INTRODUCTION

Sepsis in dogs is associated with a poor prognosis and a high mortality rate, and hence requires immediate intervention (HEILMANN et al., 2013). It is characterized by bloodstream infections resulting in systemic inflammatory response syndrome (SIRS) and organ dysfunction involving the cardiorespiratory, respiratory, hepatic, or renal systems (SINGER et al., 2016).

Blood culture is the gold standard for diagnosis of bloodstream infection that provides information about the etiology of the infection; however, its value as a diagnostic test is limited due to its low sensitivity, long turnaround time, and limited application in patients who have received antibiotic therapy (HEILMANN et al., 2013). Thus, attempts have been made to develop alternative diagnostic techniques, such as polymerase chain reaction (PCR), with a short turnaround time and high sensitivity (PAOLUCCI et al., 2010; MEURS et al., 2011; HEILMANN et al., 2013). In this study, we reviewed cases of canine sepsis at a veterinary hospital, and compared blood culture with broad-range PCR techniques in terms of their application for diagnosis of bloodstream infection in dogs and detection of antibiotic resistance in isolates.

Blood samples were collected from 88 dogs with suspected sepsis between March and December 2013. The inclusion criteria are detailed in Table 1.
used in this study were based on clinical suspicion and parameters of SIRS described by GREINER & HARTMANN (2008).

The dogs were clipped and aseptically prepped. Two equal-volume blood samples were drawn simultaneously from the jugular and cephalic veins. The volume of each blood sample ranged from 2 to 5mL depending on the weight of the animal and its clinical condition.

An aliquot of the blood in each sample was processed using a Hemobac trifásico pediátrico system® (Chocolate Agar, MacConkey Agar, and Sabouraud Agar) and incubated aerobically at 37°C for up to 7 days. An animal was considered positive for a microbial agent only when the same agent was isolated from two samples drawn from the animal. Biochemical characterization was performed according to QUINN et al. (1994).

Five hundred microliters of blood was used for phenol-chloroform DNA extraction (SAMBROOK & RUSSEL, 2001). PCR was performed using 27f (AGAGTTTGATCCTGGCTCAG) and 1492r (GGTTACCTTGTTACGACTT) universal primers for 16S rDNA (bacteria), as described by LANE (1991), and internal transcribed sequences (ITS-fungi) ITS-4 (TCCTCCGCTTAATGATATGC) and ITS-5 (GGAAGTAAAAGTCGTAACAAGG), as described by WHITE et al. (1990). Bacterial PCR mix had a final volume of 25μL, which included 10 ng DNA, 1 UTaq DNA polymerase (Invitrogen),0.2 mM of each dNTP, 2.5mM MgCl₂, 1× PCR buffer, and 20 pmol of each primer. Reaction conditions were as follows: 94°C for 5min; 30 cycles of 94°C for 45s, 63°C for 45s, and 72°C for 60 s; and a final extension step of 72°C for 7min. Fungal PCR mix had the same volume and was processed using the same reaction conditions. Except concentration of MgCl₂ that was 2.0mM and the annealing temperature/time was 53°C/30s. Ultrapure water was used as a negative control, and DNA from Pasteurella multocida, Corynebacterium sp., and Aspergillus fumigatus (ATCC 204305) was used as a positive control. Five PCR-positive samples, that were hemoculture negative, were purified and subjected to DNA sequencing with the corresponding primers. The sequences obtained were analyzed using the BLAST program (<www.ncbi.nlm.nih.gov>). Descriptive statistics were used for all individual variables, and unweighted kappa tests were used for comparisons between the diagnostic tests.

The mean age of the dogs was 5.3 years (standard deviation = 4.5 years), 70.4% (n=62) were female and 29.5% (n=26) male, and they belonged to 18 different breeds. The diagnoses included pyometra (37.5%), urinary tract infection (16%), pneumonia (11%), cutaneous abscesses (7%), and other causes (28.5%) such as peritonitis, endocarditis, and open fractures.

