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Article

Mind the Gap: Knowledge, Attitudes and Practices Regarding Equine Piroplasmosis in Portugal

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Abstract

Equine piroplasmosis (EP) is a tick-borne disease caused by *Theileria equi*, *Theileria haneyi* and *Babesia caballi*. It affects equids, representing significant health and economic concerns for the equine industry. EP is endemic in Portugal, so developing and implementing preventive strategies is essential. Accessing veterinarians' knowledge, attitudes and practices (KAP) through a survey is a suitable approach, and no such studies have been conducted in Portugal until now. A KAP survey was applied to 41 Portuguese equine vets, representing mainly the Alentejo region. The average knowledge score went from medium to high, correctly identifying the causative agents, transmission routes and clinical signs. Knowledge gaps mostly concerned the identification of *T. haneyi* as an agent, transplacental transmission, duration of infection and diagnostic methods. Reported practices were appropriate overall, including enhancing breeders' awareness of the disease and its prevention. Diagnostic and treatment protocols were generally consistent with current recommendations; however, these protocols are not yet fully standardized. Our findings highlight key areas where increasing expertise is needed and could serve as a foundation for future evidence-based guidelines to improve EP control in Portugal.

Keywords: equine; tick-borne diseases; *Theileria equi*; *Babesia caballi*; *Theileria haneyi*; KAP survey; veterinarians

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1. Introduction

Equine piroplasmosis (EP) is a tick-borne disease (TBD) of equids caused by the haemoprotozoans *Theileria equi*, *Theileria haneyi* and *Babesia caballi* [1–3]. It is transmitted by ixodid ticks of genera *Dermacentor*, *Hyaloma*, *Rhipicephalus* and *Amblyoma*, although iatrogenic and vertical transmission can occur. These ticks are found in tropical, subtropical and some temperate climates. The distribution of the parasites coincides with that of vectors [4], and it is estimated that 90% of the global horse population resides in EP-endemic areas [1,5]. EP represents a threat to equine health and has a significant economic impact on the equine industry, raising concerns among veterinarians and breeders.

Clinical presentation can vary from acute to chronic or carrier, depending on the region, the strain of the parasite and overall equine health status [2]. In the acute phase, clinical signs are often nonspecific and mainly related to intravascular haemolysis and thrombocytopenia, such as icterus, pyrexia, anorexia and lethargy. EP can even lead to death in peracute cases and to abortion in pregnant mares. Chronically infected horses can exhibit signs of chronic infection, including poor body condition, mild anaemia, decreased performance and splenomegaly. The most common clinical presentation is inapparent carrier, in which a seropositive horse with no signs of infection serves as a reservoir for parasite transmission. These animals can relapse after stressful situations, immunosuppression or steroid administration [4]. Diagnosis can be achieved by serological and molecular methods. A combination of both is recommended by the WOAH (World Organisation for Animal Health) for horse movement to free zones or countries [6]. The economic impact of this TBD is related not only to the costs of diagnosis, treatment, career pause and death, but also to restrictions on international trade and participation in equestrian events.

In Portugal, as in other Mediterranean countries, EP is endemic [7–11]. Developing and implementing effective and sustainable strategies to control EP is essential. To do so, it is important to analyse equine veterinarians' knowledge about EP, identifying gaps that can be worked out to raise awareness of the disease, its risk factors and control measures. Knowledge, attitude and practice (KAP) surveys are appropriate tools to access knowledge, practices and perceptions (attitudes) concerning a disease [12]. This type of survey has been conducted on TBDs in humans [13,14], cattle [15,16] and equine [17]. However, to the best of our knowledge, no such survey has ever been conducted on EP in Portugal. This study aimed to assess Portuguese veterinarians' knowledge, practices and perceptions of EP, identifying gaps that may guide future recommendations for equine vets to improve control of EP in Portugal.

2. Materials and Methods

A cross-sectional survey using the KAP methodology was developed, aiming at Portuguese equine veterinarians. The inclusion criteria included being a veterinarian working with equines, either mixed or full practice, in Portugal. Data were collected from November 2023 until June 2024 through an online self-administered questionnaire implemented with Microsoft Forms©. The survey was voluntary and anonymous, with informed consent at the beginning. It was approved by the Ethics Committee of Évora University, document 22198 (25 November 2023).

Each survey had four sections: the first one consisted of the participant's sociodemographic characterization; sections two, three and four comprised questions on knowledge, practices and perceptions regarding the disease. A total of 35 questions were asked, with different typologies, including multiple-choice, "Yes/No/Don't know"; a few open-ended questions; and some frequency/Likert scale questions. Validation was ensured by two equine veterinarians and approved by a psychologist.

After approval, the survey was released through social media and email, targeting veterinarians and vets' associations. After the initial invitation, a reminder was sent to associations and veterinarians' forums to increase the number of respondents. A translated version of the survey is available in Supplementary Material S1.

The raw data collected were exported directly from Microsoft Forms©. Descriptive data analysis was conducted using R software (version 4.3.2.), including frequency distributions for categorical variables and means (Ms) and standard deviations (SDs) for numerical ones. Whenever appropriate, the chi-square or Fisher's exact test was applied. Statistical significance was set at the 95% confidence level ($p < 0.05$). When the answers were incomplete, each question was analysed individually, since the missing values varied. In such cases, results were displayed using proportions and not percentages. Graphics were plotted in RStudio (Posit Software, PBC, version 2025.05.0). Knowledge questions were punctuated with a maximum knowledge score of 12 points, which was categorized as follows: low (<4 points), medium (4–8 points) and high (>8 points).

