Haematological and Biochemical Alterations in Sub Clinically Affected Dromedary Camels with Trypanosoma evansi During Breeding Season in Egypt

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ABSTRACT

Little is known about the prevalence of subclinical Trypanosoma evansi (T. evansi) infection and its effects on hematological and serum biochemical parameters in relation to different periods of breeding season in dromedary camels. Out of two hundred males during of breeding season, a total of 26 (13%) of microscopically positive flagellated protozoan and 27 (15.5%) of card agglutination test (CATT) positive trypanosomiasis were considered as sub clinically infected animals. The results revealed some decreased measured values related to erythrocyte in the periods of breeding season of infected animals. The leukogram results showed significant increase in total leukocytic count (TLC) and lymphocyte % in infected group comparing with non-infected group during periods of breeding season. Monocyte% and neutrophil% were significantly lower while, eosinophil % showed increase (P<0.01) in infected camels in initial and mid breeding season. Results showed decrease in total proteins (P<0.05) and albumin (P<0.01) during mid-breeding while increased values (P<0.05) of globulin level in initial breeding period were recorded in infected animals. Triglyceride and total cholesterol significantly increased in T. evansi infected camels in mid and late breeding periods. Increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine kinase (CK) and total bilirubin were recorded (P<0.01) in infected camels in the different breeding periods. However, alkaline phosphatase (ALP) level was significantly decreased (P<0.01). The present study concluded that subclinical infection of camels with T. evansi can affect some hematological and biochemical parameters related to liver, kidney, hematopoiesis and immune status during late period of breeding season.

KEY WARDS: Trypanosoma, Camel, Hematology, Biochemical, Subclinical.

1. INTRODUCTION

Trypanosomes are blood and tissue parasites of order Kinetoplastida and family Trypanosomatidae which are found in different mammals including humans (Coura and Borges-Pereira, 2010; Dyary, 2014). Trypanosomiasis in camels is a vital disease, widely distributed all over the world in tropical and sub-tropical areas. It is characterized by high mortality and morbidity and also anemia which have been considered as a consistent finding in infected animals (Dyary, 2014). A protozoan parasite T. evansi is the causative agent of trypanosomiasis in camels which called (sarra) (Sazmand, 2011) which considered the most widely spread endemic protozoan disease of camels and different domestic animals throughout the world (Elhaig, 2013; Shahid, 2013). This parasite affects various species of wild and domestic animals in Latin America, Africa and Asia (Ismael, 2014). In Middle East and Africa, camels are the most often affected (Sow, 2014). However, in Egypt T. evansi is an endemic disease of camels and other domestic animals (Elhaig, 2013). T. evansi has the ability to periodically change the variable surface glycoprotein (VSG), producing deteriorations of parasitaemia (Herrera, 2004). More advanced antibody detection assays for T. evansi been performed in various studies on camel trypanosomiasis such as the card agglutination test (CATT/T. evansi), the immune trypanolysis (TL) assay, the ELISA/VSG RoTat 1.2 assay have (Tehseen, 2015).

Trypanosoma, are mainly transmitted mechanically to animals by some arthropod blood-sucking flies (Tabanus, Stomoxys and Liperornis) in which they undergo a biological cycle (Reid, 2002). T. evansi multiplies in the blood and body fluid that both acute and chronic forms of the disease may develop, but commonly the chronic form is more prevalent in which the mortality reach up to 90% (Gutierrez, 2005). Infection with T. evansi induced anemia, fever, depression, dullness, weakness, nervous symptoms (Padmaja, 2012; Narnaware, 2016). In addition, T. evansi infection was responsible for a diverse range of symptoms in the susceptible mammalian hosts including generalized oedema, spleenomegally, liver, renal hypertrophy (Cadioli, 2006) and weight loss in case of no treatment causing major economic loss in infected camels in terms of poor production (Derakhshanfar, 2010). T. evansi could cross the placental barrier in pregnant camels causing some pathological events in the fetus that lead to abortion (Narnaware, 2016). Several changes in the cellular and biochemical constituents of blood may be produced due to the presence of these invading parasites in the blood (Sivajothi, 2015).

