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Prevalence and factors associated with *Leishmania* spp. and *Toxoplasma gondii* infections in apparently healthy horses in Eastern Spain

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ABSTRACT

Leishmaniasis and toxoplasmosis are two of the most common parasitic zoonoses. Leishmaniasis is endemic to 98 countries around the world, whereas toxoplasmosis is widely distributed throughout the world, causing significant health expenditure. Horses can play a relevant role in the transmission of the disease, being a silent reservoir, as clinical signs are not common. Serum samples from 166 horses living in eastern Spain (Mediterranean basin) were analysed to determine the presence of antibodies against *Leishmania* spp. and *T. gondii* by ELISA (Enzyme-linked Immunosorbent Assay.) The risk factors evaluated were the geographical area and the relative humidity and average temperature, and epidemiological factors such as sex, reproductive status, age, breed, morphotype, living with other domestic animals, use and access to the outdoors. Seroprevalence of *Leishmania* spp. and *T. gondii* infection was found 28.92%, and 16.27% respectively, whereas co-infection of the two parasites was found only in two males. *Leishmania* seroprevalence was high in castrated males and several mesodolichomorphic equine breeds used for teaching, as well as in outdoor animals. The most elevated seroprevalence was found in winter with higher levels of rainfall, whereas high seroprevalence of *T. gondii* was found in crossbreeding animals and those used for breeding. High seroprevalence of *Leishmania* spp. and *T. gondii* was found in horses of the Mediterranean basin. These data suggest that horses can act as a silent reservoir and that this species has high potential for transmission to humans, outdoor animals and in geographical areas with high average rainfall.

1. Introduction

Leishmaniasis and toxoplasmosis are two of the most important parasitic zoonosis diseases. The first is caused by *Leishmania* spp., an intracellular protozoan parasite, transmitted by sandflies. This vector-borne disease is endemic to 98 countries, including the Mediterranean basin (Alvar et al., 2012). Traditionally, the domestic dog (*Canis lupus familiaris*) has been considered the main reservoir (Ready, 2010), but in recent years the parasite has been found in different domestic species, including cats (Ahuir-Baraja et al., 2021) and horses (Gazzonis et al., 2020). Although the relevance of horses as possible reservoir, given the closeness between this species and humans, especially in peri-urban areas, studies in this regard are scarce. To date, studies conducted in Europe have shown a seropositive prevalence in apparently healthy equids ranging from 0.3% in Greece (Kouam et al., 2010) to 36.7% in Italy (Nardoni et al., 2019). The number of clinical cases is low in this species, and clinical manifestations (mainly cutaneous lesions) are mild and tend to self-recover without treatment (Koehler et al., 2002; Rolão et al., 2005; Solano-Gallego et al., 2003). An exhaustive review by Mhadhbi and Sassi (2020) describes the main clinical signs of equine leishmaniasis which includes papules or nodules in eyes, muzzle, neck, pinnae, scrotum and legs. In many cases, therefore, infection by *Leishmania* spp. in horses goes unnoticed, which makes it a silent reservoir.

For its part, toxoplasmosis is widely distributed throughout the world, since the causative parasite, *Toxoplasma gondii*, can infect all warm-blooded animals and also be transmitted through many different pathways (Aguirre et al., 2019). Some studies indicate that the health burden of toxoplasmosis in humans is the highest of all parasitic diseases (Djurković-Djaković et al., 2019), as one-third of the human population worldwide is infected with *T. gondii* (Webster, 2010). Regarding

