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# Microscopic examination of internal parasites in Iraqi camels (*Camelus dromedarius*) with molecular focus on *Trichostrongylus* spp.

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**Summary**

The camel has played a role in human civilization since its inception and holds significant importance in the customs and agricultural practices of various nations. This study examined the prevalence of internal parasitic infestations in camels within the Al-Diwaniyah and Al-Najaf provinces of Iraq from December 2021 to September 2022. A total of 200 fecal samples were randomly collected from farm camels, revealing that these animals were affected by one or more types of intestinal parasites. *Nematodes* exhibited the highest prevalence at 56 %, followed by *Protozoa* at 28.5 %, *Cestodes* at 14.5 %, and *Trematodes* at 1 %. Among these parasites, *Trichostrongylus spp*. had the highest percentage at 33 %, followed by *Moneizia benedeni* (12.5 %), *Fasciola hepatica* (10.5 %), *Strongyloides spp*. (8 %), *Giardia spp*. (7 %), *Nematodirus spp*. (6 %), and *Eimeria spp*. (6 %). Furthermore, mixed-species or single-species infections in camels were observed, including *Anoplcephala perfoliata* (4 %), *Haemonchus spp*. (3.5 %), *Dictyocaulus spp*. (3 %), *Trichuris trichura* (2.5 %), *Entamoeba spp*. (2 %), and *Balantidium coli* (1 %). Additionally, nested PCR was employed to identify *Trichostrongylus spp*., with 45.4 % of camels testing positive for this particular parasite.
**Keywords:** Internal parasites; Molecular detection; Camel; Iraq

## Introduction

One humped camel species, the camel (*Camelus dromedarius*), is a significant livestock species that is ideally suited to hot, arid environments (Guliye *et al.*, 2007). Camels were infected by a large number of parasites, many of which are the cause of enteric infection (Wahba & El-Refaii, 2003; Parsani *et al.*, 2008). In the gastro-intestinal tract of camelids, helminthic infections can be either frequent or infrequent. Some helminthes are only found in camelids, but others are also found in other hosts, particularly domestic ruminants and wild animals (Wernery & Kaaden, 2002). The significance of parasites in camel husbandry may be much more significant because they have an impact on camel productivity and performance, as well as their susceptibility to other infectious diseases. Knowledge on controlling parasitic diseases and managing camel husbandry is still very unreliable and insufficient (Borji *et al.*, 2010). Considering the economic importance of camels' participation in the production of meat in Iran's Mashhad slaughterhouse, extensive research on gastrointestinal parasites in camels is required (Borji *et al.*, 2010). Common camel parasite infections result in significant economic losses due to reduced working capacity, growth, and productivity (Parsani *et al.*, 2008). The ability of camels, among other domestic animals, to survive a range of economically relevant parasitic illnesses is noteworthy (Soulsby, 1968), and those infected with internal parasites are known to significantly reduce production, usually in severe cases

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(Abubakr *et al.*, 2000). For those who work closely with camels, some gastrointestinal parasites also have zoonotic significance (Tajik *et al.*, 2011; Lone *et al.*, 2012).

The purpose of this research is to shed light on the main parasites that affect camels. Considering their importance to livestock and the paucity of previous studies on the topic, we have decided to look into this area.

**Materials and Methods:**

*Sample Collection*

200 fecal samples were collected using sterile techniques from camels in Al-Diwaniyah and Al-Najaf farms in the provinces. Each camel's dung weighs 20 to 30 grams. All samples are taken to Al-Qadisiya University Faculty of Veterinary Medicine Laboratory in a sterile container for the necessary laboratory tests:

Direct smear: To examine helminthes and other helminths, including Protozoa oocyst (Markell *et al.*, 1999).

1. Sedimentation technique: examining the eggs of some nematodes and trematodes (Hendrix & Robinson, 2022).
2. The Flotation method (Scheathers solution) was used to look into the protozoan oocyst and nematode eggs (Hendrix & Robinson, 2022).