Despite the importance and worldwide distribution of T. evansi, very little is known about its subclinical prevalence during breeding season in Egypt. As well as, blood and serum biochemical parameters are known as essential indicators of health status in animals, the present study was designed to investigate these alterations in sub-clinically infected dromedary camels with T. evansi associated with breeding season in Egypt.

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2. MATERIAL AND METHODS

Samples collection: A total of 200 apparently healthy one–humped male camels of 4-10 years old were randomly selected at the Warak slaughter house, Giza Governorate, Egypt. Two blood samples were collected from each camel immediately after slaughter during the period of breeding season (from November 2014 to April 2015). Breeding season was divided into three periods according to Abdalla (2011), with some modifications into initial breeding (November to December, 2014, n= 57), mid breeding (January to March, 2015, n= 73) and from late breeding (April to May, 2015, n=70). One blood sample from each camel was collected into EDTA containing vacutainer tubes for hematological study and to prepare thin blood smear for parasitological examination and differential leukocytic count. The second blood was collected in plain tubes and allowed to clot for serum extraction by centrifugation at 3000 rpm 10 minutes and stored at 0℃ for biochemical studies.

Detection of *T. evansi* infection: Giemsa stained thin blood smears were prepared from each camel using glass slides to be examined microscopically for the presence of trypanosome in circulation based on standard procedures (Soulsby, 1982).

*T. evansi* negative samples by parasitological examination method were subjected to serological diagnosis for detection of anti-*T. evansi* antibodies using Card agglutination test for trypanosomiasis CATT/T (Institute of Tropical Medicine, Laboratory of Serology, Antwerp, Belgium). Almost, 45 µl of the antigen were added onto the test card and mixed with 25 µl of the test sera diluted at (1:4) with PBS (PH 7.2) according to manufacturer’s instructions. The card was agitated for 5 min and positive reaction was confirmed on recording blue granular agglutinations (Bajyana Songa and Hamers, 1988).

Hematological studies: Total erythrocytes and total leukocytes (TLC) were counted by Neubauer’s haemocytometer. The blood samples were analyzed for hemoglobin (Hb) determination by cyanomethemoglobin method according to Kuwahara (1974), Packed Cell Volume (PCV) determination was performed by microhematocrit method according to Schalm (1975). Erythrocyte sedimentation rate (ESR) was determined as Westergren’s method. The erythrocyte indices as Color index (CI), mean corpuscular volumes (MVC), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentrations (MCHC) were estimated manually according to the method described by Tornquist (2010). Differential leukocytic count such as monocyte, lymphocyte, neutrophils and eosinophils were estimated as cross sectional method according to Ghaffar (2014).

Biochemical studies: Different serum biochemical parameters were determined in the prepared serum. Briefly, Total protein was determined by colormetric method (Biuret reagent) according to Cannon (1974), using SPECTRUM kits (BioMerieux, SA). Albumin globulin and albumin globulin (A/G) ratio was determined by Modified bromocresol green colorimetric method according to Doumas (1971) using SPECTRUM kits (BioMerieux, SA). Determination of lipogram biochemical values were performed by enzymatic colorimetric method (Zohreh, 2012) using a Linear Chemicals Kits to measure serum cholesterol and triglycerides. Various enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, total bilirubin and alkaline phosphatase (ALP) were determined by UV enzymatic colorimetric method according to the standardized method described by Winn-Deen (1988) using Linear Chemicals.S.L. kits. The colorimetric reaction was measured using spectrophotometer.

Statistical analysis: Significance between data of hematological and serum biochemical parameters were analyzed by Student’s t-test at level P<0.05 according to Petrie and Watson (1999) using Statistical Package for Social Science (SPSS) for Windows version 15 computer program.

3. RESULTS

Parasitological examination and card agglutination test for trypanosomiasis (CATT): Table (1) showed animals profile analysis according to microscopical examination and CATT test. Results revealed that out of 200 total blood samples there were 26 (13%) were presumed to be positive for *T. evansi* flagellated protozoan parasite between RBCs. However, 174 (87%) blood smears were considered negative (Fig.1).

