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ORIGINAL PAPER

Piroplasmosis in an endemic area: analysis of the risk factors and their implications in the control of Theileriosis and Babesiosis in horses

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Abstract *Theileria equi* (Laveran 1901) and *Babesia caballi* (Nuttall and Strickland 1910) are the causative agents of Equine Piroplasmosis (EP), a severe and problematic disease compromising international movement of horses. Infected horses usually become asymptomatic carriers and, for this reason, their movement across borders may become restricted. The aim of this study was to assess the seroprevalence of EP in Southern France and to evaluate risk factors associated with these parasites. In 2002, we performed a complement fixation test (CF) with blood samples from 443 horses stabled at 95 different farms located in the region of Camargue. Two epidemiological questionnaires have been used: one for each single horse (individual and management factors) and one for each place where horses were sampled (environment, presence of other species, etc.) to identify risk factors for seropositivity. *T. equi* and *B. caballi* had a seroprevalence of 58 % and 12.9 %, respectively. For *T. equi*, sex, age, activity, management, and living with or near cattle were identified as risk factors, while for *B. caballi*, only living in wetlands was recognized as a risk factor in the bivariate analysis. In the multivariate analysis, the best model for *T. equi* included as variables age, breed, and deworming, while the best model for *B. caballi* included the type of housing during day and the contact with cows.

Keywords *Theileria equi* · *Babesia caballi* · Horse · Seroprevalence · Complement fixation test · Risk factors

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Introduction

Equine Piroplasmosis (EP) is the most prevalent tick-borne disease in equids (horses, mules, donkeys, zebras) in certain areas of the world and not only causes important economic losses but also leads to movement restrictions (Knowles 1996a). The disease is caused by two hemoprotozoan parasites of the phylum Apicomplexa: *Babesia caballi* (intraerythrocytic, Fig. 1) and *Theileria equi* (intraerythrocytic and intralymphocytic, Fig. 2). Both parasites are transmitted by ixodid ticks of the genera *Rhipicephalus*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, and *Boophilus* (Thompson 1969; Klinkmann 1981; Friedhoff 1982; Waal 1992; Walker and Keirans 2000; Bautista et al. 2001; Battsetseg et al. 2001; Jongejan and Uilenberg 2004). These two parasites have in fact very different life cycles (Mehlhorn and Schein 1984). Parasites of the Babesia species have a transovarial transmission in the vectors and then enters, as sporozoites, directly into the host red blood cells where they develop into piroplasms. *T. equi*, formerly called *Babesia equi*, was reclassified as

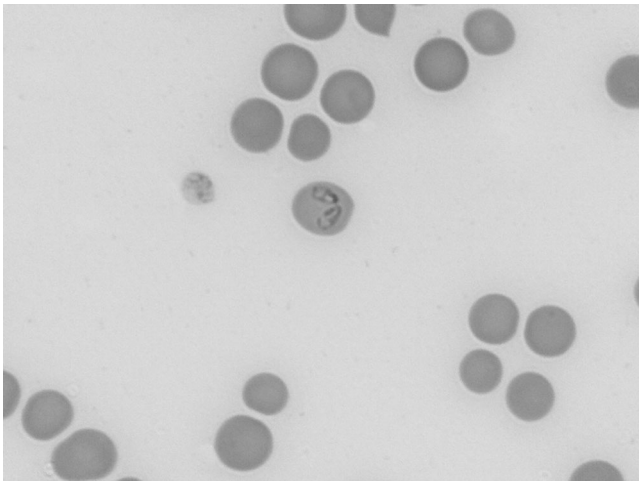

Fig. 1 *Babesia caballi* in a red blood cell (courtesy of Benoit Rannou, VetAgro-Sup)

Theileria species (Mehlhorn and Schein 1998) because of the transstadial transmission in the vector and because sporozoites do not infect red blood cells but penetrate a lymphocyte (or macrophage) where they develop into schizonts (Schein et al., 1981). The merozoites are released from the schizonts then enter the red blood cells where they grow into piroplasms.

Equine Piroplasmosis can be acute, subacute, or chronic (Rampersad et al. 2003; Uilenberg 2006). Both parasites cause severe hemolytic anemia with fever, icterus, hemoglobinuria, and edema in the distal limbs (Knowles 1996b); in case of intrauterine infection by *T. equi*, abortion and neonatal death can occur (Potgieter et al. 1992). In naïve horses, the clinical disease is particularly aggressive producing a mortality rate of up to 50 % (Waal 1992). Infections with *B. caballi* are usually less severe than those with *T. equi* (which is more frequently reported), but it is impossible to make the difference between the two parasites based on clinical signs only. Subacute and

