Experimentally induced tick toxicosis in rats bitten by *Ornithodoros brasiliensis* (Chelicerata: Argasidae): A clinico-pathological characterization

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

*Ornithodoros brasiliensis*, also known as the mouro tick, is an argasid tick only found in the highlands of Southern Brazil. *O. brasiliensis* parasitism is associated with severe reactions in its hosts ranging from local pruritus and pain to systemic disturbances. Recently, the re-emergence of *O. brasiliensis* parasitism in humans and dogs drew attention to the clinical findings induced by its bite, which are poorly understood and described. Moreover, rare experimental data about tick bite effects under controlled conditions were available. Thus, this study aimed to describe clinical and pathological findings induced by *O. brasiliensis* bites in experimentally parasitized rats. Ticks feed for ~40 min in rats, and their weight increased by approximately four times after the blood meal. Rats bitten by five adult ticks showed hyperemia of the oral/ocular mucosa, piloerection, tachypnea, claudication, ocular and nasal discharge, pruritus, and swollen and erythemic lesions. A large hemorrhagic lesion was observed on rat skin in tick attachment sites, reaching ~17 mm in diameter 12 h after a bite. Bitten rats also presented an increased bleeding tendency (~50%) 6 h after a tick bite, evaluated by the tail-cut rat model of bleeding. Blood samples of bitten rats were taken, and clinical pathology analysis showed significant alterations in the eosinophil and basophil counts, in creatine phosphokinase (CPK) and CPK MB fraction, and lactate dehydrogenase (LDH) activity, and fibrinogen level. Histopathological analysis revealed marked subcutaneous hemorrhage, edema and slight muscle degeneration at the bite site. Also, muscle degeneration and necrosis were observed in the myocardium of bitten rats 72 h after bites by histopathology and immunohistochemistry against troponin C. This work showed the ability of *O. brasiliensis* to cause severe disturbances in experimentally parasitized rats, compatible with a tick toxicosis syndrome. This observation associated with the re-emergence of *O. brasiliensis* parasitism makes this parasite as a public health hazard in southern Brazil.

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1. **Introduction**

Ticks are hematophagous parasites distributed worldwide. Its parasitism could cause severe deleterious
consequences both to humans and animals. These consequences can be related to the transmission of tick-borne pathogens or directly associated with the tick bite. The non-infectious noxious consequences related to tick bite are generally referred to as tick toxicosis (Walker, 1998; Sharifi et al., 2003; Mans et al., 2008).

Ticks of the Ornithodoros genus are well known because their bite can cause severe non-infectious disturbances in their hosts. The effect of Ornithodoros spp. bite may range from local pain and pruritus to severe toxicosis that can even progress to death (Hoogstraal and Gallagher, 1982; Mans et al., 2008; Venzal et al., 2007).

Ornithodoros brasiliensis is a tick species only found in southern Brazilian highlands. Since it was first reported, this tick has been associated with severe host reactions after bites, particularly in humans. The O. brasiliensis bite in humans is frequently followed by pruritus, the development of a slow-healing lesion at the site of tick bite, edema, erythema and focal skin rash. In some cases, victims also reported local pain, induction of blisters, limb edema, malaise, headaches and dyspnea (Martins et al., 2011; Reck et al., 2011, 2013b). In dogs, O. brasiliensis parasitism was associated with apathy, skin rash, petechiae, and mucosal hyperemia (Reck et al., 2011, 2013a). This information makes O. brasiliensis a public health concern in southern Brazil because of its potential to cause toxicosis.

With the exception of one clinical case report about a dog bitten by O. brasiliensis (Reck et al., 2011), most information available concerning clinical effects of O. brasiliensis bite are from epidemiological/retrospective analyses (Reck et al., 2013a) or dated anecdotal reports (Pinto and di Primio, 1931; Aragão, 1936; di Primio, 1937). Moreover, experimental data about this tick bite effects under controlled conditions is rarely available (Reck et al., 2013b). Thus, the present study aims to describe the clinical and pathological effects induced in rats experimentally parasitized by O. brasiliensis.

