Occurrence of *Hepatozoon* sp. in dogs in the urban area originating from a municipality in southeastern Brazil

Paula Virgínia Gomes\(^a\), Maria José Santos Mundim\(^a\), Antonio Vicente Mundim\(^b\), Diego Fernando de Ávila\(^b\), Ednaldo Carvalho Guimarães\(^c\), Márcia Cristina Cury\(^a,\)\(^*\)

\(^a\) Institute of Biomedical Sciences, Federal University of Uberlândia, Minas Gerais, Brazil
\(^b\) Faculty of Veterinary Medicine, Federal University of Uberlândia, Minas Gerais, Brazil
\(^c\) Faculty of Mathematics, Federal University of Uberlândia, Minas Gerais, Brazil

**A R T I C L E  I N F O**

Article history:
Received 15 April 2010
Received in revised form 7 July 2010
Accepted 22 July 2010

*Keywords:* Prevalence
*Hepatozoon* sp.
Blood smears
Dogs

**A B S T R A C T**

The occurrence of *Hepatozoon* sp. infection in dogs was evaluated in the urban area of Uberlândia, Minas Gerais, Brazil. The study involved 300 animals, 120 from the Veterinary Hospital of the Federal University of Uberlândia’s Faculty of Veterinary Medicine, 80 from private clinics, and 100 from the Animal Protective Association (APA). Among these animals, 7.66% presented *Hepatozoon* sp. gamonts inside neutrophils. No statistically significant difference was found among the diagnoses based on capillary and venous blood smears, but a statistical significance was identified in age range and breed. The main signs and symptoms the animals presented were pale mucous membranes, swollen lymph nodes, ocular discharge and pain in response to renal palpation. All the ticks collected were of the species *Rhipicephalus sanguineus* and no oocyst of the parasite was found in the hemolymph of the ixodidae. The findings of this study reinforce the presence of *Hepatozoon* sp. infecting dogs in Uberlândia, MG, with age and breed possibly related to the infection. The symptoms presented by the animals were consistent with those reported by other researchers.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Canine hepatozoonosis a disease transmitted by the ingestion of ticks infected with the protozoon of the genus *Hepatozoon* sp., which belongs to the phylum Apicomplexa (Rubini et al., 2008; Mundim et al., 2008; Spolidorio et al., 2009).

Dogs are infected by two species, *Hepatozoon canis* and *H. americanum*, with distinct clinical, pathological, genetic, antigenic aspects and vectors of transmission (Mathew et al., 2000; Spolidorio et al., 2009). The distribution of *H. americanum* is restricted to the United States, while *H. canis* has been reported in Africa, southwestern Asia, southern and eastern Europe, and South America (Baneth et al., 2003; Oyamada et al., 2005; Spolidorio et al., 2009).

The genetic characterization of the species of *Hepatozoon* sp. that infect dogs in Brazil has revealed that *H. canis* is the etiological agent of canine hepatozoonosis in the country (Rubini et al., 2009). The main vector of *H. canis* in Brazil appears to be *Rhipicephalus sanguineus*, although transmission of the protozoon has also been achieved from experimental infections with *Amblyomma ovale* (Forlano et al., 2005; Rubini et al., 2009).

The pathogenicity and clinical manifestations of hepatozoonosis have been studied by many authors, and vary according to the age of the host, the degree of infection and association with other diseases (Eiras et al., 2007; Rubini et al., 2008; Spolidorio et al., 2009). Symptoms may vary from asymptomatic cases (70–80% of infected animals) to grave and potentially fatal signs (Mundim et al., 2002; Baneth...
et al., 2003) such as intermittent or persistent fever, mus-
cle hyperesthesia, lethargy, difficulties of locomotion, eye
and nose discharge, diarrhea – sometimes bloody, ane-
mia, weight loss and terminal anorexia (Allen et al., 2008;
Mundim et al., 2008; Little et al., 2009; Sakuma et al., 2009).

The definitive diagnosis usually requires a variety of lab-
oratory examinations, the most common of which involves
detecting the parasite in leukocytes in blood smears
(Almosny et al., 2002; Gonen et al., 2004).