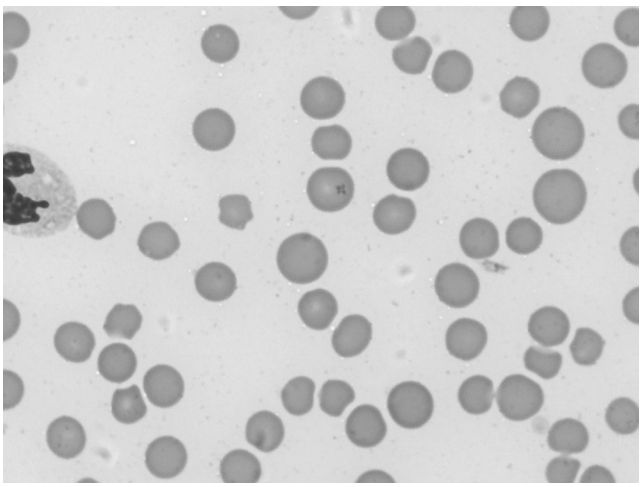

Fig. 2 *Theileria equi* in a red blood cell (courtesy of Benoit Rannou, VetAgro-Sup)

chronic forms are associated with less specific clinical signs including inappetence, weight loss, exercise intolerance, and depression (Ristic 1985; Waal et al. 1987; Camacho et al. 2005). Drugs used to treat EP have variable efficacy depending on the goal of treatment: attaining a complete eradication is much more difficult than just resolving clinical signs. Some horses may clear themselves spontaneously of *B. caballi* (Friedhoff et al. 1990; Waal 1992; Friedhoff and Soule 1996) and sterilization with treatment can be achieved (Schwint et al. 2009), while at times it is impossible to completely clear a horse from *T. equi* infection (Kirkham 1969; Friedhoff et al. 1990; Friedhoff and Soule 1996; Schwint et al. 2009; Grause et al. 2013).

Once recovered from an acute episode, a horse remains a carrier for up to 4 years with *B. caballi* and for life in the case of a *T. equi* infection (Waal and Heerden 1994; Bashiruddin et al. 1999; Sellon 2004), thereby serving as a source of infection for ticks. Detection of the areas where EP is endemic and of individual carriers before introduction in non-endemic countries or areas is of paramount importance and can be done using direct and indirect tests (OIE 2008). Equine Piroplasmosis is of importance for international trade, and a specific chapter in "The OIE Terrestrial code" (www.oie.com) describes the standard procedure for Babesia control in horses. In non-endemic countries like the USA, Canada, Australia, and Japan, only seronegative horses are allowed to be imported to prevent the introduction of carrier animals. For these reasons, in endemic countries, control of EP is critical for the equine industry to preserve the option of international movement of horses, because without it, horses cannot cross borders to compete in races or horse shows, be used for breeding purposes, or be sold abroad (Friedhoff et al. 1990).

Due to global warming and ecological changes facilitating the growth of wildlife host populations (e.g., the wild boar, and deer populations increased by 10-fold within the past 30 years), permissive ticks are expanding to hitherto non-endemic countries (Sreter et al. 2005). Of the 58 millions horses bred worldwide, 90 % live in "at-risk" regions (Ristic 1988). Equine Piroplasmosis is endemic in Europe and tropical/subtropical regions (Asia, South and Central America, Africa), and the increasing movement of horses between countries contribute to the spread of the disease from endemic to non-endemic areas (Ristic 1988; Kappmeyer et al. 2012). Despite strict control protocols for the importation of horses into the USA, evidence of introduction of equids carrying piroplasms has been found by Hall and colleagues in 2013. Currently, OIE-prescribed diagnostic tests are the indirect fluorescent antibody test (IFAT), the indirect or competitive enzyme-linked immunosorbent assay (iELISA, cELISA), and the complement fixation (CF) assay (Brüning et al. 1997; Shkap et al. 1998; Ikadai et al. 2000; Hirata et al. 2002; Asgarali et al. 2007; Acici et al. 2008; Sigg et al. 2010; Mujica et al. 2011; Seo et al. 2011). Molecular

techniques have been developed in recent years (Nagore et al. 2004; Criado et al. 2006; Alhassan et al. 2007; Adaszek and Winiarczyk 2008) and may contribute to improving test specificity and sensitivity. Despite the importance of EP in Southern Europe, studies on epidemiology and risk factors in France are lacking (Fritz 2010). Therefore, we conducted a serological cross-survey in an endemic area of Southern France to describe the seroprevalence and the risk factors associated with EP in the Camargue. A better understanding of the distribution and risk factors for EP can help identify at-risk populations and improve isolation and control measures when horses from these areas are moved to a disease-free zone.

Material and methods

Study design A detailed description of the study area and selection of horse samples has already been published in Leblond et al. (2005a). Briefly, a cross-sectional study approach was employed to determine the seroprevalence and risk factors associated to *T. equi* and *B. caballi* infections in horses bred in the Camargue or living in that area for at least 1 year. Veterinarians with an equine practice in the region volunteered to participate in the study. They were asked to provide a list of their clients who owned horses that were living in the study area. Sampling of horses was performed in two steps: a random sample of stables was selected based on the list provided by the veterinarians, then the number x of horses to be selected in each stable was defined according to the total number N of horses in that stable: if $N<5$, then $x=N$; if $5\leq N<20$, then $x=5$; and if $N\geq 20$, then $x=15$.

Sample and data collection Blood samples were collected between November 2001 and February 2002 (after a season of exposure to tick bites) by jugular venipuncture and blood collected in anticoagulant free tubes. The samples were then immediately centrifuged to harvest serum, which was subsequently stored at −18 °C until serological analysis. Data on each sampled horse (physical characteristics, use, housing, deworming program, and health status) and on the environmental conditions in which horses lived (one for each group) were collected using two questionnaires filled in at the time of blood sampling. All the locations where horses were sampled were georeferenced; they were defined as the place where horses spend most of the year, especially during the season of tick activity. Depending on the type of stable, the place was classified as a riding arena, breeding farm, or pasture. The questionnaire on the environment aimed at collecting variables indicating the presence of biotopes favorable for ticks and wild fauna. A detailed description of the questionnaires is given in Leblond et al. (2005b). An informed consent form was signed by the owner at the time of visit.

