PARASITOLOGICAL AND MOLECULAR DETECTION OF CANINE TRYPANOSOMIASIS FROM RIYADH PROVINCE, SAUDI ARABIA

Abdullah D. Alanazi

Department of Biological Sciences, Faculty of Science and Humanities, Shaqra University, P.O. Box 1040, Ad-Dawadimi 11911, Saudi Arabia.
Correspondence should be sent to: aalanazi@su.edu.sa

ABSTRACT: Trypanosoma evansi is the most widespread of the pathogenic salivarian trypanosomes; it causes a serious disease called surra that affects domestic animals such as camels, horses, and dogs, and often leads to reduced productivity and economic losses. Therefore, the objectives of the present study were to determine the prevalence rates of trypanosomiasis using 3 parasitological tests (wet blood film, Giemsa staining, and microhematocrit centrifugation technique) and polymerase chain reaction (PCR) among stray dogs from Riyadh Province, Saudi Arabia. In the current study, 117 dog blood samples collected from certain districts of Riyadh Province showed that 5 of 117 dogs (4.3%) were positive for the genus Trypanosoma. In addition, the findings indicated no effect of dog gender or age on parasite infection. For a more specific diagnosis, PCR amplification of the RoTat 1.2 VSG gene in 5 internal transcribed spacer1-positive samples diagnosed with Trypanosoma indicated that 2 were positive for RoTat 1.2 T. evansi. The absence of the RoTat 1.2 VSG gene in 3 of the 5 T. evansi-positive samples could be explained by the circulation of T. evansi type B in dogs from Saudi Arabia. Thus, this is the first study demonstrating T. evansi type B outside of Africa.

Trypanosoma evansi is the most widely distributed pathogenic species, affecting domestic and wild animals in tropical and subtropical countries, and it causes a significant disease called surra (OEIE, 2010; Desquesnes et al., 2013). These parasites can be transmitted mechanically by several genera of hematophagous flies such as Tabanus and Stomoxys species (Luckins and Dwiner, 2004; Desquesnes et al., 2009). Canine trypanosomiasis is characterized by progressive anemia, anorexia, cachexia, fever, generalized weakness, edema of the head and throat, and finally, staggering gait (Chaudhuri et al., 2009; Eloy and Lucheis, 2009).

The diagnosis of T. evansi infection is still difficult because the clinical signs are varied and nonspecific (Reid, 2002; Herrera et al., 2004). Typically, T. evansi diagnosis is mainly based on finding the parasites in smears, wet blood films (WBFs), or by the microhematocrit centrifugation technique (MHCRT) (Chowdhury et al., 2005; Gunaseelan et al., 2009; Prasad et al., 2015); however, these techniques are specific but less sensitive, particularly in detecting parasites during low levels of parasitemia. Serological methods have also been used for screening of T. evansi infection; nevertheless, antibodies may be missing because of serological latency (Reid, 2002; Birhanu et al., 2015).

Recently, DNA-based technologies including polymerase chain reaction (PCR) have been shown to be very specific and sensitive and have been widely used for the diagnosis of trypanosomiasis infection in camels, horses, cattle, and companion animals (Njiru et al., 2005; Ravindran et al., 2008; Salim et al., 2011; Rjeibi et al., 2015; Hussain et al., 2016). The rRNA internal transcribed spacer 1 (ITS1) and ITS2 have been found to be reliable for phylogenetic separation of closely related species including piromastigotes and their subspecies and trypanosomes (Fazaeei et al., 2000; Cox et al., 2005; Njiru et al., 2005). In addition, a variable surface glycoprotein gene of trypanosomes, RoTat 1.2 VSG, has been used as a confirmation test for T. evansi infection (Claes et al., 2004; Ngaira et al., 2004; Salim et al., 2011; Barghash et al., 2014; Tehseen et al., 2015).

In Saudi Arabia, T. evansi is enzootic in camels, with a prevalence up to 40% (Omer et al., 1998; Al-Khalifa et al., 2009; El Wathig and Faye, 2013; El Wathig et al., 2016) and has a prevalence of 3.0% in equines (D. A. Alanazi et al., unpubl. data). Since prevalence studies of canine trypanosomiasis are lacking in Saudi Arabia, the current study was designed to document the prevalence of trypanosomiasis in stray dogs from Riyadh Province using parasitological and molecular methods.