*Measuring the egg sizes using ocular micrometer lens*

To determine the size of the parasite eggs, an ocular micrometer lens was installed in the microscope eyepiece and a stage micrometer slide was utilized. The equation that was used to calculate egg dimensions was:

$$\text{Egg size } (\mu m) = \frac{\text{Size measured on ocular micrometer (mm)}}{\text{Size measured on stage micrometer (mm)}} \times \text{Value of stage micrometer } (\mu m) \quad \ldots\ldots(1)$$

This allowed conversion of the visualized egg size from millimeter units viewed under magnification to the actual micron dimension scale by comparing against a calibrated stage micrometer standard. The measurements accounted for magnification and field of view to provide accurate microscopic sizing data for the parasite eggs.

*Polymerase chain reaction (PCR)*

The DNA samples were extracted from the adult worms of the *Trichostrongylus spp*. by using the Takapouzist Co. DNA extraction kit as instructed by the Manufacturer. The DNA was preserved under - 20°C to analyze PCR test after that. PCR analysis was used to identify the nematodes of the genus *Trichostrongylus* by targeting ITS-2 region of the ribosomal DNA characteristic in the *Trichostrongylus spp*.

The ribosomal DNA ITS (internal transcribed spacer) II region was amplified using the (F-NC1: *5'*-ACGTCTGGTTCAGGGTTGTT-*3'*) and (R-NC2: *5'*-TTAGTTTCTTTTCCTCCGCT-*3'*) primers (Ghasemikhah *et al.,* 2012). The PCR was performed in a 20 µl final reaction volume that contained 1 µl template DNA (20 ng), 0.25 µl of each dNTP (0.1 mM each), 0.6 µl of MgCl2 (1.5 mM), 0.25 µl of each primer (25 pmol), 0.4 U of Taq DNA Polymerase (2U), 15.25 µl of double-distilled water (DDW), and 2 µl of a 1X PCR reaction. In a thermocycler (MWG, Germany), the reaction mixtures were subjected to the following conditions: denaturation at 94°C for five minutes, followed by 35 cycles of one minute at 94°C for denaturation, one minute at 52°C for annealing, one minute at 72°C for extension, and five minutes at 72°C for final extension. The PCR products were run at 100 V for one hour on a 1.5 % agarose gel. Following ethidium bromide staining (0.1 g/mL), gels were visible on a transilluminator (Syngene, UK). Using a 290 bp DNA ladder from Thermo Fisher Scientific, the size of the DNA in each gel was determined.

*Statistics analysis*

*Chi-square* ($x^2$) was utilized to determine statistical differences between data on illness prevalence and other factors' effects; the differences were deemed statistically significant at $P \leq 0.05$ (Manly *et al.*, 2007).

**Ethical Approval and/or Informed Consent**

Ethical regulations and procedures are strictly evaluated in the treatment of animals used in scientific research, with special attention to the health of camels. The regulatory agency and the Institutional Review Board (IRB) approved the collection of stool samples from Governorates (Al-Diwaniya and Al-Najaf). This ensures that all procedures comply with international animal welfare regulations and standards. The study used a variety of tools to handle, collect samples and monitor camels to reduce pain and stress in the camels. In addition, permission was obtained from the authorities or guardians to check the health of the camels in the designated area.

Table 1. Proportional Distribution of Nematodes, Protozoa, Cestodes, and Trematodes in Sampled Camels Populations

| Parasites | Number of samples | Percentage (%) |
|---|---|---|
| Nematodes | 112 | 56% |
| Protozoa | 57 | 28.5% |
| Cestodes | 29 | 14.5% |
| Trematodes | 2 | 1% |
| **Total** | **200** | **100%** |

Table 2. Percentage of Parasite Genera in infected Camels.

