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Occurrence and risk factors of equine piroplasmosis in Portugal: A five-year retrospective study

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ABSTRACT

Equine piroplasmosis (EP) is a tick-borne disease of equids caused by *Theileria equi*, *Theileria haneyi,* and *Babesia caballi.* EP is endemic in most tropical and subtropical regions worldwide, and there is a likelihood that it is also endemic in Portugal. This retrospective study aimed to determine the seroprevalence, prevalence, and potential risk factors of EP in our country over the past five years. A total of 3063 diagnostic test records were analysed. Results from the competitive enzyme-linked immunosorbent assay (cELISA) revealed a seroprevalence of 32.7 % and 15.7 % for *T. equi* and *B. caballi*, respectively, with a coinfection rate of 7.4 %. For the indirect fluorescent antibody test (IFAT), 38.8 % of the samples were positive for *T. equi*, 45.7 % for *B. caballi,* and 23.1 % for both parasites. Prevalence determined using quantitative polymerase chain reaction (qPCR) showed 40.5 % *T. equi*-positive cases, 8.3 % *B. caballi*-positive cases, and 3.2 % mixed infections in the studied population. Considering risk factors, age and season appear to be associated with higher seropositivity, and location was also found to play a significant role. This study represents the first retrospective analysis carried out in Portugal, confirming the endemicity of EP in the country. Further studies are needed to corroborate our findings, to determine actual prevalence and seroprevalence in the Portuguese general equine population, and to identify risk factors better, helping breeders and owners to minimise the health and economic impact of EP.

1. Introduction

Equine piroplasmosis (EP) is a tick-borne disease caused by the hemoprotozoan *Theileria equi*, *Theileria haneyi* and *Babesia caballi* (Wise et al., 2013; Knowles et al., 2018). It is transmitted by ixodid ticks, mainly from the genera *Dermacentor, Hyalomma, Rhipicephalus, Amblyoma* and *Haemaphysalis* (Scoles and Ueti, 2015). Transplacental and iatrogenic transmission are also possible but do not have a leading role (Tirosh-Levy et al., 2020). EP affects horses, donkeys, mules, and zebras, with a significant health and economic impact (De Waal, 1992; Wise et al., 2013). Clinical signs are usually nonspecific and include lethargy, anorexia, pyrexia, icterus, and peripheral oedema. In severe cases, it can lead to death. Chronic presentation includes weight loss, poor body condition and decreased performance (Wise et al., 2014a). Inapparent carriers are the most common cases. These animals are seropositive and have low parasitaemia, thus serving as reservoirs in the presence of a competent vector (De Waal, 1992; Ueti et al., 2008; Wise et al., 2013).

Diagnosis can be performed using molecular and serological tests, depending on the clinician's objective. Molecular tests, such as qPCR, are suited for assessing active parasitaemia or patients presenting clinical signs and subsequent follow-up since they detect current infection (Giubega et al., 2022; OIE, 2021). Serological diagnosis (either cELISA or IFAT) is better for detecting inapparent carriers due to its higher

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sensitivity and specificity (Tirosh-Levy et al., 2020). cELISA is the recommended and approved test for international horse travelling (OIE, 2021; Tirosh-Levy et al., 2020)

EP is endemic in most subtropical and tropical regions worldwide, including the Mediterranean countries, of which Portugal is part (Tirosh-Levy et al., 2020). Non-endemic countries, such as the United States of America, limit the entrance of seropositive horses (Rothschild, 2013; USDA APHIS, 2023). Restrictions to exportation, travelling, participation in equestrian sporting events, and costs related to diagnosis, treatment, loss of performance and death create a significant economic impact for horse breeders, plus the welfare impact on horses.