MATERIALS AND METHODS

Study areas

The investigation was conducted from January 2016 to December 2017 in the Riyadh Province of Saudi Arabia. This province has an area of 404,240 km² and is located in the central part of Saudi Arabia (24°–38°N, 46°–43°E) (Fig. 1). Dogs from the stray populations in this province were trapped by live bait traps and randomly selected for inclusion in the study. A field examination station was set up at each location so the dogs could be examined for trypanosomiasis. Additionally, dogs visiting veterinary clinics were included in this study.

Sampling and blood collection

A total of 117 dogs (61 males and 56 females) varying in ages from 0 to ≤ 2 yr to > 8 yr old was examined for trypanosomiasis. All the dogs were apparently healthy at the time of blood collection. Blood samples were collected from each dog (2–5 ml) from the cephalic vein into ethylenediamine tetra-acetic acid (EDTA) vacutainer tubes (BD Vacutainer® Tube, Gribbles Pathology, Victoria, Australia) and transported to the parasitological laboratory, Department of Biological Sciences, Faculty of Science and Humanities, Shaqra University, for DNA extraction.

Parasitological examinations

All blood samples were subjected to parasitological examinations by WBF, blood smear techniques using Giemsa staining as described by Jain (1986), and the MHCT as described by Schalm et al. (1975).
DNA extraction

Total genomic DNA (gDNA) was isolated from the blood samples using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) and eluted in 50 or 100 μl of elution buffer as per the manufacturer’s instruction. An aliquot between 50 and 100 μl of gDNA from each of the samples was stored at −80°C before being sent to the Molecular Laboratory, School of Biological and Marine Sciences, Plymouth University for PCR analysis. At the Molecular Laboratory, gDNA was stored at −20°C for up to 1 mo before molecular diagnostics.

Molecular analysis

Two-step PCR protocols were used to analyze the Trypanosoma spp. in DNA samples. First, the DNA samples were used to amplify a 250–700-base pair (bp) region of the ITS gene using the forward primer ITS1 CF (5′-CCGGAAGTTCACCGATATTG-3′) and reverse primer ITS1 BR (5′-TGCTGCGTTCTTCAAC GAA-3′); these primers are specific for parasites belonging to the genus Trypanosoma (Njiru et al., 2005). Then, all species-positive ITS1 T. evansi isolates (480 bp) were further subjected to a PCR test specific for T. evansi, in which a primer set that amplifies 151 bp of the T. evansi Rotat 1.2 VSG gene fragment was used (Konnai et al., 2009): TeRoTat920F (5′-CTGAAGAGGTTGGAAATGGAGAAG-3′) and TeRoTat1070R (5′-GGTGGTCTGTGTGTGTTA-3′). All PCRs were performed in a final volume of 50 μl containing 25 μl of Dream Taq DNA polymerase master mix 2X (Thermo Scientific™, Nalgene, U.K.), 0.4 μM (1 μl) of each primer, and 2 μl of DNA template. The reaction was brought to 50 μl total volume with PCR-grade water (Invitrogen, Paisley, U.K.). Positive and negative controls were included in all assays. Thermal cycling conditions for Trypanosoma species consisted of an initial 2-min incubation at 95°C; followed by 40 cycles of denaturation at 95°C for 30 sec, primer...
Infection with dogs is shown in Table I. All dog ages and the prevalence of amplicons specifically generated using the ITS1 CF/ITS1 BR primer set.