3. Results

3.1. Sociodemographic and Job-Related Characteristics of Respondents

The questionnaire had a total of 41 respondents. The participants were mainly from Alentejo (46.3%), this region being significantly more represented ($p < 0.01$). The Lisbon metropolitan area (24.4%) was second, followed by the centre (14.6%) and the north (12.2%). Only the Algarve and the Autonomous Region of Madeira had no respondents. There were more women (61.0%) than men (39.0%), and most of the vets were >30–40 years old (yo) (46.3%), followed by the >40–50 yo (24.4%), 20–30 yo (19.5%) and >50 yo (9.8%) age groups, this difference being statistically significant ($p < 0.05$). Regarding professional areas, 53.7% of the participants worked solely with equines ($p < 0.05$), 9.8% worked both in equine clinics and academic areas, and 36.6% worked in mixed practice. In total, 75.6% of the participants spent >50% of their time in equine practice, 80.6% being fully dedicated to horses. The main areas of activity within equine practice were general practice (43.9%), orthopaedics (14.6%) and reproduction (14.6%). Internal medicine practitioners represented 9.8% of the respondents.

3.2. Knowledge About EP

The mean score for knowledge was 7.6 ± 1.8 out of 12, corresponding to a medium/high level of knowledge. The knowledge scores did not significantly correlate with region, age, professional area or time dedicated to equine practice. Nevertheless, most medium- (19.5%) and high-scoring participants (24.4%) belonged to the >30–40 age group; considering practitioners 100% dedicated to equines, 16 had medium knowledge scores and 8 reached high scores.

Most participants (97.6%) classified EP agents as protozoans. All the vets recognized *T. equi* and *B. caballi* as causative agents. A total of 90.2% selected only those two, 2.4% selected also *T. haneyi*, and 7.3% chose *Anaplasma* spp. and/or *Ricketsia* spp. as responsible for EP. Regarding transmission, all the participants identified ticks as vectors. A total of 39.0% of them considered ticks as the only route of transmission; 24.4% selected ticks and transplacental and iatrogenic transmission (sharp/cutting objects); 22.0% chose both ticks and transplacental transmission; 4.9% chose ticks and iatrogenic transmission. The other five participants picked combinations that included lice, haematophagous flies and fomites. Considering the types of vectors, 80.5% identified only Ixodidae, and 7.3% of the respondents thought they could be both Ixodidae and Argasidae.

The vets were asked whether they considered certain clinical signs typical of EP. The most affirmative answers were fever, anaemia, jaundice, low performance, anorexia, colic, peripheral oedema and weight loss (Figure 1). Then, the practitioners were questioned about various factors on which clinical presentation could depend ($n = 86$ responses)—"equine immune status" (24.4%) and "all of them" (23.3%) were the most selected ones. The other options were parasite load (22.1%), parasite species (17.4%), nutritional status (11.6%) and environment vector load (1.2%). Regarding usual EP manifestation in endemic countries, 36.6% chose the asymptomatic status, 24.4.% thought it was subacute, 22.0% chose acute status, 14.6% picked chronic presentation and 2.4% chose hyperacute presentation. As for the incubation period, 41.5% of the vets opted for 2–4 weeks, 34.1% answered that they did not know, 22.0% chose 1 week and 2.4% said >4–6 weeks. The question about duration of infection was correctly answered by 48.8% of the participants ("Life-long for *T. equi* and 1–4 years for *B. caballi*"). In total, 46.3% of the vets thought infection was life-long for both parasites, and 4.9% considered it to be

1–4 years. Finally, when questioned about the diagnostic methods the WOAH recommends for exportation/travelling (*n* = 40), 50.0% chose cELISA, 37.5% said PCR and 12.4% of the respondents answered IFAT.

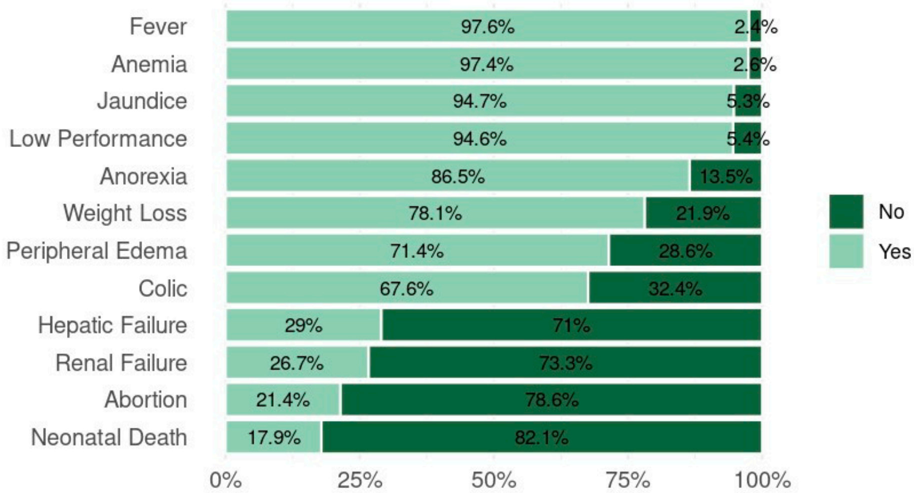

Figure 1. Clinical signs considered typical of equine piroplasmosis by respondents.

