Equine piroplasmosis: An updated review

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Keywords: horse; piroplasmosis; Theileria equi; Babesia caballi; imidocarb propionate

Summary
Equine piroplasmosis (EP) is a tick-borne protozoal disease. The causative agents are Babesia caballi and Theileria equi. Horses infected with T. equi remain carriers for life. Iatrogenic means can also be factors for transmission. Typical clinical signs of acute EP can include fever, anorexia, anaemia, icterus, congested mucous membranes, tachycardia, sweating, and limb and supraorbital oedema. In severe cases, haemoglobinuria and bilirubinuria are present as well as a variety of atypical presentations due to organism damage and dysfunction. Because clinical pathology is not specific of EP, accurate diagnosis requires specific diagnostic tests. The value and the pertinence of blood smears, polymerase chain reaction and serological tests are presented. Imidocarb propionate is considered as the drug of choice against EP. However, treatment strategies differ greatly between endemic and nonendemic regions. In endemic regions the goal is to reduce clinical disease because premunition plays an important role in the protection of horses, while in nonendemic regions the goal of treatment is to eliminate the risk of transmission with sterilising treatment protocols. As there is no effective vaccine available to date, prevention relies mainly on drug therapy, restriction in the movement of infected horses, and control of tick vectors.

Introduction
Equine piroplasmosis (EP) is a tick-borne protozoal disease that can be transmitted by ticks to equids. Horses, donkeys and mules are susceptible to infection, although clinical disease is rare in donkeys, mules and zebras. There are 2 distinctive EP causative agents, Babesia caballi and Theileria (Babesia) equi. It has been proposed that Babesia equi be reclassified as Theileria equi (Mehlhorn and Schein 1998). Because both Babesia and Theileria are piroplasms the term equine piroplasmosis will be used for this review. Typical EP infections are characterised by acute haemoletic disease of varying severity. However, many horses become subclinically infected with low level parasitaemia and apparent disease. The peculiarity of T. equi infection compared to B. caballi infection is that infected horses remain carriers for life, and serve as reservoirs for transmission to naïve horses (Schein 1988; DeWaal 1992; Rothschild and Knowles 2007; USDA-APHIS 2010a). The worldwide prevalence of EP is consistent with worldwide distribution of competent tick vectors. EP is endemic in tropical, subtropical and some temperate regions and has been reported to occur in Southern Europe, Middle East, Asia and almost all of Latin America (OIE 2013). For example, a survey conducted in 2002 in the south of France showed that the seroprevalence was 67% for T. equi and 23% for B. caballi which is consistent with most surveys establishing T. equi as the more frequently encountered EP agent (Mealey et al. 2011). In these endemic regions most horses are exposed to EP within the first year of life with case fatality rates of 5–10% in naïve horses depending on the parasite, the transmission dose, the health of the horse, and the treatment while this rate can exceed 50% when infected horses are imported into regions where populations of naïve horses and vectors are present (Maurer 1962; Rothschild and Knowles 2007). Equine piroplasmosis is a disease reportable to the World Organization for Animal Health (WHO) but depends on the willingness of governmental bodies to report adequately the EP information to WHO. There are then some obvious discrepancies between the published literature and the official countries status (Heuchert et al. 1999; Sevinc et al. 2008; Moretti et al. 2010; Mujicca et al. 2011; OIE 2013). In continental Europe, where most neighbouring countries have no geographical barriers and horse movements are free, there is a trend for EP to move towards northern countries from endemic to nonendemic countries as reported in Belgium, Germany, Switzerland (Boch 1985; Mantran et al. 2004; Sigg et al. 2010) and more recently in The Netherlands (Butler et al. 2012).

