MOLECULAR DIAGNOSIS OF THEILERIA SPP. AND BABESIA SPP. INFECTIONS IN CAMELUS DROMEDARIUS IN AL-NAJAF AL-ASHRAF PROVINCE, IRAQ

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Abstract

The present study was goal to confirm the presence of piroplasmosis in venous blood of Camelus dromedaries camel from the slaughtered at the main slaughterhouse of Al-Najaf abattoir at AL-Najaf Al-Ashraf province using microscopically (blood film) and molecular technique (PCR). A total of 94 male camels were blood collected for microscopic examination and PCR assay to detect the most common of piroplasma parasites which include Thereria spp. and Babesia spp. the study was beginning from October 2019 till the end of March 2020. Clinical signs and symptoms were recorded in camels, the prevalence rates using microscopic examination and molecular assay were 8.5 % and 18 %, respectively. The state of being prevalent rates was varied significantly between the clinical signs (P<0.05) and their causing agents “Theileriosis and Babesiosis”, except for one clinical sign oculo-nasal discharge, no significant differences were reported (P> 0.05). In conclusion: Polymerase chain reaction test was showed a higher sensitivity than microscopic examination, and a significant association of some clinical signs with piroplasma infection in dromedary camels in Iraq.

Key words: Theileriosis and Babesiosis, Camelus dromedaries, PCR

Introduction

Babesia are intraerythrocytic organisms of domestic animals and are the cause of anaemia and abnormal high concentrations of hemoglobin urine haemoglobinuria (Zintl et al., 2003). They are transported by ticks in which the protozoa pass through the egg, from one generation to another. The disease, babesiosis, is particularly severe in naive animals that are introduced to endemic areas and is a major constraint on Livestock farming in various countries in the world (Irwin, 2009). The most widespread and pathogenic species in camels is the large Babesia canis found in mainland Europe, Africa, Asia and the Americas. Rhipicephalus sanguineus is the principal vector, in which transovarian and transtadial transmission occurs.

The diseases caused by many species of theleria are a serious impediment to livestock development in Asia, Africa and the Middle East. Tick-borne parasites undergo frequent schizophrenia, eventually leading to the release of small mirozoite cells that invade red cells into piroplasms (Morrison et al., 2015). Theleria spp. are widely distributed in camels in several very large landmasses e.g. Europe, Asia and Australia have a diverse range of tick carriers and are associated with a parasite agent that ranges from mild clinical cases to deadly cases (Qablan et al., 2012). Very few studies about piroplasma parasite infections on camels have recently been found in the one-humped camel such as Egypt (Abd-Elmaleck et al., 2014) and Iran (Branch et al., 2015).

Serious economic losses causing by Theileriosis and Babesiosis still remained to be a most significant threats to camel livestock industry in Iraq, where ticks and flies played a large role in the spread of the disease, the emergence of Camel thelariais and babesiosis poses a serious health effect to animal in highly endemic areas, this parasites can causes a zoonotic disease known as Theileriasis and Babesiosis, Polymerase chain reaction assay have proven to be a very specific and detectible for detection of Thelaria and Babesia in the venous blood of Camelus dromedarius, one-humped camel. The goal of the present study was planned to confirm the
presence of *Theileria* spp. and *Babesia* spp in camels from the slaughtered at the main slaughterhouse of AlNajaf abattoirat AL-NajafAL-Ashraf province using microscopically (blood film) and molecular technique (PCR).

**Materials and Methods**

**Collection of camel blood samples**

A ninety four blood samples were collected from venous blood from the jugular vein of Camel in slaughter house in al Al-Najaf governorate by tubes containing anticoagulant agents, the samples including different ages 16 sample were recorded as <4 years old and 78 samples were recorded as >4 years old.