2. Materials and methods

2.1. Ticks

The ticks used in this study were collected in the field in the municipality of São Francisco de Paula (29° 20’ 00” S, 48° 30’ 21” W) in highlands region of southern Brazil, Rio Grande do Sul state, Brazil. They were collected in summer season and were maintained under laboratory conditions (20 °C, ±80% humidity) in Petri dishes with sterile sand (1 mm depth) for three months until the experiment was performed. Ticks used in this work had never been fed under laboratory conditions before this experiment. They were identified as O. brasiliensis ticks according to Aragão’s descriptions (1923, 1931).

2.2. Animals

Male Wistar rats (~300 g) were utilized in this work. The rats were maintained in temperature-controlled (21–24 °C, in 12-h light/dark cycles) rooms and had access to water and food ad libitum. All experiments performed in this work were carried out in accordance with ethical guidelines for animal experimentation, and all procedures were approved by the local ethics committee (CEUA/IPVDF 04/2011). All procedures were also in accordance with the NIH Animal Care Guidelines. The study design was adjusted to follow ethical guidelines, which involved reducing to the minimum the number of animals per group and at each time interval.

2.3. Study design

Rats were divided into control (18 animals) and experimental groups (36 animals). Rats of the experimental group were submitted to an experimental protocol in which they were bitten by five adult female specimens of O. brasiliensis (~10 mm length). Rats of the experimental group were slightly sedated with acepromazine (6 mg/kg, i.p.) to avoid tick removal, and 10 min later ticks were placed on the rat’s skin (one tick on each hind-limb, and three on the abdomen) to feed. Rats of control group only received acepromazine and were not submitted to any other treatment.

From these animals cited above, nine rats of control group (three for each time interval) and 18 of experimental group (six for each time interval) were used for determination of bleeding effect (tail-cut bleeding model), as further described. Since the results of the control group are quite homogeneous, they were presented as the means ± SEM of all control animals (n = 9) used for of tail-cut bleeding model. The tail cut was performed 6 (n = 3), 24 (n = 3), or 72 (n = 3) hours after acepromazine administration.

All other animals (nine rats from control group and 18 from experimental group) were utilized for the observation of clinical findings, determination of clinical pathology parameters, and histopathological analysis. Blood samples for clinical pathology tests were taken from rats of experimental group at 6, 24 and 72 h after tick bites (six animals at each time). Blood samples were obtained by puncture of the femoral veins (after inguinal incision) of previously anesthetized rats (xylazine/ketamine, 15/90 mg/kg, i.p.). Blood samples of the control group were obtained 6 (n = 3), 24 (n = 3) or 72 (n = 3) hours after acepromazine administration. After blood collection, rats were killed with a xylazine/ketamine overdose.

2.4. Clinical findings

Rats were observed at indicated times, and all clinical findings were recorded. The main characteristics observed in rats were the traditional clinical signs used for rodent clinical visual examination, as mucosa coloration, piloerection, respiratory frequency, deambulation, presence of itching, self-grooming, aspect of lesion, and general conditions. Rats from the experimental group were inspected 6, 24 and 72 h after tick bites. Animals from the control group were inspected 6, 24 and 72 h after acepromazine administration. The record of clinical findings was performed by the inspection of the animals for 30 min. Rats were maintained in their habitual cages and rooms during the observation, and inspections were always made by the same observer.
2.5. Skin hemorrhagic focus diameter

Skin hemorrhage at the site of tick bite was measured at indicated times using a high-precision pachymeter (Mitu-toyo, São Paulo, Brazil). The hemorrhagic focus was measured considering the larger hemorrhagic diameter (mm) of the skin lesions. This measurement was performed after the record of clinical findings, to avoid alterations in animal behavior due to handling.

