Morphological and molecular identification of Parabronema skrjabini of camels (Camelus dromedary) in Najaf province

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Abstract

The current study was conducted during the period from September 2019 to December 2019, the number of examined samples 150 abomasums isolated from males 127 and females 23 to identify the species of Parabronema skrjabini that infected one-humped camel slaughtered in Al-Ashraf Najaf slaughterhouse. The microscopic examination of the worms was properties compared to other worms of the digestive system, and then confirmed using genetic markers with the polymerase chain reaction (PCR). Finally, the use of sequencing and phylogenetic analysis technologies relative to those that are predominant in world regions registered in the Gene bank. The results of the microscopic examination showed that Parabronema skrjabini distinguished by a red color, females are curved dorsally and longer than males with a vertically curved and head features that resemble a horseshoe for both sexes. The number of infected samples is 53 (35.33%) and the non-infected samples 97 (64.64%). The highest rate of infection during December month 63.41%. On the other hand, the prevalence rate has been reported 65.21% females and 29.92% males with significant differences. In this research, PCR technique was used the molecular examination with the selection of the highest DNA samples, which were 10 samples to determine the alignment range according to the ITS2 gene, all samples were well suited to primer in length 783 bp and confirmed the diagnosis of these nematodes.

Keywords: Camelus dromedary Parabronema skrjabini Najaf Iraq

Introduction

Nematodes (roundworms) have cylindrical body (unsegmented) variable in color and size, the length from millimeters to centimeters and usually female longer than male (1). Parabronema skrjabini: one of the spirurida nematodes that infect abomasum of domesticated ruminants such as camel, cattle, sheep and goats. Baylis in 1921 was identified a genus Parabronema, first time described from Russia in 1924 by Rosawaska, this nematode was spread in different areas of the world as Mediterranean, Asia, central and east Africa (2). Most abomasal nematodes are caused diarrhea and lack of absorption, weakness, low weight and histopathological changes in abomasum mucosa lead to reducing the production or working efficiency of the infected animal and in case of blood-feeding nematodes may anemia and edema due to escape of plasma protein (3,4). Parabronema skrjabini is known with an indirect biological cycle, which require an intermediate host (flies) such as Stomox y bengalensis, Paregle spp. and Lyperosia spp (5), after 1-2 days fly's egg are begin to hatching and feed on the contaminated dung. Finally, parasite's is penetrating the intestine wall of the flies by the anterior lancet (6-8). Ali et al. (9) diagnosed from camel's abomasum or form other host: goat. In small ruminants, Parabronema skrjabini is isolated from sheep in Al-Kut and recorded high prevalence rate of gastrointestinal nematodes during December 100%, however less in March 44.47% (10). Parasite is firstly isolated from goats at Turkly (11). Yagoob et al. (12) recorded 4.3% in sheep at baneh town/India. Most researchers in their studies were depended on microscopic examination of faeces and adult worms (4). Recent research was used molecular
techniques to clarify the genotype of *Parabronema skrjabini* in many host (7-9). Different factors is affecting on the prevalence rate of abomasal worms (age, sex, climate and immunity status), the results of the study in Al-Kut, raq by Al-Dahar and Al-Amery (10) observed high prevalence of gastrointestinal nematode in sheep, females 75.37% males 66.5%, because the adult sheep provided with high ability to resistance nematode's invasion compared to the young ages due to development of immunity response, on other hand the seasonal fluctuation associated with the activation of the intermediate host (6). Epidemiological studies of abomasal nematodes are documented an annual temperature during year, which play an important role of larval development or arrested (9,11,12).

Arabian Camels (*Camelus dromedaries*) are important livestock animal live in harsh environments (13), the number of camels according to FAO, 2003 is around 12.5 million camels 70% of the world population (14). It's an increased the economic importance of camels with utilization of their products such as milk, wool, meat and skin (3,15,16). Few studies have focused on intestinal worms in single-humped camels due to the difficulty of sampling and increased nomadic migration to remote areas (17). Al-Taif (18) was first study of one-humped camel's infection in Iraq. Few information is their prompted us to identify this nematode by morphologically distinguishing and sequence of its genotype.

