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USE OF POLYMERASE CHAIN REACTION FOR DETECTION OF *TRYPANOSOMA VIVAX* INFECTION IN CATTLE

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ABSTRACT

Trypanosoma vivax is a protozoan that infects a wide range of wild and domestic ungulates causing important economic losses on the livestock industry. Considering a recent outbreak in bovines from the state of Pernambuco, the aim of this study was to utilize the PCR as a tool to detect *T. vivax* DNA in the blood of animals from different regions (i.e., Litoral, Zona da Mata, Agreste and Sertão). Based on previous serological survey, the cities in each region which had the highest frequency of reagent cattle to antibodies IgG anti-*T. vivax* by Immunofluorescence Antibody Test, were selected. A total of 127 bovine blood samples were obtained in heparin vacuum tube for further DNA extraction and PCR. The PCR was carried out using primers 18STnF2 and 18STnR3, which delimit a fragment of 659 *bp* of the 18S *rRNA* gene in Brazilian isolates of *T. vivax*. Out of 127 analyzed samples, 44.88% (57/127) presented amplicons with 659 bp compatible with *T. vivax*.The PCR proved to be a good tool for the diagnosis of infection by *T. vivax* in bovines, being important in the detection of infected animals independently from the clinical status.

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INTRODUCTION

Trypanosoma vivax is a protozoa that infects a wide range of wild and domestic ungulates causing important economic losses on the livestock industry (Gardiner 1989; Jones and Dávila, 2001; Silva *et al.,* 2004; Osório *et al.,* 2008). Several diagnostic methods have been utilized to detect this parasite in bovines, such as: parasitological methods that are based on direct visualization of the parasites (Silva *et al.,* 1999), the serological method (Nantulya, 1987), and the molecular methods based on the detection of nucleic acids of the protozoa (Madruga *et al.,* 1999). Among these techniques, the Polymerase Chain Reaction (PCR) has demonstrated high sensitivity when compared to parasitological (Clausen *et al.,* 1998) and serological techniques (Garcia *et al.,* 2006).

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Considering a recent outbreak in bovines from the state of Pernambuco (Pimentel et al., 2012), the aim of this study was to utilize the PCR as a tool to detect *T. vivax* DNA in the blood of animals from different regions (i.e., Litoral, Zona da Mata, Agreste and Sertão).

MATERIAL AND METHODS

Selection of the study area and animals

Based on previous serological survey (Guerra *et al.,* 2013), the cities in each region which had the highest frequency of reagent cattle to antibodies IgG anti-*T. vivax* by Immunofluoresce Antibody Test (IFAT) (Silva *et al.,* 2002), were selected. The cities were Itamaracá (Litoral), Palmares (Zona da Mata), Bezerros (Agreste), and São José do Belmonte (Sertão). A total of 127 bovine blood samples were obtained in heparin vacuum tube (Heparina Vacuette®) for further DNA extraction and PCR.

DNA extraction

Genomic DNA was extracted from 200µL of blood using a commercial Kit (Qiagen DNeasy Blood & Tissue Kit), following the manufacturer's instructions. Extractions were carried out in an appropriate environment using ***plugged tips*** to avoid cross contamination.

Polymerase Chain Reaction (PCR)

The PCR was carried out according to the protocol previously described by Geysen *et al.,* (2003), using primers 18STnF2 (5'- CAACGATGACACCCATGAATTGGGGA-3') and 18STnR3 (5'- TGCGCGACCAATAATTGCAATAC -3'), which delimit a fragment of 659 *bp* of the 18S *rRNA* gene in Brazilian isolates of *T. vivax* (Madruga *et al.,* 2003). The reactions were performed in a final volume of 25 µl, using a commercial Kit Top Taq Master Mix (Qiagen). The amplification consisted of one cycle of denaturation at 94° C for 4 min, followed by 40 cycles of denaturation at 94° C for 60 seconds, annealing at 58 °C for 90 seconds and extension at 72°C for 120 seconds. The amplification products were viewed under ultraviolet light following electrophoresis on agarose gel (2%) stained with Blue Green (LGC). DNA extracted from blood of a naturally infected bovine (Pimentel *et al.,* 2012), as evidenced by optical microscopy, was used as positive control and water DNAse free (LGC®) was utilized as negative control. *Plugged tips were utilized* to avoid cross contamination.