Serological test The test chosen for detection of *B. caballi* and *T. equi* was the complement fixation (CF) assay since it was the screening test used in many EP-free countries at that time. Sera have been tested with CF using a dilution of 1:5 for both *T. equi* and *B. caballi* following the method described in the OIE Terrestrial Manual 2002. Results have been divided into six categories: negative, slightly positive (0.5), 1+, 2+, 3+, and 4+. Since CF has a good specificity (few false positives), all horses from 1+ onwards have been considered positive for further statistical analysis. Since high titers in antibodies against one of the two parasites can cause cross-reactivity, only horses with the same titers for *T. equi* and *B. caballi* were considered positive for both (i.e., a horse with 4+ for *T. equi* and 4+ for *B. caballi* was considered positive for both parasites); if one of the two titers was higher than the other, the horse was considered negative for the lower one (i.e., a horse with 4+ for *T. equi* and 1+ for *B. caballi* was considered positive only for *T. equi*).

Statistical analysis To identify which variables were associated with equine seropositivity, generalized linear mixed models were used. The individual serological status was the binomial response variable. Risk factors considered included seven individual variables (breed, age, gender, activity, mosquito control, deworming, and housing) as well as the following eight environmental variables: presence of cattle, log-transformed surface of grazing area for horses, log-transformed percentage of wetlands, grassland, forests, trees, crops, and buildings in the location where horses where sampled.

All the putative risk factors were screened in univariate regression models. Variables identified as significant factors (at the level $p\leq 0.25$) in univariate phase were selected for the full multivariate starting model. Stepwise selection of the final model was carried out based on the Akaike criteria (AIC) (Burnham and Anderson 2002). Clustering of horses in stables was taken into account by introducing the stable as random effect in the logistic model.

Goodness-of-fit of the final models for *T. equi* and *B. caballi* was assessed by computing the area under the curve (AUC) of the receiver operating characteristic plots.

Spatial analysis A spatial analysis was conducted to look for clustering in the distribution of positive vs. negative stables. For this analysis, a positive stable was defined as a stable comprising at least two positive horses, and the model we used is a bivariate location point process. The Khat bivariate function was used (Leblond et al. 2005a). This function uses a distance criterion between positive and negative points to determine if a spatial structure exists or if points are randomly distributed. This method has the advantage to eliminate the population-at-risk confounding (Carpenter 2001).

All analyses were performed using R software version 3.0.2.

Results

Eventually, 443 horses from 95 different farms were enrolled into the present study and tested. Among them, 257/443 were positive for *T. equi* and 57/443 for *B. caballi*, which represents a seroprevalence of 58 % and 12.9 %, respectively. Of the horses tested, 36 were positive for both parasites, 221 just for *T. equi*, and 21 only for *B. caballi*. In the Camargue, the presence of *T. equi* was more important than that of *B. caballi* (Figs. 1 and 2), but the difference in geographical location was not statistically significant.

Bivariate analysis Statistical results ($p<0.05$) of the bivariate analysis of the risk factors are reported in Table 1 for *T. equi* and in Table 2 for *B. caballi*. For *T. equi*, geldings among the Camargue horses living in large areas, within pastures or marshes, near or with cattle, and being used for breeding or tourism-related pleasure riding activities were at a significantly higher risk of contracting the infection than were horses younger than 4 years old, treated against mosquito bites, and being on a regular deworming regimen, which all carried a lower risk. For *B. caballi*, the only risk factor identified was being kept in wetlands.

Multivariate analysis For *T. equi*, the full starting model included 11 variables: breed, gender, age, activity, mosquito control, deworming, housing, presence of cows, log-transformed area for horses, log-transformed percentage of wetlands, and log-transformed percentage of crops. The best model had an AIC value of 549.7 and six models had AIC values between 549.7 and 551.7. Age, breed, and deworming were included in all the