Transmission
Because B. caballi and T. equi are transmitted by many of the same tick vectors they frequently coinfect equids. Both parasites can be transmitted by more than 15 species of the genera Dermacentor, Hyalomma and Rhipicephalus, while Rhipicephalus (Boophilus) microplus, which is a tick more specific to cattle can also transmit T. equi to horses (Rothschild and Knowles 2007). The reservoir of parasites differs greatly between the 2 types of EP agents. For B. caballi, ticks serve as a reservoir because the organism persists in the ticks through several generations, with transtadial and transovarial transmission. For T. equi horses are the primary reservoir because T. equi is transmitted transstadially so that the parasite is not transmitted to further tick generations; in addition horses are generally carriers for life if not submitted to sterilising treatments, which are not implemented in endemic regions (Ueti et al. 2005). Transovarial transmission of T. equi has been reported but the role of this transmission route in natural transmission is still unclear (Ikadai et al. 2007). Iatrogenic means can also be factors of EP transmission in case of blood contaminated needles and syringes reuse (Gerstenberg et al. 1999), or blood transfusion from EP carriers’ donors. This route represents a major cause of transmission when introducing an inapparent carrier to a nonendemic region with or without competent tick vectors.

Summary of life cycles
Babesia caballi
Like most Babesia species, the B. caballi life cycle targets only erythrocytes. Infected ticks infect the host while feeding and
sporozoites invade erythrocytes developing immediately into trophozoites that divide into 2 large pyriform shaped merozoites. These merozoites serve as infected material for uninfected ticks, which feed on the infectious animal. Most of the parasites ingested are destroyed within the feeding tick but some survive and start to develop and infect a variety of tissues and eggs. The small pyriform bodies produced in the salivary glands of the next generation of ticks serve as infective material (Schein 1988; Rothschild and Knowles 2007) (Fig 1).

**Clinical signs and differential diagnoses**

Clinical signs of EP are similar for both infectious agents. In the endemic regions, seropositive inapparent carriers, which represent the majority of infected horses, have low level of parasitaemia that may not be detectable on blood smears while they represent a transmission risk. In addition these horses are at risk of developing clinical EP in case of concurrent disease or stress such as anaesthesia or strenuous exercise. Breeding mares that act as inapparent (or silent) carriers can also transmit the infection to their fetuses across histologically normal placenta and/or abort during the last trimester of pregnancy. The mechanism of this transmission and abortion is not clearly understood (Allsopp et al. 2007). The foals born to infected mares in endemic regions are protected from clinical disease through ingestion of protective colostral antibodies, which may persist for 4–5 months (Friedhoff and Soule 1996; Heuchert et al. 1999). There is a range of clinical presentations from acute and severe disease to chronic and mild, the acute forms being the more severe and carrying the poorer prognosis due to organ damage complications while

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the chronic forms present the less specific clinical signs such as mild inappetence, poor performance, weight loss, splenomegaly and malaise. High parasitaemia as well as low protective immunity status are incriminated in the more severe cases. Typical clinical signs of acute EP can include fever (exceeding 40°C), anaemia, icterus, congested mucous membranes, tachypnoea and tachycardia, sweating, limb and supraorbital oedema, anorexia and occasionally petechial and ecchymoses (Figs 2–6). In severe, cases haemoglobinuria and bilirubinuria are present as well as a variety of atypical presentations due to organ damage and dysfunction (oedema and inflammation) caused by obstruction to capillaries or other small vessels with parasitised erythrocytes (Rothschild and Knowles 2007), the advocated mechanism being changes of the erythrocytes membrane’s biochemical structure leading to reduced deformability and aggregation at capillary level (Ambawat et al. 1999). In addition, severe haemolysis can be associated with acute liver or renal failure (haemoglobin induced pigment nephropathy) as well as disseminated intravascular coagulation as a systemic response to inflammation (DeWaal 1992). Therefore, in severely affected horses, the complications may vary with the organ affected: inflammation of mucous membranes and blood vessels in various organs, pneumonia, renal and liver failure, digestive tract signs including colic, constipation, diarrhoea or catarrhal enteritis, central nervous system involvement with encephalitis or ataxia (Schein 1988; Rothschild and Knowles 2007; USDA-APHIS 2010a). Death may occur especially in these severe cases. Equine piroplasmosis is considered as a routine disease in endemic areas and thus the veterinarian has always in mind this diagnosis when facing such clinical signs. However, the clinical signs of EP are consistent with other diseases including equine infectious anaemia, purpura haemorrhagica, idiopathic immune
mediated anaemia and intoxications. Immune mediated haemolytic anaemia can also be a complication of EP in a number of horses. Despite treatment based on positive EP testing the anaemia worsens and may reach sometimes less than 10% packed cell volume (PCV) with associated icterus and elevated bilirubinaemia. Coomb's test is usually positive and high doses of corticosteroids are required to induce an immunosuppressive effect aimed at controlling the process (unpublished personal data).

