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Seroprevalence of *Babesia caballi* and *Theileria equi* in the Swiss horse population

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ABSTRACT

In Switzerland, the prevalence and incidence of equine piroplasma parasite (EPP) infections are unknown. In order to obtain a first insight into the prevalence, a representative sample of 689 sera of horses from Switzerland was serologically tested for the presence of antibodies directed against *T. equi* and *B. caballi* using the Indirect Fluorescence Antibody Test (IFAT). A total of 50 (7.3%) horses were seropositive for EPP: overall, the seroprevalence of *T. equi* was significantly higher than that of *B. caballi* ($p = 0.002$). The seropositivities in indigenous horses (animals bred and raised in Switzerland) and in imported horses were 4.8% (11/230) and 8.5% (39/459), respectively. Unlike in indigenous horses, where no significant difference in seroprevalences could be observed between the two parasite species, the seroprevalence of *T. equi* was significantly higher ($p<0.001$) than that of *B. caballi* in imported horses. Horses imported from France, Spain and Portugal exhibited a significantly higher seroprevalence, and horses imported from Germany a significantly lower seroprevalence of EPP compared to indigenous horses. There were no associations between sex, age, weight loss, surgery or blood transfusions with *T. equi* and *B. caballi* seroprevalences.
The overall seroprevalence of 7.3% clearly shows that infection with EPP is a threat to the health of the horses in Switzerland. With the presumed expansion of permissive tick vectors, EPP infections will potentially increase in importance in the future. Therefore, continuous monitoring is mandatory.

1. Introduction

Equine piroplasmosis (EP), caused by *Theileria equi* (*T. equi*) and *Babesia caballi* (*B. caballi*), ranges among the most important tick borne diseases of horses. Piroplasmosis occurs in regions and environments (pasture) where horses are exposed to vectors — ticks, especially of the genera *Dermacentor*, *Hyalomma* and *Rhipicephalus* [1–7]. The disease is mainly endemic in tropical and subtropical areas, but regions with a moderate climate may be affected as well. Thus, EP is prevalent in parts of Africa, the Middle East, Asia, Central- and South-America, the Caribbean, but also in Europe [8,9]. In the 1980s EP was tentatively eradicated from the United States, but focal outbreaks have been regularly reported. Rigorous import regulations are in place to prevent re-emergence of the infection in many non-affected countries.

Dermacentor sp. and *Rhipicephalus* sp., important vectors of both *T. equi* and *B. caballi*, have been sporadically observed in parts of Switzerland for decades and seem to progressively expand their habitat [10–12]. Several factors may play an important role in the spread of the disease: the relocation of infected horses and ticks through national and international movement together with the geographic expansion of the tick vectors due to climate warming and respective creation of new areas, which are ecologically permissive for these vectors. Since the first description of an autochthonous case in Switzerland in 1994 [13], EP has been considered as an 'emerging disease'.

To date, information on the epidemiology of equine piroplasmosis is very limited in Switzerland. The goal of this study was therefore to determine the *T. equi* and *B. caballi* seroprevalences in a representative number of sera from adult imported and adult indigenous horses, i.e. horses bred and raised in Switzerland.

2. Materials and methods

2.1. Sample collection

To achieve a diagnostic detection limit of 0.5% disease prevalence with a certainty of 95% in a population of 85,000 horses in Switzerland, a minimum required sample of 596 randomly selected animals was calculated (WinEpiscope v_2, Module Disease Detection). For the purpose of threshold prevalence estimation, we considered factors such as breed, sex, intended use and husbandry to be irrelevant. The horse owners participated voluntarily in this study. The imported animals originated from countries from which horses are regularly introduced to Switzerland, including Germany, France, Spain, Portugal, Austria, Italy, Hungary, Czech Republic, Poland,