**Materials and methods**

**The samples collection**

A total of 150 abomasum were directly collected from camel carcasses after slaughtering in Al-Najaf slaughterhouse. Data of animals were collected for the purpose of determining risk factors like sex, age, and time period. Transmission of samples in a cold box to the parasitology laboratory in the Veterinary Medicine College, Al-Qadisiyah University, the worms were isolated by forceps to diagnose the species of abomasal nematodes and depending on the diagnostic characteristics described by Soulsby (1). The worms placed on a glass slide and the clearing by a few drops of lacto phenol, and then soft pressure was applied to allow the body to lie flat without damage.

**Microscopically**

Macroscopical examination by using a light microscope to distinguishing the target nematode according to Ali *et al.* (4). The measurements of *Prabronema* were taken for morphological diagnosis (1).

**Extracted rDNA-ITS2 of Parabronema skrjabini**

The PCR technique and the extraction of rDNA depend on the package (gSYAN DNA Extraction / Gene aid / USA) was designed as a diagnostic marker for the presence of the ITS2 gene (17). Primer pair were Para-Ir-F 5'- GTA GGT GAA CCT GCG GAAGG -3 and Para-Ir-R 5'- CTGAGC TGA GGT CAA CGA AT-3. In an automatic thermocycler, the overall volume of the PCR mixture was 100μL, containing 1 standard buffer, 100 mMol MgCl₂, 100 μM dNTP mix (AccuPowerTM PCR PreMix/Korea), 20 pmol of each primer, 1U Tag DNA polymerase (Cinaclone) and 1 μl of DNA template (100 ng DNA). The PCR pathway was used the following protocol: first step 5 min incubation at 94°C to denature the double stranded DNA, 33 cycles at 94°C (denaturing), second step 45s at 59°C (annealing), third step 45s at 72°C (extension). Ultimately, the PCR was done at 72°C with 5 min extension stage. Agarose gel (1.5%) prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C, then 3μl of ethidium bromide stain were added into agarose gel solution. Agarose gel is poured into the tray after placing the comb in appropriate place after which, left to solidify at room temperature within 15 minutes, then removed the comb gently from the tray and inserted wells 10μl of PCR product and 5ul of (100bp Ladder) into one well. The gel tray was put in the electrophoresis chamber and filled in by 1X TBE buffer, the electric field was conducted for 1 hour at 100 volts and 80 gpm. Using UV Trans illuminator detection of positive bands.

**Statistics analysis**

The findings of this review have been analyzed using SPSS application to evaluate statistical differences with the Chi-square test (χ²) and the P Values P≤ 0.05 (19).

**Results**

**Microscopically**

The parasite is firmly embedding in the mucous layer of the camel's abomasa. Their mouth is provided with a pair of lateral pseudolabia and indicated in figures 1-4.
Males

Body 26-30 mm in long, 104-119 μm in wide. Buccal cavity 90-110 (100) μm deep. The anterior section of the esophagus is 140-150 (145) μm and the posterior section is 1.52-1.70 (1.61) mm in long. No excretory pore has been observed, Tail is coiled ventrally, with some lateral alae at the posterior extremity. Spicules are distinctly unequal.

Females

Body 35 mm in long and 192 μm in wide, unobserved pore excretory. Tail is pointed or blunt and distinctively curved on the dorsal side. Eggs with elongated form, anal opening 155 μm from the posterior end.

Infection rate of Parabronema skrjabini

The results showed that among 150 camels, 53 positives for parasites with total infection rate 35.33%. The highest rate in December were 63.41% while the lowest in September were 10 (18.42%). Statistical analysis found that there were significant differences during study period of infection rates which showed in table 1.