2.6. Clinical pathology

Blood was drawn from rats using EDTA and sodium citrate as anticoagulants. The following parameters were determined: complete blood cell examination (erythrocyte and platelets count; differential leukocytes count; hemoglobin content; hematocrit; mean corpuscular hemoglobin concentration, MCHC; mean corpuscular volume, MCV), platelet count, creatine phosphokinase (CPK) activity, creatine phosphokinase MB fraction (CPK-MB) activity, total lactate dehydrogenase (LDH) activity, aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, plasma urea and creatinine levels, fibrinogen, prothrombin time (PT), and thromboplastin time (aPTT). CPK, CPK-MB, LDH, AST, and ALT activities were determined by spectrophotometry using commercial kits (Labtest Diagnostica, Lagoa Santa, Brazil). Plasma urea and creatinine levels were also determined by commercial kits (Labtest Diagnostica, Lagoa Santa, Brazil). Fibrinogen level, PT and aPTT were evaluated as previously described (Reck et al., 2009). Other mentioned blood tests (erythrocytes, platelets and leukocytes count, hemoglobin content, hematocrit, MCHC, and MCV) were done in an automated veterinary hematology analyzer (Bio-1800 Vet®, Bioeasy Diagnostica S/A, Belo Horizonte, Brazil). Samples were kept at −20 °C until determination of plasma parameters. The blood cell count was performed immediately after blood collection. Rat blood samples were also analyzed for the presence of hemoparasites by Giemsa staining. Since the results of the control group were quite homogeneous, they were presented as the mean ± S.E.M. of all control animals (n = 9).

2.7. Histopathology and immunohistochemistry

Histopathological investigation consisted of hematoxylin-eosin standard (HE) staining in thin sections of rat organs embedded in paraffin blocks. The organs were collected immediately after rat death and were placed in 10% buffered formalin solutions until use.

Heart tissue of experimental and control rats underwent immunohistochemistry (IHC) analysis against troponin C. For IHC, thin sections of heart tissue were prepared using Immunostride® slide glass (EasyPath, São Paulo, Brazil). For antigen detection a monoclonal mouse antibody against troponin C (Novocastra Reagents, Newcastle, UK) diluted to 1:40 was employed. IHC was performed using the streptavidin–biotin–peroxidase complex technique (DakoCytomation Inc., Carpinteria, USA) and revealed using diaminobenzidine (DakoCytomation Inc, Carpinteria, USA). Endogenous peroxidase was blocked using 10% H2O2 in methanol. Contra-staining was carried out using Harris hematoxylin. The troponin C IHC technique stains normal/healthy myocardial cells, and a diminished or absent staining was observed in injured cells (Ortmann et al., 2000).

2.8. Tail-cut bleeding model (bleeding effect)

The bleeding effect, i.e., systemic hemorrhagic tendency was evaluated using a classical rat tail-cut model. The methodology was performed according to a previously described protocol (Nazareth et al., 2006), with minor modifications. Briefly, rats were anesthetized with sodium thiopental (85 mg/kg, i.p.), and their tails were put in a warm bath (37 °C) for 5 min. A segment of 5 mm of the tail was excised using a scalpel, and then the tail was placed inside a beaker with distilled water (40 mL) at 37 °C for 30 min. After that rats were killed with a sodium thiopental overdose. The blood loss, as an index of bleeding effect, was evaluated by spectrophotometry to determine the solution absorbance at a wavelength of 540 nm (Abs540). For data comparison, the Abs540 values of the experimental group were expressed as fold of control.

2.9. Statistical analysis

The results were expressed as mean ± S.E.M. of n samples. The statistical analyses were carried out using a one-way analysis of variance (ANOVA) followed by the Dunnett post hoc test. Values of P less than 0.05 were considered statistically significant. The statistical analyses were conducted using the GraphPad Prism 3.0 software (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Tick feeding characteristics

Immediately after the contact with rat skin, ticks attached and started to feed. The average time to complete a blood meal and spontaneous detachment (as an engorged tick) from rat was ~40 min (ranging from 21 to 110 min). During feeding (~10 min after the blood meal starts), ticks start the production of a large amount of coxal fluid, a colorless liquid excreted by coxal glands. Most ticks excreted coxal fluid for an additional 60 min after detachment. The average volume of coxal fluid excreted was ~120 μL per tick (ranging from 30 to 200 μL). After a blood meal, a tick increased to about four times its original weight. The estimated blood intake average per tick was ~245 mg, calculated based on the following equation: (coxal fluid weight + final tick weight) – initial tick weight.