Table 1. Number and percentage of trypanosoma infected camels according to microscopical examination and CATT test

<table>
<thead>
<tr>
<th>Total blood samples</th>
<th>No. of microscopically examined +ve samples</th>
<th>No. of +ve CATT test</th>
<th>CATT test grade</th>
<th>Total subclinical infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grad 1 (+)</td>
<td>Grade 2 (++)</td>
<td>Grade 3 (+++))</td>
</tr>
<tr>
<td>200</td>
<td>27 (15.5%)</td>
<td>13 (13%)</td>
<td>81.5%</td>
<td>11.1%</td>
</tr>
</tbody>
</table>
As showed in table 1, CATT test recorded that out of 174 examined camels there were 27 (15.5%) were found to be seropositive to T. evansi. The seropositive camels rate was shown at three grades according to anti-Trypanosoma antibody titters; grade 1 (+), grade 2 (++) and grade 3 (+++). Most seropositive sera were observed at grade 1 (22; 81.5%). However, grade 2 was shown in a relatively low rate (3; 11.1%). The lowest rate (2; 7.4%) was detected at grade 3. The total number of both positive trypanosomiasis according to microscopically and serologically examined 200 animals was 53 that represent 26.5% (P<0.05).

Hematological studies: As shown in table 2 results of haematological profile showed significant decrease in RBcs count, Hb concentration, PCV, MCV whereas ESR showed significant increase (P<0.01) in Trypanosoma infected camels in initial, mild and late breeding. In addition, CI showed significant increase (P<0.01) in late breeding than that in initial and mid breeding respectively.

Comparing the haematological changes in T. evansi infected group within the different breeding season periods, it was revealed that a significant reduction in Hb concentration in late breeding than that in initial and mid breeding. However, CI significantly higher in late breeding than mid breeding period (table.2). MCH and MCHC did not show any significant difference neither in infected group nor within different breeding season periods.

Mean (±SE) values of leucocytic picture of T. evansi infected and non-infected dromedary camels were presented in table 3. The results of leucogram showed significant increase TLC and lymphocytes in infected group.
It was found that total protein significantly increased (P<0.01) in Trypanosoma infected camels than non-infected camels during mid and late breeding periods. Results of lipid profile showed significant increase in total cholesterol levels than non-infected camels. However, acidophil % of infected camels showed significant increase (P<0.01).

The difference in the mean values in infected camels among the different periods of breeding showed significant decrease (P<0.01) in lymphocyte% in initial than mid and late breeding whereas, value of acidophil% was significantly lower in late breeding than that in initial (P<0.01) and mid (P<0.05) breeding (table.3).

### Table.3. Leukocytic picture of *T. evansi* sub-clinically infected and non–infected dromedary camels (Mean±SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial breeding</th>
<th>Mid breeding</th>
<th>Late breeding</th>
<th>Initial breeding</th>
<th>Mid breeding</th>
<th>Late breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total leukocytic count (10^3/mm^3)</strong></td>
<td>25.08±1.10</td>
<td>20.78±1.71</td>
<td>19.20±1.86</td>
<td>29.81±1.28**</td>
<td>32.15±2.52**</td>
<td>34.09±2.41**</td>
</tr>
<tr>
<td><strong>Lymphocyte %</strong></td>
<td>40.20±2.01</td>
<td>43.60±1.21</td>
<td>41.40±1.44</td>
<td>62.03±1.52**</td>
<td>69.09±1.14**</td>
<td>70.20±1.77**</td>
</tr>
<tr>
<td><strong>Monocyte %</strong></td>
<td>5.94±0.76</td>
<td>5.60±0.41</td>
<td>5.28±0.83</td>
<td>3.40±0.51*</td>
<td>2.80±0.37**</td>
<td>3.60±0.51</td>
</tr>
<tr>
<td><strong>Neutrophil %</strong></td>
<td>44.20±0.74</td>
<td>41.42±0.93</td>
<td>41.23±1.10</td>
<td>36.60±2.06**</td>
<td>33.20±2.20**</td>
<td>37.40±1.29</td>
</tr>
<tr>
<td><strong>Eosinophil %</strong></td>
<td>3.45±0.14</td>
<td>2.63±0.24</td>
<td>3.51±0.19</td>
<td>6.62±0.51A**</td>
<td>7.80±0.37b**</td>
<td>4.33±1.29 Ab</td>
</tr>
</tbody>
</table>

*and ** values in Trypanosoma infected group are significantly different than their corresponding in the non-infected group at P<0.05 and P<0.01 respectively.