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Belgium, Netherlands, Denmark and Ireland. To select imported horses, the federal customs authority provided a list of horses imported between 2003 and 2007 with the names and addresses of horse-importers, as well as the number of imported horses and their country of origin. The goal of the sample selection for imported horses was to accurately represent the proportions of the countries of origin of actual horse imports. For indigenous horses, the goal of sample selection was to obtain a representative geographical distribution of the horse population in Switzerland. Because to date equidae have not been yet registered in a central database, horses were identified with the help of the databases of the equine clinic at the Vetsuisse-University in Berne and the Swiss Association for Horse Sports (Schweizerischer Verband für Pferdesport, SVPS). Eligible horse owners were phoned – and if they complied with the study – visited between December 2007 and December 2008 to collect blood samples of their animals. We collected and tested 689 serum samples from 459 (66.6%) imported horses and 230 (33.4%) indigenous horses. At the day of sampling, all owners considered their horses to be healthy. For each horse, the owner completed a questionnaire, providing information on issues such as how long the horse has been in the owner's possession, whether weight loss or other signs of disease had been observed, and if the horse had travelled outside of Switzerland. Other questions were related to housing conditions, (intended) use, feeding, vaccination and deworming practices. The collected sera were also used in a separate study on the prevalence of Equine Infectious Anaemia (EIA) [14].

During the visit, a 9 ml sample of blood was collected from the jugular vein and placed in sterile serum tubes (Monovette® 9 ml Z; Sarstedt AG and Co., D-51588 Nümbrecht). The samples were stored at 4 °C for a maximum of 4 days and then spun at 4 °C and 1160 ×*g* for 11 min (Centrifuge 5810 R, Eppendorf). The serum was then placed in sterile 9 ml-Vacuettes® (Z No Additive; Greiner Bio-One GmbH, A-4550 Kremsmünster) and stored at −80 °C until serological analysis.

Overall, the median age of horses in the study was 11 years (range 2–31; Fig. 1). The median age of Swiss-bred horses was 18 years (range 13–31), the median age of imported horses was 8 years (range 2–29). 9.4% (65) of the horses were stallions, 42.7% (294) were mares, and 47.9% (330) were geldings.

2.2. Serological test (IFAT)

The serum samples were analyzed with an Indirect Fluorescent Antibody Test (IFAT) as described by Tenter and Friedhoff [15]. Slides were coated with either *T. equi* or *B. caballi* merozoites, respectively (laboratory of Dr. Böse GmbH, Harsum, Germany). The Institute of Parasitology in Berne provided positive and negative control sera. As secondary antibody, a fluorescein-conjugated anti-horse antibody was used at a dilution of 1:90 (rabbit-anti-horse-IgG-FITC, ICN Pharmaceuticals Inc., Ohio, USA). The IFAT had been previously standardized and validated to obtain an accreditation status according to ISO 17025. In the first screening step the samples were diluted 1:40 (*T. equi*) and 1:80 (*B. caballi*), respectively. Sera that tested positive in this assay were subsequently analyzed by titration with two-fold dilutions of up to 1:320 (*T. equi*) and 1:640 (*B. caballi*).

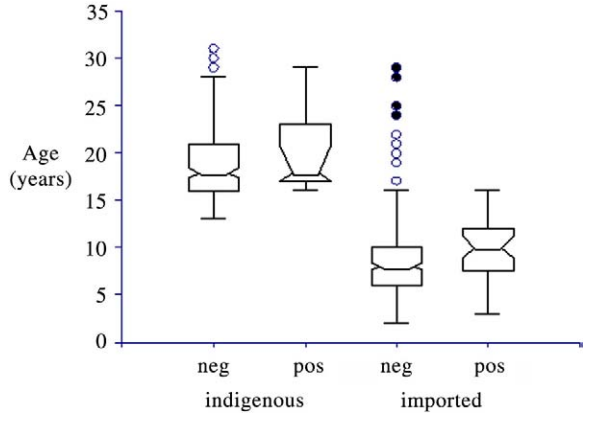

Fig. 1. Age distribution of indigenous and imported horses on the basis of seroreactivity. In the box plot, open dots indicate moderate and filled dots strong outliers.

2.3. Statistics

The seroprevalences of *T. equi* and *B. caballi* were determined with exact 95% confidence intervals (CI), and compared between various groups such as parasites, horse origin (import, indigenous as well as various countries of origin), gender and age classes using cross tabulation and the Chi-Square or Fishers Exact Test, whenever appropriate. Median age was compared between groups (serological status, origin) using the Mann–Whitney U/Wilcoxon Rank-Sum Test, and the association between piroplasma seropositivity and import status with age was evaluated using a 2-way analysis of variance (ANOVA) model. A p value of <0.05 was considered significant. All analysis were performed with the software NCSS 2007 (NCSS Statistical Software, Kaysville, Utah, USA).