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domestic animals, cats present high prevalence and are typically asymptomatic and, in dogs, the subclinical disease is common (Dubey et al., 2009). In horses, the seroprevalence of *T. gondii* infection varies depending on the detection method and country, and ranges from 18% to 43% (Dubey et al., 2020). In Spain, García-Bocanegra et al. (2012) found a *T. gondii* seroprevalence of 14.7% in horses (García-Bocanegra et al., 2012) and, more recently, Cano-Terriza et al. (2023) carried out a larger study, which included 1085 horses in four different countries. In the latter study, the authors found an overall seroprevalence of 18.9% and, specifically in Spain, seroprevalence was 16.6% (Cano-Terriza et al., 2023). The *T. gondii* infection in horses is typically asymptomatic (García-Bocanegra et al., 2012), therefore, like *Leishmania* spp., can act as a silent reservoir. Otherwise, the ingestion of uncooked or undercooked meat containing cysts of *T. gondii* is one of the major form of transmission and horse meat is consumed in several countries (Pomares et al., 2011), so determining the prevalence of this infection in horses is highly relevant for human health.

Determining the prevalence of *Leishmania* spp. and *T. gondii* infection in horses and associated risk factors of infections is of vital importance to control the spread of these parasites and reduce the number of infections in humans.

The aim of this study was to analyse the seroprevalence of *Leishmania* spp. and *T. gondii* infection and co-infection in horses, and to evaluate the risk factors associated with these infections in a geographical area where *Leishmania* spp. is endemic.

2. Material and methods

2.1. Geographical areas of study

Samples and data were recovered from December 2022 to June 2023, in two periods [December 2022–January 2023 (winter), and May–June 2023 (spring)] in eastern Spain (Mediterranean region). The climate in this region is characterised by hot and dry summers and mild winters, with irregular rainfall, concentrated in spring and autumn (Garijo-Toledo et al., 2023). The study took place in three areas of this region: Castellon, Valencia and Alicante. Table 1 shows the location, latitude, longitude, temperature, rainfall and elevation of these areas.

2.2. Animals and epidemiological data

A total of 166 horses (*Equus caballus*) were included in the study. None of the animals presented any clinical signs compatible with *Leishmania* or *Toxoplasma* infection. All animals were apparently healthy. For all animals, a structured questionnaire for owners was applied during recovery sample collection. The close-ended questions asked included sex (male, female), reproductive status in males (castrated or not), age (foal, less than five years old; young, between five and twelve years old; adult, between thirteen and twenty-one years old; and elder, more than twenty-one years old), breed, purebred or crossbred, housing system (outdoor, indoor), presence of other domestic animals in the household (mainly dogs or cats), use (teaching, breeding, dressage, hitching, walking and jumping). Breeds were classified based on their morphological types as mesomorphic, meso-brachymorphic, meso-dolichomorphic or dolichomorphic (Dall'Olio et al., 2010), except ponies.

2.3. Sample collection and serological analysis

Ten millilitres of whole blood were aseptically collected via jugular venipuncture using Vacutainer tubes without anticoagulant from each horse. The samples were kept at room temperature and serum was separated by centrifugation at 3000 rpm for 10 min, transferred into cryovials, labelled and stored at −80 °C until the laboratory analysis was carried out.

2.4. Serological analysis

Serum samples were used to determine the presence of specific antibodies for *Leishmania* spp. and *Toxoplasma gondii* in all samples. *Leishmania* spp. specific antibodies were detected using the ELISA test for anti-*Leishmania* specific immunoglobulin G (IgG) antibodies (*Leishmania* vet ELISA®, Demeditec Diagnostic GmbH, Bonn, Germany), following the manufacturer's instructions. The animals with ELISA titre > cut-off was considered seropositive. Positive and negative controls were included in each test. According to the manufacturer, the sensitivity and specificity of the kit are >98%.

T. gondii specific antibodies were checked using the ELISA commercial kit for antibody detection ID Screen® Toxoplasmosis Indirect Multi-Species (TOXO-MS, ID. Vet, Grables, France). This ELISA test uses the P30 *T. gondii* tachyzoite surface protein as antigen. The tests were performed following the manufacturer's instructions. A sample-to-positive ratio (S/P%) was calculated for each serum sample according to $S/P\% = \frac{ODsample - OD\ NC}{OC\ PC - OD\ NC}$ x 100, where OD is the optical density either of the samples, the positive controls (PC) or the negative controls (NC). According to the manufacturer, animals with S/P% ≤ 40% were considered negative, inconclusive if 40% < S/P% < 50%, and positive with S/P%≥ 50%. Samples with results that were inconclusive were reanalysed. If the result was inconclusive a second time, they were withdrawn from the study.