<table>
<thead>
<tr>
<th>Months</th>
<th>Exam No</th>
<th>Positive No</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>38</td>
<td>7a</td>
<td>18.42%</td>
</tr>
<tr>
<td>October</td>
<td>28</td>
<td>9b</td>
<td>32.14%</td>
</tr>
<tr>
<td>November</td>
<td>43</td>
<td>11c</td>
<td>25.58%</td>
</tr>
<tr>
<td>December</td>
<td>41</td>
<td>26d</td>
<td>63.41%</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>53</td>
<td>35.33 %</td>
</tr>
</tbody>
</table>

The different letters show significant differences (P<0.05).

The highest percentage of infection observed in 5 years group of age as 52.17% and the lowest rates were observed in age ≤ 2 years 9.75%, with significant differences as shown in the table 2.

The results have shown that there are significant differences between males and females, females more affected by Parabronema skrjabini 65.21% than males 29.92% and referred into the table 3.

Molecular Examination

The ITS2-rDNA region of P. skrjabini, after amplifying and sequencing within ten samples, PCR amplicons were 783bp in the length, and showed in Figure 5. Lane (1-10) positive samples of P. Skrjabini isolates at 783bp.
The present study shows *Parabronema skrjabini* in camels in Al-Najaf slaughterhouse, during the four months of the current study, the total infection rate is 35.33% the higher than the survey of Ali et al., (4) for the same location was 25.56% during the months of March, followed by January and February, with an emphasis on their research period (nine-months), suggesting they attribute the reasons for increased intermediate host (flies) prevalence in this time and the coinciding with temperature changes. Liu et al. in (20) recorded 86% higher in the Bactrian camel. The maximum infection reaches 81% and identical to the higher finding in the current study is 63.41% during the month of December. Some studies have been interesting in seasonal changes and their effect on infection rates during the year, where the seasonal prevalence of gastrointestinal nematodes in the Mashhad slaughterhouse / Khorasan province (15) had increased in certain seasons (summer and spring). Yakhchali (21) showed the climatic conditions are played an important role in infection rates, such as the spring and autumn seasons, researchers in Turkey have reported peak rates of parasites (11). El-Azazy (22) reported increased infections of small ruminants between December and October. Conversely, Borji et al. (15) confirming with his study of camel and other researchers for infection of small ruminants in Iran no they’re indicating to a seasonal effect on the prevalence of nematode infection (23,24), Barghandan et al. (25) after testing for sheep in three areas, the prevalence rates were similar for different seasons.

The results in the current study shows that females infection is higher than males in view of the small number of females slaughtered during the study time due to veterinary procedures in the Najaf slaughterhouse and the prevalence rate is 56.52% females, 42.51% males and it was associated with the Iranian researchers of gastrointestinal camel nematodes with a *P. skrjabini* total infection rate of 34.7% and a higher female infection than male infection rate of 54% and 45% respectively (26). In small ruminants, the occurrence was 9.15% higher for females compared to 5.91 % males in sheep, and this difference in goats was not present at Basra city during *P. skrjabini* investigation (22). Hernández-Castellano et al. (27) assumed that the increase in ewes infection due to immune-modulatory responses was higher with parturition. Al-Dahar and Al-Amery (10) estimated the prevalence of *P. skrjabini* in sheep in December at a high rate of 100%, with the variation in the

### Table 2: Infection rate of *Parabronema skrjabini* according to age

<table>
<thead>
<tr>
<th>Months</th>
<th>Camel ≤ 2 year</th>
<th>2-5 year</th>
<th>Camel ≥ 5 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exam n</td>
<td>Infect n</td>
<td>Infect %</td>
</tr>
<tr>
<td>September</td>
<td>11</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>October</td>
<td>9</td>
<td>1a</td>
<td>11.11%</td>
</tr>
<tr>
<td>November</td>
<td>13</td>
<td>2</td>
<td>15.38%</td>
</tr>
<tr>
<td>December</td>
<td>8</td>
<td>1</td>
<td>12.5%</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>4</td>
<td>9.75%</td>
</tr>
</tbody>
</table>

The different letters show significant differences (P≤0.05).

