculation of sporozoites	(Tenter and Friedhoff 1986a)
	2 weeks		3 weeks		8 weeks		>115 weeks	Inoculation	(Knowles, Kappmeyer et al. 1992)
	2 weeks		7 weeks		10->115 weeks		>115 weeks	Experimental tick transmission	(Knowles, Kappmeyer et al. 1992)
4.7 days			1 week	11 days			>22 weeks	Inoculation of erythrocytes	(Cunha, McGuire et al. 2006)
13 days			3 weeks	7 weeks			>22 weeks	Experimental tick transmission	(Cunha, McGuire et al. 2006)
2-4 days	2-10 days	3-20 days	2-19 days	4-17 days	>220 days	>220 days	>220 days		(Weiland 1986)
	30.4 days	21.9 days			540->540 days	>540 days		Inoculation of erythrocytes	(Kuttler, Goff et al. 1988)

Organism: *B. caballi*										
		Time to Detection				Time to Negative Test				
Sample Size	Blood Smear	CF	IFAT	cELISA	Blood Smear	CF	IFAT	cELISA	Infection Method	Reference
3		13-15 days	10-11 days			80-140 days	>190 days		Inoculation of erythrocytes	(Tenter and Friedhoff 1986a)
6	10-15 days	12-17 days		5-15 days	11-15 days	>30 days		>30 days		(Ikadai, Osorio et al. 2000)
9	2-4 days	2-10 days	3-20 days	2-19 days	4-17 days	67 days	>220 days	>220 days		(Weiland 1986)

Maps by Andrew M. Fox, Cartographer
Edit/document design by Carol LoSapio, Technical Editor
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USDA:APHIS:Veterinary Services
Centers for Epidemiology and Animal Health
National Center for Risk Analysis
2150B Centre Avenue, Mail Stop 2W4
Fort Collins, CO 80526-8117
(970) 494-7000
E-mail: CEAH@aphis.usda.gov

NCRA Ref #: EP_DomesticPathwaysAssessment
http://www.aphis.usda.gov/about_aphis/programs_offices/veterinary_services/ceah.shtml