Existing studies about the prevalence of the parasite in
Brazil and around the world affirm that it may vary in terms
of geographical regions and the breeding conditions of the
animal (O’Dwyer et al., 2001; Rubini et al., 2008; Spolidorio
et al., 2009). Most of these studies report prevalence data of
the parasite in dogs living in rural areas, and those of urban
areas to a lesser extent. Researches carried out by Massard
(1979) and Rubini et al. (2008) demonstrated that infection
occurs more frequently in rural areas.

The objective of this study was to evaluate the occur-
rence of the infection in dogs from private clinics, the
Veterinary Hospital and the Animal Protective Association
in the urban area of Uberlândia, comparing positivity at the
different sources and correlating the infection to some epi-
demiological aspects such as age, sex, breed and habits of
the infected animals and clinical symptoms found.

2. Materials and methods

2.1. Study animals

In the period of August 2008 to June 2009, 300 dogs
living in the urban area of the municipality of Uberlândia
were evaluated, 120 (40%) being treated at the Veterinary
Hospital of the Federal University of Uberlândia’s Faculty
of Veterinary Medicine, 80 (26.7%) at private clinics, and
100 (33.3%) from the Animal Protective Association (APA),
because it is located in the urban perimeter and houses
stray dogs picked up off the streets. The number of an-
imals was established based on data of other researches
conducted in several regions in Brazil. After performing
this study and ascertaining the occurrence of infection, the
error of the estimated proportion (Vieira, 2008) was calcu-
lated, yielding a result of 0.09%, thus demonstrating that
the error was low and the sample size sufficient.

Regardless of the reason, all the animals treated at the
Veterinary Hospital and at private clinics were subjected to
a complete clinical examination by the veterinary doctor in
charge of the shift. Body temperature, cardiac and respira-
tory frequency were measured, the color of mucosae (oral
and nasal) was observed, and the lymph nodes, spleen, liver
and kidneys were palpated, following semiologic examina-
tion and containment criteria. The dogs at the APA were
examined clinically by the researcher, a veterinary doc-
ator; due to the shelter’s infrastructural deficiencies, these
examinations were limited to observation of the color of
the mucosae and palpation of the lymph nodes, spleen, liver
and kidneys.

The information about the animals’ age, sex, breed, and
clinical signs were recorded on individual protocols.

In order to correlate the infection with several data, an
investigative questionnaire was drawn up for the owners
of the dogs treated at the Veterinary Hospital and pri-
vate clinics, containing questions about the animals’ habits
and living standards, their contact with other animals and
whether they lived with and shared the same environment
and reported symptoms.

2.2. Blood collection and preparation of blood smears

Blood samples were collected from the marginal ear
veins of each dog using disposable needles, and the
blood smears were prepared immediately after the col-
lection. Two milliliters (mL) of blood were also taken by
venipuncture of the cephalic or radial vein, using pre-
identified sterile vacuum tubes (Vacutainer) containing an
antiocoagulant 0.1 mL of ethylenediaminetetraacetic acid
tripotassium salt (EDTA K3) in 10% solution. The blood was
homogenized and a drop of sample was deposited on a
slide to prepare the blood smears. Two slides were pre-
pared for each collection point, making a total of four slides
per animal.

The smears were fixed and stained by the method of
May Grunwald–Giemsa (MGG), as described by Ferreira
Neto et al. (1981). They were then examined under a light
microscopy equipped with a 100× lens to determined
positivity for Hepatozoon sp. and other hemoparasites, if
present.

The level of parasitemia was determined directly in
the smears, counting the neutrophils and monocytes paras-
itized by gametocytes of Hepatozoon sp. from a total of
200 leukocytes. The smears were analyzed by two trained
people to ensure the highest possible confidence in the
results.

The entire procedure was carried out at the Veterinary
Hospital’s Laboratory of Clinical Pathology at FAMEV-UFU.

2.3. Ticks: identification and analysis of hemolymph

The dogs were examined for ticks during their visit
to the vet and/or when blood samples were collected.
Ectoparasites from infested dogs were collected, seeking
to obtain the evolutive adults forms. The ticks were placed
in jars labeled with each animal’s number and were taken
to the Laboratory of Parasitology of UFU. Genera and/or
species were identified based on the dichotomous key
developed by Aragão and Fonseca (1961).

