Clinical aspects and diagnosis of leishmaniasis in equids:
a systematic review and meta-analysis

Aspectos clínicos e diagnóstico da leishmaniose em equídeos: uma revisão sistemática e meta-análise

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Abstract

Leishmaniasis are a group of diseases of zoonotic importance caused by over 20 species of protozoa of the genus *Leishmania*, in which domestic dogs are considered to be the main reservoir for the disease. However, the involvement of other vertebrates as reservoirs for these parasites has also been investigated. Therefore, the objective of the present study was to carry out a systematic review with meta-analysis on occurrences of leishmaniasis in equids. The case reports described animals with cutaneous symptoms of leishmaniasis (papules, nodules, ulcers or crusts) that regressed spontaneously, located mainly on the head and limbs, from which three species of protozoa were identified in the lesions: *Leishmania braziliensis*, *Leishmania infantum* and *Leishmania siamensis*. In turn, the meta-analysis showed a combined prevalence of 25%, although with high heterogeneity among the studies, which was attributed to the use of different methods for diagnosing the disease. Leishmaniasis in equids is a benign disease but it should be included in the differential diagnosis of cutaneous diseases among these species. Seroepidemiological studies are important in investigating and monitoring suspected exposure of these hosts to the parasite, especially in endemic areas. However, there is also a need to standardize diagnostic methods.

Keywords: Horse, diagnosis, *Leishmania*, meta-analysis, zoonosis.

Resumo

As leishmanioses são um grupo de doenças de importância zoonótica causadas por mais de 20 espécies de protozoários do gênero *Leishmania*, sendo o cão doméstico considerado o principal reservatório da doença. No entanto, diversas pesquisas têm investigado o envolvimento de outros vertebrados como reservatórios do parasita. Portanto, o objetivo do presente estudo foi realizar uma revisão sistemática com meta-análise da ocorrência de leishmaniose em equídeos. Os relatos de caso descreviam animais com sintomas cutâneos de leishmaniose (pápulas, nódulos, úlceras, crostas) que regrediram espontaneamente, localizadas principalmente na cabeça e membros, sendo identificadas três espécies do protozoário nas lesões: *Leishmania braziliensis*, *Leishmania infantum* e *Leishmania siamensis*. Em seguida, a meta-análise evidenciou uma prevalência combinada de 25%, porém com alta heterogeneidade entre os estudos, atribuída às diferenças nos métodos utilizados no diagnóstico da doença. A leishmaniose em equídeos é uma doença benigna, porém deve ser incluída no diagnóstico diferencial de doenças cutâneas nessas espécies. Os estudos soroepidemiológicos são importantes para investigar e monitorar a suspeita de exposição desses hospedeiros ao parasita, principalmente em áreas endêmicas, porém há necessidade de padronização dos métodos de diagnóstico.

Introduction

According to the World Health Organization (WHO, 2017), leishmaniasis are a group of parasitic diseases caused by over 20 different species of *Leishmania* spp., protozoa that are transmitted mainly through the bite of sandflies. Four forms of the disease are known: visceral leishmaniasis (or kala-azar), post-kala-azar dermal leishmaniasis, cutaneous leishmaniasis and mucocutaneous leishmaniasis.

In humans, the most common clinical manifestation is the cutaneous form, which is considered endemic in 44% of the countries that notified cases of the disease in 2015 (WHO, 2017); while the visceral form is the most severe, nearly always fatal if untreated, and is endemic in 38% of the countries that reported occurrence of the disease in that same year (WHO, 2017). In addition to affecting humans, leishmaniasis also affects several domestic mammals. Infected dogs are the most important reservoirs for the parasite in urban areas, which makes them the main source of infection for people living in endemic areas (PACE, 2014).

