ABSTRACT
The present study was designed to compare the molecular diagnosis and light microscopy for detection of *Trypanosoma evansi* in camels in the AL-Najaf province. Blood samples of 100 camels were collected during the period from October 2016 to April 2017. Results revealed that PCR using *T. evansi* specific primers was the highest test of *T. evansi* diagnosis (90%) followed by the light microscopy (28%). Amplification of DNA from animals infected with *T. evansi* allows the diagnosis of parasite levels far lower than detectable by microscopic microscopy diagnosis.

KEYWORDS: Microscopic examination, Molecular diagnosis, *Trypanosoma evansi*, Camels, Iraq.

INTRODUCTION
Camel Trypanosomosis (surra), caused by the protozoan parasite *Trypanosoma evansi* (Eyob and Matios, 2013). The *Trypanosoma evansi* infects different hosts species and transmitted mechanically by biting flies (Tabanidae and Stomoxys species) also by Vampire bats (Desquesnes et al., 2013). Surra disease that causes considerable economic losses due to weight loss, weakness, abortion, estrus cycle disorders, reduced productivity and death (Reid, 2002; Sazmand et al., 2011). Currently, laboratory diagnosis of Trypanosomasis is usually performed by direct parasitological examination and/orserological methods, which are time consuming and may lack accuracy (Ashour et al., 2013). The polymerase chain reaction (PCR) is nowadays a simple and valid molecular test to diagnosis the parasite species, strains and genotypes (Elhaig et al., 2013, Barghashet al., 2014). The aim of this study is identify *T. evansi* infection in camels by direct microscopy examination and molecular assay.

MATERIALS & METHODS
One hundred venous blood samples were collected from camels during the period from October 2016 to April 2017 in AL-Najaf province. Five ml of blood sample were collected from each animal and kept in anticoagulant ethylene diamine -tetra acetic acid (EDTA) tubes. Samples were transferred in cooling box to the laboratory to conduct the necessary tests to determine the infection with *T.evansi*.

**Microscopic examination**
Blood films were prepared from each sample, stained by Giemsa and subjected to light microscopic examination to investigate the parasite (Beaver and Jung, 1985).

**DNA isolation and PCR assay**
DNA was extracted from blood samples using the whole blood DNA extractions as described by Sazmand et al. (2016).

**Primers**
Two primers were used for detection of the *T. evansi* in this study (Table 1).

<table>
<thead>
<tr>
<th>Primers</th>
<th>Primer sequence (5 to 3 )</th>
<th>Product size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RoTat 1.2</td>
<td>F GCG GGG TGT TTA AAG CAA TA</td>
<td>205</td>
<td>(CLAES et al.,2004)</td>
</tr>
<tr>
<td></td>
<td>R ATT AGT GCT GCG TGT GTT CG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F GAA TAT TAA ACA ATG CGC AG</td>
<td>164</td>
<td>(Muieed et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>R CCA TTTATT AGC TTT GTT GC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS
The percentage of infection was showed that among (100) *T. evansi* suspected cases were examined microscopically 28%, whereas 90 % by PCR (Table 2), (Figure 1, 2).
TABLE 2: Showed the examined camels and positive cases.

<table>
<thead>
<tr>
<th>Diagnostic methods</th>
<th>Total number</th>
<th>positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic examination</td>
<td>100</td>
<td>28</td>
<td>28%</td>
</tr>
<tr>
<td>PCR examination</td>
<td>100</td>
<td>90</td>
<td>90%</td>
</tr>
</tbody>
</table>

Chi-Square --- --- 13.562 **

** (P<0.01).

FIGURE 1: Gel electrophoresis of PCR product of Rotat (205bp), for T.evansi using 2% agarose gel. Lane 1-6: PCR product positive for Rotat genes.

FIGURE 2: Gel electrophoresis of PCR product of TBR (164bp), for T. evansi using 2% agarose gel. Lane 1-5: PCR product positive for TBR genes.
DISCUSSION

Molecular methods for diagnosis of *T. evansi*, as more sensitive and specific diagnostic tools, have been increasing used to detect *T. evansi* in carrier animals. It is recognized even relative amount of DAN of the parasite (Clausen *et al.*, 2003; Masiga *et al.*, 2001). There was no comprehensive data on the use of PCR for diagnosis of infection in Iraqi breed of dromedary Camels. Parasitological prevalence, assessed by Giemsa stained thin smear was much lower (28%) than molecular prevalence (90%). The result of this study agreed with some studies, Clausen *et al.* (1998) who showed that the detection rate of PCR was about x 2 higher compared with (HAT) and (MAECT) for diagnosis of *Trypanosoma* infections in cattle and also Ravindran *et al.* (2008) found a higher prevalence of *T. evansi* in dogs (7.7%), camels (34.4%), and in donkeys (6.8%) during PCR than blood film examination. In Kenya Njiru *et al.* (2004) reported the PCR the considered the most sensitive technique than other methods to detect trypanosomiasis. Aslam *et al.* (2010) reported that PCR test be a superior assay over ELISA and MHCT for the diagnosis of trypanosomiasis in horses. During this study, number of samples was negative by light microscopic but were found positive when using PCR. The ability of this tool are very low false-positive and insensitive to other blood parasite species (Sazmand *et al.*, 2016).

CONCLUSION

There is a significant difference in the incidence rate when using different diagnostic tools. The molecular assay for detection of Trypanosomiasis is a best test (sensitive and specific). This test is suited for detect of Trypanosomiasis in both mammalian blood and insect vectors and beneficial for epidemiological survey.

REFERENCES