Table 1 Qualitative and quantitative variables for *T. equi*

Qualitative variables		Negative no. of horses (%)	Positive no. of horses (%)	*p*	OR	95 % CI
Breed	Other	26 (5.9)	25 (5.6)		1	
	Camargue	61 (13.8)	165 (37.2)	0.004	2.7	1.4–5.3
	Iberic	57 (12.9)	46 (10.4)	0.494	NS	
	Saddle	42 (9.5)	21 (4.7)	0.067	NS	
Gender	Stallion	42 (9.5)	34 (7.7)		1	
	Mare	60 (13.5)	55 (12.4)	0.979	NS	
	Gelding	84 (19)	168 (37.9)	0.009	2.2	1.2–3.9
Age	<1991	59 (13.3)	119 (26.9)		1	
	1991–1996	88 (19.9)	104 (23.5)	0.004	0.5	0.3–0.8
	1997–2001	39 (8.8)	34 (7.7)	0.001	0.4	0.2–0.7
Control measures against mosquitos on the horse	No	123 (27.8)	195 (44)		1	
	Yes	63 (14.2)	62 (14)	0.029	0.5	0.3-0.9
Deworming	No	5 (1.1)	24 (5.4)		1	
	Yes	181 (40.9)	233 (52.6)	0.019	0.2	0.1-0.8
Housing during day	Box	28 (4.3)	25 (5)		1	
	Paddock	78 (17.6)	68 (15.5)	0.879	NS	
	Pasture	63 (14.2)	112 (25.3)	0.058	2.1	0.98–4.5
	Marshes	17 (3.8)	52 (11.7)	0.003	4.5	1.7–11.9
Activity	Leisure	87 (19.6)	107 (24.2)		1	
	Sport	34 (7.7)	23 (5.2)	0.101	NS	
	Livestock cutting	55 (12.4)	105 (23.7)	0.108	NS	
	Other	10 (2.3)	22 (5)	0.055	2.6	0.98–7.02
Presence of cows with horses in the stable	No	72 (16.3)	75 (16.9)			
	Yes	114 (25.7)	182 (41.1)	0.051	1.8	1.0–3.2
Quantitative variables				*p*	OR	95 % CI
Area for horses in the stable (log-transformed)				0.006	1.62	1.2–2.3
Land cover in the stable (log-transformed percentage of)						
Wetlands				0.212	NS	
Grassland				0.371	NS	
Forests				0.796	NS	
Trees				0.856	NS	
Crops				0.166	NS	
Building				0.914	NS	

Table 2 Qualitative and quantitative variables for *B. caballi*

Qualitative variables		Negative no. of horses (%)	Positive no. of horses (%)	*p*	OR	95 % CI
Breed	Other	45 (10.2)	6 (1.4)		1	
	Camargue	183 (41.3)	43 (9.7)	0.342	NS	
	Iberic	99 (22.3)	4 (0.9)	0.398	NS	
	Saddle	59 (13.3)	4 (0.9)	0.580	NS	
Gender	Stallion	73 (16.5)	3 (0.7)		1	
	Mare	105 (23.7)	10 (2.3)	0.423	NS	
	Gelding	208 (47)	44 (9.9)	0.138	NS	
Age	<1991	158 (35.7)	20 (4.5)		1	
	1991–1996	164 (37)	28 (6.3)	0.459	NS	
	1997–2001	64 (14.4)	9 (2)	0.867	NS	
Control measures against mosquitoes on the horse	No	278 (62.8)	40 (9)		1	
	Yes	108 (24.4)	17 (3.8)	0.801	NS	
Deworming	No	22 (5)	7 (1.6)		1	
	Yes	364 (82.2)	50 (11.3)	0.239	NS	
Housing during day	Box	52 (11.7)	1 (0.2)		1	
	Paddock	136 (30.7)	10 (2.3)	0.341	NS	
	Pasture	144 (32.5)	31 (7)	0.074	NS	
	Marshes	54 (12.2)	15 (3.4)	0.080	NS	
Activity	Leisure	171 (38.6)	23 (5.2)		1	
	Sport	54 (12.2)	3 (0.7)	0.298	NS	
	Livestock cutting	130 (29.3)	30 (6.8)	0.213	NS	
	Other	31 (7)	1 (0.2)	0.407	NS	
Presence of cows with horses in the stable	No	136 (30.7)	11 (2.5)			
	Yes	250 (56.4)	46 (10.4)	0.071	NS	
Quantitative variables				*p*	OR	95 % CI
Area for horses in the stable (log-transformed)				0.097	1.81	0.9–13.2
Land cover in the stable (log-transformed percentage of)						
Wetlands				0.121	1.76	0.86–3.58
Grassland				0.300	NS	
Forests				0.705	NS	
Trees				0.748	NS	
Crops				0.131	NS	
Building				0.253	NS	

selected models. Gender, activity, mosquito control, presence of cows, and wetlands were each selected once in the five other models, but these variables did not improve significantly the best model. Camargue horses and older horses were more at higher risk of contracting EP. Deworming decreased the risk of being seropositive.

For *B. caballi*, the full starting model included eight variables: gender, deworming, housing, activity, presence of cattle, log-transformed area for horses, log-transformed percentage of wetlands, and log-transformed percentage of crops. The best model had an AIC value of 306.8 and included the variables "housing" and "presence of cows." Nine other models had AIC values between 306.8 and 308.8. The variable housing was included in all 10 models. Presence of cows was included four times, deworming and wetlands three times, and crops twice in the 10 selected models, but these variables did not improve significantly the best model. Living outdoor, in pasture or in marshes, was positively associated with seropositivity of horse to *B. caballi*. Even if not significant, the presence of cattle was associated with a higher risk of being positive to *B. caballi*.

The area under the ROC curve calculated for the final model was 0.78 for *T. equi* and 0.93 for *B. caballi*, indicating a reasonable ability to discriminate between the two infectious agents (Brooker et al. 2002).