However, several studies have sought to identify other vertebrates that can host and participate in the cycle of these protozoa (QUARESMA et al., 2011; GAO et al., 2015; KENUBIH et al., 2015; ROHOUSOVA et al., 2015). Among the investigations that have sought new possible reservoirs, there have been reports on equids showing clinical manifestations of cutaneous leishmaniasis (KOEHLER et al., 2002; SOLANO-GALLEGO et al., 2003; MÜLLER et al., 2009; SOARES et al., 2013). Furthermore, prevalence studies conducted in Europe (FERNÁNDEZ-BELLON et al., 2006; LOPES et al., 2013; SGORBINI et al., 2014), Asia (GAO et al., 2015; AHARONSON-RAZ et al., 2015), Africa (MUKHTAR et al., 2000; KENUBIH et al., 2015; ROHOUSOVA et al., 2015) and South America (AGUILAR et al., 1989; FEITOSA et al., 2012; TRUPEL et al., 2014; OLIVEIRA et al., 2017; BENASSI et al., 2018) have demonstrated that horses (*Equus caballus*), donkeys (*Equus asinus*), mules (*Equus asinus caballus*) and ponies (*E. caballus*), are parasitized by different species of *Leishmania*, such as *Leishmania braziliensis*, *Leishmania infantum* and *Leishmania siamensis*, including cases of mixed infections.

The importance of research on leishmaniasis in equids lies in the fact that these species of domestic animal, just like dogs and cats, are in close contact with humans, which may be through use as a means of transportation or for work or leisure activities. Moreover, the low socioeconomic level of the population living in endemic areas for the disease could generate risks of infection across the zoonotic cycle, such that equids would play the role of either potential reservoirs or sources of food for sandflies in peri-domestic areas.

Therefore, the objective of the present study was to carry out a qualitative and quantitative synthesis (with meta-analysis) based on a systematic review of the literature. Greater clarification regarding the main clinical aspects of leishmaniasis in equids and methods for diagnosing it was sought, with a view to enabling support for future studies on this subject.

Materials and Methods

Study design

The present study consisted of a systematic review of the literature, with synthesis and analysis of clinical findings and meta-analysis on the quantitative data available in articles from indexed journals, both from Brazil and from other countries. The study was conducted based on the methodological recommendations of PRISMA: the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (MOHER et al., 2009).

Article eligibility

Articles published in indexed journals were considered eligible if they consisted of case reports and cross-sectional studies describing the clinical and epidemiological characteristics (prevalence, species, sex and age) and diagnostic methods that are used to identify equids naturally infected with *Leishmania* spp. There were no restrictions regarding the year in which the study was developed or published, the language or the country where the study was conducted.

The types of publications included were complete articles, short communications and case reports that addressed issues within the following criteria: (I) information on the clinical presentation of leishmaniasis in equids; (II) prevalence of the disease in equid populations; or (III) diagnosis of leishmaniasis in equids. Reviews of the literature, research notes, editorials, experimental assays and other types of publications not within the inclusion criteria were excluded.

Information sources and search strategies

Considering the pre-established inclusion criteria, the process of identifying articles was developed using the PubMed, SciELO, ScienceDirect, Scopus and Web of Science databases. The following combination of search terms in English was used: [leishmania OR leishmaniosi] AND [equids OR equine OR horses OR donkeys OR mules]. The citations of studies thus identified, containing title and abstract, were saved in BibTeX format and were exported to a bibliographic manager for subsequent selection. The searches were conducted between October 19 and 26, 2018.

Selection of studies and data extraction

A bibliographic manager tool was used to exclude duplicate records. After this stage, two researchers selected studies independently based on an analysis of titles and abstracts, followed by a full reading of the text. Through this full evaluation of the texts, other studies were excluded because they did not meet the eligibility criteria. Occurrences of divergences between the two researchers were resolved by reaching a consensus.

To make it easier to extract and analyze the data, the articles selected were divided into two groups: the first included all case reports of leishmaniasis in equids, for qualitative synthesis; while the second included cross-sectional studies, for quantitative synthesis and meta-analysis.

Data extraction was conducted independently by two researchers and the information was added to a previously developed electronic spreadsheet. The qualitative data extracted from the first group of articles comprised the authors, year of publication, country, clinical characteristics (type and location of lesions), etiological agent and identification of animals (species, sex and age). In turn, the quantitative data extracted from the second group of articles comprised the references (authors and year of publication), country...
where the study was conducted, sample size, number of positive animals, prevalence (%) and diagnostic methods.