Animals of slaughter house (n = 94) has recently noticed clinical examination of piroplasms infestations and a history of tick infestation, some of them typically have abnormal mucous membrane color, anorexia, increased respiratory rate tachypnea, discolored urine, as well as pertaining of eyes and nose oculo-nasal discharge. Some rare cases showed superficial lymph nodes enlargement; while the other apparently have healthy upon clinical examination, all samples collection was obtained from males of Camel at period beginning from October 2019 to the end of March 2020, then the samples directly transport in ice box into molecular laboratory college of veterinary medicine university of Kufa to performing direct smear examination and molecular assays.

**Microscopic Examination**

The blood smears were performed through fixed it in 99% methanol for five minutes and quickly stained with Giemsa stain 10% solution (RXMarine International, India) for thirty minutes and directly examination under microscopic apparatus by oil immersion at 100X magnification power.

**Blood DNA Extraction**

Genomic DNA from blood samples were extracted by using High Pure PCR Template Preparation Kit and done according to company instructions, the Blood and tissue cells were lysed special Lysis Buffer and Proteinase K in short incubation with the presence of a chaotropic salt such as guanidine-HCl. The DNA extraction was read using Thermo Scientific NanoDrop spectrophotometers, the ratio at 260/280nm absorbance was used to assess the purity of nucleic acids, and then kept at deep freezer for molecular assay.

**Polymerase chain reaction (PCR)**

Polymerase chain reaction (PCR) amplification was performed with two sets of oligonuclotied sequences primers table 1 which used to detect *Theileria* spp. and *Babesia* sp. protozoa with two amplicon size 694 bp and 383 bp, respectively. These primers were originate from conserved regions of 18S rRNA genes of both parasites in blood of camel, the method was accomplished according to procedure (Shayan and Rahbari, 2005), the oligonuclootid sequences primers in this study were designed in by using Genbank data base NCBI , primers sequences were obtained from company (Macrogen company, Korea) as table 1.

**PCR master mix preparation**

AccuPower PCR PreMix Kit PCR total volume master mix was prepared according to company instructions as following mix of 20 micoliter volume: 5µl of NA target, 1.5 µl for 10 picomoles forward primer, 1.5 µl for 10 picomoles reverse primer,12 µl of nuclease free water.

Then, all the PCR tubes centerfuged into vortex centrifuge for 2 minutes. Then transferred into thermocycler (MJ-Mini BioRad. USA), the PCR Thermocycler Condition was recorded as 95°C initial Denaturation for 5 minutes, followed by 30 cycles (95°C for 30 seconds, 58°C for 30 seconds and 72°C for 1 minute) and finally, the extension step was recorded at 72°C for 5 minutes. PCR amplicons (694 bp and 383 bp) were examined by agarose 1.5% gel electrophoresis, stained with fluorescent tag “Ethidium bromide” nucleic acid stain and the results were showed by UV transilluminator.

**Results and Discussion**

In this study, microscopic and molecular screening techniques were used to test the blood of Arabian dromedary camels (*Camelus dromedarius*) for protozoan parasites which causes (Theileriosis and Babesiosis). The taxonomic status of these species remains unclear due to a lack of molecular characterization and experimental infection (Sazmand *et al.*, 2016), therefore, our results considered that the study be exclusively at the species level, not the genus of parasites. In current study, Housekeepinggene-diagnosed primers were used to identify 18rRNA of these parasites in camel blood, these parasites were detected in blood of camels by using both

| Table 1:                                                                 |
|-----------------|-----------------|-----------------|
| **PCR product** | **Sequence**     | **oligonuclotied** |
| 694bp           | ATTGGAGGGCAAGTCTGGTG  | 18SrRNA gene    |
|                 | TGCACCACCCAAAAGAAT  | *Theileria* spp. |
| 383bp           | AGGAAATGGAGGGAAAGGCAC | 18SrRNA gene    |
|                 | CTAGTGATCCGGGCCCTT  | *Babesia* sp.   |
Molecular Diagnosis of *Theileria* spp. and *Babesia* spp. infections in *Camelus dromedarius*. Modified Giemsa stain of thin blood smears showed different morphological and developmental features piroplasms in red blood cell by using light microscope, some of samples were showed comma and signet-ring with large vacuoles ranged from 0.5 to 1.5 µm in diameter shaped as intra-cytoplasmic bodied which was seems to be *Theileria*, some of them have tetrad form, pairs or ameboid aligned shape which was seems to be *Babesia* spp. (Abd-Elmalek et al., 2016), PCR Reaction was used to examine piroplasma infection in camel that was slaughtered after DNA was extracted from its blood, the result showed a 694 base pair size of PCR product belongs to *Theileria* spp. Fig. 1 and a 383bp PCR product size belongs to *Babesia* spp. Fig. 2.