Discussion

This is the first study to evaluate the prevalence and the risk factors for EP in Southern France. Since many countries

worldwide have banned the entry of seropositive horses, the attempt to establish control measures against the parasites in endemic areas has become fundamental to the effort to eradicate or at least control the disease. Serological tests are widely accepted as surveillance tools because of their ease of use (Brüning et al. 1997). In 2002, standard serological tests available for EP were the complement fixation (CF) and the indirect fluorescent antibody (IFA) tests, respectively, but problems were associated with each of the these serological assays (Friedhoff 1982). The IFA test for *T. equi* was considered more sensitive than that of the CF test (89 % vs. 63 %), while the estimated specificity was the same (96 %) (Ogunremi et al. 2007). The IFA test for *B. caballi* was more sensitive than that of the CF test (92 % vs. 28 %) but less specific (95 % vs. 99 %) (Ogunremi et al. 2008). Problems associated with the IFA test included cross-reactivity, subjective judgment of the reader, and the high cost of the antigen (Bakheit et al. 2007). In this study, we decided to use the CF test because it was the test of choice to detect EP according to OIE recommendations in 2002. Moreover, as we expected high levels of prevalence for both parasites, we preferred to choose the CF test because of its higher specificity and ability to distinguish between the two parasites.

The sampling scheme we used in this study was designed to accurately represent the population of indigenous horses in the Camargue with respect to their geographical distribution in the study area. Indeed, the Camargue breed and livestock cutting horses, which are very specific to the study area, were well represented in our sample. To avoid bias arising from imported horses or maternal transfer of colostrum, horses present in the area for less than 1 year or born in 2001 were not included in the study population. However, as the database used for stable sampling accorded with those horses that were on file in veterinary practices, we cannot exclude a selection bias due to the exclusion of horses which were not patients of the veterinarians in the area. This may have influenced the results for EP prevalence in horses, but not the analysis of risk factors, provided that the selection bias was homogeneously distributed across the study area. Getting a better insight into the size and geographic distribution of the equine population should make it easier to design improved sampling schemes for future studies.

In our study, the seroprevalence of *T. equi* and *B. caballi* was 58 % and 12.9 %, respectively, and 8.1 % of the horses were positive for both parasites. Seroprevalence of *T. equi* was significantly higher than *B. caballi*, in agreement with most of the previous reports from other countries like Trinidad (Asgarali et al. 2007), Brazil (Heim et al. 2007), Greece (Kouam et al. 2010a), Switzerland (Sigg et al. 2010), Italy (Grandi et al. 2011), and Spain (Garcia-Bocanegra et al. 2013). This wide variability in seroprevalence can be explained by the fact that horses are usually able to clear themselves from *B. caballi* in 4 years while they commonly remain carriers of *T. equi* for life (Waal and Heerden 1994).

Several studies on the seroprevalence of *T. equi* and *B. caballi* have been conducted in different countries. High infection rates were predominantly found in subtropical and tropical regions: in Brazil, between 80 % and 90 % for both parasite species have been found (Pfeifer Barbosa et al. 1995; Heuchert et al. 1999; Heim et al. 2007; Santos et al. 2011; Peckle et al. 2013; Vieira et al. 2013). In Europe, lower overall seroprevalences were found in Switzerland (7.3 %; Sigg et al. 2010) and Netherlands (4 %; Butler et al. 2012), while in Portugal and Greece, seroprevalence varied between 11 % to 17.9 % for *T. equi* and 2.2 to 11.9 % for *B. Caballi* (Kouam et al. 2010a; Ribeiro et al. 2013). In central (Hungary; Farkas et al. 2013) and Southern (Spain, Italy, and Turkey) Europe, seroprevalence was found to vary much more, ranging from 32 % to 68 % (Acici et al. 2008; Moretti et al. 2010; Camacho et al. 2005; Garcia-Bocanegra et al. 2013). Overall, these studies clearly indicate that Mediterranean countries are at much higher risk for EP than that of Northern and thus colder regions in Europe, although seroprevalence data among studies should be compared with caution due to the different diagnostic tests used and differences in sampling schemes.

A review of the risk factors for EP identified in other studies is presented in Table 3. In our study, just being a Camargue horse was recognized as being a risk factor in the case of both parasites. While a genetic predisposition has never been studied in detail in this breed, other studies seem to indicate that breed might well be a risk factor for EP. Arabs in the study of Sevinc et al. (2008) and Quarter horses and local breeds in the study by Steinman et al. in 2012 were identified as breeds being more susceptible of contracting EP. Probably, the higher prevalence found in these breeds reflects differences in management practices such as access to pasture for longer periods of time and/or living in pastures in close proximity to cows. Grazing has been reported as a risk factor in other studies without a breed predisposition (Rapoport et al. 2014; Steinman et al. 2012; Moretti et al. 2010; Ribeiro et al. 2013). In accordance with those studies, also in our investigation, horses housed on pastures or marshes during the day were at a 2.1 and 4.5 times higher risk of being seropositive for *B. caballi* and *T. equi*, respectively.

Gender was identified as a protective factor in our study with stallions being less likely positive for EP. This has also been reported in other studies (Shkap et al. 1998; Ruegg et al. 2007; Moretti et al. 2010) and is probably due to the fact that stallions are generally kept in stalls instead of being left grazing freely in pastures, in part because of their greater value and typical male behavior. In contrast, two studies, one conducted in Mongolia and the other in Turkey (cf. Table 3; Sevinc et al. 2008; Munkhjargal et al. 2013), found significantly more seropositive animals among male horses, which most likely is due to different breeding and housing conditions in these countries.