Clinical pathology
Clinicopathological abnormalities in EP horses usually include reduced red blood cell count, platelet count and haemoglobin concentration. The PCV rarely falls below 20% but in severely affected horses profound anaemia may occur with lower PCV values (Zobba et al. 2008). Eosinopenia and monocytosis may be observed in the early stages of the disease followed by lymphocytosis. In acute forms neutropenia and lymphopenia with occasional left shift may also be present (Rothschild and Knowles 2007). Thrombocytopenia with prolonged clotting time may also occur (DeWaal 1992; Zobba et al. 2008). Fibrinogen may be decreased, even if rare in horses, in case of acute liver failure or disseminated intravascular coagulation or more frequently elevated in case of systemic response to inflammation (Allen et al. 1975; Tamzali et al. 2001). Albumin concentration may be also decreased or elevated depending on length duration of the disease, associated conditions causing the protein loss and the hydration status (Allen et al. 1975; Rothschild and Knowles 2007). Hyperbilirubinaemia and liver enzymes increase may also occur (Zobba et al. 2008). Haemoglobinuria can also be observed to varying degrees. In chronic forms only mild anaemia is present with no change in serum bilirubin concentration (Rothschild and Knowles 2007). Most of these abnormalities are not specific of EP and occurs to varying degrees. Thus more specific tests are required for diagnosis.

Diagnosis
Blood smears
Careful microscopic examination of Giemsa, Wright or Diff-Quick stained blood smears is important because the level of parasitaemia can be very low even in acute cases. In B. caballi infections the percentage of parasitised erythrocytes does not usually exceed 1% and may be lower than 0.1% (Friedhoff et al. 1990; Rothschild and Knowles 2007), while in T. equi infections it usually ranges from 1–7% but may exceed 20% (Rothschild and Knowles 2007; Donnellan and Marais 2009) and reach 80% in severe cases (Friedhoff et al. 1990). The parasites appear with powder-blue stained cytoplasm containing red stained chromatin and a central vacuole. B. caballi trophozoites appear either as single ring forms or oval or elliptical. B. caballi merozoites (dividing forms) occur in pear shaped pairs that are longer than the radius of the erythrocyte and linked by a common pole with a length of 2–5 µm (Fig 7). T. equi trophozoites are predominantly oval organisms up to 3 µm. T. equi merozoites usually occur as 4 piriform parasites, 1.5 µm long, in a characteristic Maltese cross formation (Fig 8). In chronic or inapparent carriers, blood smear examination may not be rewarding because the level of parasitaemia can be very low. Thick blood smear technique as well as centrifugation both increases significantly the number of erythrocytes to be screened and may help to increase the sensitivity of the technique in cases of very low parasitaemia (Rothschild and Knowles 2007; OIE 2008; USDA-APHIS 2010a).

Given the difficulty in blood detection of the parasites serological methods have been developed for diagnosis.

Serological tests
Three types of serological tests are used to detect previous exposure to infection but also for inapparent chronic carriers having low level of parasitaemia, which may not be detectable by blood smears. However, these tests are of low value for clinical diagnostic purposes in endemic regions where most equids have been in contact with EP agents and are positive. Both B. caballi and T. equi antibodies can be detected with these tests.

These include the complement fixation test (CFT), the competitive inhibition enzyme-linked immunosorbent assay (cELISA) test and the indirect immunofluorescent antibody test (IFAT). Currently the recognised tests for purposes of equine
importation by the World Organization for Animal Health (OIE) are the IFAT and cELISA tests (OIE 2008).