Although EP status in Portugal was reported as prevalent by Tirosh-Levy et al. (2020), this classification relied on the limited number of studies available on *T. equi* and *B. caballi* in our country (Baptista et al., 2013; Fuehrer et al., 2020; Ribeiro et al., 2013) which are not representative of the whole territory and current situation. According to the perception of Portuguese equine veterinarians, EP prevalence may be higher than previously reported, suggesting that EP is endemic in Portugal. The objectives of this study were to determine the serological and molecular prevalence of EP over the last five years in Portugal and to access potential risk factors for diagnostic test positivity.

2. Material and methods

2.1. Data collection

This retrospective study utilised data provided by a specialised veterinary laboratory in Portugal. It encompassed all diagnostic tests (serological and molecular) for *T. equi* and *B. caballi* conducted between January 2019 and December 2023. A total of 3066 equidae samples were tested, including 3053 horses, 12 donkeys, and 1 mule. Epidemiological information included in the records, namely sex, age, breed, and animal location, was analysed. The available data were anonymised. Only three horse samples were excluded, leaving 3063 records.

Age, location, and months were organised into categories. We considered three groups regarding age: 0–5 years old (yo), 6–10 yo, and older than 10 yo. Location groups were assigned according to the Portuguese NUTS ("Territorial Units' Nomenclature") system, namely NUTS II: North, Centre, Lisbon Metropolitan Area (LMA), Alentejo and Algarve. Months were organised in seasons according to Mediterranean climate: spring (March, April, May), summer (June, July, August), autumn (September, October, November), and winter (December, January, February).

2.2. Diagnostic tests

Diagnostic tests consisted of competitive enzyme-linked immunosorbent assay (cELISA – *T. equi* and *B. caballi* antibody test kit, VMRD® Inc., Pullman, WA, USA), indirect fluorescence antibody test (IFAT – antibody test kit, MegaFLUO® THEILERIA equi and MegaFLUO® BABESIA caballi, Diagnostik GmbH, Hörbranz, Austria) and real-time/quantitative polymerase chain reaction (qPCR – antigen kit TheSpp MONODOSE and BabSpp MONODOSE dtec-qPCR, genetic PCR solutions™, GENETIC ANALYSIS STRATEGIES SL, Alicante, Spain). For cELISA, the cut-off is 40 % inhibition, defined by the manufacturer, with all samples above this value considered positive. IFAT results were categorised as "negative", "slight reaction", "weak positive", and "positive". Samples classified as "negative" and "slight reaction" were considered negative. For qPCR, positivity was determined according to the manufacturer's instructions.

2.3. Statistical analysis

Seroprevalence and prevalence were assessed based on cELISA/IFAT and qPCR results, respectively. Seroprevalence and prevalence were analysed using the chi-square test (χ^2) at a probability of $P < 0.05$ and a confidence interval of 95 %. Considering the sample size, this test assessed the independence between categorical variables, such as sex, age, location, and season. Chi-square components were employed when a variable's difference was unclear (McHugh, 2013). Whenever applicable, the odds ratio (OR) was used to measure association. Breed was not included for statistical analysis due to a high diversity of breeds, part of them with a small sample size. The agreement between different test results for the same parasite in the same patient (when available) was assessed with Choen's *Kappa* test (Landis and Koch, 1977). Statistical analysis was conducted using R Software, version 4.3.2.

3. Results

3.1. Diagnostic tests

Data from 3570 cELISA, 1564 IFAT, and 1067 qPCR tests were collected (the number of tests per parasite is available in Table 1). From those, 1758 samples were tested for both *T. equi* and *B. caballi* using cELISA, 754 with IFAT, and 408 using qPCR methods (Supplementary Table 1).

Considering cELISA, a seroprevalence of 32.7 % (592/1810) and 15.7 % (276/1760) was found for *T. equi* and *B. caballi*, respectively. Mixed infection occurred in 7.3 % (129/1758) of the samples. For IFAT, 38.8 % (295/761) and 45.7 % (367/803) of the samples were positive for *T. equi* and *B. caballi*, respectively. 23.1 % (174/754) were positive for both parasites. As for qPCR, a prevalence of 40.5 % (257/635) and 8.3 % (36/432) was observed for *T. equi* and *B. caballi*, respectively. Mixed infection comprised 3.2 % (13/408) of the samples.