**Data analysis**

The qualitative data were analyzed using descriptive statistics, by means of absolute and percentage distributions, to characterize the clinical aspects of leishmaniasis in equids and methods for diagnosing it. The primary outcome for the quantitative data was the crude prevalence of leishmaniasis in equids, with a 95% confidence interval (95% CI).

Heterogeneity was assessed using Cochran’s Q test and was quantified through the I² test of Higgins and Thompson. The combined estimates and 95% confidence interval were calculated based on the random-effects model through the inverse of variance, using the DerSimonian-Laird method. Funnel plots were also analyzed visually and Egger’s test was applied, as alternatives for identifying possible biases. All analyses were conducted using the R statistical software (version 3.5.1), through the RStudio software interface (version 1.1.463).

**Results**

The initial search of databases and study selection are presented in Figure 1. Out of the total number of studies searched (n = 531), 29 met the eligibility criteria and were divided as follows: 11 studies described the clinical characteristics of leishmaniasis in equids, and thus were included in the qualitative synthesis; and another 18 were considered to be cross-sectional studies (prevalence studies), with sufficient data for quantitative synthesis and meta-analysis.

**Qualitative synthesis of the clinical aspects of leishmaniasis in equids**

The 11 studies included in the qualitative synthesis were conducted in seven different countries (Germany, Brazil, Spain, United States, Puerto Rico, Portugal and Switzerland) and reported occurrences of cutaneous leishmaniasis in a total of 22 equids (E. caballus), of which 15 were male (68.18%) and 7 were female (31.82%), with ages between 0 and 2 (n = 2), 2 and 5 (n = 8), 5 and 10 (n = 9) and over 10 years (n = 3).

The major clinical manifestations described in the studies were limited to the skin, and included ulcers (n = 10), nodules (n = 7), crusts (n = 2), papules (n = 1), areas of alopecia (n = 1), presence of exudate (n = 1) and pruritus (n = 1). Most lesions were described as multiple, although single lesions were also observed in some studies, distributed over different parts of the body. The protozoa identified in the studies were *Leishmania braziliensis*, *Leishmania infantum*, *Leishmania siamensis* and also mixed infection by *L. braziliensis* and *L. infantum*. Further details of the clinical factors (characteristics and locations of lesions), methods used for diagnosis and etiological agents are described in Table 1.

**Quantitative synthesis and meta-analysis on leishmaniasis prevalence in equids**

The studies included in this stage were conducted in Brazil (n = 10), China (n = 1), Spain (n = 1), Ethiopia (n = 2), Greece (n = 1), Israel (n = 1), Italy (n = 1), Portugal (n = 1), Sudan (n = 1) and Venezuela (n = 2). In one of these studies (AGUILAR et al., 1989), data collected in both Brazil and Venezuela were published. In turn, in two other studies (TRUPPEL et al., 2014; BENASSI et al., 2018), two different diagnostic techniques were used. We therefore considered the use of these different techniques to constitute different studies and, for this reason, the initial total of 18 studies identified was then counted as 21 in the quantitative analysis phase.

Table 2 summarizes the main characteristics of the studies included in the meta-analysis. In evaluating the prevalence results through Cochran’s Q test (in which p = 0) and the I² statistic of Higgins and Thompson (in which I² = 99.5%), high heterogeneity was observed among the studies. Therefore, a random-effects model was used, according to subgroup, to conduct the meta-analysis. This model yielded a combined prevalence of leishmaniasis in equids of 25% (CI: 15-35%), among the studies included (Figure 2). To assess the possible causes of heterogeneity, the studies were divided into subgroups according to the technique that had been used for the diagnosis: parasitological; enzyme-linked immunosorbent assay (ELISA); direct agglutination test (DAT); indirect fluorescence
antibody test (IFAT); and polymerase chain reaction (PCR). These results are shown in Figure 2.

The visual analysis on the funnel plot (Figure 3) showed that there was asymmetrical distribution among the 21 studies, thus demonstrating the possibility of publication bias, which was confirmed through applying Egger’s test (p = 0.01).