3.3. Practices Regarding EP

Veterinarians were asked if they informed their clients about the importance and prevention of EP (*n* = 40): 70.0% said "yes", 27.5% said "sometimes" and 2.5% said "no". Those who responded affirmatively were requested to rate how often they advised some preventive practices. The results are presented in Table 1. Then, the survey had some questions on diagnosis: how many asymptomatic (seropositive) and symptomatic cases the participants had diagnosed in the last five years, which parasite was found, which diagnostic methods were applied, and what were the reasons for testing (see Table 2 for the results on the last two). Most respondents (*n* = 41) diagnosed <25 cases in the last five years: 70.7% were asymptomatic and 63.3% showed clinical signs. *T. equi* was the most frequently detected parasite (27/33: >25–100% of cases), followed by *B. caballi* (27/33: <50% of cases) and coinfection (13/19: <50% of cases).

Table 1. Frequency of recommended prevention measures for EP (equine Piroplasmosis). The answers are presented as percentages. *n* = number of respondents.

Prevention Measures	*n*	Never	Rarely	Sometimes	Frequently	Always
Applying acaricides on pasture	35	48.6	14.3	14.3	17.1	5.7
Applying tick repellent	39	2.6	2.6	2.6	38.5	53.8
Search and removal of ticks	39	10.3	5.1	17.9	30.8	35.9
Do not share sharp/cutting objects	38	31.6	21.1	7.9	7.9	31.6
Quarantine of newly arrived equids	38	23.7	18.4	15.8	23.7	18.4
Treatment of animals with clinical signs compatible with EP	38	5.3	0.0	5.3	21.1	68.4
Treatment of confirmed cases (aiming sterilization)	36	30.6	22.2	16.7	16.7	13.9

Table 2. Frequency of diagnostic tests requested (n = 41) and reasons for testing over the last five years (n = 84, corresponding to the total number of responses given to this question, since participants could choose more than one option; PPE-pre-purchase exam).

Diagnostic Tests Requested More Frequently	*n* (%)
cELISA	22.0
PCR	24.4
cELISA and PCR	48.8
Other [1]	4.9
Reasons for Requesting EP Diagnostic Test	**n (%)/Total No. of Responses**
Clinical or subclinical manifestations	41.7
Before sports events outside of Portugal	15.5
PPE for endemic countries	9.5
PPE and/or exportation to EP-free countries	32.1
Other [2]	1.2

cELISA—Competitive Enzyme-Linked Immunosorbent Assay; PCR—Polymerase chain reaction; PPE—Pre-purchase exam; EP—Equine piroplasmosis; [1] IFAT (1/41), blood smear (1/41); [2] Assessing herd prevalence (1/84).

After diagnosis, the veterinarians were questioned about treatment. Most vets said they frequently/always treat horses with acute clinical presentation (38/40) and subacute cases (36/38). Asymptomatic horses are never (12/39) or rarely (11/39) treated, although some participants said that sometimes (6/39) or frequently/always (10/39) they treat these cases. The least treated cases (never/rarely) are those where the diagnosis is made prior to travelling (24/38), exportation (21/39) or sterilization (24/38). As for the drug of election (n = 41), imidocarb dipropionate (ID) (78.0%) and oxytetracycline (75.6%) are the most frequently used, in contrast to diminazen or buparvaquone, which are never/rarely used (97.6% and 90.2%, respectively). Doxycycline is also used by 7.3%. The side effects more frequently reported include sweating (25/38), agitation (24/38), colic (32/39) and diarrhoea (22/38). Participants frequently use flunixin (32/38) and dipyrone (17/35) to prevent them. Detailed information on previously described results for drugs used, observed side effects and their prevention can be found in Appendix A (Table A1). Regarding treatment outcomes (n = 38), 50% reported a complete remission of their cases, without relapses, and 39.5% said that horses treated relapsed. In total, 10.5% reported improvements but not a complete remission of clinical signs. When questioned about whether they retest treated cases (n = 39), 53.8% said they never do, 43.6% said they retest when justified and 2.6% said they always repeat the diagnostic test. The time after treatment and the method applied varied (cELISA or PCR, with a minimum time of two weeks and a maximum of eight months).

3.4. Perceptions of EP

The participants were asked about their perceptions of breeders/horse owners' knowledge about EP and its prevention (Figure 2). Most respondents (38/40) agreed that breeders are informed about EP and consider it important. Twenty-six of thirty-nine vets agreed that they recognize ticks as vectors and often remove them. Regarding prevention practices, 26/39 participants considered that breeders apply external parasite control and ask for vets' advice. Most participants (34/39) believe it is important to know vectors' distributions, competences and habitats to implement prevention protocols. Grazing horses are considered to be at higher risk of infection when compared to stabled ones (34/39), and climate change impacts EP distribution (32/39). Also, participants agreed that persistently infected horses serve as reservoirs for infection (32/39).