Table 3 Review of the risk factors of Equine Piroplasmosis in the literature

Study	Country	Number of horses	Prevalence	Positively associated risk factor	Non-associated risk factor	*T. equi* vs. *B. caballi*	Test
Sigg et al. 2010	Switzerland	689	Overall: 7.3 %	Imported: 8.5 % (France, Spain, Portugal > Swiss > Germany)	Gender, age, weight loss, surgery, transfusion	T > B for imported horses	IFAT
Kouam et al. 2010a	Greece	544	Overall: 11.6 % *T. equi*: 11 % *B. caballi*: 2.2 %	Species (mules), farming > racing > recreation, region, country of origin (Greece), age (older)	Gender	T > B	ELISA
Garcia-Bocanegra et al. 2013	Spain	537	Equids: Overall: 58.4 % *T. equi*: 56.1 % *B. caballi*: 13.2 % Both: 10.8 % Horses: Overall: 53.3 % *T. equi*: 50.3 % *B. caballi*: 11.4 % Both: 8.4 %	*T. equi*: age (older), ticks presence, no vaccination plan *B. caballi*: ticks		T > B	ELISA
Heuchert et al. 1999	Brazil	140	Mares: *T. equi*: 49.2 % *B. caballi*: 79.4 % Foals: *T. equi*: 36 % in 12 months *B. caballi*: 100 % in 10 months	*B. caballi*: cattle contact, ticks presence			IFAT
Sevinc et al. 2008	Turkey	481	ELISA: *T. equi*: 16.21 % *B. caballi*: 0.8 % Both: 1.46 %	Activity (racing), gender (male > mares > foals), breed (Arab)			Blood smears ELISA
Camacho et al. 2005	Spain	60	*T. equi*: 40 % *B. caballi*: 28.3 % Both: 20 %	Wild horses arrival (?)			IFAT
Shkap et al. 1998	Israel	361	*T. equi*: 33 %	Pasture access, gender (mare, gelding)			IFAT CELISA
Acici et al. 2008	Turkey	153	Equids: *T. equi*: 21.5 % *B. caballi*: 36.4 % Both: 5.4 % Horses: *T. equi*: 23.8 % *B. caballi*: 38 % Both: 5.6 %		Age		
Grandi et al. 2011	Italy	294	IFAT: Overall: 8.5 % *T. equi*: 8.2 % *B. caballi*: 0.3 % Both: 0 %		Age, sex		IFAT PCR

Table 3 (continued)

Study	Country	Number of horses	Prevalence	Positively associated risk factor	Non-associated risk factor	*T. equi* vs. *B. caballi*	Test
			PCR (on + horses): *T. equi*: 33 % *B. caballi*: 0 %				
Rosales et al. 2013	Venezuela	694	ELISA: Overall: 50.2 % *B. caballi*: 23.2 % *T. equi*: 14 % Both: 13 % PCR (136 horses) Overall: 66.2 % *T. equi*: 61.4 %	Livestock cutting, exposure to ticks			ELISA PCR
Asgarali et al. 2007	Trinidad	93	Overall: 82.8 % *T. equi*: 33.3 % *B. caballi*: 68.8 % Both: 19.4 %		Age, sex	T > B in > 5 years B > T in foals and yearlings	IFAT
Karatepe et al. 2009	Turkey	125	Overall: 18.4 % *T. equi*: 12.8 % *B. caballi*: 9.6 % Both: 6.2 %	Location, age (older, *B. caballi*)	Sex, age (*T. equi*)	T > B	IFAT
Mujica et al. 2011	Venezuela	360	*T. equi*: 50.3 % *B. caballi*: 70.6 % Both: 35.56 %		Sex, age		ELISA
Abutarbush et al. 2012	Jordan	253	ELISA: *T. equi*: 14.6 % *B. caballi*: 0 % Blood smear: Overall: 0 %	Grazing			ELISA Blood smear
Bahrami et al. 2014	Iran	105	PCR: *T. equi*: 22.8 % Blood smear: *T. equi*: 4.7 %		Age, gender, breed		PCR Blood Smear
Vieira et al. 2013	Brazil	198	Overall: 97.5 % *T. equi*: 78.3 % *B. caballi*: 69.2 % Both: 50 %	Rural area, age (older)	Sex, ticks		cELISA
Santos et al. 2011	Brazil	714	*T. equi*: 81 %	Geographic area, altitude (<500 in), poor farming conditions			IFAT
Farkas et al. 2013	Hungary	324 (PCR 101)	cELISA, IFAT: Overall: 32 % PCR: Overall: 49 %	Age (younger, PCR)	Age (serology), gender		cELISA IFAT PCR
Peckle et al. 2013	Brazil	314	*T. equi*: 81 %	Work, walking, reproduction activity, ticks (*Amblyomma cajennense* ++)			PCR

Table 3 (continued)