After identification, each tick’s capitulum was removes
and the idiosome was lightly pressed to force out the
hemolymph (Baneth et al., 2001). To ascertain the possi-
ble presence of oocysts, smears of this hemolymph were
prepared, stained by the May Grunwald–Giemsa method,
and examined under a light microscopy with a 100× lens.
Two slides were prepared for each tick and were ana-
lyzed by two trained people to ensure the reliability of the
results.

2.4. Animal ethics committee

The project was analyzed and approved by the Ethics
Committee for the Use of Animals (CEUA) under Protocol
no. 038/08.
Table 1

Occurrence of *Hepatozoon* sp. in naturally infected dogs from three different sources in the urban area of Uberlândia, MG, from August 2008 to June 2009.

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of animals</th>
<th>Positive for Hepatozoon sp.</th>
<th>Occurrence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veterinary hospital</td>
<td>120</td>
<td>6(a)</td>
<td>5.00</td>
</tr>
<tr>
<td>Private clinic</td>
<td>80</td>
<td>1(a)</td>
<td>1.25</td>
</tr>
<tr>
<td>APA</td>
<td>100</td>
<td>16(b)</td>
<td>16.00</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>23</td>
<td>7.66</td>
</tr>
</tbody>
</table>

Different lower case letters in the column indicate a statistically significant difference ($p < 0.05$), Binomial test.

2.5. Statistical analysis

The occurrence of infection was analyzed based on data such as age, sex and breed, as well as behavioral characteristics related to the parasitosis, using the nonparametric Binomial test and considering a 5% level of significance. The association of two proportions was analyzed using the Chi-squared test, also with a 5% level of significance. Descriptive statistics were applied to the variables of clinical signs and symptom, using the BIO ESTAT 4.0 software program (Ayres et al., 2005).

3. Results

At the Veterinary Hospital and private clinics, the animals were divided into four age brackets, with 47 (23.5%) from zero to one year, 18 (9%) from one to two years, 83 (41.5%) from two to eight years, and 52 (26%) older than eight years of age. Due to the difficulty of estimating the exact age of the dogs from the APA, they were divided into groups based on the dentition pattern, making a total of 30 (30%) puppies and 70 (70%) adults.

Of the total number of animals that participated in the study, 180 (60%) were females and 120 (40%) were males. As for their breeds, 159 animals (53%) were mixed breeds and 141 (47%) were purebreds.

Twenty-three (7.66%) of the animals tested positive for *Hepatozoon* sp., six (5%) from the Veterinary Hospital, one (1.25%) from a private clinic, and 16 (16%) from the APA. The statistical analysis indicated a significant difference between the positive animals from the APA, which had a higher proportion of infected animals than the Veterinary Hospital and the private clinic ($p < 0.05$) (Table 1).

Of the 23 infected animals, eight (34.79%) presented infections concomitant to *Hepatozoon* sp., two by *Ehrlichia* sp. and two by *Babesia* sp., while four presented infection by *Ehrlichia* sp., *Babesia* sp. and *Hepatozoon* sp. (Table 2).

Among the animals from the Veterinary Hospital and private clinic ($n = 200$), the highest prevalence was found in the age group of zero to one year, with four positive animals (8.51%). A statistically significant difference ($p < 0.05$) was found between the dogs in this age group when compared with those in the age group of one to two years and of two to eight years (Table 3). Among the positive dogs from the APA, 15 (21.43%) were adults and one (3.33%) was a puppy, with a statistically significant difference ($p < 0.05$) (Table 4).

Of the total of positive animals, 18 (10%) were females and five (4.16%) were males, with no statistically significant difference found between the sexes ($p > 0.05$).

With regard to breed, 20 (12.57%) were mixed breeds and three (2.12%) were purebreds, i.e., a pinscher, a pit bull and a border collie, showing a statistically significant difference ($p < 0.05$) (Table 5).

Among the main clinical alterations observed in the infected animals from the Veterinary Hospital and private clinic ($n = 7$), one animal (14.28%) showed elevated

Table 2

Occurrence of *Hepatozoon* sp. in naturally infected dogs in association with other hematozoons, Uberlândia, MG, from August 2008 to June 2009.