### Table 3: Infection rate of *Parabronema skrjabini* according to sex

<table>
<thead>
<tr>
<th>Months</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exam No.</td>
<td>Infect No.</td>
</tr>
<tr>
<td>September</td>
<td>31</td>
<td>3</td>
</tr>
<tr>
<td>October</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>November</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td>December</td>
<td>33</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td>38</td>
</tr>
</tbody>
</table>

Significant differences at (P≤0.05).

Figure 5: Electrophoresis image showed PCR product analysis ITS2-rDNA for *P. Skrjabini* Isolates from abomasum samples of one humped camel. Marker ladder 1500-100bp. Lane (1-10) positive *P. Skrjabini* isolates at 783bp product size.

**Discussion**

The present study shows *Parabronema skrjabini* in camels in Al-Najaf slaughterhouse, during the four months of the current study, the total infection rate is 35.33% the higher than the survey of Ali et al., (4) for the same location was 25.56% during the months of March, followed by January and February, with an emphasis on their research period (nine-months), suggesting they attribute the reasons for increased intermediate host (flies) prevalence in this time and the coinciding with temperature changes. Liu et al. in (20) recorded 86% higher in the Bactrian camel. The maximum infection reaches 81% and identical to the higher finding in the current study is 63.41% during the month of December. Some studies have been interesting in seasonal changes and their effect on infection rates during the year, where the seasonal prevalence of gastrointestinal nematodes in the Mashhad slaughterhouse / Khorasan province (15) had increased in certain seasons (summer and spring). Yakhchali (21) showed the climatic conditions are played an important role in infection rates, such as the spring and autumn seasons, researchers in Turkey have reported peak rates of parasites (11). El-Azazy (22) reported increased infections of small ruminants between December and October. Conversely, Borji et al. (15) confirming with his study of camel and other researchers for infection of small ruminants in Iran no they’re indicating to a seasonal effect on the prevalence of nematode infection (23,24), Barghandan et al. (25) after testing for sheep in three areas, the prevalence rates were similar for different seasons.

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incidence of infection between females and males in the province of Al-Kut, as the female was more affected by prolactin and its suppression of immunity, leading to an increase in the newly acquired larval fixation and their developing inside the animal.

On the other hand, other studies were rejected the relationship to some parasitic prevalence factors, as in Iran Hamadan, researchers observed the parasite infection by up to 22%, but without affecting the sheep's gender (23), and this was identical to the results of other parasitologists during the study of abomasal nematodes in sheep at Banah town (12,25) no significant age, gender and season differences were observed. Al-Dahar and Al-Amery (10) suggested that infection rates in small ruminants have risen in young animals (1-2 years) due to repeated exposure to infection during grazing, this evidence was consistent with the other abomasal nematodes study in cows, which confirmed infections at 2-3 years of age (23).

The current study find that the seasonal fluctuations were influential and consistent with the results of the researchers looking for a disease in the camel (4,6) or small ruminant (9,11,28) by taking along with the difference in the number of samples and the period for the of their studies, which related to the spread of the intermediate host (flies). But, it did not agree with (15,25) and the difference in results according to sex and age are consistent with the results in camel (4,26) and small ruminant (10,28), where females were more susceptible to nematodes as a result of lack of the examined numbers or immunosuppression, unlike what was suggested (12,25). In the present study, the morphological results showed both male and female worms in infected camels, the length of the body was male 26-30 mm and female 35 mm, horse shoe- shields in the cephalic region. The length of spicules is distinctly unequal and thus their similarity to the morphological characteristics with another studies (4,7,22). The nuclear rDNA's first and/or ITS2 is suitable for diagnosis (29,30). Some of the reports of Strongylida, Ascaridida, Diofilaria, Dipetalonema and Thelazia (31-34) based on the gastrointestinal nematodes ITS regions. Molecular study of the genus Parabronema focuses mainly on the genes found in Genbank. The positive samples were similar to those reported were consistent with the molecular review of other hosts (7,22,35-38).

The current data ensure the importance of the molecular techniques with morphological character in differentiating between Parabronema skrjabini and another species of nematodes that were identified for their presence in the abomasum of camels.

Conclusion

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Conflict of interests

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