The agreement between diagnostic tests was substantial for *T. equi* regarding cELISA and qPCR ($k = 0.71$) and for *B. caballi* ($k = 0.72$) when crossmatching cELISA and IFAT. A slight agreement was found between IFAT and qPCR for *T. equi* ($k = 0.2$). No agreement could be determined for the remaining combination of diagnostic tests due to insufficient cross-sampling. The exact number of common tests and respective results are in Supplementary Table 2.

3.2. Risk factors

Sex information was available for all 3063 records, 2232 males and 831 females. There were statistically significant differences between sex in cELISA and qPCR results for *T. equi*. Females have decreased chances of being positive for cELISA ($p < 0.01$, OR 0.62) and higher chances of qPCR-positivity ($p = 0.012$, OR 1.55). For *B. caballi*, there were differences in IFAT results ($p < 0.01$), with females being 1.62 more prone to be positive (Table 2).

Regarding age, information was available on 1771 samples. Of those, 986 (55.7 %) had 0–5yo, 459 (25.9 %) 6–10yo, and 326 (18.4 %) equids had more than 10yo. When looking for an association between age and test results, the p-value was < 0.001 for *T. equi* IFAT, < 0.01, and < 0.05 for *B. caballi* cELISA and IFAT, respectively. Chi-square components were analysed and are presented in Table 3, along with frequency distribution by diagnostic test.

Table 1
Frequency distributions of *T. equi* and *B.caballi* by diagnostic test and respective 95 % confidence intervals (CI95).

	Theileria equi			*Babesia caballi*		
	Tested	Positive (%)	CI95	Tested	Positive (%)	CI95
cELISA	1810	32.7	30.6 – 34.9	1760	15.7	14.1 – 17.5
IFAT	761	38.8	35.4 – 42.3	803	45.7	42.3 – 49.2
PCR	635	40.5	36.7 – 44.3	432	8.3	6.11 – 11.3

CI95 – Confidence interval at 95 %

Table 2
Sex frequency distribution by diagnostic test and respective odds ratio.

		Theileria equi		*Babesia caballi*		
	Tested	Positive	OR	Tested	Positive	OR
cELISA						
F	479	121 (25.3 %)	0.62[a]	461	76 (16.5 %)	1.08
M	1331	471 (35.4 %)		1299	200 (15.4 %)	
IFAT						
F	196	80 (40.8 %)	1.12	209	114 (54.5 %)	1.61[b]
M	565	215 (38.1 %)		594	253 (42.6 %)	
PCR						
F	192	92 (47.9 %)	1.55[c]	127	8 (6.3 %)	0.66
M	443	165 (37.2 %)		305	28 (9.2 %)	

OR – odds ratio
[a] $p < 0.001$
[b] $p < 0.01$
[c] $p < 0.05$

As for the geographic origin of samples, 55.1 % came from LMA (1689/3062), 25.8 % (789/3063) from Alentejo, 9.0 % (277/3063) from Centre, 6.6 % (201/3063) were sent from Algarve and 3.5 % (107/3063) from North NUTS. A significant association between NUTS and positivity was found for cELISA and IFAT for *T. equi* (p-value <0.05), with the North and Algarve regions showing higher seroprevalences. Considering *B. caballi*, cELISA and IFAT results were also significant (p-value <0.01 and 0.05, respectively), being LMA and Algarve the regions with higher percentages of antibodies (Table 4).

When analysing results by seasons, ELISA and IFAT results for *T. equi* were influenced ($p < 0.01$ and <0.05), with winter and spring showing a higher and similar percentage of seropositive results. Chi-square analysis of IFAT results for *B. caballi* also yielded a p-value < 0,05, with spring exhibiting the highest positive results. Chi-square components revealed significantly fewer cases in autumn than expected (Table 5).