In the same row values with the same small or capital letters are significantly different at P<0.05 and P<0.01 respectively.

### Biochemical studies:

The mean (±SE) values of serum biochemical parameters of *T. evansi* sub-clinically infected dromedary camels and non–infected camels are presented in Table.4. It was found that total protein significantly decreased (P<0.05) during mid breeding in infected than non-infected camels. Albumin concentration of *T. evansi* infected camels was significantly lower (P<0.01) in mid and late breeding than their corresponding in the non-infected groups. However, globulin level was significantly higher (P<0.05) in infected camels than non-infected camels in initial breeding period. While A/G ratio showed significant increase in infected camels than non-infected (P<0.01) in all breeding periods. Results of lipid profile showed significant increase in *T. evansi* infected camels in both triglyceride and total cholesterol levels than non-infected (P<0.05) in mid and late breeding periods.

### Table.4. Serum biochemical parameters of *T. evansi* sub-clinically infected and non–infected dromedary camels (Mean±SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial breeding</th>
<th>Mid breeding</th>
<th>Late breeding</th>
<th>Initial breeding</th>
<th>Mid breeding</th>
<th>Late breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Protein (gm%)</strong></td>
<td>6.71±0.56</td>
<td>7.39±0.49</td>
<td>6.92±0.50</td>
<td>5.38±0.48</td>
<td>5.59±0.47*</td>
<td>4.94±0.46</td>
</tr>
<tr>
<td><strong>Albumin (gm%)</strong></td>
<td>3.74±0.26</td>
<td>3.49±0.26</td>
<td>3.78±0.28</td>
<td>3.17±0.12 ab</td>
<td>2.15±0.22 a**</td>
<td>2.58±0.19 b**</td>
</tr>
<tr>
<td><strong>Globulin (gm %)</strong></td>
<td>3.01±0.25</td>
<td>3.30±0.21</td>
<td>3.54±0.27</td>
<td>3.87±0.19*</td>
<td>2.95±0.34</td>
<td>3.16±0.42</td>
</tr>
<tr>
<td><strong>Albumin globulin ratio</strong></td>
<td>1.07±0.01</td>
<td>1.06±0.02</td>
<td>1.11±0.01</td>
<td>1.68±0.14 a**</td>
<td>1.57±0.07 A**</td>
<td>1.26±0.03 A**</td>
</tr>
<tr>
<td><strong>Triglyceride (mg %)</strong></td>
<td>45.71±2.28</td>
<td>51.95±3.44</td>
<td>50.48±1.45</td>
<td>40.76±1.77 AB</td>
<td>61.67±1.96 A**</td>
<td>67.96±3.32 B**</td>
</tr>
<tr>
<td><strong>Cholesterol (mg %)</strong></td>
<td>52.66±8.45</td>
<td>52.61±2.73</td>
<td>44.50±4.11</td>
<td>53.32±1.25 A</td>
<td>63.98±1.49 A**</td>
<td>59.79±3.05 A**</td>
</tr>
<tr>
<td><strong>ALT/GPT (U/L)</strong></td>
<td>26.69±0.82</td>
<td>24.74±1.55</td>
<td>27.96±0.48</td>
<td>27.64±2.73 a</td>
<td>41.09±4.40 A**</td>
<td>35.12±3.40</td>
</tr>
<tr>
<td><strong>AST/GOT (U/L)</strong></td>
<td>118.44±4.25</td>
<td>129.83±3.58</td>
<td>161.84±5.63</td>
<td>156.14±10.87 A**</td>
<td>202.72±6.66 A**</td>
<td>179.95±11.30</td>
</tr>
<tr>
<td><strong>Creatinine kinase (U/L)</strong></td>
<td>112.71±3.37</td>
<td>102.34±6.63</td>
<td>130.29±3.45</td>
<td>292.63±38.36 **</td>
<td>263.04±20.61 **</td>
<td>210.37±19.28 **</td>
</tr>
<tr>
<td><strong>Bilirubin (mg/dl)</strong></td>
<td>0.45±0.09</td>
<td>0.56±0.09</td>
<td>0.51±0.12</td>
<td>2.15±0.30 AB</td>
<td>0.80±0.08 A**</td>
<td>0.98±0.06 B**</td>
</tr>
<tr>
<td><strong>ALP (U %)</strong></td>
<td>31.48±4.69</td>
<td>37.10±1.81</td>
<td>52.61±2.45</td>
<td>20.71±1.76</td>
<td>16.84±2.25 **</td>
<td>23.49±2.29 **</td>
</tr>
</tbody>
</table>
DISCUSSION