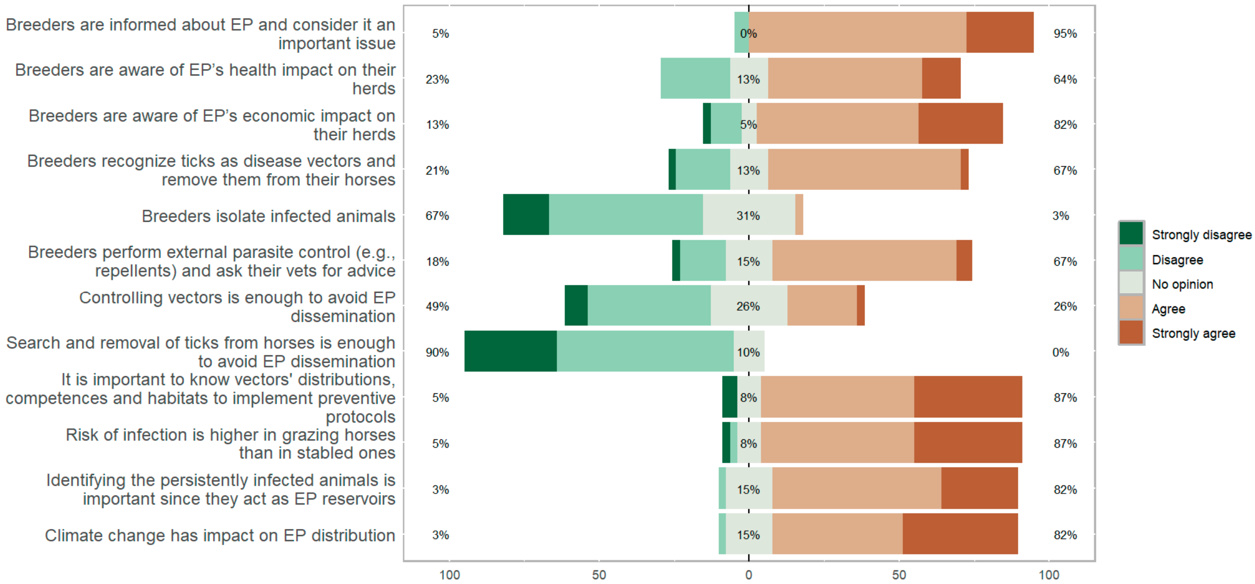

Figure 2. Divergent stack bar representing the results on perceptions of breeders/owners' knowledge and EP prevention. Percentages on the left correspond to "strongly disagree" and "disagree" answers, and percentages on the right correspond to "agree" and "strongly agree".

The veterinarians were also questioned about their attitudes towards EP diagnosis (Figure 3). Most (35/40) disagreed that only animals with clinical signs should be tested and agreed that intermittent fever is a reason for testing (37/39). Thirty-two of thirty-nine did not consider that only horses whose destiny is not Europe should undergo laboratory diagnosis. Regarding breeding mares, opinions were divided on whether all breeding mares should be tested. When confronted with the statement "Only mares with abortion history should be tested for EP", 23/39 disagreed. Considering foals born from infected mares, 23/39 vets agreed they may be seropositive even if not infected. When it comes to diagnostic methods, most participants (30/39) disagreed that cELISA is enough for diagnosis, considering that PCR is a complement to serology (25/39).

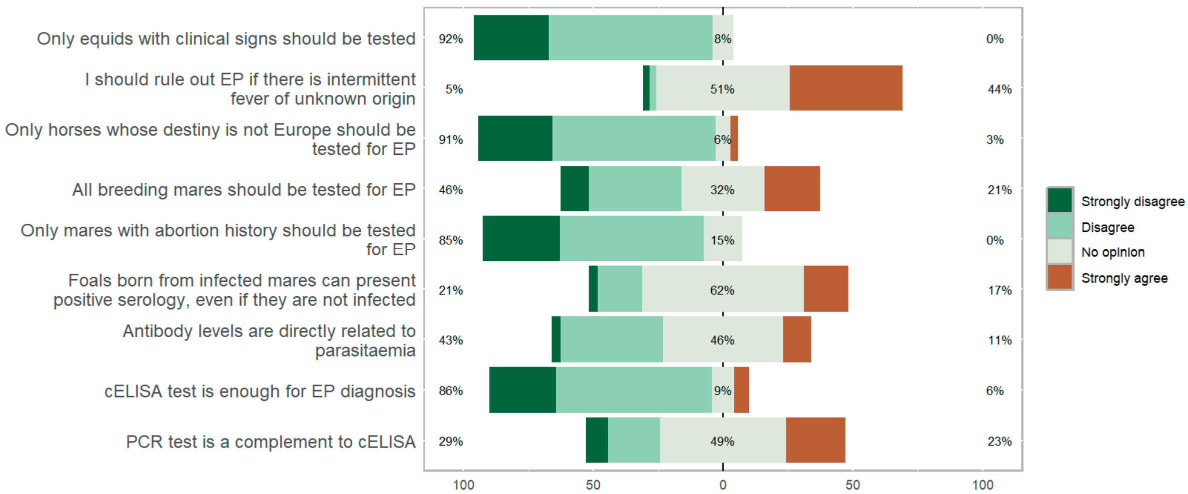

Figure 3. Divergent stack bar representing the results on perceptions of EP diagnosis. Percentages on the left correspond to "strongly disagree" and "disagree" answers, and percentages on the right correspond to "agree" and "strongly agree".

The last part of this section assessed attitudes towards treatment (Figure 4). Most of the vets (25/39) agreed that ID is the most used drug. Even though 19 participants thought that some colleagues may not be comfortable with treatment due to its side effects, 29/38 considered that most vets use preventive drugs. Nevertheless, 12/39 knew of or had witnessed cases of death post-treatment. Regarding ID dosage, there was agreement between 20 participants that 2 mg/kg q24 or 48 h is the most used dosage; when questioned about 4 mg/kg q24 or 48 h, fewer vets agreed (15/38). A consensual opinion could not be found regarding the sterilization dosage for *T. equi*. When questioned about the goal of the treatment, 31/39 agreed that in endemic countries it is to improve clinical signs, and 20/39 participants agreed that in countries with free status the goal is sterilization.