| Parasites | Number of samples | Percentage (%) |
|---|---|---|
| | ***Nematodes*** | |
| *Trichostrongylus spp* | 66 | 33% |
| *Strongyloides spp* | 16 | 8 |
| *Nematodirus spp* | 12 | 6 |
| *Haemonchus spp* | 7 | 3.5 |
| *Dictyocaulus spp* | 6 | 3 |
| *Trichuris spp* | 5 | 2.5% |
| | ***Cestodes*** | |
| *Fasciola hepatica* | 21 | 10.5% |
| *Anoplcephala spp.* | 8 | 4% |
| | ***Trematodes*** | |
| *Paramphistomum cervi* | 2 | 1% |
| | ***Protozoa*** | |
| *Moneizia benedeni* | 25 | 12.5 |
| *Giardia spp* | 14 | 7% |
| *Eimeria spp* | 12 | 6% |
| *Entamoeba spp* | 4 | 2% |
| *Balantidium coli* | 2 | 1% |
| **Total** | **200** | **100%** |

**Results**

In the study, a total of 200 parasite samples were analyzed across four categories. The majority of samples belonged to the *Nematodes* category, with 112 samples, accounting for 56 % of the total. *Protozoa* constituted the second largest category, with 57 samples, representing 28.5 %. *Cestodes* comprised 29 samples, making up 14.5 % of the total. *Trematodes* were the least prevalent, with only 2 samples, accounting for 1 %. Overall, the combined total of all categories equaled 200 samples, representing 100 % of the analyzed population. (Table 1)

Various parasites were examined across different categories. Among the *Nematoda*, *Trichostrongylus spp*. was the most prevalent, with 66 samples accounting for 33 % of the total. *Strongyloides spp*. followed with 16 samples (8 %), while *Nematodirus* spp. had 12 samples (6 %). *Haemonchus spp*., *Dictyocaulus spp*., and *Trichuris trichura* were also identified, with 7(3.5 %), 6 (3 %), and 5 (2.5 %) samples, respectively. In the Cestoda category, *Fasciola hepatica* was the most prevalent, with 21 samples representing 10.5 % of the total. Anoplcephala *perfoliata* and *magna* followed with 8 samples (4 %). In the Trematoda category, *Paramphistomum cervi* was found in 2 samples, accounting for 1 %. Among the Protozoa, *Moneizia benedeni* was the most prevalent with 25 samples (12.5 %), followed by *Giardia* spp with 14 samples (7 %),

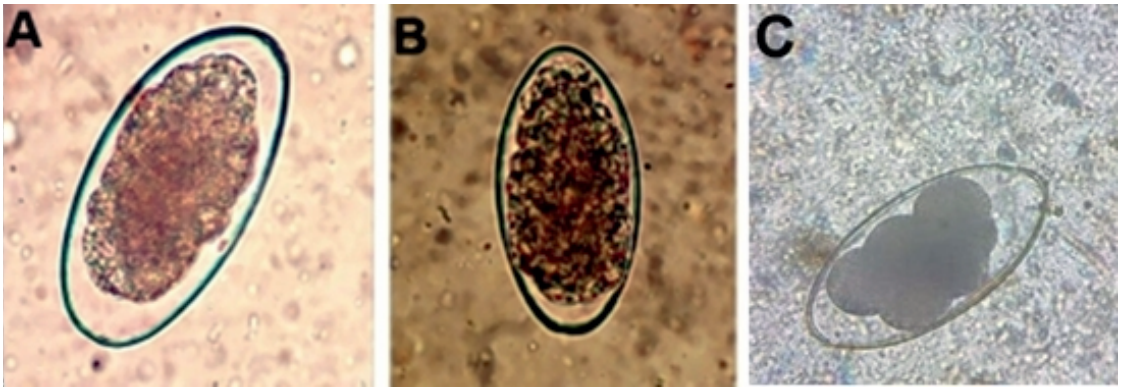


Fig. 1. Eggs of (A) *Strongyloide* spp., *Trichostrogylus* spp., and *Nematodirus* spp. X40.