In this study, 26 (13%) were presumed to be positive for *T. evansi* flagellated protozoan parasite by microscopic examination. However, 27 (15.5%) were found to be sub-clinically infected by CATT test. These results might indicate that antibodies to *T. evansi* could persist even after the elimination of the parasite from the circulation (Thesen, 2015). Most seropositive sera were observed at grade 1 (+) (81.5%; 22/27) which might be due to the differences in the immune system between the examined camels, weakness of immune reaction due to serological latency which usually accompanies the chronic phase of infection (Wernery, 2001), host susceptibility to infection (Herrera, 2004). The total number of positive trypanosomiasis according to microscopically and serologically examined animals was 53 that represented 26.5% (P<0.05). However, this percent was considered high as recorded previously (Hussain, 2016). Camel surra disease is one of the most important diseases in camels which causes many economic losses. Anemia and leukocytosis are common features of trypanosomiasis in camels (Hussain, 2016).

The lower haematological values of RBCs, Hb concentration, PCV% were considered as indication for anemia as reported previously in the study of Padmaja (2012) and Eyob and Matios (2013). The lower PCV % and RBCs count values in the sub-clinically infected camels in this study, were consisted with previous results of Argungu (2014) and Hussain (2016) using camels infected with *T. evansi* in Sokoto and Pakistan respectively. Reduction in MCV values considered as indicator for anemia as reported previously in the study of Padmaja (2012) and Eyob and Matios (2013). This finding was similar to the results recorded in different areas by Eyob and Matios (2013) and Hussain (2016). This increase in TLC was in agreement with previous work of Argungu (2014) that attributed the increase to the elevation of cell mediated response to fight the infection.

It was observed that monocyte % and neutrophil % were significantly declined while, acidophil % increased (P<0.01) in infected camels in initial and mid breeding season compared to non-infected camels. Eosinophilia could be also occurred as a result of specific parasitic disease and allergic reaction (Al-Ani, 2004). As well as, several changes in metabolic system of the host might be changed by trypanosome infection and these changes associated with the severity of parasitaemia specially, in initial phase of the disease and beside tissues and organs injury (Kwem, 2000). The physiological responses of animals were also affected by the seasonal changes specially in the thermal environment. Generally, the haematological profile could be considered as one of the important indicators of determining the physiological changes and the adaptation of animals to the environment (Kumar and Pachaura, 2000). In recent years, a considerable number of researches has been carried out the haematological and biochemical parameters of camels but few papers study the effect breeding season change and its effect on the haematological and biochemical values in camels (Salem, 2012). This study revealed the changes during the initial, mid and late breeding season periods in *T. evansi* infected camels. A significant reduction in Hb concentration was observed in late breeding period. However, CI significantly higher in late breeding than mid breeding period. As well as, significant increase...
was seen in lymphocyte values during mid and late breeding periods than in initial period of breeding season. Whereas, value of acidophil% was significantly higher in initial and mid breeding periods than during late breeding. This results come in agree with the findings of El-Bahrawy and El-Hassanein (2011) and Salem (2012) who reported that Hb value decreased while TLC and lymphocytes values increased during rutting (winter season) than the pre-rut season and post-rut (late spring).