<table>
<thead>
<tr>
<th>Hemoparasites</th>
<th>No. of positive animals</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hepatozoon</em> sp.</td>
<td>15(a)</td>
<td>65.21</td>
</tr>
<tr>
<td><em>Hepatozoon</em> sp. and <em>Ehrlichia</em> sp.</td>
<td>2(b)</td>
<td>8.70</td>
</tr>
<tr>
<td><em>Hepatozoon</em> sp. and <em>Babesia</em> sp.</td>
<td>2(b)</td>
<td>8.70</td>
</tr>
<tr>
<td><em>Hepatozoon</em> sp.; <em>Ehrlichia</em> sp. and <em>Babesia</em> sp.</td>
<td>4(b)</td>
<td>17.39</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>100</td>
</tr>
</tbody>
</table>

Different lower case letters in the column indicate a statistically significant difference ($p < 0.05$), Binomial test.

Table 3

Occurrence of *Hepatozoon* sp. in naturally infected dogs from the Veterinary Hospital and private clinic in Uberlândia, MG, by age group, from August 2008 to June 2009.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>No. of animals</th>
<th>No. of infected animals %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>47</td>
<td>4(a)</td>
</tr>
<tr>
<td>1–2</td>
<td>18</td>
<td>0(b)</td>
</tr>
<tr>
<td>&gt;8</td>
<td>52</td>
<td>2(a)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>7</td>
</tr>
</tbody>
</table>

Different lower case letters in the column indicate a statistically significant difference ($p < 0.05$), Binomial test.

Table 4

Occurrence of *Hepatozoon* sp. in naturally infected dogs from the APA of Uberlândia, MG, by stage of life, from August 2008 to June 2009.

<table>
<thead>
<tr>
<th>Stage of life (APA)</th>
<th>No. of animals</th>
<th>No. of infected animals %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puppy</td>
<td>30</td>
<td>1(a)</td>
</tr>
<tr>
<td>Adult</td>
<td>70</td>
<td>15(b)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>16</td>
</tr>
</tbody>
</table>

Different lower case letters in the column indicate a statistically significant difference ($p < 0.05$), Binomial test.

Table 5

Occurrence of *Hepatozoon* sp. in naturally infected dogs in the urban area of the municipality of Uberlândia, MG, by breed, from August 2008 to June 2009.

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. of animals</th>
<th>Number of animals infected by Hepatozoon sp. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed breeds (Pinscher, Pit Bull and Border Collie)</td>
<td>159</td>
<td>20(a)</td>
</tr>
<tr>
<td></td>
<td>141</td>
<td>3(b)</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>23</td>
</tr>
</tbody>
</table>

Different lower case letters in the column indicate a statistically significant difference ($p < 0.05$), Binomial test.
cardiac frequency, three (42.85%) showed pale mucous membranes, two (28.57%) had swollen submandibular, prescapular and popliteal lymph nodes, one (14.28%) presented serous ocular discharge, three (42.85%) displayed pain in response to renal palpation, and one (14.28%) presented splenomegaly (Table 6). Among the symptoms reported by the dogs’ owners, one animal (14.28%) had diarrhea, one (14.28%) obstantipation, two (28.57%) showed reduced appetite, and one (14.28) presented vomiting and weight loss (Table 7).

Among the animals from the APA, seven (43.75%) showed pale mucous membranes, nine (56.25%) had swollen submandibular and popliteal lymph nodes, five (31.25%) suffered from serous ocular discharge, two (12.5%) showed nasal discharge, and three (18.75%) presented splenomegaly (Table 8).

No statistically significant difference (p > 0.05) was found between the locations to which the animals from the Veterinary Hospital and the private clinic had access, with three (42.85%) frequently walking in the proximities of their homes, three (42.85%) not only walking on the street but also frequently being taken to a farm, and one (14.28%) staying exclusively at home. All these animals lived in homes with yards and four of them had contact with other dogs.

In the analysis of the blood smears of the positive animals, nine (3%) had gametocytes only in the capillary blood smears, two (0.66%) only in the venous blood smears, and 12 animals (4%) tested positive in the two blood smears performed. No statistically significant difference was found between the two techniques employed (p > 0.05).