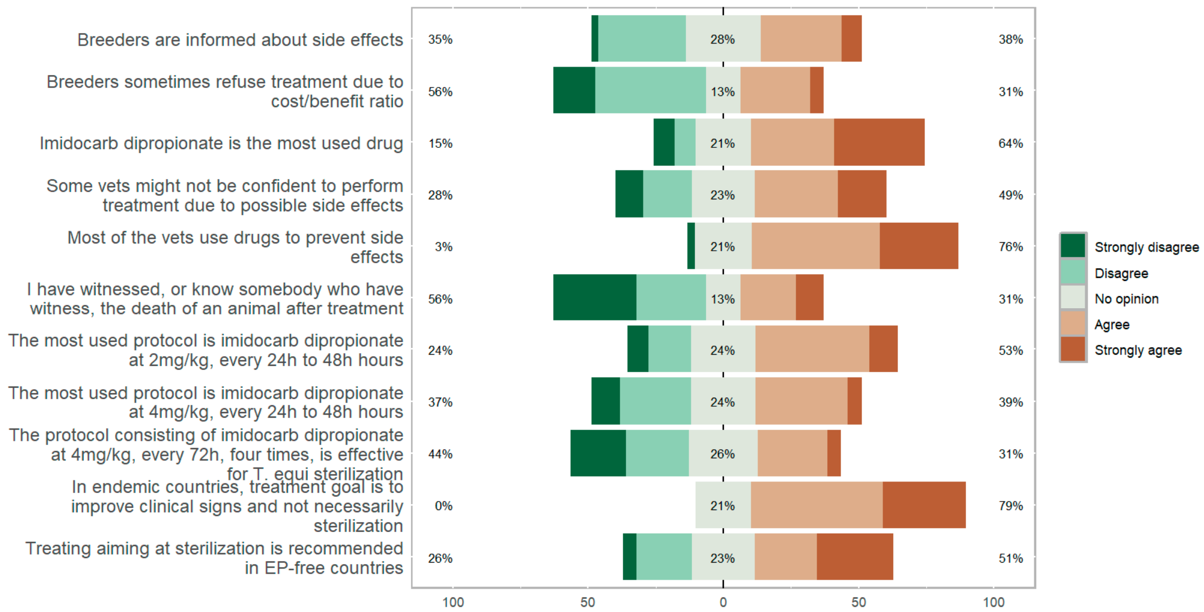

Figure 4. Divergent stack bar representing the results on perceptions of EP treatment. Percentages on the left correspond to "strongly disagree" and "disagree", and answers and percentages on the right correspond to "agree" and "strongly agree".

4. Discussion

Ticks and tick-borne diseases have an important impact on human and animal health. This impact is increasing worldwide due to climate change. To prevent equine TBDs such as EP, veterinarians and all equestrian-related professionals should have enough knowledge and apply adequate prevention measures. Considering equine vets, this is the first study in Portugal to assess knowledge, practices and perceptions of EP. The number of respondents corresponds to approximately 30% of the equine vets in the country, considering that the Portuguese Association of Equine Veterinarians (APMVE) has approximately 135 members. The Alentejo region was significantly more represented, strengthening our results, since it was also the region with the highest equine population when the questionnaire was available.

The knowledge scores of Portuguese equine vets were medium to high. Even though it was not statistically significant, the participants who were 100% dedicated to equine medicine had higher knowledge, reflecting the efforts these professionals put into their practice. However, considering this subpopulation, there is still an opportunity for improvement, since the number of participants with medium scores was twice that of those with high scores.

Most participants correctly identified EP agents, including their classification as protozoans. However, only one identified *T. haneyi* as a cause of EP. This participant was 100% dedicated to horses, specifically internal medicine, which might justify their higher knowledge of EP agents. *T. haneyi* was reported for the first time eight years ago [3], and since then it has been identified in only a few countries [18,19]. Although there are no reports of *T. haneyi* in Portugal, its potential occurrence should not be ignored. This parasite lacks EMA-1 (*Equine Merozoite Protein 1*), a surface protein that is the target of commercial cELISA kits and some PCR reactions, leading to false negative results [3]. Regarding transmission, all participants recognized EP as a TBD; however, only nine vets correctly picked all the possible transmission routes. It is important to strengthen knowledge on this topic, as transplacental and iatrogenic transmission might occur, even though not so commonly [5,20–22]. Transplacental transmission can result in an infected foal developing haemolytic anaemia, eventually causing death; thus, EP should be considered as a differential diagnosis in those cases [4,20]. Blood transfusions are often performed without a previous EP molecular test of the donor, which represents a risk to the recipient, especially if it is a naïve horse already immunocompromised.

Most respondents identified correctly the typical clinical signs of EP. The least identified were indeed the less common, such as hepatic and kidney failure, abortion, and neonatal death. Considering the factors on which clinical presentation depends, practitioners are aware of the importance of equine immune status and other factors, which they selected correctly as "all of them" [23,24]. "Environment vector load" is important, especially in endemic areas, since it increases the possibility of vector contact and thus of getting infected.