Complement fixation test
The CFT was the previous official test for EP and has been used worldwide for testing horses before entry in EP-free countries. Based on the complement fixation during the reaction between specific antigen and antibody, it is very specific and considered positive when the reaction is positive at a dilution of 1:5 (Fig 9). The antibody titres can be detected from Day 8 after infection and decline after 2–3 months. In addition to the need for production of large quantities of antigens the CFT presents many disadvantages such as the occurrence of false negative results, cross-reactivity between B. caballi and T. equi sera and low sensitivity in chronic cases (Rothschild and Knowles 2007; OIE 2008; USDA-APHIS 2010a). CFT is negativated within 2 months once B. caballi infection has been successfully cleared with a sterilisation protocol (Schwint et al. 2009).

Indirect immunofluorescent antibody test
The IFAT is more sensitive than CFT and has been used as supplementary test when CFT was inconclusive. Parasite antigens are bound to glass slides with a fluorescein-labelled antiequine serum and allowed to react with test sera. Bound antibodies are visible under ultraviolet light. Sera are considered positive if strong parasite fluorescence is shown at a dilution of 1:80 and higher (Fig 10). Antibody response can be detected 3–20 days after infection and remain during the latent period of infection. IFAT provides more consistent results than CFT. In addition to the fact that it needs large amounts of antigen and that it is time consuming, the IFAT is difficult to standardise due to the subjectivity in the interpretation of fluorescence (Rothschild and Knowles 2007; OIE 2008; USDA-APHIS 2010a). To the author’s knowledge there is no information about the negativation of the IFAT after clearing EP infection in horses.

Competitive inhibition enzyme-linked immunosorbent assay
The cELISA test was approved in 2004 by OIE for both B. caballi and T. equi as the prescribed test for international horse trading. It detects antibodies to both EP agents using specific monoclonal antibodies. It has been greatly improved by the use of a recombinant EMA-1 protein instead of culture derived whole parasites. This facilitates the standardisation of the assay. It overcomes the problem of antigen purity because specificity depends only on the monoclonal antibody used. The cELISA tests are available as kits for detection of antibodies to either B. caballi or T. equi. The cELISA test has demonstrated improved performance compared to CFT and IFAT especially in cases of inapparent carriers (latent infections) (Rothschild and Knowles 2007; OIE 2008; USDA-APHIS 2010a). However, recent clinical cases of EP in the USA have illustrated that in acute infection the CFT may be positive while the cELISA test is negative until several weeks after clinical disease. Thus, within an outbreak, multiple tests may be necessary to determine a horse’s infection status accurately (Mealey et al. 2011) and this test may not be as sensitive as expected for exportation if a horse has been recently infected. cELISA is negativated within 6–7 months once B. caballi infection has been successfully cleared with a sterilisation protocol (Schwint et al. 2009).
cleared with a sterilisation protocol (Schwint et al. 2009). In a recent study aimed at evaluating a sterilisation protocol for T. equi natural infection cELISA remained positive up to 24 months after the horses have been successfully cleared as demonstrated with nested polymerase chain reaction (PCR) and elimination of the transmission risk (Ueti et al. 2012). These results will be presented later in this article but the validity of the current cELISA test in such circumstances is questioned.

**Polymerase chain reaction**

Several types of PCR tests have been developed for research purposes including quantitative (or real-time) PCR, nested PCR and nested PCR with hybridisation (Butler et al. 2008; Schwint et al. 2009). While the latter are still used mainly for research, quantitative PCR is becoming routinely available for diagnostic purposes in specialised laboratories. Nested PCR has been demonstrated to be more sensitive than primary PCR (Rothschild and Knowles 2007; USDA-APHIS 2010a). PCR allows the detection of parasite DNA. It has been also demonstrated to be more sensitive than blood smears for the diagnosis of inapparent infections in many studies. Interestingly, PCR has also allowed the detection of both B. caballi and T. equi in the bone marrow of asymptomatic horses (Pitel et al. 2010). Finally, PCR testing may be interesting for the early evaluation of sterilising treatment especially when treating a B. caballi infected horse for the purpose of exportation to an EP free country because the official cELISA test required for horse trading remains positive for a long period (up to 6 months) after successful treatment.