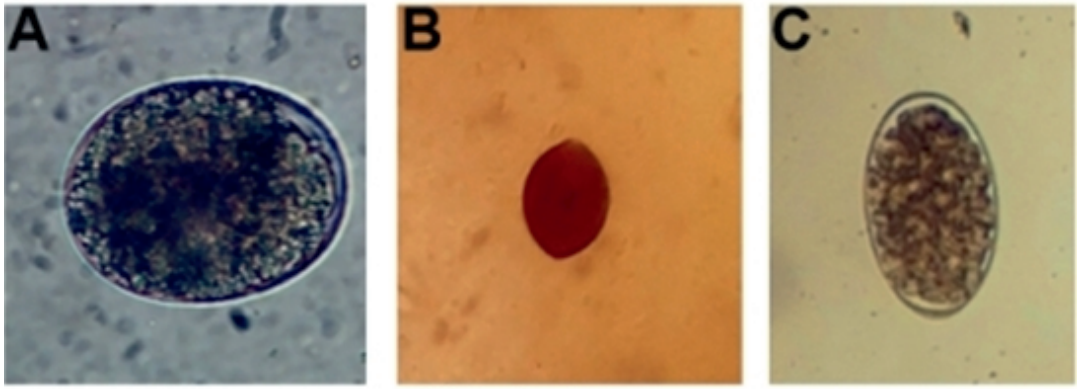


Fig. 2. eggs of *Dictyocaulus* spp., *Trichuris trichura*, and *Fasciola hepatica*. X40.

*Eimeria spp*. with 12 samples (6 %), *Entamoeba spp*. with 4 samples (2 %), and *Balantidium coli* with 2 samples (1 %). The total number of samples analyzed was 200, representing 100 % of the studied population. In summary, nematode infections predominated, with *Trichostrongylus* spp. being the most prevalent specific parasite genus. Multiple parasite genera were common as mixed infections in the camels. (Table 2)

*Microscopic and molecular examination of Trichostrongylus spp.*

*The prevalence of infected camels with Trichostrongylus spp. depends on the microscopic examination*

After the microscopic examination as shown in Figures 1B, 4A, 5B, and C, *Trichostrongylus* comprised the highest proportion of parasites, accounting for 33 % (66 out of 200). The egg of the *Trichostrongylus* characterized by small, oval-shaped eggs with a smooth outer shell. Besides *Trichostrongylus* spp., these figures also depicted the presence of various other parasites, as detailed below: (A) Trophozoite of *Entamoeba spp*.: A single-celled organism with an irregular shape and movement, varying in size and appearance. (B) Cyst of *Giardia spp*.: A small, oval or pear-shaped structure with a characteristic "face" appearance due to the presence of two nuclei and other internal structures. (C) Eggs of *Strongyloid* Small, oval-shaped eggs with a smooth outer shell, and containing larvae.

Depending on the observations from figure 6, the microscopic examination revealed the following egg characteristics: (A) *Moniezia benedeni* (Cestode tapeworm) eggs were large oval eggs with a thick outer membrane, an oncosphere visible inside, and typically measured 80 – 100 µm in length by 60 – 80 µm in width. (B) *Paramphistomum cervi* (Ruminal fluke) eggs were operculated eggs with a small lid or cap at one end, measuring typically 140 – 150 µm by 90 – 100 µm, and containing a fully developed miracidium when laid. (C) *Haemonchus spp*. eggs were barrel-shaped rather than oval, with a thin outer membrane, measuring around 70 – 90 µm in length by 40 – 50 µm in width, and may have had a visible larva inside.

*The prevalence of infected camels with Trichostrongylus spp. depends on the polymerase chain reaction (PCR) technique*

The PCR test results shown that 45.5 % (30/66) of the samples were positive for *Trichostrongylus* spp. and observing a clear bands at 250bp target size. Breakdown of Positive Bands showed positive bands were scored using band intensity relative to the each other. 15 samples had band intensities ≥80 %, 10 samples between 50 – 70 % intensity, and 5 samples showed faint bands <50 % intensity. Sequencing Confirmation using 12 positive PCR products sequenced via Sanger sequencing and BLAST analysis indicated >98 % homology to reference *Trichostrongylus*