Study	Country	Number of horses	Prevalence	Positively associated risk factor	Non-associated risk factor	*T. equi* vs. *B. caballi*	Test
Ribeiro et al. 2013	Portugal	162	Blood smear: *T. equi*: 3.1 % *B. caballi*: 1.9 % cELISA: *T. equi*: 17.9 % *B. caballi*: 11.1 %	Outdoor/mixed outdoor-indoor housing			cELISA Blood smears
Munkhjargal et al. 2013	Mongolia	250	ELISA: Overall: 81.6 % *T. equi*: 19.6 % *B. caballi*: 51.6 % Both: 10.4 % PCR: Overall: 51.2 % *T. equi*: 6.4 % *B. caballi*: 42.4 % Both: 2.4 %	Gender (male), age (middle age)			ELISA PCR
Moretti et al. 2010	Italy	412	IFAT: Overall: 68.4 % *T. equi*: 12.4 % *B. caballi*: 17.9 % Both: 38.1 %	Grazing			Blood smears IFAT PCR
Steinman et al. 2012	Israel	590	Overall: 26.4 %	Grazing, breed (quarter horse, local), geographic location	Age, gender		PCR
Rapoport et al. 2014	Israel	273	*B. caballi*: 9.3 %	Pasture, age (younger)			PCR

T Theileria equi, *B* Babesia caballi, *IFAT* Indirect fluorescent antibody test, *ELISA* Enzyme-linked immunosorbent assay, *PCR* Polimerase chain reaction, *cELISA* Competitive Enzyme-linked immunosorbent assay

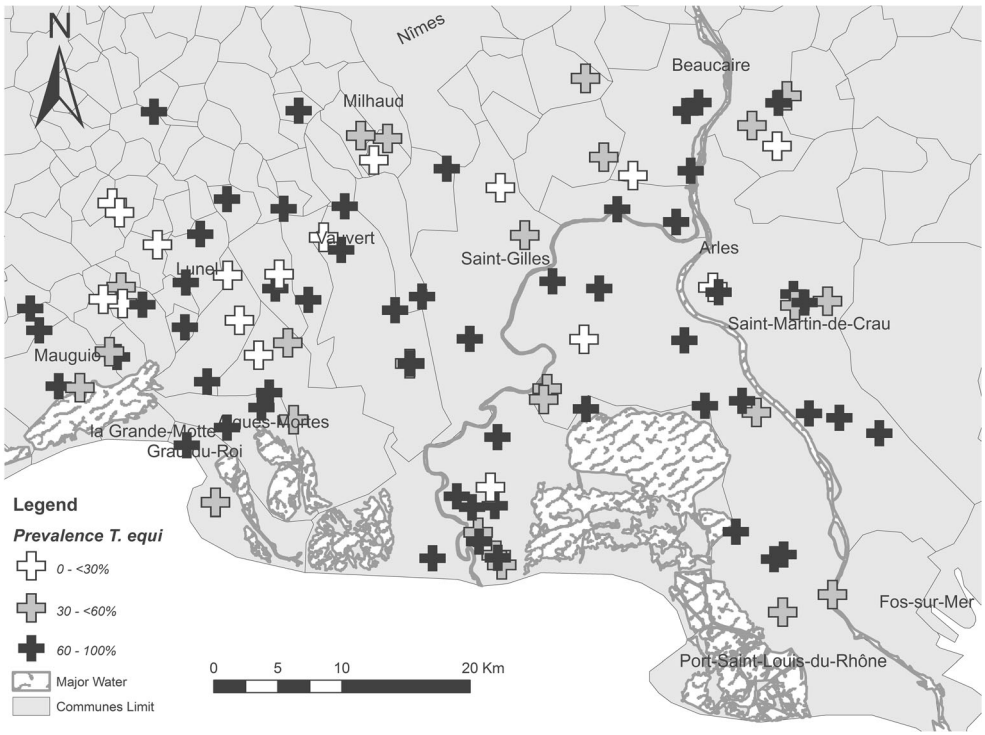

Fig. 3 Geographic distribution of *T. equi*

As in our study, in most reports, older age is considered a risk factor for seropositivity. This is easily explained by the longer exposure to vector ticks and to the carrier condition established in parasite-infested horses (cf. Table 3; Kouam et al. 2010a; Garcia-Bocanegra et al. 2013; Karatepe et al. 2009; Vieira et al. 2013; Munkhjargal et al. 2013; Steinman et al. 2012; Rapoport et al. 2014).

In our study, deworming and flies control measures were protective factors. Herds without a vaccination program were more seropositive also in the study of Garcia-Bocanegra et al.