Determination of the functional status of various organs was investigated by serum biochemical analysis. In this study, the mean values of serum total protein and albumin were significantly lower in *T. evansi* sub-clinically infected dromedary camels. However, globulin level and A/G ratio of infected camels were significantly higher. These results have been recorded previously by Hussain, (2014) and could be due to hepatic degeneration and damages accompanying with hypoxia (Enwezor and Sacky, 2005) or might be due to parasite antigen (Azza, 2008). The decline in total serum protein and albumin beside the increase in globulin might be due to stresses and also could be explained as a response to the parasitic antigen (Hilali, 2006). As well as, this hyperglobulinemia might be due to the stimulation of the immune system and production of immunoglobulins against infection (Singh and Jayprakasan, 2001). Results of lipid profile in this study showed significant increase in both of triglyceride and total cholesterol in *T. evansi* infected camels comparing to non-infected camels in mid and late breeding. Hypertriglyceridemia and hypercholesterolemia were observed previously in several animals infected with various species of trypanosomes such as in *T. brucei* infection in rats (Igokwe, 2009) and *T. evansi* in camels (Alireza, 2011). It was reported that infection with *T. evansi* in camel developed lipid metabolism abnormalities (Savino, 2003). Development of hypertriglyceridemia could be due to disturbed plasma triglyceride degradation that possibly cause free fatty acid unavailable for importation into hepatocytes (Alireza, 2011). In this study, a significant elevation of different hepatic enzymes activity such as ALT during mid breeding season and AST in initial and mid breeding periods were also recorded in *T. evansi* infected camels. However, ALP activity was significantly decreased in infected camels. Our findings were in accordance with the study of Seleim, (2003). This elevation in ALT and AST activities might be attributed to hepatic toxicosis (Seleim, 2003). As well as, Kwem, (2000) reported that AST elevated activity in trypanosoma experimentally infected cattle could be because the parasites secreted it as a part of their metabolites into blood circulation, as ALT and AST have been detected in homogenates and suspensions of trypanosome. This elevation in AST and ALT activity could be also attributed to the cellular damage caused by the trypanosomes lysis or host destruction of trypanosomes respectively (Enwezor and Sacky, 2005). Creatinine kinase and total bilirubin concentrations were significantly higher in infected camels compared to non-infected camels in the different breeding periods. Kidney affection also reported by Azza (2008) and manifested by increase of creatinine kinase and these may be attributed to acidosis or primary renal insufficient caused by decreased renal blood flow (Seleim, 2003).

Concerning the impact of the breeding periods on the changes of serum biochemical parameters in trypanosoma infected camels, it was seen that albumin recoded significant increase in initial breeding than mid and late breeding while A/G ratio showed significant decrease in late breeding. Similar results were recorded previously by (Ahmadi-hamedani, 2014) where albumin and globulin concentrations were higher during hot-humid months than hot-dry months or breeding season. These results suggested the camels have a great ability to adapt to heat stress. On the other hand, Amin, (2007) showed that the serum level of globulin increased significantly during the dry season than in the green season of one-humped camels. In addition, triglycerides level increased in mid and late breeding than that in initial while cholesterol showed significant increase in mid breeding period than in initial breeding period. These results are in agreement with those reported by Salem (2012) as cholesterol values increased during rutting (winter season) than the pre-rut season and post-rut (late spring) (Salem, 2012). In addition, it was found that the cholesterol concentration of the dromedary camels during breeding was significantly higher than non-breeding season in the hot-dry or hot-humid months. This seasonal variation in serum cholesterol concentration may be attributed to the type of feed and environmental temperature during different seasons. During breeding season (winter) the green fodder was berseem since berseem is a rich source of steroids Zeidan, (2008). Serum ALT and AST enzymes activities of the male dromedary camels were higher during mid breeding season than initial breeding whereas total bilirubin level was significantly higher in initial breeding than both mid and late breeding seasons. Exposure to high heat load may result in oxidative stress and cellular damage, and consequently may lead to hepatic dysfunction when coupled with dehydration. This may lead to elevation of ALT and AST activities (Gaughan, 2011).

4. CONCLUSION

Results of the present study revealed unexpected high prevalence of subclinical trypanosomiasis (26.5%) in male camels associated with breeding season. Our study supports the using of antibody detection test rather than microscopical detection of flagellated protozoon. Finally, the present study concluded that hematology and biochemical parameters related to liver, kidney, hemapoiesis and immune status can be altered by subclinical infection with *T. evansi* in camels mainly during late period of breeding season. It is clearly that further studies are
needed in order to investigate the impact and pathogenesis in camel subclinical trypanosomiasis on a wide range in Egypt.

REFERENCES