In the PCR analysis, 46 (52.3%) dogs tested positive for microbial agents. Twenty-three bacterial isolates were observed, namely, Staphylococcus sp. (n=9), Escherichia coli (n=7), Enterobacter sp. (n=2), Micrococcus sp. (n=2), Streptococcus sp. (n=1), Pseudomonas sp. (n=1), and Salmonella sp. (n=1). From three dogs (3.4%), two distinct bacteria were isolated. No fungal isolates were detected in blood cultures.

In the PCR analysis, 46 (52.3%) dogs tested positive for bacteria and only 1 (1.1%) for fungi. A comparison between results of the PCR analysis and blood culture showed a lack of concordance between the two tests (Table 2). The DNA sequences of samples that tested negative on the blood culture but positive on bacterial PCR showed identity with Candidatus mycoplasma haematoparvum (99%), Anaplasma platys (99%), Pseudomonas sp. (97%), and Flavobacterium sp. (97%). DNA sequences of

<table>
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<th>Criteria</th>
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<tbody>
<tr>
<td>Tachypnea</td>
<td>Respiratory rate &gt; 20 per minute</td>
<td>Heart rate &gt; 120</td>
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<td>Tachycardia</td>
<td>Temperature &gt; 39.2°C</td>
<td>Temperature &lt; 38.1 °C</td>
</tr>
<tr>
<td>Fever</td>
<td>&gt; 16 × 10³μL</td>
<td>&lt; 6 × 10³μL</td>
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<tr>
<td>Leucopenia</td>
<td>Band neutrophils &gt; 3%</td>
<td>Band neutrophils &gt; 3%</td>
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Table 1 - Criteria to diagnose of systemic inflammatory response syndrome (SIRS) in dogs.
samples that tested positive on fungal PCR had 98% identity with *Phoma* sp.

Pyometra and urinary infection were the most common diagnoses. Similar studies conducted in the past have shown that these conditions are associated with a high risk of sepsis (HAUPTMAN et al., 1997). Blood culture had a low sensitivity, detecting only 22.7% of cases, however clinical conditions could interfere in results, since, in human, sepsis rates were different from septic shock (30%), pneumonia (30%) and overall condition (5-15%), and in dog from pyometra (51.5%) (KALENSKI et al., 2012).

In a previous study, more cases of sepsis are detected by PCR than by blood culture because of the high sensitivity of the former (LIESENFELD et al., 2014). Together with DNA sequencing, it can be used to complement identification of uncultivable, pernicious or slow-growing microorganisms (PAOLUCCI et al., 2010), such as *Candidatus mycoplasma haematoparvum*, *Anaplasma platys* and *Phoma* spp. that cause opportunistic infections in immunosuppressed dogs (CABAÑES et al., 1996; MESSICK 2004). Blood culture; however, is still necessary to determine the correct antibiotic therapy (PAOLUCCI et al., 2010) but some improvement as multiplex PCR could detect both bacteria DNA and resistance genes (*mecA*, *vanA/B*, and *blaKPC*) of sepsis in humans (SALIMNIA et al., 2016).

Thus, PCR allows for early diagnosis of sepsis and rapid treatment (antibacterial or antifungal), which lowers the treatment/hospital cost (LIESENFELD et al., 2014). Although, microbial DNA can be found even in healthy individuals, a previous study showed that the presence of microbial DNA in the blood of human patients with sepsis indicated a high risk of death (O’Dwyer et al., 2017).

Sepsis in dogs is a life-threatening condition and thus diagnostic tools are important to determine a rapid treatment protocol. In this study, PCR testing was shown to have diagnostic value for canine blood infections because it has a shorter turnaround time and higher sensitivity than traditional blood culture.

**ACKNOWLEDGEMENTS**

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**BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL**

All procedures were approved by the Ethics Committee for Animal Use UFMT 23108.01329/13-4.

**DECLARATION OF CONFLICTING INTERESTS**

We have no conflict of interest to declare.

**REFERENCES**


**Table 2 - Comparison of PCR (bacteria/fungi) and isolation technique results in dogs with suspected sepsis (Kappa test = 0.06 [95% CI, –0.14 to 0.25]).**

<table>
<thead>
<tr>
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