2.5. Statistical analysis

Prevalence of parasitic infection was analysed using Pearson's Chi-square test to determine the association of seroprevalence of two parasitic infection studied and putative risk factors, and the association between these two infections. Seroprevalence for *Leishmania* spp. and *T. gondii* were considered as dependent variables. Sex, reproductive status in males, age, purebred or crossbred, housing system, presence of other domestic animals, use, morphological types of equine breed and season (winter or spring) of the recovery sample were the independent variable analysed. Differences were considered statistically significant at p-value <0.05.

3. Results

Seroprevalence of *Leishmania* spp. infection in horses studied was found to be 28.92% (CI: 26.04–31.81%), whereas for *T. gondii* infection it was 16.27% (CI:14.64–17.89%). Co-infection of two parasites was found only in two animals (1.20%, CI, 1.08–2.33). The rate of two

Table 1
Prevalence of *Leishmania* spp. and *T. gondii* single infection and co-infections in the study areas (CI = confidence interval).

Area	No. tested	*Leishmania* spp.			*T. gondii*			Co-infections *Leishmania* spp. + *T. gondii*		
		No. positive	% prevalence (95% CI)	*p*-value	No. positive	% prevalence (95% CI)	*p*-value	No. positive	% prevalence (95% CI)	*p*-value
Castellon	16	1	6.25 (5.63–6.88)	**< 0.05**	5	31.25 (28.13–34.38)	0.16	1	6.25 (5.63–6.88)	0.85
Valencia	89	8	8.99 (8.09–9.89)		15	16.85 (15.17–18.54)		1	1.12 (1.01–1.23)	
Alicante	61	39	63.93 (57.54–70.33)		7	11.48 (10.33–10.33)		0	–	
Overall	166	48	28.92 (26.04–31.81)		27	16.27 (14.64–17.89)		2	1.20 (1.08–1.33)	

analysed infections and co-infection is shown in Fig. 1. Only *Leishmania* spp. infection prevalence is different between the geographical areas included, with the high prevalence being found in Alicante ($p < 0.05$) (Table 1). The main differences regarding the weather in these geographical areas in related to rainfall, which is higher in Alicante during the winter than in the other areas (Table 2).

Regarding risk factors, *Leishmania* spp. infection was higher in spring than winter, and was related to reproductive status of males, with higher prevalence of infection in uncastrated horses than in castrated ($p < 0.05$). Haflinger, Irish cob, Koninklijk Warmbloed Paard Nederland (KWPN) and ponies are the breeds with greater prevalence, whereas more mesodolichomorphic horses are infected than any other morphological type. The use of animals and the type of housing affected the prevalence of this infection, which was higher in horses used for teaching and those living outdoors (Table 3).

Use and crossbreeding are related to *T. gondii* infection, which presented higher prevalence in animals used for breeding and crossbred horses (Table 4). Finally, the only risk factor of co-infection seems to be the sex, with higher co-infections in males than in females ($p < 0.05$). Analysing the *Leishmania* spp. infection as a risk factor for *T. gondii* infection, horses infected with *Leishmania* spp. showed low prevalence of *T. gondii* infection (p-value <0.05).

4. Discussion

In this study, the seroprevalence and risk factors for *Leishmania* spp. and *T. gondii* infections were assessed in 166 horses in eastern Spain, to determine the epidemiology and possible control measures to diminish the role of this species of these two zoonotic diseases caused by parasites. The results revealed that the seroprevalence of these two zoonotic parasites was elevated in this Mediterranean region.