After infection, EP can manifest in different forms: the most common is the unapparent carrier (asymptomatic) state [4,25]. Although 36.6% of participants chose this option, 24.4% and 22.0% of vets selected subacute and acute manifestations. It is important to reinforce the difference between asymptomatic status and subacute disease. In endemic countries, due to early and repeated contact with the parasite, equines develop a competent immune response. A good health status is maintained unless immunosuppression occurs, allowing the protozoans to multiply. These animals serve as reservoirs for infection. Subacute disease is similar to acute, but it is accompanied by weight loss and intermittent fever; mucous membranes can vary from pale pink to pink or pale yellow to bright yellow, and signs of mild recurrent colic might be present [25,26]. Regarding the incubation period, 41.5% of respondents chose the correct option, which was 2–4 weeks [1,6]. Knowing the incubation period is very important to understanding the course of disease and to interpreting diagnostic results. Duration of infection is another aspect that needs to be clarified: although 48.8% knew that infection is life-long for *T. equi* and 1–4 years for *B. caballi* [1,23], 46.3% thought both were life-long. It is important to understand that only *T. equi* is life-long, so clearance of infection is highly unlikely. Contrarily, *B. caballi* infection can be cleared by the animal's immune system and with proper treatment. An animal that is positive for *B. caballi* might still have a chance of being exported to a free country/zone after treatment, keeping him in a vector-free environment; in *T. equi*-positive animals, cure is hardly ever achieved. Finally, WOAH recommendations for exportation/travelling include serological and molecular tests, namely, IFAT and cELISA, and PCR, respectively [6]. IFAT has limitations, such as the difficulty of differentiating weak positives from negative results and decreased sensitivity [26]. Portuguese vets are aware of this fact, since 50% chose cELISA and only 12.4% chose IFAT. A total of 37.5% picked PCR—the respondents preferred serological methods instead of molecular ones. It is indeed advised to use both, especially for EP-free countries [6,27].

Regarding the practices section, most of the vets inform their clients about the importance of EP. Frequently recommended prevention measures were applying tick repellent and searching for and removing ticks, which is in accordance with previous recommendations [23,28]. Applying acaricides on pasture was the least recommended method. Although described in the literature [29,30], it is not practical. It should be combined with pasture rotation, requiring division of pastures and adding costs to the owner. "Treatment of animals with clinical signs compatible with EP" was the most recommended prevention measure. Treating these animals considers their welfare, but it is not a preventive measure per se. First, if there is a suspicion, it should be confirmed before treatment; second, the animals remain reservoirs—even if the parasitaemia falls below the detection limit, ticks can still get infected and transmit the disease. A novel, safe and effective therapy that could control acute clinical manifestations and promote a radical cure would have a major impact on equine health and industry worldwide [27].

Considering the Portuguese vets' experience with EP cases over the last 5 years, most respondents diagnosed slightly more asymptomatic than symptomatic cases. Since Portugal is an endemic country, it was expected that there would be more asymptomatic cases compared with clinical ones. Seropositive horses with no clinical signs are usually diagnosed when undergoing a pre-purchase exam, pre-travelling or assessing herd prevalence. On the other hand, clinical cases are confirmed when horses display compatible clinical signs. Being mostly general practitioners, the respondents experienced both cases in their practice. Regarding the agents responsible for infection, the majority were due to *T. equi*, followed by *B. caballi*, and coinfection was the least reported. This aligns with previous studies in endemic areas [8,9,31,32], including Portugal [11].

Most respondents frequently request a combination of cELISA and PCR to diagnose EP. This is in accordance with the literature and the responses given in the knowledge section. The most frequently stated reasons for testing were clinical and subclinical manifestations, pre-purchase (PPE) exams, and/or exportation to EP-free countries. These results were expected: first, because when there are compatible clinical signs, it is imperative to confirm infection prior to treatment, given its described side effects [27,33,34]; second, because tests can be requested by the buyer in the PPE and/or they are demanded for exportation/travelling to free countries/zones [26].

Considering the treatment of confirmed cases, Portuguese vets mainly treat cases with acute or subacute clinical presentations. Treatments for travelling, exportation or sterilization were the least selected options. In endemic countries such as Portugal, treating diagnosed horses with clinical manifestations is a good practice in order to improve their health status and not for clearance of the infection. A cure is not desirable, as in such zones horses will be exposed to vectors and parasites. Hence, protective immunity is ideal instead [24,27]. Some vets treat animals before travelling or exportation: even if clearance is not the goal in these cases, treatment might be beneficial if there is a positive molecular result. Transportation can be stressful, leading to clinical disease, which could be prevented by previous treatment.

Imidocarb dipropionate and oxytetracycline are the drugs of election for Portuguese veterinarians. Diminazene and buparvaquone are sometimes or rarely used. These two drugs are frequently referred to in the literature. However, they lack the EMA (European Medicines Agency)'s authorization (Art. 115(5), Reg. (EU) 2019/6 [35]). ID is effective against both parasites in different dosage regimens [1,2,27]. Care should be taken when using ID in donkeys and mules since they are more susceptible to side effects [24]. Oxytetracycline is reported to be more effective against *T. equi* than *B. caballi* [2], but a cure is rarely obtained after treatment. Combining this drug with ID reduces its dosage, which can be helpful in donkeys and mules to minimize side effects [27]. Thus, there is evidence that both drugs are effective, but ID is still the most effective one and is recommended for EP treatment. Its efficacy does not apply to *T. haneyi* and *T. haneyi* coinfection, as demonstrated by Sears and colleagues [36]. Side effects of ID are common and triggered a few minutes after administration. They include agitation, hyperhidrosis and colic, corresponding to the side effects most frequently reported by the respondents in this study. Side effects cannot be overlooked, so the administration of a preventive drug is advised. There are various drugs described, such as anticholinergic drugs, like atropine or glycopyrrolate [37,38]. However, there is a risk of ileus due to their long-lasting effects. Drugs with analgesic properties, such as flunixin meglumine or dipyrone, can also be used and are indeed the most used by the surveyed vets.