### Table 1. Characteristics of the studies included in the qualitative synthesis regarding leishmaniasis in equids.

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Country</th>
<th>Clinical manifestation</th>
<th>Location</th>
<th>Method</th>
<th>Etiological agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoshida et al. (1988)</td>
<td>Brazil</td>
<td>Ulcers</td>
<td>Prepuce</td>
<td>Parasitological</td>
<td>Not identified</td>
</tr>
<tr>
<td>Yoshida et al. (1990)</td>
<td>Brazil</td>
<td>Ulcers</td>
<td>Unknown</td>
<td>Parasitological</td>
<td>Leishmania braziliensis</td>
</tr>
<tr>
<td>Barbosa-Santos et al. (1994)</td>
<td>Brazil</td>
<td>Nodules and ulcers</td>
<td>Genital organs, limbs, nasal cavity, neck and jaw</td>
<td>Parasitological + IFAT</td>
<td>Leishmania braziliensis</td>
</tr>
<tr>
<td>Rama et al. (1996)</td>
<td>Puerto Rico</td>
<td>Ulcers, crusts and nodules</td>
<td>Pinna, neck, maxilla and shoulder</td>
<td>Parasitological</td>
<td>Not identified</td>
</tr>
<tr>
<td>Koehler et al. (2002)</td>
<td>Germany</td>
<td>Nodules and ulcers</td>
<td>Eyelid</td>
<td>Parasitological</td>
<td>Leishmania infantum</td>
</tr>
<tr>
<td>Solano-Gallego et al. (2003)</td>
<td>Spain</td>
<td>Papules, nodules, alopecia, ulcers and crusts</td>
<td>Face, axillary and inguinal regions</td>
<td>Parasitological + ELISA</td>
<td>Leishmania infantum</td>
</tr>
<tr>
<td>Rolão et al. (2005)</td>
<td>Portugal</td>
<td>Ulcers</td>
<td>Pelvic limb</td>
<td>Parasitological + PCR</td>
<td>Leishmania infantum</td>
</tr>
<tr>
<td>Müller et al. (2009)</td>
<td>Germany</td>
<td>Nodules</td>
<td>Head, flank, axilla, ear and thorax</td>
<td>Parasitological + PCR</td>
<td>Leishmania siamensis</td>
</tr>
<tr>
<td>Reuss et al. (2012)</td>
<td>United States</td>
<td>Nodules and ulcers</td>
<td>Pinna, neck, shoulders and withers</td>
<td>Parasitological + PCR</td>
<td>Leishmania siamensis</td>
</tr>
<tr>
<td>Soares et al. (2013)</td>
<td>Brazil</td>
<td>Ulcer, exudation and pruritus</td>
<td>Pelvic limb and vulvar region</td>
<td>Parasitological + PCR + ELISA + IFAT</td>
<td>L. braziliensis and L. infantum</td>
</tr>
<tr>
<td>Gama et al. (2014)</td>
<td>Portugal</td>
<td>Nodules and ulcers</td>
<td>Face</td>
<td>Parasitological + DAT + PCR</td>
<td>Leishmania infantum</td>
</tr>
</tbody>
</table>