The *Leishmania* spp. seroprevalence (28.92%) in horses was included in this study. Previous studies have been limited in Europe and the seroprevalence found ranged from 0.3% in Greece (Kouam et al., 2010) to 14.3% in Spain (Fernández-Bellon et al., 2006) and similar prevalence (27.5%) was found recently in the same region (Martínez-Sáez et al., 2023), which could indicate an increase in the prevalence of this parasite in horses in this region. More recent studies carried out in South America showed a seroprevalence of 27% in apparently healthy horses (Biral et al., 2021). The higher seroprevalence was found in Alicante, where the average rainfall was higher in this geographical area. Heavy infections of sandfly females appear at 20 °C (Hlavacova et al., 2013), and this temperature was observed in the three areas studied during the spring. However, the incubation period of the parasite in the vector is shorter at higher temperatures, which could explain why the highest number of seropositive animals was found in spring. Average rainfall

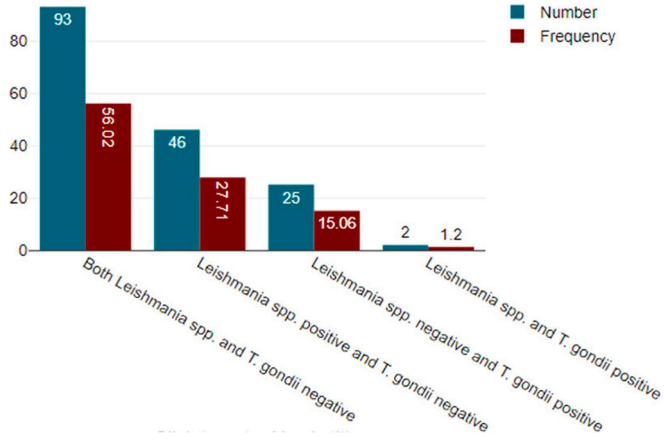

Fig. 1. Rate of co-infections of *Leishmania* spp. and *T. gondii* in horses of the study.

Table 2
Description of the study areas (W: winter; S: spring).

Descriptions	Study areas		
	Castellon	Valencia	Alicante
Latitude and longitude	39°58′59″N 0°1′59″W	39°28′11’N 0°22′38″W	38°20′43″N 0°28′59″W
Elevation (metres above sea level)	37	23	49
Average temperature during study (°C)	14.1 (W) and 19.6 (S)	15.3 (W) and 19.4 (S)	14.9 (W) and 19.6 (S)
Average rainfall during study (l/m^2)	16.2 (W) and 229.8 (S)	12.7 (W) and 30.8 (S)	18.0 (W) and 96 (S)

also influences distribution of the vector, so that a higher relative humidity, due to greater rainfall, facilitates the dispersion of sandflies and, therefore, increases the number of seropositive cases found in the Mediterranean basin (Ntais et al., 2013). However, the low number of animals included in this study is a limitation, so further studies analysing different seroprevalence between regions are necessary.

Seroprevalence in apparently healthy horses found for *T. gondii* was 16.27%. It is worth noting that to date no valid report of clinical toxoplasmosis in horses in Europe exist. Previous studies showed similar results in the Mediterranean countries, and ranged the seroprevalence of this parasite in horses from 14.7% in Spain (García-Bocanegra et al., 2012) to 13.29% in Portugal (Lopes et al., 2013) or 17.20% in Italy (Papini et al., 2015). The ELISA kit used in this study was the multi-species ID Screen® Toxoplasmosis Indirect kit (IDVET, Montpellier) for the detection of antibodies against the *Toxoplasma* P30 protein (Dubey, 2021). Only two studies have realized with this ELISA kit, and seroprevalence of *T. gondii* was estimated around 16.0% in New Caledonia and 43.07% in Algeria (Roqueplo et al., 2011), whereas Ouslimani et al. (2019) found a seroprevalence of 43.07% in Algeria.