4. Discussion

4.1. Diagnostic tests

This study presents serological and molecular results collected over five years from 3063 equids residing in Portugal. The reasons practitioners requested each test could not be determined. However, according to the laboratory, cELISA is frequently required for travelling or trade. Some breeders also test their foals before selling them. Clinicians request IFAT not only for exportation purposes but sometimes also to rule out infection in clinical cases since it is more affordable than qPCR, and strong positives are related to higher parasitaemia. qPCR is mainly used for the diagnostic confirmation of clinically suspected cases. Consequently, we cannot exclude some related bias because our

Table 3
Age frequency distributions by diagnostic test. χ^2 values are presented when $p < 0.05$ for the respective component.

	Theileria equi					*Babesia caballi*				
	Tested	Positive (%)	E.V.	χ^2	*p*	Tested	Positive (%)	E.V.	χ^2	*p*
cELISA										
0–5yo	700	228 (32.6)	-	-	0.58	676	135 (20.0)	115	3.47	< 0.01
6–10yo	269	95 (35.3)				268	31 (11.6)	46	4.67	
> 10yo	67	25 (37.3)				61	5 (8.2)	10	2.79	
IFAT										
0–5yo	236	68 (28.8)	95	7.54	< 0.001	245	100 (40.8)	115	1.88	< 0.05
6–10yo	102	57 (55.9)	41	6.30		106	55 (51.9)	50	0.58	
> 10yo	98	50 (51.0)	39	2.89		104	58 (55.8)	49	1.78	
PCR										
0–5yo	146	57 (39.0)	-	-	0.24	108	12 (11.1)	-		0.55
6–10yo	97	48 (49.5)				64	4 (6.2)		-	
> 10yo	162	66 (40.7)				114	12 (10.5)			

yo = years-old
E.V. = expected value

Table 4
NUTS frequency distributions by diagnostic test. χ^2 values are presented when $p < 0.05$ for the respective component.

	Theileria equi					*Babesia caballi*				
	Tested	Positive (%)	E.V.	χ^2	*p*	Tested	Tested	E.V.	χ^2	*p*
cELISA										
North	91	41 (45.1)	30	4.24	< 0.05	19	9 (47.4)	14	1.76	< 0.01
Centre	175	48 (27.4)	57	1.49		118	54 (45.8)	27	3.07	
LMA	997	326 (32.7)	326	0.00		394	199 (50.5)	153	6.06	
Alentejo	509	162 (31.8)	167	0.12		226	82 (36.3)	76	3.09	
Algarve	38	15 (39.5)	12	0.53		46	23 (50.0)	6	0.15	
IFAT										
North	14	8 (57.1)	5	1.22	< 0.05	19	9 (47.4)	9	0.01	< 0.05
Centre	117	50 (42.7)	45	0.48		118	54 (45.8)	54	0.00	
LMA	366	136 (37.2)	142	0.24		394	199 (50.5)	180	1.99	
Alentejo	213	73 (34.3)	83	1.11		226	82 (36.3)	104	4.39	
Algarve	51	28 (54.9)	20	3.43		46	23 (50)	21	0.19	
PCR										
North	9	4 (44.4)	-	-	0.67	5	0 (0.0)	-		0.80
Centre	3	2 (66.7)				1	0 (0.0)			
LMA	380	148 (38.9)				274	26 (9.5)		-	
Alentejo	134	60 (44.8)				71	5 (6.6)			
Algarve	109	43 (39.4)				71	5 (6.6)			

LMA = Lisbon Metropolitan Area
E.V. = expected value

Table 5
Season frequency distributions by diagnostic test. χ^2 values are presented when $p < 0.05$ for the respective component.