3.2. Clinical findings

The main clinical findings observed in bitten rats were oral/ocular mucosa hyperemia, piloerection, tachypnea, claudication, ocular and nasal discharge, pruritus, and swollen and erythemic lesion. The relative frequency of clinical findings is summarized in Table 1.
3.3. Skin hemorrhagic focus diameter and tail-cut bleeding

The hemorrhagic focus at the tick bite site in rat skin progressively increased until 12 h post-bite. At this time, the lesion’s mean diameter ($n = 6$) was ~17 mm. Fig. 1 shows the progression of hemorrhagic focus diameter after tick bite. Fig. 2 shows the aspect of typical tick bite lesions in a rat’s hind limb.

The tail-cut bleeding time significantly increased (~50%) at 6 h after tick bite, compared to control animals (Fig. 3). No statistical differences in tail bleeding were observed at any other tested time.

3.4. Clinical pathology

The clinical pathology analysis showed significant alteration (at least at one tested time) of the following blood parameters: eosinophil and basophil count, CPK, CPK-MB and LDH activity, and fibrinogen level. All other measured clinical pathology parameters were not significantly altered. Clinical pathology parameters significantly altered in experimental group are summarized in Table 2. No hemoparasites or tick-borne microorganisms were found in rat blood smears stained with Giemsa.

3.5. Histopathology and IHC against troponin C

Histopathological analysis revealed an extensive subcutaneous hemorrhage and edema at the site of the tick bite at all times (6, 24 and 72 h). At 72 h after tick bite, in addition to marked subcutaneous hemorrhage and edema, mild muscle fiber degeneration was also observed. Muscle degeneration was characterized by increased cytoplasmic eosinophilia and reduction of characteristic muscle striations. Also, intra-epidermal hemorrhage with slight epidermal detachment was observed. Fig. 4 shows the typical histological findings of a tick bite lesion at 72 h after bite. Polymorphonuclear infiltration was rarely seen or absent at the site of tick bite. When present, infiltrated cells were predominantly eosinophils.

Morphological alterations were also seen in heart tissue of bitten rats 72 h after tick bites (Fig. 5). The main alteration observed was myocardium degeneration and necrosis, characterized by cell retraction, enhanced cytoplasmic eosinophilia, lack of cytoplasmic striations, nuclear pyknosis and occasional nuclear karyorrhexis and karyolysis. The lesions were found mainly in papillary heart muscles and in areas near the inner (left) ventricle wall. Several incidents of subendocardial hemorrhage, particularly in left ventricle, were also observed in heart tissue (data not shown). Other organs and tissues presented no alterations.

Myocardial injury was confirmed by IHC against cardiac troponin C. Areas of myocardial necrosis and degeneration in HE sections co-localized with non-stained areas in IHC sections (Fig. 5). In heart tissue from all control rats, a marked and homogenous staining for troponin C was observed (data not shown).

4. Discussion

*O. brasiliensis* parasitism has been frequently associated with severe reactions to tick bite, particularly in humans (Reck et al., 2013a). These noxious reactions to tick bite are also frequently observed after the bite of other *Ornithodoros* species. The bite of the Arabian tick *Ornithodoros muesebecki* may lead to blisters, pruritus, fever and headache in humans (Hoogstraal and Gallagher, 1982). In laboratory and

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**Table 1**

Clinical findings induced by *O. brasiliensis* tick bite in Wistar rats.

<table>
<thead>
<tr>
<th>Clinical finding</th>
<th>Control*</th>
<th>Time after tick bites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6 h&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyperemia of oral/ocular mucosa</td>
<td>0/9</td>
<td>61% (7/18)</td>
</tr>
<tr>
<td>Piloerection</td>
<td>0/9</td>
<td>72% (13/18)</td>
</tr>
<tr>
<td>Tachypnea</td>
<td>0/9</td>
<td>28% (5/18)</td>
</tr>
<tr>
<td>Claudication</td>
<td>0/9</td>
<td>17% (3/18)</td>
</tr>
<tr>
<td>Ocular and nasal discharge&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0/9</td>
<td>0% (0/18)</td>
</tr>
<tr>
<td>Pruritus&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0/9</td>
<td>72% (13/18)</td>
</tr>
<tr>
<td>Swollen lesion</td>
<td>0/9</td>
<td>89% (16/18)</td>
</tr>
<tr>
<td>Erythemic lesion</td>
<td>0/9</td>
<td>100% (18/18)</td>
</tr>
</tbody>
</table>