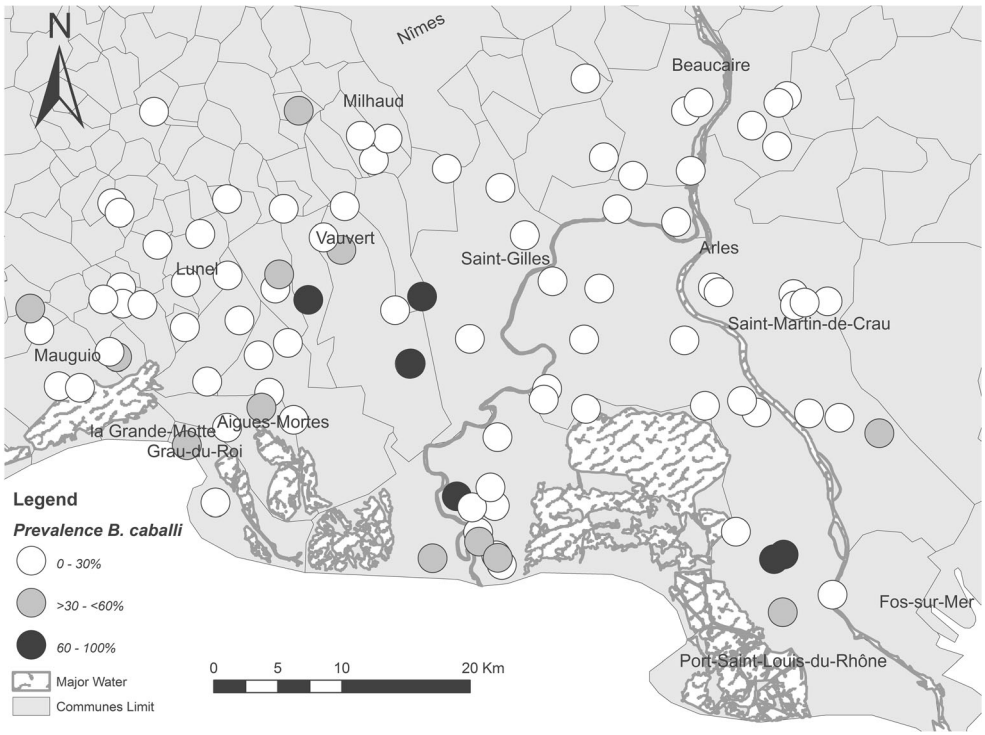

Fig. 4 Geographic distribution of *B. caballi*

(2013). Deworming, vaccination, and flies control are indicators of good herd management practices and imply a reduced exposure to tick infestation (Moretti et al. 2010; Santos et al. 2011; Vieira et al. in 2013).

We also found that working or living with cows was associated with a positive serologic result as previously reported in Brazil by Heuchert et al. (1999). This is caused by the fact that tick vectors have limited abilities to disperse and their presence depends on the abundance of appropriate hosts. Other studies also showed that for Ixodes ticks, higher numbers of ticks were collected in pastures where cows were grazing as well (Léger et al. 2013).

As is clearly depicted in Figs. 3 and 4, in our study, seropositive horses for *T. equi* and *B. caballi* did not follow any geographical distribution pattern worth further statistical exploration by cluster analysis (Chadoeuf et al. 2004). This finding was quite surprising as a geographical clustering was observed when we studied the prevalence of *Anaplasma phagocytophilum*, another tick-borne disease, in horses in the same area (Leblond et al. 2005a). The absence of cluster observed for EP made any further studies with regard to environmental risk factors using satellite imaging, as previously done in this area for other vector-borne diseases such as West Nile virus disease (Leblond et al. 2007) useless (Carpenter 2001). The study area was probably too small (around 250 km^2=96.5 $mile^2$) and ecologically uniform to observe geographical variations of seroprevalence, in contrast to what was observed in other studies conducted in Greece, Spain, or Italy in which different climatic zones were studied (Kouam et al. 2010b; Garcia-Bocanegra et al. 2013; Moretti et al. 2010).

Climate and biotopes of the Mediterranean region are particularly favorable for several species of ticks, including the main vector species involved in the transmission of EP (*Dermacentor*, *Hyalomma*, and *Rhipicephalus* spp.). At the time of the study, in 2002, we had sparse knowledge of their prevalence and distribution in the Camargue. Further studies were conducted to investigate the prevalence and diversity of ticks in this area (Chastagner 2013). Questing ticks from horse pastures and feeding ticks from horses were collected in the spring of 2007–2008 and 2010. A total of 406 adult ticks were collected, representing six species: *Rhipicephalus bursa* (n=258), *Rhipicephalus sanguineus* (n=117), *Rhipicephalus turanicus* (n=4), *Rhipicephalus pusillus* (n=11), *Dermacentor marginatus* (n=14), *and Hyalomma marginatum* (n=2) (Chastagner, 2012). All these species are potential vectors for EP. Moreover, no cluster was identified when a spatial analysis was conducted for the geographical distribution of the different species. These results could explain why no geographical variation could be identified with regard to the seroprevalence of horses for EP. It is known that ticks are very sensitive to local conditions of ecological niches (Halos et al. 2010). Further studies should probably aim at looking for finer resolution to identify environmental risk factors and vectors for EP in the Camargue.

Conclusion

To our knowledge, this is the first report of a large-scale serological investigation on EP in France. We showed that the Camargue area can be considered, as much as other Mediterranean regions, as a highly endemic area for EP. Occurrence of piroplasmosis in horses is amplified by frequent movement of the animals and the high number of natural hosts and vectors favored by recent ecological and climatic changes (Porchet et al. 2007). Therefore, further studies should be conducted to identify areas at risk in France. Moreover, it is crucial to obtain more reliable and timely estimates of the numbers of cases of EP and the related economic losses. For this purpose, the ability to diagnose infected and chronic carrier horses with accuracy is critical and molecular tools have to be used for diagnosis and identification of the various strains circulating in different areas.

Nationally, there is a need for surveillance and further research on the epidemiology of EP in France since testing of horses for EP is mandatory for the international movement of horses. This applies even more so as France has one of the largest equine populations in Europe and, moreover, hosts the 2014 World Equestrian Games. For that reason too, a continuous monitoring had recently been implemented by the RESPE (French network for the surveillance of equine diseases; www.respe.net), which will help establish long-term preventative and control measures against the spreading of EP.

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