		Theileria equi						*Babesia caballi*		
	Tested	Positive (%)	E.V.	χ^2	*p*	Tested	Positive (%)	E.V.	χ^2	*p*
cELISA										
Spring	431	158 (36.7)	141	2.06	< 0.01	418	67 (16.0)	-	-	0.26
Summer	355	106 (29.9)	116	0.88		354	44 (12.4)			
Autumn	594	170 (28.6)	194	3.03		583	94 (16.1)			
Winter	430	158 (36.7)	141	2.14		405	71 (17.5)			
IFAT										
Spring	111	51 (45.9)	43	1.48	< 0.05	127	68 (53.5)	58	1.71	< 0.05
Summer	208	82 (39.4)	81	0.02		203	95 (46.8)	93	0.05	
Autumn	280	89 (31.8)	109	3.52		286	111 (38.8)	131	2.97	
Winter	162	73 (45.1)	63	1.66		187	93 (49.7)	86	0.66	
PCR										
Spring	155	67 (43.2)	-	-	0.81	88	10 (11.4)	-	-	0.40
Summer	182	74 (40.7)				117	6 (5.1)			
Autumn	168	67 (39.9)				136	13 (9.6)			
Winter	130	49 (37.7)				91	7 (7.7)			

E.V. = expected value

sampling technique is not randomised.

The seroprevalence found in this study aligned closely with that of Camino et al. (2021), who found seropositivity of 35.8 % for *T. equi*, 15.6 % for *B. caballi* and 8.9 % for both parasites in Spain. In Northern Portugal, Ribeiro et al. (2013) reported a seroprevalence of 17.9 % for *T. equi*, 11.1 % for *B. caballi*, and a coinfection rate of 4.9 %. It is noteworthy that our samples were predominantly from Lisbon and Alentejo, which has the largest equine population, according to Portuguese Agriculture and Veterinary Authority (DGAV) records (unpublished data). Additionally, horses in these southern regions of Portugal are commonly kept in pasture or mixed housing, increasing their exposure to ticks and consequently heightening the risk of infection.

When comparing IFAT seropositivity to cELISA, it is higher for each parasite individually as well as for coinfection (*T. equi*: 32.7 % cELISA *vs* 38.8 % IFAT | *B. caballi* 15.6 % cELISA *vs* 45.7 % IFAT | coinfection 7.3 % cELISA *vs* 23.1 % IFAT). Kamyingkird and colleagues (2014) also reported a twofold higher IFAT seropositivity than ELISA. They ascribed this to IFAT nonspecific reactions and a higher specificity of the ELISA method. Nardini et al. (2022) obtained similar results; however, contrarily to Kamyingkird et al. (2014), they suggest a potentially lower detection capacity of cELISA, considering the highest agreement between IFAT and PCR methods in their research. The disparity between this study and the present retrospective analysis is that previous researchers compared results from the same sample pool, whereas this study primarily examines results from distinct equid samples. Among sera tested by both methods, there was no agreement in *T. equi* cases ($k = 0.08$). In contrast, substantial agreement was observed for *B. caballi* ($k = 0.72$), with 8 IFAT-positive results negative by cELISA out of 137 cELISA-negative samples.

One possible reason practitioners choose IFAT over cELISA is the cost of the test, particularly when considering "herd testing" prior to selling. Another factor could be the laboratory turnaround time for test results, with IFAT providing results considerably faster than cELISA. Nevertheless, cELISA remained the preferred test (3570 cELISA *vs* 1564 IFAT), aligned with the WOAH (former OIE) recommendations for travelling.

Molecular diagnosis reveals a positivity rate of 40.5 % for *T. equi* and 8.3 % for *B. caballi*, which agrees with other studies in the Mediterranean region, where *T. equi* is more prevalent than *B. caballi* (Camino et al., 2021; Nardini et al., 2022; Rocafort-Ferrer et al., 2022). Of the 408 samples tested for both parasites, 13 (3.2 %) were positive. Coinfection rates are generally low but usual in endemic countries. Fuehrer et al. (2020) tested 101 healthy military horses from Lisbon, with a *T. equi* prevalence of 32.7 % (33/101), but could not find PCR positives for *B. caballi*. Another study (Barros, 2018) tested 27 horses. From those, *T. equi* DNA was detected in 15 (56 %) samples and *B. caballi* DNA in 2 (7 %) samples. No coinfection was identified. Although this last study has a small sample size, it also presents a higher prevalence of *T. equi* over *B. caballi*.