The seroprevalence of *T. gondii* in horses is lower than in other livestock species (Li et al., 2021), being higher in domestic than in wild horses (Webster, 2010). Given that horse meat consumption is common in Mediterranean areas, these results indicate that control measures should be carried out to avoid *T. gondii* infection in this species. As in previous papers, the co-infection of two parasites in not common in horses (Lopes et al., 2013) or other species such as dogs (Gebremedhin et al., 2021), cats (Neves et al., 2020) or hares (Tsokana et al., 2019). Other authors found a significant association between *Leishmania* spp. infection and *T. gondii* in dogs (de Paulan et al., 2013; Zulpo et al., 2012), in areas with high prevalence of both infections. Some previous infections with virus or other parasites can increase these co-infections. For example, cats with feline immunodeficiency virus infection present high levels of co-infections for *Leishmania* spp. and *T. gondii* (Bezerra et al., 2023).

Low co-infection prevalence could be a consequence of a greater activation of the Th1 immune response, with a higher production of interferon gamma (IFN-γ) activated by protozoan infection (Deng et al., 2023). This cytokine actives the associated receptor JAK1, which phosphorylates STAT1 and STAT2, two cytosolic transcription factors (Ivashkiv, 2018). This cascade activation and the production of nitric oxide synthase 2 mediated to IFN-γ high levels seems to be critical to control intracellular protozoan infections, reducing the parasite burden. This effect has been observed in infections for different parasites such as *Plasmodium* spp. (Doolan and Hoffman, 2000; van der Heyde et al., 1997), *Trypanosoma* spp. (Hertz et al., 1998), *Leishmania* spp.(Mirzaei et al., 2021; Pinheiro and Rossi-Bergmann, 2007; Swihart et al., 1995) or *T. gondii* (Scharton-Kersten et al., 1996).

Uncastrated males presented higher *Leishmania* spp. infection than castrated animals. Similar results were found previously, being the seroprevalence in uncastrated males around 3.70% and in castrated males around 24.23% (Ouslimani et al., 2019). Uncastrated males have low levels of testosterone (Inoue et al., 1993) and studies in rodents have shown parasitic burden regulation in *Leishmania* spp. infection

Table 3
Seroprevalence of *Leishmania* spp. and risk factors related to infection (KWPN: Koninklijk Warmbloed Paard Nederland).

Variable	Categories	N°. tested	N°. pos. (% prevalence)	95.5% CI	*p*-value
Sex	Male	108	31 (28.70)	25.83–31.57	0.93
	Female	58	17 (29.31)	26.38–32.24	
Reproductive status (males)	Castrated	71	17 (23.94)	21.55–26.34	**<0.05**
	No castrated	52	29 (55.77)	50.19–61.34	
Age	Foal (< 5 years)	21	8 (38.09)	34.29–41.90	0.36
	Young (5 ≤ years ≤12)	40	8 (20.00)	18.00–22.00	
	Adult (13 ≤ years ≤21)	80	26 (3.25)	2.93–3.58	
	Elder (> 21 years)	25	6 (24.00)	21.60–26.40	
Breed	Anglo-Arabian	1	0	–	**<0.05**
	Belgian Warmblood	2	0	–	
	Spanish Sport Horse	73	24 (32.88)	29.59–36.16	
	Connemara	1	0	–	
	Haflinger	1	1 (100.00)	90.00–110.00	
	Hannoverian	4	0	–	
	Hispano-Breton	8	0	–	
	Hispanic-Arabic	6	0	–	
	Holsteiner	1	0	–	
	Irish Cob	2	2 (100.00)	90.00–110.00	
	Jaca Navarra	2	0	–	
	KWPN	5	0	–	
	Percheron	1	1 (100.00)	90.00–110.00	
	French Saddle Horse	4	0	–	
	Arabian	1	0	–	
	Pony	4	4 (100.00)	90.00–110.00	
Purebred	Yes	101	29 (28.71)	25.84–28.71	0.94
	No	65	19 (29.23)	26.31–32.15	
Morphological type	Dolichomorphic	6	0	–	**<0.05**
	Mesomorphic	17	2 (11.76)	10.59–12.94	
	Mesobrachymorphic	4	2 (50.00)	45.00–55.00	
	Mesodolichomorphic	93	30 (32.26)	29.03–35.48	
Use	Teaching	27	26 (96.30)	86.67–105.93	**<0.05**
	Breeding	4	0	–	
	Dressage	74	20 (27.03)	24.32–29.73	
	Hitching	12	1 (8.33)	7.51–9.17	
	Walking	31	0	–	
	Jumping	18	1 (5.56)	5.00–6.11	
Type of housing	Outdoor	111	45 (40.05)	36.49–44.59	**<0.05**
	Indoor	55	3 (5.45)	4.91–6.00	
Living with dogs	Yes	158	48 (30.38)	27.34–33.42	0.06
	No	8	0	–	
Period of the year	Winter	105	9 (8.57)	7.71–9.43	**<0.05**
	Spring	61	39 (63.93)	57.54–70.33	