### Table 2. Quantitative synthesis regarding the main characteristics of the studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Sample</th>
<th>Positive</th>
<th>Prevalence (%)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aguilar et al. (1984)</td>
<td>Venezuela</td>
<td>28</td>
<td>6</td>
<td>21.43</td>
<td>Parasitological</td>
</tr>
<tr>
<td>Aguilar et al. (1989)</td>
<td>Brazil</td>
<td>26</td>
<td>8</td>
<td>30.77</td>
<td>Parasitological</td>
</tr>
<tr>
<td>Aguilar et al. (1989)</td>
<td>Venezuela</td>
<td>32</td>
<td>9</td>
<td>28.13</td>
<td>Parasitological</td>
</tr>
<tr>
<td>Mukhtar et al. (2000)</td>
<td>Sudan</td>
<td>96</td>
<td>66</td>
<td>68.75</td>
<td>DAT</td>
</tr>
<tr>
<td>Fernández-Bellon et al. (2006)</td>
<td>Spain</td>
<td>112</td>
<td>16</td>
<td>14.29</td>
<td>ELISA</td>
</tr>
<tr>
<td>Vedovello et al. (2008)</td>
<td>Brazil</td>
<td>55</td>
<td>42</td>
<td>76.36</td>
<td>DAT</td>
</tr>
<tr>
<td>Kouam et al. (2010)</td>
<td>Greece</td>
<td>773</td>
<td>2</td>
<td>0.26</td>
<td>ELISA</td>
</tr>
<tr>
<td>Feitosa et al. (2012)</td>
<td>Brazil</td>
<td>466</td>
<td>68</td>
<td>14.59</td>
<td>ELISA</td>
</tr>
<tr>
<td>Lopes et al. (2013)</td>
<td>Portugal</td>
<td>173</td>
<td>7</td>
<td>4.05</td>
<td>DAT</td>
</tr>
<tr>
<td>Sgorbini et al. (2014)</td>
<td>Italy</td>
<td>277</td>
<td>18</td>
<td>6.50</td>
<td>DAT</td>
</tr>
<tr>
<td>Truppel et al. (2014)</td>
<td>Brazil</td>
<td>227</td>
<td>25</td>
<td>11.01</td>
<td>ELISA</td>
</tr>
<tr>
<td>Truppel et al. (2014)</td>
<td>Brazil</td>
<td>227</td>
<td>37</td>
<td>16.30</td>
<td>PCR</td>
</tr>
<tr>
<td>Acosta et al. (2014)</td>
<td>Brazil</td>
<td>20</td>
<td>0</td>
<td>0.00</td>
<td>IFAT</td>
</tr>
<tr>
<td>Aharonson-Raz et al. (2015)</td>
<td>Israel</td>
<td>319</td>
<td>6</td>
<td>1.88</td>
<td>DAT</td>
</tr>
<tr>
<td>Kenubih et al. (2015)</td>
<td>Ethiopia</td>
<td>15</td>
<td>5</td>
<td>33.33</td>
<td>DAT</td>
</tr>
<tr>
<td>Rohousova et al. (2015)</td>
<td>Ethiopia</td>
<td>20</td>
<td>2</td>
<td>10.00</td>
<td>PCR</td>
</tr>
<tr>
<td>Oliveira et al. (2017)</td>
<td>Brazil</td>
<td>257</td>
<td>62</td>
<td>24.12</td>
<td>IFAT</td>
</tr>
<tr>
<td>Evers et al. (2017)</td>
<td>Brazil</td>
<td>398</td>
<td>183</td>
<td>45.98</td>
<td>IFAT</td>
</tr>
<tr>
<td>Benassi et al. (2018)</td>
<td>Brazil</td>
<td>40</td>
<td>40</td>
<td>100.00</td>
<td>PCR</td>
</tr>
<tr>
<td>Benassi et al. (2018)</td>
<td>Brazil</td>
<td>40</td>
<td>1</td>
<td>2.50</td>
<td>IFAT</td>
</tr>
</tbody>
</table>

**Discussion**

The qualitative analysis on the case reports showed that all the equids were affected by the cutaneous form of leishmaniasis (Table 1), presenting lesions that began with papules and nodules and progressed to ulcers, with presence of crusts, alopecia, exudate
and pruritus as a result of the evolving inflammatory process that was underway. There is a consensus among researchers that the common infection site of *L. braziliensis* is the skin, while *L. infantum* is responsible for visceral infection (KOehler et al., 2002). However, the present review shows that this has not been observed among equids, given that all the reports cited in this review showed predominance of the cutaneous form of the disease, regardless of which species of *Leishmania* was identified.

When an etiological diagnosis of the disease was possible, the most frequent species found was *L. infantum* (n = 8), followed by *L. siamensis* (n = 5), *L. braziliensis* (n = 2) and mixed infection of *L. infantum* and *L. braziliensis* (n = 1). In the remaining cases (n = 6), the agent was not identified. Occurrences of species with higher pathogenicity towards humans (*L. infantum* and *L. siamensis*) infecting equids is a reason for greater concern, given that vectors use this mammal species as a source of food and could consequently ingest and become contaminated by the protozoa, although this type of transmission has not yet been demonstrated in equids in a general manner.