Figure 11 shows a summary of diagnostic tests.

**Immunity**

The protective immunity acquired by horses in endemic regions is the result of persistent EP infection, which results in continuous stimulation of immunity by the parasite. In this aspect foals need a certain amount of tick exposure to allow them to develop immunity. Thus horses are protected for life and resistant to clinical disease in most cases. The exact mechanism of this protective immunity is unknown to date and further research is needed to define clearly the determinants of immune protection against EP infection. There is no cross protection between B. caballi and T. equi. Innate immunity also plays a role in the control of EP but the precise role of cells involved in parasite control (neutrophils, macrophages and natural killers) is not exactly defined yet. The spleen plays a central role in the control and elimination of EP agents. Horses with intact spleen are able to control the parasite infection and to survive while splenectomised horses are unable to control the infection and die (Frerichs et al. 1969; Kuttler et al. 1986). However, adaptive immunity appears of primary importance. Foals with severe combined immunodeficiency are unable to control experimental infection with T. equi; they develop severe parasitaemia and anaemia and die. But these foals have an intact spleen and competent innate immune system. This demonstrates that the spleen is unable to control T. equi infection in the absence of adaptive parasite-specific immune response (McGuire et al. 1975; Knowles et al. 1994; Wiler et al. 1995; Shin et al. 1997).

Equine piroplasmosis infection produces a strong humoral response. ELISA identification of antigen-specific antibody titres has shown that IgGa and IgGb correlate with control of T. equi during the acute stage of infection while IgG(T) increases during chronic infection (Cunha et al. 2006).

Several experimental immunisation strategies have been tested for EP but at present there is no effective vaccine. Concepts for developing an effective vaccine would be as follows (Mealey et al. 2011): since sporozoites are the infective forms entering the equine host, immune responses directed against them could prevent infection. Relevant humoral responses would include antibodies capable of blocking sporozoite entry into lymphocytes (T. equi) or erythrocytes (B. caballi), and antibodies capable of eliciting antibody-dependent cellular cytotoxicity of schizont-containing lymphocytes (T. equi). Important cell-mediated responses would include CD8+ cytotoxic T lymphocytes that eliminate schizont-containing lymphocyte target cells in an MHC class I-restricted manner, and CD4+ helper T lymphocytes that promote IgG class switching, activation of macrophages, and enhancement of cytotoxic T lymphocyte killing (Fig 1).

**Treatment**

In endemic regions, the goal of treatment is not to clear infection but to reduce clinical disease because EP prevalence is high and premunition (protective immunity) by persistent subclinical infection plays a role in the protection of horses against subsequent infection and disease. In other words it would be deleterious to sterilise a horse because this would result in waning the immunity such that the horse would become susceptible to developing severe clinical disease similarly to a naive horse. Maintaining a low level of
parasitaemia maintains sufficient immunity to provide clinical protection. This is different from nonendemic regions where the goal of treatment is generally to clear infection and eliminate the risk of transmission. It is also the case when a seropositive horse has to be moved from an endemic region to an EP-free region.

While several different antiprotozoal drugs and treatment protocols have been used to treat EP, imidocarb propionate appears as the drug of choice for EP treatment because it is effective against erythrocytic stages of both B. caballi and T. equi (Rothschild and Knowles 2007).

**Imidocarb propionate in horses**

Imidocarb dipropionate is a diamine of the carbamidie series of antiprotozoal compounds. It has been recently delisted for horses in Europe because of the lack of minimum residue limits for horsemeat but is still labelled for cattle and/or dogs in most of the countries throughout the world so that it can be used under the cascade system according to local veterinary regulations. The mechanism of action is not precisely known but it is active on the erythrocytic stages of the parasites. When injected i.m., imidocarb is rapidly sequestered in tissues and can remain in the body for a prolonged period with high levels in storage tissues such as the liver and kidneys, leading to a prolonged antiprotozoal effect (Belloli et al. 2002). Imidocarb has a narrow safety margin with an LD50 of 16 mg/kg bwt. Death occurs within 6 days after injection of this dose and is attributed to acute renal cortical tubular necrosis and acute periportal hepatic necrosis (Adams 1981). Donkeys are more susceptible to imidocarb propionate than horses (Donnellan and Marais 2009; USDA-APHIS 2010a).