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>No. examine</th>
<th>Parasitological examinations</th>
<th>PCR methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Smear</td>
<td>WBF</td>
</tr>
<tr>
<td>&lt;2</td>
<td>43</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>2-4</td>
<td>28</td>
<td>1</td>
<td>3.6</td>
</tr>
<tr>
<td>4-6</td>
<td>21</td>
<td>1</td>
<td>4.8</td>
</tr>
<tr>
<td>6-8</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>3</td>
<td>5.6</td>
</tr>
</tbody>
</table>

DISCUSSION

This is the first parasitological and molecular diagnosis of trypanosomiasis in dogs in Middle Eastern countries. Nevertheless, T. evansi is a common protozoan infection in camels in this part of the world (Barghash et al., 2014). Previous studies from Saudi Arabia have estimated trypanosomiasis infection levels in camels ranging between 0 and 40% (Omer et al., 1998; Al-Khalifa et al., 2009; El Wathig and Faye, 2013; Al-Afaleq et al., 2015). Unfortunately, there were no available data to compare the results of the present study with local or regional studies on T. evansi infection in dogs because infection in dogs is not frequent; contamination could occur through the exposure of dogs to several hematophagous vector species or orally by the ingestion of contaminated meat (Raina et al., 1985).

In the current study, the results showed that diagnosis with parasitological examinations was insufficient to confirm trypanosomes to the species level, in contrast with PCR methods. The ITS1 PCR test is increasingly used in surveys for T. evansi confirmation in dogs (Aref et al., 2013; Rjeibi et al., 2015; Jaimes-Dueñez et al., 2017). However, this method has drawbacks such as the low analytical sensitivity of the ITS1 CF and BR primers, which reach approximately 10 pg (100 trypanosomes) for Trypanozoon, and their inability to differentiate between the different subgroups of Trypanosoma (Cox et al., 2005; Njiru et al., 2005). Therefore, the variable surface glycoprotein of trypanosomes, RoTat 1.2 VSG, was used as a target to distinguish T. evansi from other Trypanosoma species (Urakawa et al., 2001; Salim et al., 2011, 2014; Barghash et al., 2014). Therefore, the variable surface glycoprotein of trypanosomes, RoTat 1.2 VSG, was used as a target to distinguish T. evansi from other Trypanosoma species (Urakawa et al., 2001; Salim et al., 2011, 2014; Barghash et al., 2014; Birhanu et al., 2016; Tehseen et al., 2017). The low positive numbers of T. evansi...
infections detected in the current study based on the RoTat 1.2 V5G gene compared with the results of the ITS1 PCR agreed with the results of previous studies (Njiru et al., 2005; Salim et al., 2011; Elhaig et al., 2013; Barghash et al., 2014; Tehseen et al., 2015). Nevertheless, mixed infection by T. evansi type A and type B is also possible, since these strains have been identified from Kenya (Nigeria et al., 2004; Njiru et al., 2006), Sudan (Salim et al., 2011), and Ethiopia (Birhanu et al., 2015, 2016). This study reported for the first time T. evansi type B outside of Africa; the reason could be related to the export of livestock from eastern Africa, particularly from Sudan, Djibouti, and Somalia, to Saudi Arabia.

In the present study, the results revealed that T. evansi infection was not affected by the sex of dogs; both male and female were equally susceptible. These results are in agreement with those of previous studies from other parts of the world (Chowdhury et al., 2005; Prasad et al., 2015). In addition, the results of the present study showed that all ages were similarly susceptible to T. evansi infection, in agreement with the findings of Rashid et al. (2008), Gunaseelan et al. (2009), Prasad et al. (2015, and Rjeibi et al. (2015).

CONCLUSION

In the present study, T. evansi infection in Saudi dogs was reported for the first time, and the detailed diagnostic PCR protocol was provided. Additionally, this study demonstrated the presence of T. evansi type B outside of Africa for the first time. Further studies are necessary to estimate the prevalence rates of T. evansi infection in Saudi dogs and to prevent parasite infection.

ACKNOWLEDGMENTS

This study was revised and approved by the Ethics Committee, Department of Biological Science, Shaqra University, according to the ethical principles of animal research. This project was kindly supported by Shaqra University, Deanship of Scientific Research. The author thanks the staff members of the Biological Science Department, Faculty of Science and Humanities, Shaqra University, for technical support.

LITERATURE CITED


HAZAN, N. 1986. Schalm’s veterinary haematology, 4th ed. Lea and

HUSSAIN, M., Z. SAEED, M. GULSER, R. S. SHAIKH, M. ALI, M.