The level of parasitemia in the smears varied from one to 20 gametocytes in 200 leukocytes analyzed, with the highest levels found in dogs from the APA. In 18 infected animals (78.26%), the level of parasitemia observed in the capillary blood smears was significantly higher than in the venous blood smears (p < 0.05) (Figs. 1 and 2).
Of the 300 animals participating in this study (n = 300), 78 (26%) had ticks, all of the species *Rhipicephalus sanguineus*. As for the animals infected with *Hepatozoon* sp., ixodid ticks were found in 10 (43.5%) and no statistically significant association was found between infection and tick infestation (p > 0.05).

No evolutive forms of hematoparasites were found in the analysis of the hemolymph of the collected ticks.

### 4. Discussion

Canine hepatozoonosis is a cosmopolitan disease (O’Dwyer et al., 2001; Mundim et al., 2008; Rubini et al., 2008) that is gaining in importance in Veterinary Medicine as an emerging infection due to the increasing number of clinical cases.

The prevalence of the disease varies in different regions of Brazil and studies have shown that in urban areas it has been observed in lower proportions, and in most cases is diagnosed in laboratory examinations (Gondim et al., 1998; O’Dwyer et al., 2001; Spolidorio et al., 2009).

The presence of the protozoon in the municipality of Uberlândia was reported by Mundim et al. (2008), who evaluated naturally infected dogs. It should be pointed out, however, that the present study is the first survey of *Hepatozoon* sp. in the urban area of the city of Uberlândia.

In this study, the number of parasitized animals (7.66%) was similar to the numbers reported by Gondim et al. (1998) and O’Dwyer et al. (2004) in dogs living in urban areas of other Brazilian regions. However, other studies carried out in different regions of Brazil and worldwide have reported higher rates of prevalence (Paludo et al., 2003; Karagenc et al., 2006; Spolidorio et al., 2009; Vojta et al., 2009). The findings of those studies presented variable results which can be ascribed to differences in diagnostic methodologies, sampling number, geographical location, and origin of the animals (from rural or urban zones).

The occurrence of positive dogs found at the APA was higher than at the Veterinary Hospital and the private clinic, possibly because of the large number of animals sharing the same space, and also due to the absence of tick infestation control, which facilitates transmission of the ectoparasite from one dog to another, thereby increasing infections by *Hepatozoon* sp. (Almosny et al., 2002; Baneth et al., 2003).

Many conditions predispose to the development of hepatozoonosis, including concomitant infection with other hematozoans commonly observed in dogs, such as *Ehrlichia* sp. and *Babesia* sp. (Mundim et al., 2002; Yabsley et al., 2005). In the present study, these two hemoparasites were found in association with *Hepatozoon* sp., and can be attributed to the presence of the vector tick *Rhipicephalus sanguineus*, which is also the transmitter of *Ehrlichia* sp. and *Babesia* sp. (Mundim et al., 2008).

Hepatozoonosis may affect dogs of all ages, although it is more prevalent in young animals (Mundim et al., 1994, 2008). In the present study, positive animals were found in almost all the age groups, but the highest occurrence was in dogs less than one-year old. These findings are similar to those reported by Ezeokoli et al. (1983), Baneth and Weigler (1997), Mundim et al. (1994) and O’Dwyer et al. (2001). According to Gal et al. (2007), the immune system of puppies is still immature, which predisposes them to infections through environmental exposure and contact with adult and parasitized dogs. O’Dwyer et al. (2001) state that hepatozoonosis, a disease with intermittent parasitemia, can infect dogs of all ages, but the higher prevalence in young animals may be due to the acute phase of the disease, when more gametocytes can be detected in the peripheral blood. In the dogs from the APA, a higher occurrence of the infection was observed in adult animals (93.75%), which is probably the result of the greater contact among these dogs that live together, albeit in kennels separated from the puppies.

In this research, sex did not influence the infection, which is consistent with Beaufils and Martin-Granel’s (1988) report that infection may occur independently of the animals’ sex.

In our study, most of the infected animals were of mixed breeds. Murata et al. (1993) and Hérvas et al. (1995) mention that breed does not seem to lead to a greater predisposition to infection, although they state that the frequency is higher in hunting or guard dogs, probably due to their lifestyle and their probability of being in contact with ticks.