Considering treatment outcomes, all 38 respondents chose an option in which the horse improved: in half of the cases there was a complete remission, in some there was a relapse, and a few cases never experienced a complete remission. A possible reason for treatment failure or relapse is the choice of an inadequate dosage regimen, either due to insufficient drug concentration or treatment duration, which may favour drug resistance [27]. Treatment failure in symptomatic cases can prevent a complete remission of clinical signs, where a chronic status is maintained. Regarding retesting after treatment, if the diagnosis was made solely based on serology, a paired serum analysis 15–21 days apart or a combination with PCR is necessary to confirm the diagnosis [27]. One of the respondents always repeated the test two weeks after treatment; the test of choice was PCR rather than cELISA. Retesting is also a common practice when therapeutics aim to decrease antibody titres aiming at exportation. In such cases, the serological analysis is repeated after three months: if it has not decreased, a second round of EP treatment is recommended [39,40]. When justified, some of the surveyed vets said they would retest, where one of the previous situations might occur. The majority said they never repeated the test. We found this practice risky since at least PCR should be repeated to assess the efficacy of the treatment.

Regarding perceptions of breeders'/horse owners' knowledge of EP and its prevention, most participants considered that breeders are informed and concerned about EP. This is in accordance with the practices section, where most vets said that they inform their clients on EP. However, economic impact awareness seems to surpass health impact. That is reasonable, since health impacts will generate costs with vet assistance and losses due to athletic career breaks. Additionally, Portugal being an endemic country, most cases are asymptomatic and seropositive, which will not impact health directly but can block international trade. Portuguese vets find that their clients practice adequate prevention measures, such as tick removal and external parasite control, and some even ask for advice on these matters. Most surveyed professionals do not think that breeders isolate infected animals. These equids will only represent a risk if competent tick vectors are present. Good grooming, performing a tick check, applying acaricides and maintaining a tick-free environment will help to prevent infection. A tick check alone, without any other measures, is not enough, as agreed by most respondents. To elaborate and implement proper prevention protocols, it is important to know the vectors' distributions, competences and habitats [24]. Climate change plays an important role in distribution, with ticks starting to quest early in spring until later in autumn [39,40]. Horses kept on pasture are at higher risk of EP infection due to an increased probability of contact with ticks [23]. If there are persistently infected animals, they will serve as reservoirs, maintaining the infection in the population. Most of the vets surveyed were aware of these facts.

Concerning attitudes towards EP diagnosis, it is consensual that testing should not be carried out only in animals with clinical signs. This is a positive attitude, especially in breeders whose goal is to export their horses. Testing young horses can have a tremendous economic impact, as this provides the opportunity to move negative horses to "free zones" inside the country, allowing them to grow far from ticks and reservoirs. Another positive attitude is to test cases in which there is intermittent fever. This clinical sign is compatible with EP subacute manifestation and needs to be ruled out. Laboratory diagnosis should be based on molecular methods (PCR) to confirm infection [2]. Serological methods are suitable for detecting previous exposure. When considering the international movement of horses, the surveyed vets agreed that a laboratory diagnosis should occur even within Europe. This is a good perception, particularly within the European Union (EU). In the EU, an EP test is not mandatory for the introduction and movement of horses, only a clinical evaluation and a report to attest the absence of clinical signs compatible with any transmissible disease (Council Directive 2009/156/EC [41]). EP prevalence differs in Mediterranean countries compared to northern ones, so this practice can contribute to spreading this TBD to northern Europe [27]. Perception regarding diagnostic tests is in accordance with knowledge and practices, with cELISA considered not enough for diagnostics alone and PCR being a complement to cELISA. This combined serological–molecular approach is the most appropriate.

The diagnostics for breeding mares showed high discordance amongst participants. Transplacental transmission can occur, resulting in abortion or neonatal piroplasmosis [1,42]. It is also possible that carrier mares gave birth to healthy carrier foals [43,44]. The previous authors suggest that parasitaemia levels influence the outcome of foetal infection. They also speculate that the presence of maternal antibodies and innate immune responses might control parasitaemia levels, leading to an apparently healthy foal. Phipps and Otter [44] reported the presence of seropositive equines in the United Kingdom born from infected mares imported from Portugal, suggesting congenital infection. Considering the possibility of transplacental infection and the different outcomes, it is advisable to screen breeding mares so their foals can be closely monitored in the first days of life [20]. Assistant vets should be aware that clinical signs of neonatal piroplasmosis are similar to those of neonatal isoerythrolysis. An early diagnosis and treatment can lead to a better prognosis. Thus, it would be good practice to test all breeding mares, not only the ones with an abortion history. Also, it should be noted that not all foals born from infected mares are PCR-positive but may be seropositive since they acquire antibodies via colostrum ingestion [43].