(Anuradha et al., 1990; Mock and Nacy, 1988). In fact, Anuradha et al. managed to reduce the parasitic count in male hamsters by administering testosterone (Anuradha et al., 1990). Another risk factor found related to *Leishmania* spp. infection was the equine breed and morphotype. Related to equine breed, the higher prevalence was found in Spanish Sport Horses, Haflinger, Irish Cob, Percheron and Ponies. Previous studies showed high seroprevalence in ponies (Gazzonis et al., 2020), but the number of different equine breeds included in our study could be a bias to obtain conclusions. However, the effect of breed in this parasitic infection was observed previously in dogs (Edo et al., 2021). In horses, Gazzonis et al. found an effect of morphotype in the seroprevalence of *Leishmania* spp. and showed higher prevalence in mesodolichomorphic breeds and lower prevalence in dolichomorphic breeds, according to our results (Gazzonis et al., 2020). These results, along with the highest seroprevalence found in horses used for walking, are in agreement with Lopes et al., which indicates that the *Leishmania* spp. infection is higher in animals used for recreational purposes (Lopes et al., 2013). Access to the outdoors is also a risk factor for infection by this parasite, in accordance with results obtained by other authors in dogs (Symeonidou et al., 2021). Therefore, these results suggest that the use and management of horses could be involved in the spread of *Leishmania* spp. infection, like results described for other vector borne infections, as *Babesia caballi* or *Theileria equi* (Onyiche et al., 2019).

Seroprevalence of *T. gondii* is higher in crossbred horses and stallion (used for breeding), whereas other authors found no statistical differences between mixed breed or purebred, or different activities, including breeding (Cano-Terriza et al., 2023). Although this is the first study where these risk factors are determined, other authors have shown that equine breed influences the seroprevalence of *T. gondii* infection (Marzok et al., 2023; Ouslimani et al., 2019). Some studies found higher levels of infected horses in cage-free farms than in captive farms (Liang et al., 2022), higher in horses living in farms than those living in equestrian centres (Ouslimani et al., 2019) and in rural and larger herds than in urban and smaller herds (Alvarado-Esquivel et al., 2012), which could explain our results, inasmuch as the horses used for breeding were usually living in cage-free rural farms. Our results also reveal sex as a risk factor for co-infection. However, the low number of co-infected horses found is a result that should not be considered.

One limitation of this study is the fact that all horses included were apparently healthy, which can represent an underestimation of the infections in horses. This could mean that the seroprevalence data values would be even higher, meaning that horses might be an important reservoir of both intracellular parasites analysed.

5. Conclusions

The results of this study showed high infection rates for *Leishmania* spp. and *T. gondii* in horses living in the Eastern Spain, Mediterranean basin. The main risk factors found were rainfall and temperature, reproductive status in males, equine breed and morphotype, use and outdoor access for *Leishmania* spp. infection, and only crossbred and use for *T. gondii* infection. Further studies are necessary to genotype and

Table 4
Seroprevalence of *T. gondii* and risk factors related to infection.