The clinical signs found in the infected animals of this study were similar to those reported by other authors (Paludo et al., 2003; Mylonakis et al., 2005; Mundim et al., 2008), with mild clinical manifestations, the most common ones being pale mucosae and enlarged lymph nodes. These findings differ from the clinical signs of infection by *H. americanum*, which causes a debilitating and often fatal disease (Baneth et al., 2003).

Factors such as locations of access and housing may play a fundamental role in the transmission of hepatozoonosis. In the present study, among the positive animals from the Veterinary Hospital and private clinic, three (42.5%) had frequent access to farms. Dogs with access to rural areas may easily be infested with different species of ticks, and thus become infected by different agents (Rubini et al., 2008). It is also worth noting that animals living in houses, especially homes with yards, are at greater risk of being parasitized by ticks and thus catching diseases transmitted by them (Soares et al., 2006).

Our comparison of blood smears using capillary and venous blood in this research did not reveal a statistically significant difference, and did not increase the probability of finding the parasite, as also reported by Paludo et al. (2003) and Rubini et al. (2008). However, this finding is not consistent with the study of O’Dwyer et al. (2004), who reported that the probability of detecting gametocytes increased when combining the two types of smears.

The level of parasitemia in the blood smears found in this study is consistent with the findings of other authors in Brazil (O’Dwyer et al., 2001; Paludo et al., 2003; Mundim et al., 2008). According to Baneth and Weigler (1997), parasitemia can be considered high when 800 gametocytes of *H. canis* /μl of blood are found; hence, the parasitemia found in the present study can be considered low. According to Eiras et al. (2007), the absence of parasitemia in blood smears does not indicate absence of infection, and these animals can be considered false negatives. Baneth and Weigler (1997) emphasized the temporary state of the
absence of parasitemia, which occurs when the parasite lodges in the visceral organs in the meront stage.

*Rhipicephalus sanguineus* is the main tick species that infests dogs in Brazil, especially in urban areas, and is considered the principal vector of hepatozoonosis in urban areas in the country (Forlano et al., 2005; Rubini et al., 2008, 2009). However, experimental studies by Forlano et al. (2005) and Rubini et al. (2009) demonstrated that the parasitosis may also be transmitted by *Amblyomma ovoide*, although it is more prevalent in rural areas. All the ixodid ticks collected in the present study were of the species *R. sanguineus*, confirming the higher prevalence of this species in urban areas in Brazil. However, it was not possible to observe an association between animals infected by *Hepatozoon* sp. and infested with ticks.

Current techniques for researching *Hepatozoon* sp. oocysts are based on the analysis of the hemolymph of ticks. In this research, no oocysts were found in tick hemolymph, possibly because the ixodids were not actually infected due to the low parasitemia observed in the dogs. Using the same methodology as the one employed in this study, Baneth et al. (2001) found oocysts in 85% of ticks infected by their methodology as the one employed in this study, Baneth et al. (2001) found oocysts in 85% of ticks infected by their methodology as the one employed in this study. Perucchini and Nascimento (2008) also demonstrated that the parasitosis may occur in 61% of the ticks that were infected by percutaneous injection with blood from the same dog.

The results of this study reconfirm the presence of *Hepatozoon* sp. infecting dogs in Uberlândia, MG, but with a low occurrence, and suggest that age and breed may be related to the infection. Future researches about the epidemiological and clinical aspects of hepatozoonosis are essential to add to the body of knowledge about this parasitosis.

**Acknowledgments**

The authors gratefully acknowledge the collaboration of the professors, technicians and medical veterinarians of the Laboratory of Clinical Pathology of the Veterinary Hospital and the Laboratory of Parasitology of the Federal University of Uberlândia, as well as the private veterinarian clinics where all the practical part of this research was carried out, and Ms. Beatrice Allain for proofreading this manuscript.

**References**


Gouinon, L., Aratere, P.V., Gomes et al. / Veterinary Parasitology 174 (2010) 155–161


plexa: Hepatozoidae) by the tick *Amblyomma ovale* (Acari: ixodidae).