Finally, regarding perceptions of treatment, approximately one-third of the vets believe that breeders are informed about its side effects. It is important to alert breeders/horse owners about what is expected, even when using preventive drugs. Yet half of the participants disagreed that breeders would refuse to treat their horses due to the cost/benefit ratio, which is good considering the Portuguese economy. Regarding the drug of choice, there is a perception that ID is the most used. However, in the practice section, a considerable number of vets said they use tetracyclines instead. A negative attitude towards the side effects of ID, with almost half of the respondents thinking that colleagues are not comfortable with its use because of them, might justify the use of tetracyclines. However, their intravenous use is risky [45], requiring careful and slow administration. Nonetheless, there is a positive attitude towards the use of preventive drugs, which is reflected in practice. Death post-treatment, either due to side effects or treatment failure, is still a reality and has been witnessed by some of the surveyed vets. Regarding the treatment protocol, the perception of which dosage should be used is controversial. Some agreed that the less aggressive protocol is the most used, and others agreed that 4 mg/kg q24-48 h is preferred. If the lower and shorter protocol is preferred following an empiric diagnostic based on clinical signs, this is a dangerous practice. This protocol is adequate for *B. caballi* but not for *T. equi*, which might lead to recrudescence or drug resistance [27]. The sterilization protocol also raised disagreement amongst vets. Treatment protocols seem to be an *Achilles' heel*.

The treatment goals differ from endemic to free countries, as previously stated, and the surveyed vets had correct perceptions on this matter, agreeing that in endemic countries the aim of treatment is to improve clinical signs, while in free ones sterilization is intended.

This research has certain limitations that should be acknowledged. First, the response rate: although higher participation was expected, a 30% response rate is considered adequate for professional surveys of this kind [46]. Many equine veterinarians in Portugal are self-employed or have additional roles, such as teaching, which limits their availability to respond to questionnaires. Some respondents did not complete all the questions, resulting in some partial gaps in the dataset. Additionally, individual experience may be influenced by workload and geographical area of activity, as management systems differ across the country—particularly between breeding farms and sport horse facilities. Most respondents were based in southern regions, where equine populations are highest, potentially limiting the generalizability of the findings to areas with lower horse densities or different epidemiological contexts. As previously reported [11], the prevalence of EP in Portugal varies across regions, which can influence veterinarians' clinical experience and confidence in managing the disease. These factors should be considered when interpreting the results. Finally, raising awareness about the importance of participating in such surveys is essential, as high-quality data collection depends on robust and representative response rates.

5. Conclusions

The KAP survey applied in this study served its purpose of assessing knowledge, attitudes and practices of Portuguese equine practitioners. Although the response rate was not as high as expected, a considerable proportion of Portuguese equine vets are represented in our study. The knowledge level of our respondents was medium to high, their attitudes towards the disease are satisfactory, and in general they apply good practices.

A chance for improvement still exists, though. We recommend strengthening knowledge on the following specific topics: (1) the novel species *T. haneyi*, which may be underdiagnosed due to practitioners' limited awareness and the current lack of available diagnostic tests; (2) routes of transmission, especially transplacental transmission; (3) different clinical manifestations; (4) duration of infection; (5) appropriate selection of diagnostic methods based on the specific purpose of testing (e.g., clinical diagnosis, export or screening); (6) testing of breeding mares and its importance; (7) medications and treatment protocols available. Acquiring expertise on these topics will enhance our capacity to implement even better practices towards EP in Portugal, contributing to overall capacity building in this field. The insights gained from this study could support the creation of evidence-based guidelines for the prevention and control of equine piroplasmosis.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/parasitologia5030038/s1: Supplementary Material S1: Survey—Knowledge, attitudes and practices of equine veterinarians regarding equine piroplasmosis.

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Abbreviations

The following abbreviations are used in this manuscript:

EP	Equine piroplasmosis
KAP	Knowledge, attitudes and practice
TBD	Tick-borne disease
WOAH	World Organisation for Animal Health
cELISA	Competitive Enzyme-Linked Immunosorbent Assay
PCR	Polymerase chain reaction
IFAT	Indirect Fluorescence Antibody Test
ID	Imidocarb dipropionate

Appendix A

Table A1. Frequencies of drugs used for treatment, side effects observed after treatment and drugs used for their prevention.

Drugs Used for Treatment						
	n	**Never**	**Rarely**	**Sometimes**	**Frequently**	**Always**
Imidocarb Dipropionate	41	0.0	12.2	9.8	39.0	39.0
Diminazen	41	85.4	12.2	2.4	0.0	0.0
Oxytetracycline	41	4.9	4.9	14.6	46.3	29.3
Buparvaquone	41	78.0	12.2	4.9	4.9	0.0
Other:	41	Doxycicline (7.3)				
Side Effects Observed						
Sweating	38	13.2	21.1	34.2	31.6	0.0
Agitation	38	5.3	28.9	52.6	10.5	2.6
Colic	39	5.1	12.8	59.0	23.1	0.0
Diarrhoea	38	10.5	31.6	34.2	23.7	0.0
Dyspnoea	37	43.2	32.4	21.6	2.7	0.0
Lethargic State	37	24.3	24.3	37.8	5.4	8.1
Anaphylactic Shock	37	86.5	13.5	0.0	0.0	0.0
Drugs Used to Prevent Side Effects						
Flunixin Meglumine	38	0.0	2.6	13.2	31.6	52.6
Phenylbutazone	35	60.0	22.9	11.4	2.9	2.9
Dipyrone/Metamizole	35	25.7	5.7	20.0	37.1	11.4
Glucocorticoids	34	85.3	8.8	5.9	0.0	0.0
Glycopyrrolate	33	93.9	6.1	0.0	0.0	0.0
Atropine	34	94.1	5.9	0.0	0.0	0.0

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