Considering the two types of diagnostic methods, we find a higher *T. equi* positivity rate using qPCR than serological methods. This might be explained by the fact that qPCR is mainly used for diagnosis when there are compatible clinical signs. Given the origin of our dataset, this was probably the reason why practitioners requested qPCR. Care must be taken when extrapolating these results to a clinically healthy population.

A substantial agreement ($k = 0.71$) between *T. equi* qPCR and cELISA results was found, with only 2 qPCR-negative/cELISA-positive cases. For IFAT, the agreement was slight ($k = 0.2$), with 3 qPCR-negative/IFAT-positive cases and 1 qPCR-positive/IFAT-positive case. Serological positive results with no DNA detection can occur in old infections or inapparent carriers when parasitaemia is below the detection level of qPCR, but there was previous seroconversion. When DNA is detected but no measurable antibodies are present, the infection may be recent, or there may be fluctuating parasitaemia (Coultous et al., 2019). Repeating the serological test 2–3 weeks later is advisable to detect seroconversion (Nardini et al., 2022).

4.2. Risk factors

Considering sex, females have fewer chances to be *T. equi*-seropositive (cELISA) and have superior odds of being *T. equi* qPCR positive. This fact might be related to sex-specific management practices. Females are more exposed to vectors and parasites due to the common practice of having reproductive mares in pasture with their foals, thereby increasing the likelihood of sustaining detectable parasitaemia. Serological results disagree with Bartolomé Del Pino et al. (2016), where females had higher odds of being *T. equi* seropositive. Male horses, usually chosen for a sports career, kept in-house and daily groomed, may become inapparent carriers after infection, displaying minimal or no clinical signs. Consequently, their parasitaemia levels may fall below detection limits, although seropositivity remains lifelong (Tirosh-Levy et al., 2020).

Regarding *B. caballi*, females seem 1.61 times more prone to be IFAT seropositive than males, which can be assigned to management practices, as stated before. Other studies, like Kouam et al. (2010) and Axt et al. (2024), found no sex influence on respective diagnostic test results.

Considering age, a significant increase in observed IFAT-positive cases amongst older equids than estimated for both parasites may reflect a higher exposure, particularly in animals kept on pasture, or an exposure-related higher immunity level. Additionally, IFAT results appear to be better associated with the presence of a vital parasite (Nardini et al., 2022). When considering *B. caballi* cELISA, younger

equids were the most affected. It is usual to observe higher infection rates in young animals, especially in endemic regions, due to increased exposure to causative agents (Zanet et al., 2017). Moreover, *B. caballi* infection decreases with age, as it tends to be self-limiting within approximately four years (Weiland, 1986), opposing to *T. equi* infection, which persists life-long (Onyiche et al., 2019; Tirosh-Levy et al., 2020). Knowing the housing type of tested equids would be helpful to understand these findings better.

Lisbon Metropolitan Area (LMA) has more *B. caballi* seropositives, while *T. equi* seropositives are mainly in the North, considering both IFAT and cELISA. The fact that LMA had higher rates of *B. caballi* seropositives may be ascribed to the parasite life cycle. Transovarian transmission occurs in *B. caballi* but not in *T. equi*, which means that the main reservoir for the first is the tick, while for the latter, it is the equine host (Scoles and Ueti, 2015). LMA also has higher temperatures than the North region, which enhances the chances of ticks' survival from one year to another. In the North NUTS, ticks are less frequently found; horses tend to be in stables rather than in pastures, are groomed frequently, and hence are less exposed to vectors. However, *T. equi* infection is life-long, as previously referred, so they may have been infected in another geographical area or time, or even *in situ*, and still have antibodies. Information about tested equids' previous location and travelling history would be helpful.