In endemic regions, both B. caballi and T. equi infections are usually treated with i.m. administration of imidocarb at 2.2 mg/kg bwt for 2 treatments with a 24–48 h interval and it is considered sufficient and most effective (Rothschild and Knowles 2007). The protocol for clearing (or sterilising) B. caballi and/or T. equi infection consists of 4 i.m. injections of 4 mg/kg bwt imidocarb administered at 72 h intervals (Kulltter et al. 1987; DeWaal 1992; Meyer et al. 2005; Rothschild and Knowles 2007). Imidocarb is rapidly cleared from plasma but this protocol may ensure that high drug concentrations are maintained in storage tissues therefore supplying the body with delivery reservoirs for the continuous release of imidocarb; the storage areas may also be sites where circulating infected cells are exposed repeatedly to elevated drug concentrations (Belloli et al. 2002).

Adverse effects due to anticholinesterase effects are common but usually transient. These include spasmodic colic, diarrhoea, and inappetence (Meyer et al. 2005; Rothschild and Knowles 2007). Pretreatment to minimise these side effects is recommended with either atropine sulphate (Meyer et al. 2005), glycopyrrolate which has fewer side effects than atropine, N-butyloxypalamonium bromide or nonsteroidal anti-inflammatory drugs such as dipyrone or flunixin meglumine. In the author’s experience the combination of N-butyloxypalamonium bromide and nonsteroidal anti-inflammatory drugs has provided regularly a satisfactory control of anticholinesterase effects. Local injection site reactions can also occur.

**Is imidocarb propionate clearing protocol efficient?**

Treatment failure has been reported in a natural coinfection (Butler et al. 2008). Four Paso Fino horses imported to the island of Curacao on the basis of a negative CFT test were retested seropositive to B. caballi and B. equi with the IFAT test and were treated with 5 consecutive doses of imidocarb dipropionate (4.7 mg/kg bwt i.m. q. 72 h). This treatment protocol temporarily resulted in negative serological tests but samples collected 6 and 18 weeks after treatment tested positive for both B. caballi and B. equi antibodies.

In contrast, this clearing protocol has been tested with success in both species with subsequent removal of transmission risk.

For B. caballi infection (Schwint et al. 2009), horses were inoculated with B. caballi and treated with imidocarb according to the above protocol 70 days later. Five days post treatment, the treated horses were PCR-negative and remained PCR-negative over a 9 month period. They became also seronegative by CFT within 56 days and sero-negative by cELISA within 201 days. Inoculation of blood sampled from each of the treated horses 3 months after treatment did not transmit B. caballi to naïve horses and uninfected transmission-competent Dermacentor nitens ticks fed on 2 treated horses failed to transmit B. caballi when subsequently fed on naïve horses, showing that transmission risk from the treated horses was successfully eliminated.

For T. equi infection (Mealey et al. 2011; Ueti et al. 2012), numerous naturally infected horses involved in the last Texas T. equi outbreak have been treated similarly. Following imidocarb treatment, nested PCR was negativated in 24 of 25 naturally infected horses. Blood transfer from treated horses that became nested PCR negative failed to transmit to naïve, splenectomised horses. Although these results were consistent with elimination of infection in 24 of 25 horses, cELISA remained positive in the majority of treated horses. Imidocarb treatment was unsuccessful in one horse that remained infected as measured by nested PCR and retained the ability to infect a naïve recipient via intravenous blood transfer. The authors suggest that the most likely explanation for long-term T. equi-specific antibody persistence was the maintenance of long-lived plasma cells secreting antibodies in the absence of specific antigens as reported following elimination of infection in other infectious disease models. As the cELISA test is the current official test for horse movements, the extended time between chemotherapeutic elimination of T. equi in horses that no longer pose a transmission risk and the first seronegative result could be problematic and points out the need for the development of a rapid and accurate serological assay to confirm chemotherapeutic elimination of T. equi.

