HEMATOLOGICAL AND MOLECULAR STUDY OF TRYPANOSOMA EVANSI IN EXPERIMENTALLY INFECTION IN RABBITS

R. Khalid Ali I. Badr Falih
Lecturer Prof.
Email:dr.rafidkhalid@uofallujah.edu.iq

ABSTRACT
The lack of a sensitive diagnostic test for trypanosomiasis in animals may hamper epidemiological data and control program implementation. This study aimed to detection of Trypanosoma evansi in rabbits that experimentally infected by this parasite which identified from camels of iraq by polymerase chain reaction. A study was conducted on 24 males rabbit divided into two groups (12 rabbits for each group). The first group was injected intra peritoneal (i/p) with 0.5 ml containing 1x10^5 trypanosome. The second group injected with distilled water and considered as control. At 7 and 10 days post infection, blood sample were collected from these rabbits. White blood cells count was conducted after 14 and 28 days post infection. The results showed that the 18S RNA and RoTat 1.2 genes related to T. evansi were identified .Furthermore ,there was significant elevation of Neutrophils ,Monocytes,Lymphocytes ,Eosinophil and Basophils in infected rabbits compared with the control group. From this study we can conclude that PCR is a sensitive and effective technique for diagnosis of T.evansi through using 18S RNA and RoTat 1.2 genes. In addition ,WBC count was significantly elevated in an infected rabbits compared to the control. Whereas ,Lymphocyte was significantly higher in control group compared with infected group.

Key words: Hemoprotozoa, PCR, 18SRNA gene, trypanosomiasis, good health
INTRODUCTION

Trypanosomosis of camel (Surra) is the most frequent disease in the world and its caused by Trypanosoma evansi. The disease transmitted mechanically by haemotrophagus biting flies such as Stomoxys and Tabanus spp. (4). Salivaria branch, which originates in front portion of the insects digestive system, is the route through which T. evansi is spread. (6). Trypanosoma evansi uses glucose catabolism to produce adenosine triphosphate (ATP) which is necessary for parasite movement and survival. Anemia is a characteristic pathology of infections with T. evansi due to the peroxidation of the erythrocytes lipid which causes membrane damage, osmotic fragility, and RBC death (13). Males are more susceptible to infection than female (22). But T. evansi infection leading to abortion of pregnant animals and generating economic loss as a result of its propensity to generate negative feedback (2). In camels, Trpanosomiasis causes severe morbidity (16). Camels become infected with trypanosomiasis in two forms (acute and chronic). Chronic form is the most frequent, and it may be related with secondary infections and this may be due to the fact that trypanosomiasis infection causing immunosuppression and complicating clinical diagnosis (27). Fever, emaciation, anemia, ocular opacity, lacrimation, diarrhoea and edema of the dependent parts are insufficient for diagnosis and identification of this parasite in the blood is problematic due to the intermittent nature of parasitaemia (8,15). Alternative diagnostic techniques that are more delicate are therefore needed. For the identification of camel's disease, several diagnostic methods such as PCR have been utilized, including COX1 and ITS2 region (12). In a variety of hosts, PCR has been shown to be more sensitive and accurate than traditional techniques of parasitology, with the benefit of being able to identify parasites at the species level (1). Present study aimed to detection of Trypanosoma evansi in rabbits that experimentally infected by this parasite which identified from camels in Iraqi.

MATERIALS AND METHODS

From January to October 2020, twenty four adult healthy rabbits with 7-9 weeks of age were used for the present study. The rabbits divided into two groups, 12 rabbits for each. The first group was injected with 0.5 ml of blood from infected camel containing 1x10^6 trypanosome intraperitoneally (i/p). The second group injected with distilled water and considered as control. Blood sample were collected from these rabbits and the genes of 18S RNA and RoTat 1.2 related to T. evansi were identified by polymerase chain reaction (PCR). The protocol of genes detection was used according to manufacturing company (19). Blood was conducted after 14 and 28 days post infection for hematological analysis.

Microscopic finding

10 µL of blood was used , a clean slide was prepared for examination, general smear protocol applied to obtain a clear smear view using Giemsa stain(1:9) with (pH 7.2) followed by washing with clear water.

DNA extraction

The DNA of Trypanosoma evansi was extracted from infected rabbit according to the manufacturer instructions(G-spin DNA extraction kit,intron biotechnology, cat.no. 17045). All extracted DNA were kept at -20C until examined. Molecular identification of Trypanosoma evansi using specific primer of UNI18s gene (5'-GCGAACGCAAAGCTAATAC-3') (5'-ACGGCACAATACTACGTG-3'), integrated DNA Technologies company,Canada). For PCR reaction utilized 25µl and qualified in Table 1, the program of amplification is specific in Table 2.