* Control animals were observed at 6, 24 and 72 h.
<sup>b</sup> 18 animals were observed at 6 h after tick bite.
<sup>c</sup> 12 animals were observed at 24 h after tick bite, since six were killed at 6 h.
<sup>d</sup> Six animals were observed at 72 h after tick bite, since six were killed at 6 h, and other six at 24 h.
<sup>e</sup> Reddish mucous discharge.
<sup>f</sup> Animals itching and licking the lesion site.

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**Fig. 1.** Hemorrhagic focus diameter: skin hemorrhage at the site of tick bite was measured at indicated times using a high-precision pachymeter. The results are expressed as mean ± SEM of six animals per group.
domestic animals, the bite of the African sand tampan tick *Ornithodoros savignyi* induces intense local pain, dyspnea, hemorrhages and death, probably associated with anaphylactic shock or cardiac arrest (Mans et al., 2008). *Ornithodoros aff. puertoricensis* produced marked deleterious effects in laboratory mice, such as hyperemia in both nasal and ocular mucosa, dyspnea, motor incoordination, and death (Venzal et al., 2007).

Despite the evident public health importance of *O. brasiensis* and its re-emergence in southern Brazil (Reck et al., 2013a), there are no comprehensive experimental data describing the clinical-pathological effects of *O. brasiensis* feeding on controlled conditions. In this study, we addressed this issue, characterizing the clinical and pathological effects induced by *O. brasiensis* parasitism in rats. Clinical findings observed in the first 72 h after tick bite were both local (pruritus, swollen and erythemic lesion, claudication) and systemic (oral/ocular mucosa hyperemia, ocular and nasal discharge, piloerection, tachy/dyspnea). These symptoms were compatible/similar with those observed after parasitism by *O. brasiensis* in humans and dogs (Martins et al., 2011; Reck et al., 2011, 2013a), and are even quite similar to those associated with the bite of other *Ornithodoros* species (Venzal et al., 2007; Mans et al., 2008). Compared with the main clinical findings described for bitten humans, there are some similarities, particularly in local symptoms, such as pruritus and erythemic and edematous lesions. The occurrence of dyspnea, despite being rarely observed in experimental animals, was also reported by a few bitten humans. In bitten humans, discomfort and malaise is frequently reported. Despite the fact that these characteristics cannot be directly recorded in rodents, some findings, such as piloerection, indicate that rats are in a stressful situation (Carstens and Moberg, 2000). The occurrence of mucosa hyperemia seems to be a common finding in animals bitten by *O. savignyi* and *O. aff. puertoricencis* (Venzal et al., 2007; Mans et al., 2008) and was also observed in a dog bitten by *O. brasiensis* (Reck et al., 2011). Ocular and nasal discharge observed in bitten rats was characterized by a reddish mucous secretion, a clinical condition never described for animals naturally bitten by *O. brasiensis*. This finding may be associated with an increase in nasal/ocular mucous permeability, which is in accordance with mucosa hyperemia. Alternatively, this finding may be associated with a hemorrhagic tendency. However, the most reasonable cause of nasal and ocular discharge was the increased secretion of Harder’s gland, a common finding in rats under highly stressful situations or after contact with irritating agents (Quinton, 2003). The occurrence of nasal and ocular discharge was also frequently observed in mice bitten by *O. aff. puertoricencis* (Venzal et al., 2007) and domestic animals bitten by *Hyalomma truncatum*, the agent of a tick toxicosis syndrome called sweating sickness (Dolan and Newson, 1980). This set of observed symptoms in bitten rats is compatible with the definition of a tick toxicosis syndrome.