Interestingly, the horse that did not respond to treatment at the first round was successfully retreated one year later with elimination of the transmission risk. This shows that resistance to imidocarb was not the issue and that in case of treatment failure it would be worth retrying after a few weeks or months. The authors suggest that this phenomenon could be either due to individual differences in drug metabolism/distribution or to the failure of imidocarb to completely eliminate the pre-erythrocytic stages of T. equi similarly to a closely related human pathogen, Plasmodium vivax, for which the antimalaria drug chloroquine has failed to eliminate the pre-erythrocytic liver stages causing relapsing of malaria (Baird and Reckmann 2003).

**Prevention**

As there is no commercially available effective vaccine against B. caballi or T. equi to date, prevention against EP relies...
mainly on drug therapy, restriction in the movement of infected horses, and control of tick vectors.

**Restricting the movement of infected horses**
Currently, the countries that restrict the movement of serologically positive horses are the USA, Canada, Australia, Japan, Mexico and Brazil (Rothschild and Knowles 2007). For example in the USA, a horse that is B. caballi positive is quarantined and may be allowed to enter after treatment and subsequently tested negative while a horse that tests positive for T. equi is denied entrance. If a horse already resident in the USA is found positive for T. equi, it is quarantined, exported or subjected to euthanasia. Currently, most of these T. equi positive horses join the ongoing research projects. If a resident horse is tested positive for B. caballi it is simply quarantined, treated and retested for clearance. All these procedures are monitored by the federal authorities (USDA-APHIS 2010a,b).

In Europe, the WHO information shows that the approaches of government agencies vary considerably and very few countries have border controls except some insular countries that are in addition naturally protected. As there are no administrative borders between most of the European countries the introduction of inactive carriers from endemic to nonendemic countries remains difficult to control within Europe in the absence of strict controls.

**Tick-vectors control**
The control of infected ticks is a difficult or impossible undertaking, especially if neighbouring countries are EP-endemic. Quarantine of horses imported from these regions (or having travelled in these regions) with administration of pyrethroids is common sense and may help to minimise the risk of introducing infected ticks to EP-free regions (USDA-APHIS 2010a).

At major world equestrian competitions, the risk of spreading EP is prevented by biosecurity measures associating a strict segregation policy to acaricide treatment for all EP saropassive horses: natural tick barriers, designated grazing areas treated with tick retardant agents, separate stabling and frequent inspection of horses upon re-entry into the stables, treatment of all horses with an equine de-wormer that effectively kills attached ticks prior to entry but also with an acaricide prior to entry and during stay for EP-positive horses. In addition, EP-positive horses are shipped directly from their federally mandated quarantine centre to special stables within the competition site and are also required to leave the country directly from these stables.

**Conclusion**
In Europe, there is a trend for EP to move towards northern countries from endemic to nonendemic countries. However, there is a dramatic difference in EP management between endemic and nonendemic regions. So-called EP-free countries such as North American countries desire to remain nonendemic and apply very strict control measures and sterilising treatments. In endemic countries, premunition strategies are aimed at reducing clinical signs in case of overt clinical EP by reducing the level of parasitaemia but maintaining low-level infection for maintenance of protective immunity. There is then a need for development of efficient T. equi clearing methods (both treatment and diagnostic test) and protection. Ongoing research aimed at understanding immunity may help also the development of future efficient immunisation strategies.

**Author’s declaration of interests**
No conflicts of interest have been declared.

**Acknowledgements**
The author acknowledges Dr Robert H. Mealey, Associate Professor of Equine Immunology at Washington State University and Professor Philippe Jacquiet, Professor of Parasitology at Toulouse Veterinary School, for their help and advices during the preparation of this review article.

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