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq PCR PreMix</td>
<td>5µl</td>
</tr>
<tr>
<td>Forward primer</td>
<td>10 picomols/µl (1 µl)</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>10 picomols/µl (1 µl)</td>
</tr>
<tr>
<td>DNA</td>
<td>1.5µl</td>
</tr>
<tr>
<td>Distill water</td>
<td>16.5 µl</td>
</tr>
<tr>
<td>Final volume</td>
<td>25µl</td>
</tr>
</tbody>
</table>

1234
Table 2. Amplification program for polymerase chain reaction

<table>
<thead>
<tr>
<th>No.</th>
<th>Phase</th>
<th>Tm (°C)</th>
<th>Time</th>
<th>No. of cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Initial Denaturation</td>
<td>95°C</td>
<td>5 min</td>
<td>1 cycle</td>
</tr>
<tr>
<td>2</td>
<td>Denaturation</td>
<td>95°C</td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Annealing</td>
<td>58°C</td>
<td>45 sec</td>
<td>35 cycles</td>
</tr>
<tr>
<td>4</td>
<td>Extension</td>
<td>72°C</td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Final extension</td>
<td>72°C</td>
<td>7 min</td>
<td>1 cycle</td>
</tr>
</tbody>
</table>

Statistical analysis
All variable results were analysis by (one-way ANOVA) and the significant differences for the result was determined at (P≤0.01) (17).

RESULTS AND DISCUSSION
Microscopical examination of blood smear showed the protozoa of *Trypanosoma evansi*, which appeared as slender shape with either tapered or blunt ends and with central nucleus position and free flagellum (Figure 1).

Figure 1. Numerous *Trypanosome evansi* (black arrow) in blood smear of infected rabbits stained by Giemsa stain (100x).

The 18S RNA and RoTat 1.2 genes of *T. evansi* were amplified at the size of 550bp and 205 bp, respectively (Figures 2 and 3). The 18S RNA gene was registered in GenBank at NCBI by the following code: KY114580.1.

Figure 2. Fragment size(bp)of gene 18S RNA gene=550pb
White blood cell count (WBC)

Our finding showed a significant differences (p ≤ 0.01) in WBCs count mean between infected and control group, except 14 days and 28 days.

Table 3. WBC count (mean ± S. D.) at 14 and 28 days post infection in infected and control rabbit groups

<table>
<thead>
<tr>
<th>Type of cell</th>
<th>Control group</th>
<th>Infected group at 14 with 1x10^5 T. evansi</th>
<th>Infected group at 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils %</td>
<td>48.6 ± 1.8</td>
<td>67.4 ± 2.3</td>
<td>61.4 ± 10.7</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>4.7 ± 0.3</td>
<td>7.8 ± 1.6</td>
<td>6.8 ± 1.9</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>0.7 ± 0.1</td>
<td>2.6 ± 0.4</td>
<td>2 ± 0.4</td>
</tr>
<tr>
<td>Basophils %</td>
<td>0.9 ± 0.1</td>
<td>3.5 ± 0.3</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>41.7 ± 1.5</td>
<td>31 ± 3.4</td>
<td>25.5 ± 3.5</td>
</tr>
</tbody>
</table>

*Horizontal column means that there is a significant differences at P ≤0.01. Mean ± S.D.

The important of trypanosome infection in camels is attributed to the pathogenesis and clinical signs of this disease in world wide. Numerous research on the characterization of trypanosomes in general and T. evansi in particular have been published (20,25). Despite the fact that camels are generally lived in different environmental (desert), compared with livestock camels still has some cases of infection with T.evansi. Transmission via biting flies is conceivable due to the presence of vector around the susceptible camel (19). PCR methods is successfully used for identification of T. evansi in horses and buffaloes (5). Application of PCR for detection T. evansi in camels is limited, PCR contamination is also an issue. Use of clear aerosol resistant tip (nucleic acid free) and different places for both DNA extraction and amplification could help to reduce contamination. To keep high degree of sensitivity at the same time reduce the specificity, each PCR experiment should include negative and positive control. The nested PCR assays speed, specificity and sensitivity would make these test more easier to detect T. evansi infection in different species of animals (10,17). But these test is a higher sensitivity than microscopic analysis of blood smears (18,19). Epidemiological molecular analysis result range from 33.9 percent to 42.1 percent (11). PCR claimed the highest rate of infection. Indicating high sensitivity level which was 90% and these results are corresponding with Elhaig (7). 18 SRNA and Rotat1.2 are the molecular targets genes used in this study. 18S RNA results showed dromedary camels in scrupulous area were
infected with Trypanosomes, especially *T. evansi* which were also positive with RoTat 1.2 genes during this experiment, some samples were found to be negative by optical microscopy but positive by PCR. This method has a low false-positive rate and is unaffected by other blood parasite species (20). It is worth noting that the level of blood parasites was highest in the dry season, which leading to increase activity of these flies and facilitated transmission and infection due to the gathering of camels herds near water well, in addition to other predisposing factors such as dry rain and low level of animals nutrition in the last part of the dry season (24). Neutrophils, monocyte, eosinophil and subsequent basophil which observed at the present study associated with the host immune response against parasitic infection and leading to activation of mononuclear phagocytes as well as neutrophils leukocytes may be associated with the stress of Trypanosome infection result in the production of corticosteroid and these result are corresponding with Aradaib and Majid (3). Leukocytosis and lymphocytosis were recognized in the acute phase, followed by leukopenia in the chronic phase (9,14) leukocytosis is not a reliable marker of infection as revealed by different studies,(23,26).

CONCLUSIONS

PCR is an sensitive and effective technique for *T. evansi* diagnosis by using 18S RNA and RoTat 1.2 genes. In addition to, WBC count (mean ± S.D.) was significantly elevated in infected rabbit compared to control groups, except mean of Lymphocyte which was significantly higher in control group in contrast to the infected group.

REFERENCES


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