PCR assay was found more prevalence to detect both *Babesia* spp. and *Theileria* spp. infestation in camel rather than Microscopic examination Fig. 3. Noaman, 2014 was found among 52 samples, microscopic examination have 100% and 70.83% specificity and sensitivity and rather this ratio was different from PCR assay. The high prevalence of parasites disease observed in the current study may be attributed to the high availability of tick carriers, because the hot and humid environment is very favorable for ticks and ultimately for the survival of the Piroplasma (Chauhan et al., 2015). (Ranjbar, & Afshari, 2009) was showed a prevalence rates in Iranian camel have been reported as 19.47% of *Theileria* spp. In Iran.

Table 2 showed the result of most common of piroplasma infection in age more than 4 years old 62.2% with 95% confidence interval (35.4 to 84.4), it is different from other age group less than 4 years old have percentage 8.9% and 95% confidence interval as (3.6 to 17.6), this differences was significant (P<0.05) increase in age more than 4 years, the results was agreement with (Abaker et al., 2017) who was found the highest prevalence rate was 18.6% among cattle from 1 to 3 years, and the lowest prevalence was 0% among cattle.
under one year old. On the other hand, the most common cases of parasite infestation were recorded in animals have ticker servoir, one of the most studies have illustrated that protozoan parasites; *Theileria* spp. and *Babesia* spp. were mainly vertical transmitted by reservoir ticks and phylogenetically related to zoonotic species (Iori et al., 2010). Piroplasma spp. can be able to infect RBCs and/or WBCs of camels (Hamed et al., 2011), in general; *Theileria* spp. and *Babesia* spp. might mature in unattached ticks and can enter the host right after tick attaches (Iori et al., 2010).

χ² Chisquare test, DF: Degree of freedom, CI: confidence intervals

The study proved that there are cases occurring in camels who suffer from discolored urine, two thirds of the total number (10/15) of camels suffered from hematuria, the prevalence of parasite infestation 66.6% with confidence interval 38.3 to 88.1%. This significant difference (P< 0.05) with affected camels without hematuria 8.8% is due to piroplasma can cause hemolytic anemia, this type of anemia can lead to jaundice yellow-colored skin of camels and red/coffee-colored urine, this case was clarified by (Saini, & Sankhala, 2015) when he studied the relationship of disease with discolored red urine in cattle. The current study showed that there is no significant relationship (P> 0.05) between camels that have oculo-nasal discharge with other camels that do not have oculo-nasal discharge, this study was disagreement with (El-Ashker et al., 2015) who found significant clinical signs e.g. oculo-nasal discharge & hemoglobinuria and the detection of their causing agents Babesiosis and Theileriosis in cattle. Superficial lymph node is characterized by their dromedary lobule size and appearance our study was found that lymph nodes were elongated, ovoid, flattened, rounded or triangular in some cases, this study was adopted by the (Gavrylin et al., 2014) in measuring the enlarged lymph nodes in the camel. A total of 24 dromedary camels that have been slaughtered, 13 of them (54.1%) have lymph nodes enlargement with confidence interval ranged (32.8 to 74.4%). In conclusion our result found significant association of some clinical signs with piroplasma infection in dromedary camels in Iraq, also the molecular assay is more reliable than microscopic examination for detection of these parasites.

References


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