3. Results

Out of a total of 689 horses, 50 (7.3%) tested seropositive for EPP. Thirty (4.4%) were positive only for *T. equi*-antibodies, 10 (1.5%) only for *B. caballi*-antibodies and 10 (1.5%) horses were seropositive for both parasite species (Table 1). Overall, the prevalence of *T. equi* was significantly higher ($p=0.002$) than that of *B. caballi*.

Of 230 indigenous horses, 11 (4.8%) were seropositive for EPP. Four horses (1.7%) had antibodies only against *T. equi*, 6 (2.6%) only against *B. caballi* and 1 horse (0.4%) was double-positive for both species (Table 1). No significant difference ($p=0.53$) was observed between the prevalence rates. Of the 11 indigenous horses that tested positive, 5 had been abroad for periods of 1 day up to 3 years, all in France. The 6 other horses had not been abroad for as long as the owners knew the horses, but a prior stay abroad could not be completely excluded.

Out of 459 imported horses, 39 (8.5%) tested seropositive for EPP. Twenty-six (5.7%) had antibodies only against *T. equi*, 4 (0.9%) only against *B. caballi* and 9 horses (2.0%) tested positive for both parasite species (Table 2). The seroprevalence for *T. equi* was significantly higher ($p=0.0001$) than that of *B. caballi*. From 138 horses imported from France, 24 (17.4%) were found to be seropositive for EPP. Thirteen horses (9.4%) were positive only for *T. equi*, 4 horses (2.9%) were positive only for *B. caballi* and 7 horses (5.1%) had antibodies to both species. Thus, in horses imported from France, the seroprevalence for *T. equi* was significantly higher than that for *B. caballi* ($p=0.03$). Horses from Germany, Italy, Poland, Hungary, Czech Republic and Austria were found to have antibodies to *T .equi* only, whereas in horses from France, Spain and Portugal, antibodies to both species were found. Horses imported from France ($p=0.0002$), Spain ($p=0.003$) and Portugal ($p=0.002$) had significantly higher prevalences of EPP than indigenous horses. German horses had a significantly lower prevalence of EPP than indigenous horses ($p=0.044$) (Table 1). None of the horses from the Netherlands ($n=6$), Ireland ($n=4$), Belgium ($n=1$) and Denmark ($n=1$) had antibodies against *T. equi* or *B. caballi* (data not shown).

Out of 65 stallions taking part in the study, 6 (9.2%) were seropositive for *T. equi* and 2 of them had antibodies against *B. caballi* as well. Stallions had significantly more often antibodies against *T. equi* than against *B. caballi* ($p=0.046$). From 294 mares, 21 (7.1%) were piroplasma-positive; 13 (4.4%) had antibodies only against *T. equi*, 4 (1.4%) only against *B. caballi* and 4 (1.4%) mares were seropositive for both species. Mares also had a significantly higher

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Table 1
Seroprevalence and 95% confidence intervals (95% CI) of equine piroplasma parasites in Switzerland.

Group	Number of horses (*n*)	*T. equi*-positive *n* % (95%CI)	*B. caballi* positive *n* % (95%CI)	*T. equi* + *B. caballi* positive *n* % (95%CI)
Totally assessed horses	689	30[a] 4.4% (1.9%–4.4%)	10[a] 1.5% (0.8%–2.7%)	10 1.5% (0.8%–2.7%)
Indigenous horses	230	4 1.7% (0.7%–4.4%)	6 2.6% (1.2%–5.6%)	1 0.4% (0.0%–2.4%)
Totally imported horses	459	26[a] 5.7% (3.9%–8.2%)	4[a] 0.9% (0.3%–2.2%)	9 2.0% (1.0%–3.7%)
Imported horses per country of origin				
Germany	200	2[b] 1.0% (0.3%–3.6%)	0[b] 0% (0%–1.9%)	0[b] 0% (0%–1.9%)
France	138	13[a,c] 9.4% (5.6%–15.5%)	4[a,c] 2.9% (1.1%–7.2%)	7[c] 5.1% (2.5%–10%)
Italy	33	3 9.1% (3.1%–23.6%)	0 0% (0%–10.4%)	0 0% (0%–10.4%)
Poland	20	1 5.0% (0.9%–23.6%)	0 0% (0%–16.1%)	0 0% (0%–16.1%)
Hungary	16	1 6.3% (1.1%–28.3%)	0 0% (0%–19.4%)	0 0% (0%–19.4%)
Czech Republic	13	1 7.7% (1.4%–33.3%)	0 0% (0%–22.8%)	0 0% (0%–22.8%)
Spain	11	3[c] 27.3% (9.7%–56.6%)	0 0% (0%–25.9%)	1[c] 9.1% (1.6%–37.7%)
Austria	11	1 9.1% (1.6%–37.7%)	0 0% (0%–25.9%)	0 0% (0%–25.9%)
Portugal	4	1[c] 25% (4.6%–70%)	0 0% (0%–49%)	1[c] 25% (0.8%–10.5%)
Horses per gender				
Stallions	65	4[a] 6.2% (2.4%–14.8%)	0[a] 0% (0%–5.6%)	2 3.1% (0.8%–10.5%)
Mares	294	13[a] 4.4% (2.6%–7.4%)	4[a] 1.4% (0.5%–3.5%)	4 1.4% (0.5%–3.5%)
Geldings	330	13 3.9% (2.3%–6.6%)	6 1.8% (0.8%–3.9%)	4 1.2% (0.5%–3.1%)