Scientific ethics committee

The project was approved by the Ethics Committee on Animal Use of the Federal Rural University of Pernambuco with the license number 049/2012.

RESULTS AND DISCUSSION

Out of 127 analyzed samples, 44.88% (57/127) presented amplicons with 659bp compatible with *T. vivax* (Table 1).

Table 1. Polimerase chain reaction for trypanosoma vivax from diferents regions of pernambuco state, brazil

Region	Positive		Negative	
	(N°)	(%)	(N°)	(%)
Litoral	8	47,06	9	52,94
Zona da mata	22	59,46	15	40,54
Agreste	16	43,24	21	56,76
Sertão	11	30,56	25	69,44
Total	57	44,88	70	55,12

The results herein obtained are similar to those found by SALIM et al. (2011) and Enwezor *et al.* (2008), which observed a good specificity in the detection of infection by *T. vivax* using molecular diagnosis. The diagnosis of *Trypanosoma* by PCR usually has high sensitivity, enabling the detection of small amounts of the parasite in samples such as one parasite/mL (Silva *et al.,* 2002) of blood, or even 0.1pg of DNA from *Trypanosoma* (Clausen *et al.,* 1998). The PCR has been able to detect these protozoa in the blood of cattle 72-96 hours after drug administration (Clausen *et al.,* 1999), and therefore it is suitable for the detection of *T. vivax* (Desquesnes, 2004). Although PCR is generally considered more sensitive than other diagnostic techniques (Desquesnes, 1997; Masake, 1997; Bengaly *et al.,* 2001; Gonzáles *et al.,* 2006), in the acute phase of infection by *Trypanosoma* similarities have been reported with respect to sensitivity between the PCR and parasitological methods. However, in the chronic phase, the PCR can be four times more sensitive (Desquesnes, 1997; Clausen *et al.,* 1998). Few epidemiological surveys about bovine trypanosomiasis have been conducted in Brazil (Silva *et al.,* 1996; Ventura *et al.,* 2001; Madruga *et al.,* 2006; Cortez *et al.,* 2006, Baptista-Filho *et al.,* 2011), being limited to outbreak reports in the states of Paraíba, Maranhão, Minas Gerais, Pernambuco and São Paulo (Batista *et al.,* 2007; Guerra *et al.,* 2008; Carvalho *et al.,* 2008; Pimentel *et al.,* 2012 and Cadioli *et al.,* 2012). Most of these studies were carried out based on serological or parasitological techniques, which present problems of sensitivity and specificity, especially in endemic areas where infections by *T. evansi* also occur (Araújo *et al*., 1997). Serologic cross reactions with *Babesia bovis* have also been reported, difficulting the obtention of accurate results (Madruga *et al.,* 1999). In the present study, PCR was used due to its accuracy and reliability (Desquesnes and Dávila, 2002), allowing safer diagnosis of the protozoan.
Thus, PCR allows the accurate diagnosis of chronically infected animals, which have lower parasitaemia, undetectable by parasitological tests. These tests require large amounts of parasites in the blood, making the diagnosis more difficult (Dirie *et al.,* 1993). According to Desquesnes *et al.* (1997), free DNA of *T. vivax* has a short period of stability, thus the detection of DNA of *T. vivax* in the present study is indicative of active infection.

Conclusion

The PCR proved to be a good tool for the diagnosis of infection by *T. vivax* in bovines, being important in the detection of infected animals independently from the clinical status.

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