Variable	Categories	No. tested	No. pos. (% prevalence)	95.5% CI	*p*-value
Sex	Male	108	17 (15.74)	14.17–17.31	0.80
	Female	58	10 (17.24)	15.52–18.97	
Reproductive status (males)	Castrated	71	10 (14.08)	12.68–15.49	0.92
	No castrated	52	7 (13.46)	12.12–14.81	
Age	Foal (< 5 years)	21	3 (14.29)	12.86–15.71	0.90
	Young (5 ≤ years ≤12)	40	8 (20.00)	18.00–22.00)	
	Adult (13 ≤ years ≤21)	80	12 (15.00)	13.50–16.50	
	Elder (> 21 years)	25	4 (16.00)	14.40–17.60	
Breed	Anglo-Arabian	1	0	–	0.08
	Belgian Warmblood	2	1 (50.00)	45.00–55.00	
	Spanish Sport Horse	73	14 (19.18)	17.26–21.10	
	Connemara	1	0	–	
	Haflinger	1	0	–	
	Hannoverian	4	1 (25.00)	22.50–27.50	
	Hispano-Breton	8	2 (25.00)	22.50–27.50	
	Hispanic-Arabic	6	1 (16.67)	15.00–18.33	
	Holsteiner	1	0	–	
	Irish Cob	2	0	–	
	Jaca Navarra	2	1 (50.00)	45.00–55.00	
	KWPN	5	2 (40.00)	36.00–44.00	
	Percheron	1	0	–	
	French Saddle Horse	4	3 (75.00)	67.50–82.50	
	Arabian	1	0	–	
	Pony	4	0	–	
Purebred	Yes	101	22 (21.78)	19.60–23.96	**<0.05**
	No	65	5 (7.69)	6.92–8.46	
Morphological type	Dolichomorphic	6	3 (50.00)	45.00–55.00	0.34
	Mesomorphic	17	3 (17.65)	15.88–17.65	
	Mesobrachymorphic	4	1 25.00)	22.50–27.50	
	Mesodolichomorphic	93	18 (19.35)	17.42–21.29	
Use	Teaching	27	0	–	**<0.05**
	Breeding	4	2 (50.00)	45.00–55.00	
	Dressage	74	11 (14.86)	13.38–16.35	
	Hitching	12	3 (25.00)	22.50–27.50	
	Walking	31	4 (12.90)	11.61–14.19	
	Jumping	18	7 (38.89)	35.00–42.78	
Type of housing	Outdoor	111	17 (15.31)	13.78–16.85	0.63
	Indoor	55	10 (18.18)	16.36–20.00	
Living with dogs	Yes	158	25 (15.82)	14.24–17.41	0.49
	No	8	2 (25.00)	22.50–27.50)	
Period of the year	Winter	105	20 (19.05)	17.14–20.95	0.20
	Spring	61	7 (11.48)	10.33–12.62	

assess the virulence of these parasites, as well as the role of horses in the transmission of the infections to humans.

Ethics approval and consent to participate

The experimental protocol was approved by the Ethics Committee of Universidad Cardenal Herrera CEU (protocol code VCS/022/PEA/0279). The experimental protocols complied with the guidelines of the Ethic Committee. Blood samples were collected by the researchers (veterinarians) after getting informed consent from the owners of the horses. All efforts were made to minimize animal suffering during sample collection. Oral and written informed consent was obtained from all people who participated in the study.

Consent for publication

Not applicable.

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CRediT authorship contribution statement

Samuele Pala: Writing – original draft, Methodology, Data curation. **Lola Martínez-Sáez:** Methodology, Data curation, Conceptualization. **Lola Llobat:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pablo Jesús Marín-García:** Writing – original draft, Methodology, Formal analysis.

Declaration of competing interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

The data and material obtained are available to the researchers, with prior authorisation from the corresponding author upon reasonable request.

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