[a] Significant difference between *T. equi*- and *B. caballi*-seroprevalences.
[b] Significantly less piroplasma-positive horses than in indigenous horses.
[c] Significantly more piroplasma-positive horses than in indigenous horses.

seroprevalence for *T. equi* than for *B. caballi* ($p = 0.03$). Out of 330 geldings, 23 (7%) were seropositive for equine piroplasma parasites. Thirteen (3.9%) had antibodies only against *T. equi*, 6 (1.8%) only against *B. caballi* and 4 (1.2%) were positive for both parasites. No statistically significant difference could be detected in the seroprevalences for *T. equi* and *B. caballi* in geldings ($p = 0.11$). Furthermore, there was no statistically significant difference in the prevalence of EPP between male and female horses, neither for *T. equi* ($p = 0.44$) nor for *B. caballi* ($p = 0.97$) (Table 1).

With the titration of the positive samples we found 9 sera to be positive at a dilution of 1:40, 9 at 1:80, 7 at 1:160 and 15 at 1:320 for *T. equi*. For *B. caballi* 6 were positive at a dilution of 1:80, 9 at 1:160, 1 at 1:320 and 4 at 1:640 (Table 2). The 10 horses in which we simultaneously detected antibodies against *T. equi* and *B. caballi* all had different titers for both parasites, except one horse.

There was no significant difference in age distribution between piroplasma-positive and -negative horses ($p = 0.88$) (Fig. 1). However, the imported horses in the study were significantly younger when compared to the indigenous horses ($p < 0.0005$). For further analysis we divided the horses into 4 age groups representing the quartiles for indigenous and imported horses: 0–16 years, 17–18 years, 19–21 years and 22–31 years for indigenous horses and 0–6 years, 7–8 years, 9–11 years and 12–32 years for imported horses. There were no differences in the prevalence of EPP between the different age groups, neither in indigenous nor in imported horses (*T. equi* $p = 0.13$; *B. caballi* $p = 0.68$).

Horses that were positive for EPP did not show any significant weight loss when compared to EPP-negative horses ($p = 0.96$). Horses positive for EPP had not undergone surgery ($p = 0.93$) or recorded blood transfusions ($p = 0.19$) significantly more frequently compared to EPP-negative horses.

Table 2
Frequency of different antibody-titers against *T. equi* and *B. caballi*.

Theileria equi	<1:40[a]	1:40[b]	1:80[b]	1:160[b]	1:320[b]
Number of horses	649	9	9	7	15
Babesia caballi	<1:80[a]	1:80[b]	1:160[b]	1:320[b]	1:640[b]
Number of horses	669	6	9	1	4

[a] Negative sera.
[b] Positive sera.

4. Discussion

Standard serological tests for piroplasma parasites are the Complement Fixation Test (CFT) and the Indirect Fluorescent Antibody Test [2]. The IFAT for *T. equi* is more sensitive than the CFT (89% vs. 63%), while the estimated specificity is the same (96%) [16]. The IFAT for *B. caballi* is more sensitive than the CFT (92% vs. 28%) and slightly less specific (95% vs. 99%) [17]. In this study, we decided to use the IFAT because of its high sensitivity and good specificity.

The serum panel we used in this study was designed to accurately represent the horse population in Switzerland with respect both to the ratio of indigenous horses to imported horses and also to the geographical origin of both groups of horses. The same sera have previously been used to show the absence of Equine Infectious Anaemia in the Swiss horse population (14).