The lesions that were described in the studies surveyed here were located mainly on the head and limbs, although they were also observed on the neck, genital organs, abdomen and thorax, which are regions where there are few or even no hairs, which facilitates access by the vector mosquito to begin feeding and, consequently, to inoculate the parasite into the skin. Moreover, research on humans has shown that attractive volatile substances are exhaled from specific...
parts of the body, such as the ears, which attract greater numbers of insects to that region (REBOLLAR-TELLEZ et al., 1999). Although no such reports relating to equids have been conducted, presence of volatile attractants forms a plausible argument for explaining the presence of lesions on certain body parts rather than on others, thus suggesting that this occurs not just because of absence of hair in these places.

Skin diseases are common among equids, and some present characteristics similar to those described for leishmaniasis, such as equine sarcoid, squamous cell carcinoma, pythiosis, habronomiasis (KOELLER et al., 2002; SOLANO-GALLEGØ et al., 2003) or any other cutaneous disorder that causes either popular or nodular lesions and/or ulcers in equids (SOARES et al., 2013). Thus, leishmaniasis should be considered in making differential diagnoses of dermatopathies in equids, especially when the animals inhabit regions that are endemic for leishmaniasis and lesions occur in areas such as the genital organs, head, neck, ears and inguinal and axillary regions, and when there is no satisfactory response from administration of antimicrobial or antifungal therapies (RAMOS-VARA et al., 1996).

Most lesions that were described in previous studies either regressed spontaneously without the need for treatment or did not recur after surgical removal (RAMOS-VARA et al., 1996; KOELLER et al., 2002; SOLANO-GALLEGØ et al., 2003; ROLÁO et al., 2005; MÜLLER et al., 2009; GAMA et al., 2014). This emphasizes the idea that the immune response of equids against the parasite is effective (FERNÁNDEZ-BELLON et al., 2006).

Use of medication to treat the disease was reported in three studies. In the first, Barbosa-Santos et al. (1994) initially used immunotherapy and observed an increase in antibody levels and worsening of lesions, which then led them to use conventional chemotherapy (pentavalent antimony), through which regression of lesions was achieved after a second application. In the second report, Ramos-Vara et al. (1996) used sodium stibogluconate and reached complete cure for the lesions. Lastly, Solano-Gallego et al. (2003) used anti-inflammatory therapy with dexamethasone, but without any satisfactory response.

Regarding the quantitative synthesis, the meta-analysis indicated that the prevalence of leishmaniasis in equine populations was 25% (CI: 15-35%), with distribution over four different continents (Africa, South America, Asia and Europe). However, the high heterogeneity of the studies analyzed gives rise to some reflection regarding the degree of reliability of this combined prevalence among the studies.

In seeking to elucidate this high heterogeneity, we were particularly interested in the number of different diagnostic techniques that have been used to investigate leishmaniasis in equids. Thus, one meta-analysis per subgroup was conducted, with separation according to the method used for the diagnosis: parasitological examinations, IFAT, ELISA, PCR and DAT.

The forest plot (Figure 2) brings the 21 studies included in the meta-analysis, where the points inside the boxes in the center of the graph represent the prevalence of each study individually and the horizontal line the confidence interval, and the diamonds demonstrate the combined prevalence among studies. Prevalence results and confidence intervals are shown in the fourth and fifth columns, respectively. The last column represents the weight with which each study participated in the aggregate result.

The heterogeneity was high both in the aggregate outcome of the 21 studies ($I^2 = 100\%$) and in the DAT ($I^2 = 99\%$), ELISA ($I^2 = 97\%$), IFAT ($I^2 = 98\%$) and PCR ($I^2 = 100\%$) subgroups. As shown in Figure 2, heterogeneity is clearly present between the studies that used different diagnostic techniques, and it is also present among studies that used the same technique. One exception were the three studies that used parasitological techniques, which did not show heterogeneity between each other ($I^2=0\%$). Parasitological examinations are considered to be the gold standard for diagnosis, and thus their results are more reliable.

