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MICROBIOLOGY

Polymerase Chain Reaction and blood culture for diagnosis of canine sepsis

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ABSTRACT: *Sepsis is characterized by the presence of organ dysfunction secondary to the dysregulated systemic inflammatory response associated with an infection, and has high mortality rates. Traditional diagnostic techniques based on non-microbiological isolation are time-consuming and may delay treatment. Thus, this study aimed to compare bacterial and fungal broad-range polymerase chain reaction (PCR) and blood culture for diagnosis of sepsis in dogs. Blood samples from 88 dogs with suspected sepsis were analyzed by blood culture, and PCR to detect bacterial and fungal DNA. On blood culture, 20 (22.7%) samples tested positive for bacterial isolates; however, none tested positive for fungi. Through PCR analysis, bacterial DNA was detected in 46 (52.3%) animals, whereas fungal DNA was present in one (1.1%) sample. Our results showed that PCR-based testing has important diagnostic value for canine blood infections because it has a shorter turnaround time and higher sensitivity than traditional blood culture.*
Key words: *blood culture, PCR, bacteremia, fungemia.*

Reação em Cadeia da Polimerase e hemocultura para diagnóstico de sepse canina

RESUMO: *Sepse se caracteriza pela presença de disfunção orgânica secundária à resposta inflamatória sistêmica desregulada, associada a uma infecção com elevadas taxas de mortalidade. As técnicas tradicionais baseadas no isolamento microbiológico são demoradas e podem atrasar o tratamento. O objetivo deste estudo foi comparar a Reação em Cadeia da Polimerase (PCR) bacteriana e fúngica e hemocultura em cães com sepse. Foram analisadas 88 amostras de sangue de cães com suspeita de sepse por meio de hemocultura e PCR para detectar DNA bacteriano e fúngico. Nas culturas sanguíneas, 20 (22,7%) amostras foram positivas para isolados bacterianos. No entanto, nenhuma amostra foi positiva para fungos. Através da análise por PCR, o DNA bacteriano foi detectado em 46 animais (52,3%), enquanto que o DNA fúngico estava presente em uma amostra (1,1%). Neste caso, a PCR apresenta importante valor diagnóstico em cães com infeções sanguíneas devido a sua rapidez e maior sensibilidade do que a isolamento por hemocultura.*
Palavras-chave: *Hemocultura, PCR, bacteremia, fungemia.*

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 (Table 1)

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Table 1 - Criteria to diagnose of systemic inflammatory response syndrome (SIRS) in dogs.

Criteria	
Tachypnea	Respiratory rate > 20 per minute
Tachycardia	Heart rate > 120
Fever	Temperature > 39.2°C
Hypothermia	Temperature < 38.1 °C
Leucocytosis	> 16×10^3/µL
Leucopenia	< 6×10^3/µL
Increased band neutrophill with normal WBC	Band neutrophils > 3%

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 (TCCTCCGCTTATTGATATGC) 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 $MgCl_2$, 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 $MgCl_2$ 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.

On blood culture, 20 (22.7%) dogs tested positive for microbial agents. Twenty-tree 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 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]).

	Blood culture positive	Blood culture negative	Total
PCRpositive	12	35	47
PCRNegative	8	33	41
Total	20	68	88

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 (50%), 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* bla_{KPC}) 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.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All procedures were approved by the Ethics Committee for Animal Use UFMT 23108.015329/13-4.

DECLARATION OF CONFLICTING INTERESTS

We have no conflict of interest to declare.

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