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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 [18–20]). In Europe, however, published data reflecting larger geographical areas exist only from Spain (seropositivity 94% to *T. equi* and 90% to *B. caballi* by IFAT in Cordoba, [21]; 40% for *T. equi* and 28.3% for *B. caballi* by IFAT in Galicia, Spain [22]), and from Italy (12.4% for *T. equi* and 17.9% for *B. caballi* with IFAT for Italy [23]). In our study, of the 11 horses imported from Spain, 3 were seropositive for *T. equi* and 1 horse was positive for both parasites. This leads to a seropositivity rate for EPP of 36.4%. Of the 33 horses imported from Italy, 3 (9.1%) were positive for *T. equi* and none for *B. caballi*. The difference between the results reported from Italy [23] and our results may be due to the limited number of Italian horses in our study and differences in the geographical distribution within Italy. No recent data on the seroprevalence of EPP is available from other European countries, which regularly export horses to Switzerland. The relatively high numbers of horses imported from France and Germany tested in our study (138 and 200, respectively) may provide a rough estimate of the real seroprevalences in these countries. The seroprevalence of horses imported from Germany was 1%, while it was 17.4% for the horses imported from France. However, we cannot completely rule out that the horses were infected after they had been moved to Switzerland.

In indigenous horses, the prevalence rate for EPP was 4.8%. The 11 indigenous horses with antibodies against one or both parasite species are located in various regions of Switzerland. Five of the positive horses had stayed in France for durations ranging from 1 day to 3 years. Evidence of autochthonous infections in different areas of Switzerland would be important epidemiological information in order to elaborate control measures. Unfortunately, however, incomplete owner information precluded uninterrupted reconstruction of the life histories of the infected animals identified in the present study: it was not possible to confirm that the 6 remaining seropositive horses had never left Switzerland. Autochthonous infections could thus not be conclusively proven with this study. An alternative approach, searching for *T. equi* or *B. caballi* in the indigenous tick population, may be more adequate to investigate this important question.

As the age of our study population was not normally distributed, and the indigenous horses had a higher mean age than imported horses, we separated them to compare age groups. The comparison of 25%, 50% and 75% percentiles of both groups showed no difference in seroprevalences between age groups. Neither could we see a different age distribution between infected and non-infected horses. For the interpretation of the results, it is important to consider that *T. equi* persists as a life-long infection, whereas the expected persistence of *B. caballi* in its host is 1.5 years on average. Consequently, a *T. equi*-positive IFAT may reflect an actually infected (chronic and long persisting) status, and this may be the reason for the high seropositivities in older horses reported in several other studies [24–27]. On the other hand, a horse may be seropositive for *B. caballi* without still harboring the parasite [24].

The comparison of titers against *T. equi* and *B. caballi* in horses that were seropositive to both species also indicated that in three of these horses a potential cross-reaction, as shown before by others [28], might have occurred, as one of the titers was only marginally positive.

In our study the prevalences for *T. equi* vs. *B. caballi* did not differ between female and male horses. This is in agreement with some other studies [26,27], while increased prevalences in geldings and in mares were observed in Mongolia [29] and in Italy [23], respectively.

Recently, specimens of *Dermacentor reticulatus*, infected with *Babesia canis canis*, have been found in the Western part of the Lake Geneva region [12]. As these ticks are potential vectors for EPP, autochthonous cases of EP might be possible in this region. In Southern Switzerland (canton of Ticino), a study on dogs had revealed that approximately 1/3 of ticks collected on dogs belonged to the species *Rhipicephalus sanguineus* [11]. This tick species has been found on horses in Italy [30], and *Rhipicephalus* sp. are vectors for EPP. They may also be relevant to horse owners and veterinarians in this canton. Climate and ecological changes in the potential habitat of ticks, the increase in host- and vector-populations and the more frequently occurring mobility of owners and their horses may therefore, with time, favour the increased emergence of infection with *Babesia* spp. and *Theileria* spp. in horse and dog populations [12].

To our knowledge, this is the first report on a large scale serological investigation on EPP in Switzerland. Accurate diagnosis of equine piroplasma infection is essential for providing baseline information on the prevalence, incidence and distribution in the affected equine population, and is thus a prerequisite for elaborating appropriate and effective control measures. The overall seroprevalence of 7.3% clearly shows that EPP is an important threat to the health of horses living in Switzerland. Therefore, continuous and careful monitoring is needed.

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