Furthermore, looking at *T. equi* IFAT and cELISA results by geographical area, the Algarve region has close frequencies to the North. Despite *T. equi* IFAT-positive results in the North NUTS (57.1 %), the Algarve value is only slightly lower (54.9 %), with a chi-square component of 3.43. A significantly higher number of IFAT-positive than expected was found in Algarve. The same is true for cELISA results in the North region, corroborating our previously described findings (chi-square = 4.24). When analysing *B. caballi* results, the chi-square value (6.06) validates the higher seropositivity in LMA, which is larger than expected. For IFAT, 50.5 % of LMA samples had antibodies, but it is noteworthy to look at Alentejo results since it was found to have fewer cases than expected (Table 4). This finding may reflect the beginning of Portuguese breeders' awareness of EP and their starting to adopt better management practices.

Seropositivity appears to be associated with seasonal variations, with spring and winter exhibiting a significantly higher percentage of IFAT-positive results for both parasites and cELISA-positive results for *T. equi* (Table 5). In temperate climates like Portugal, equids are more exposed to vectors from late spring to early autumn, when ticks are abundant (Dantas-Torres, 2010). An increase in mean and extreme temperatures has been verified in our country in the past decades (Schleussner et al., 2019). Climate changes may anticipate the ideal conditions for ticks, starting to quest early in spring until later in autumn (Deshpande et al., 2024; Nuttall, 2022). Following infection, IFAT seroconversion occurs within 2–20 days for both parasites (Weiland, 1986). For *T. equi*, seroconversion occurs within 7–11 days after natural infection, peaking at 30–45 days (Wise et al., 2014b). Positive cELISA results for *T. equi* arise approximately five weeks after tick infection (Wise et al., 2014a). These timelines may elucidate the significant increase in seropositivity observed during spring and winter, with a first infection period in early spring and another during autumn. Also, spring positives might be infections from the previous tick season. Camino et al. (2021) found a significant increase in *B. caballi* seroprevalence during autumn and winter but not in *T. equi* seroprevalence. They attributed this finding to a higher infection rate during summer. The present chi-square analysis revealed a significantly lower seroprevalence than expected in autumn for the three diagnostic tests discussed here, contradicting their findings. This difference may be caused by an increased number of tests conducted during autumn, likely due to an equestrian sports event and a horse fair taking place in Portugal at this time.

5. Conclusion

This is the first retrospective study on EP in Portugal, encompassing a considerable number of nationwide samples. Our results confirm EP endemicity in the country. Similarly to other endemic nations, *T. equi* is more prevalent than *B. caballi*, and coinfection is less common. Sex- and age-related prevalence were not consistent among diagnostic tests. However, increased age seems to be a risk factor towards IFAT positivity. Evidence suggests an association between seasonality and serological results, with spring and winter having a higher percentage of seropositive cases. Equids in the south appear at higher risk of having *B. caballi* antibodies, while in the North, *T. equi*-seropositives are more common.

Further studies on EP in Portuguese equids are still needed to corroborate our findings, and to determine actual prevalence and seroprevalence in general equine population. Such studies should include not only the mainland but also the archipelagos (Madeira and Azores), employing random sampling methods rather than relying on practitioner-derived data. Moreover, an additional comprehensive risk factor analysis, including housing type, grooming habits, and tick prevention protocols (either in pasture or in the animal), should be undertaken to understand this disease better, helping breeders and owners minimise its impact.

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CRediT authorship contribution statement

Ana Cabete: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Jacinto Gomes:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **Telmo Nunes:** Methodology, Formal analysis. **Elisa Bettencourt:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Ludovina Padre:** Writing – review & editing, Project administration, Funding acquisition. **Ângela Xufre:** Resources, Investigation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ângela Xufre declares a professional relationship with the private laboratory which provided the data: employment (administrator). Corresponding author (Ana Cabete) has a PhD scholarship grant by the Fundação para a Ciência e Tecnologia, I.P. (FCT, Funder ID = 50110000187) under Grant 2023.02527.BD. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2024.110378.

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