A marked finding observed in both humans and animals bitten by *O. brasiensis* is the extensive skin hemorrhagic lesion induced by tick bite. Here we registered the evolution of the hemorrhagic lesion, showing it increased until 12 h after tick bite, when it reached approximately 17 mm diameter. This hemorrhagic focus can be considered an extensive hemorrhagic lesion and usually bitten rats take 7–10 days to heal. Just for comparison purposes,
researchers working with snake venoms created the concept of minimal hemorrhagic dose (MHD), which is useful to define and compare hemorrhagic snake venoms. The MHD is the minimum dose required to induce a hemorrhagic lesion of 10 mm in diameter 24 h after experimental venom injection, a kind of threshold for hemorrhagic lesions induced by venoms (Gutiérrez et al., 1985). In this case, one tick bite is able to induce a hemorrhagic lesion over the MHD threshold for hemorrhagic venoms.

The hemorrhagic lesions induced by O. brasiliensis bites and the finding that a dog bitten by this tick has a slight delay in blood coagulation (Reck et al., 2011) suggests that O. brasiliensis secretes potent anti-hemostatics. To verify whether O. brasiliensis bites are able to disrupt the host’s hemostatic system, we determined the bleeding tendency of bitten animals using the rat tail-cut bleeding model and also measured the coagulation parameters PT and aPTT in vivo. The increased bleeding time at 6 h after tick bite indicates a systemic hemorrhagic tendency. Intriguingly, no alterations in PT and aPTT tests were observed. These findings could be explained by the differences among these methodologies; while PT and aPTT tests evaluate only the activity and levels of coagulation factors (Parry, 1989), the tail-cut bleeding model evaluates the hemostasis in vivo, which includes all components of this system, such as coagulation and anti-coagulation factors, platelets and endothelium.

Clinical pathology information in victims of tick toxicosis, particularly in humans/animals bitten by Ornithodoros ticks are quite rare (Reck et al., 2013a). In this work, we conducted a complete blood cell examination and examined the most important clinical pathology biochemical markers. Increases in eosinophil and basophil counts have frequently been found in parasitic diseases and in non-infectious inflammatory skin lesions, such as allergic reactions (Costa et al., 1997). This finding was also present in a dog bitten by O. brasiliensis, as well as the increased CPK activity (Reck et al., 2011) and in some human cases of tick toxicosis caused by other tick species (Boffey and Paterson, 1973). CPK increase can be attributed to potential tick saliva toxicity to skeletal muscle or cardiac tissue, since damage to these tissues are the main causes of CPK increase (van der Veen and Willebrands, 1966). Lesions in cardiomyocytes were frequently related to the increase of specific CPK isoforms, particularly, the MB fraction (van der Veen and Willebrands, 1966). In bitten rats, a major increase in CPK-MB was observed 24 h after tick bite, suggesting a cardiac injury in bitten animals. In some cases, the increase in CPK-MB activity could be associated with a sub-clinical cardiopathy condition (Gautam et al., 2004). Cardiac injury is the most severe clinical consequence of tick toxicosis induced by O. savignyi, which contains potent cardiotoxins in its saliva (Mans et al., 2002). Heart interstitial edema and degeneration of myocytes were observed in mice bitten by O. aff. puertoricencis (Venzal et al., 2007). Despite the correlation between increased LDH activity and heart injury, this biochemical marker could be better described as a non-specific marker of cytotoxicity and tissue injury, since several pathological conditions can lead to an LDH activity increase (Sobel and Shell, 1972). Therefore, in bitten rats the increased LDH activity is possibly both a molecular marker of the extensive tissue damage at the site of tick bite and also myocardium injury.

The fibrinogen level showed a dual profile. At first, 6 h after tick bite, animals showed a reduction in fibrinogen levels, which coincides with the time of increased bleeding

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Table 2

Blood parameters of Wistar rats bitten by O. brasiliensis ticks.