Serological tests were the diagnostic techniques that presented greatest methodological differences when used to diagnose leishmaniasis in equids. Regarding DAT, for example, five studies used different antigen concentrations, serum dilutions and cutoff points (LOPES et al., 2013; AHARONSON-RAZ et al., 2015; KENUIBIH et al., 2015), along with positive control sera from either humans or dogs (MUKHTAR et al., 2000; VEDOVELLO et al., 2008). In ELISA, there were also methodological differences, especially regarding the types of antigens and conjugates used in serum dilutions (FERNÁNDEZ-BELLON et al., 2006; KOUAM et al., 2010; FEITOSA et al., 2012; TRUPPEL et al., 2014). Other serological studies used IFAT and also presented differences regarding the cutoff points and conjugates used (SGORBINI et al., 2014; ACOSTA et al., 2014; OLIVEIRA et al., 2017; EVERS et al., 2017; BENASSI et al., 2018).

Regarding molecular tests through PCR, the meta-analysis results also demonstrated high heterogeneity ($I^2 = 100\%$) among the four studies that used this technique (TRUPPEL et al., 2014; GAO et al., 2015; ROHOUSSOVA et al., 2015; BENASSI et al., 2018). Although the authors of these studies used different primers to identify parasite DNA, we do not believe that this was the source of the heterogeneity that was observed in the meta-analysis of this subgroup. In this case, we believe it is coherent to attribute this heterogeneity to the variation in real prevalence of the disease in the studies indicated, given the high sensitivity and specificity of the PCR technique as a diagnostic method and the combined prevalence and confidence interval observed among the studies in which it was applied (37%; 0 – 92%).

Publication bias is often responsible for the heterogeneity between studies that is found through meta-analysis. In Figure 3 each point on the plot represents a study, with the prevalence arranged on the X axis and the standard error on the Y axis. In the absence of bias, the points are expected to have a symmetrical distribution under the dashed area of the triangle (inverted funnel), with the most accurate studies occupying the vertex (lowest standard error) and the least accurate distributed at the base of the triangle. It was possible to verify by observing the funnel plot (Figure 3) a marked asymmetry of the points (studies), indicating possible publication bias in the present meta-analysis, a fact also confirmed by the Egger’s test ($p = 0.01$). In relation to cross-sectional studies, it is especially common to find that researchers are not interested in publishing their studies when they do not find significant prevalence in their investigations. Moreover, the editors of journals even appear reluctant to publish these negative findings. This lack of negative findings generates bias that can interfere in the results.
from a meta-analysis (Pereira & Galvão, 2014). However, we believe that the difference in the methodologies that were used for diagnosing the disease in equids was the factor that most contributed towards the high heterogeneity observed.

Conclusion

The clinical disease caused by different species of Leishmania spp. is benign to equids, thus not requiring treatment in most cases, with the exception of basic care to avoid secondary contaminations, or cases in which the lesions reach larger proportions. However, leishmaniasis is clinically important, given that it can be mistaken for other common skin diseases in equids. Thus, leishmaniasis should be considered in the differential diagnosis for these diseases. Moreover, more detailed investigations are needed regarding the pathology of the disease in these hosts, particularly if development of visceral lesions occurs, and regarding the role of the immune response in determining whether clinical signs will appear.

Hence, seroepidemiological studies are important for investigating and monitoring suspected exposure of these hosts to the parasite, especially in endemic areas, given that natural equid-vector-human transmission has not yet been either demonstrated or refuted and could, therefore, represent a risk to vulnerable populations. However, the diagnostic techniques used for this purpose have not yet been standardized for equids and this may generate conflicting results and erroneous conclusions regarding prevalence of the disease in this host population, as observed in the present study.

References


Oliveira PM, Garcia F, Evers F, Barbosa VM, Obando DCM, Nasciutti NR, et al. Seroepidemiology of Leishmania spp. in equids from Uberlândia,