<table>
<thead>
<tr>
<th>Blood parameters</th>
<th>Control</th>
<th>6 h</th>
<th>24 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils (μL)</td>
<td>380 ± 36</td>
<td>1100 ± 150**</td>
<td>980 ± 130*</td>
<td>1020 ± 180*</td>
</tr>
<tr>
<td>Basophils (μL)</td>
<td>90 ± 25</td>
<td>440 ± 110*</td>
<td>320 ± 90</td>
<td>290 ± 70</td>
</tr>
<tr>
<td>CPK (U/L)</td>
<td>135 ± 37</td>
<td>149 ± 94</td>
<td>864 ± 156***</td>
<td>729 ± 116**</td>
</tr>
<tr>
<td>CPK-MB (U/L)</td>
<td>18 ± 6</td>
<td>27 ± 9</td>
<td>91 ± 30*</td>
<td>31 ± 11</td>
</tr>
<tr>
<td>Total LDH (U/L)</td>
<td>111 ± 39</td>
<td>109 ± 28</td>
<td>326 ± 63*</td>
<td>319 ± 57*</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.8 ± 0.1</td>
<td>1.5 ± 0.1*</td>
<td>2.2 ± 0.14</td>
<td>5.1 ± 0.5***</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001.

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Fig. 4. Histological analysis of local lesions induced by O. brasiliensis bite: Histological (HE staining) findings of tick bite lesion (in rat hind-limb) at 72 h after bite. Note the marked subcutaneous hemorrhage (triangles) and edema (asterisks); muscle degeneration (filled arrows), evinced by increased cytoplasmic eosinophilia and reduction of characteristic striations; and intraepidermal hemorrhage with epidermal detachment (open arrows). Bar, 210 μm.
The causes of fibrinogen reduction are not clear to date, but may be associated with a fibrinolytic activity, already identified in some tick species (Francischetti et al., 2003). The presence of a fibrinolytic component in O. brasiliensis saliva remains as a subject for further investigations. Later, at 72 h after tick bite, fibrinogen levels increase. Although this fibrinogen increase seems paradoxical, it is a frequent finding in several inflammatory situations since this protein is also considered one of the most sensitive acute phase inflammation markers (Geiger et al., 1988).

The histopathological analysis showed a local lesion mainly characterized by severe hemorrhage, edema, and slight cell degeneration. The occurrence of myocardial injury is compatible with the CPK-MB increase and similar to what is observed for other Ornithodoros species, such as O. savignyi and O. aff. puertoricencis (Venzal et al., 2007; Mans et al., 2008). These findings support the hypothesis of a cardiac injury associated with O. brasiliensis tick toxicosis and reinforces the severity of this condition. It is important to note that histopathological analysis of animals bitten by Ornithodoros species are only rarely reported in literature.

All findings described here are compatible with an experimentally induced condition of tick toxicosis. The occurrence of disturbances and lesions immediately after tick bite and early on after bite (24–72 h) reinforces the hypothesis of a non-infectious condition. Usually, in tick-borne diseases, the onset of symptoms begins 5–10 days after tick bite (Bratton and Corey, 2005). In bitten rats, blood smears did not allow the identification of any blood microorganism. It is important to note that, recently, studying a case of a dog showing a condition compatible with tick toxicosis after natural parasitism by O. brasiliensis, the possibility of infection by Babesia spp., Hepatozoon spp., Coxiella spp., Borrelia spp., Ehrlichia spp., Anaplasma spp., and six Rickettsia species were excluded (Reck et al., 2011). In this sense, O. brasiliensis can cause severe non-infectious disturbances directly associated with its bite, which is partially reproduced in experimental conditions. Moreover, this conclusion does not rule out the possibility that O. brasiliensis could be vector of a tick-borne disease. Indeed, this possibility is under investigation.

This work showed that O. brasiliensis causes severe disturbances to its hosts. In spite of slight differences between the condition observed in humans, dogs and rats, in all cases the tick bite could lead to both local and systemic disturbances. The finding that O. brasiliensis causes tick toxicosis together with recent epidemiological data (Reck et al., 2013a) makes this parasite a serious public health hazard in southern Brazil. Also, this work reveals information to aid our understanding of the complex syndrome known as tick toxicosis.

**Ethical statement**

On behalf of, and having obtained permission from all the authors, Dr. José Reck declares that: (a) this manuscript has not been published in whole or in part elsewhere; (b) the paper is not currently being considered for publication elsewhere; (c) all authors have been personally and actively involved in substantive work leading to the report, and will
hold themselves jointly and individually responsible for its content; (d) all relevant ethical safeguards have been met in relation to animal experimentation. Dr. José Reck testifies to the accuracy of the above on behalf of all the authors.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.toxicon.2014.06.017.

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