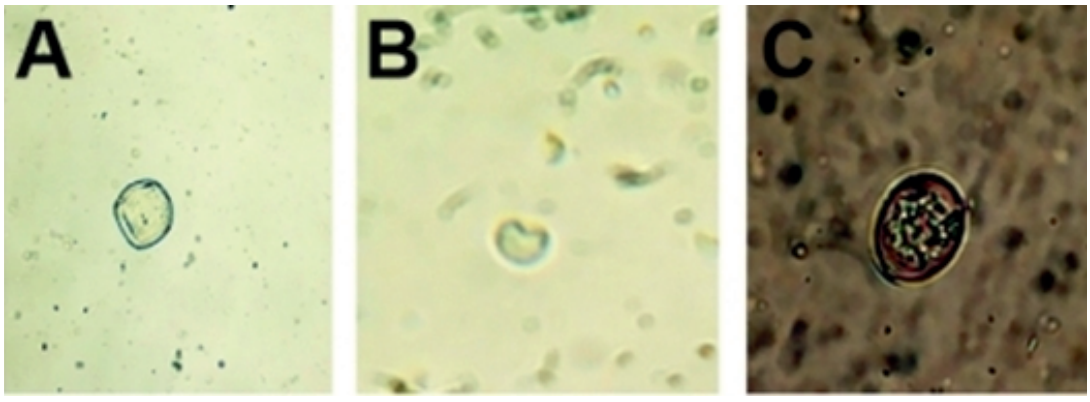


Fig. 3. (A) Egg of *Anoplcephala perfolata,* (B) Oocyst of *Anoplcephala magna,* (C) Egg of *Eimeria* spp. X100.

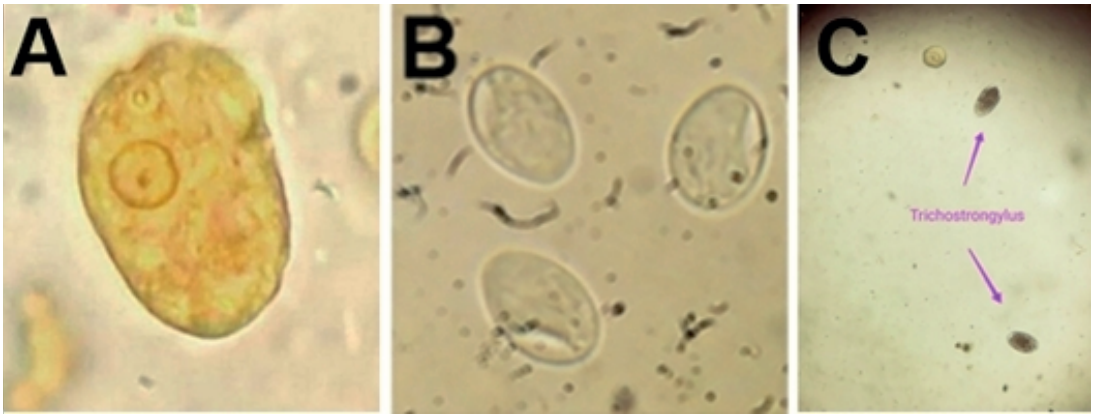


Fig. 4. (A) Trophozoite of *Entamoeba* spp. (X40), (B) cyst of *Giardia* spp.(X40) (C) eggs of *Strongyloid* and *Trichostrongylus* (X10).

sequences for 10 samples, while 2 samples showed 95 % and 92 % homology, indicating genus-level confirmation (Fig. 7).

**Discussion**

In this study, the percentage of infection with various intestinal parasites reached 88.5 %. This result is agrees with (Karawan, 2017), which reported a percentage rate of 86.36 % of internal parasites in camels in Iraq (Demelash *et al.*, 2014), recorded a percentage of 80.73 % of harbouring different gastrointestinal parasites in camels in Ethiopia. In Pakistan's Faisalabad Abattoir, results reported by (Anwar & Hayat, 1998) were also close to 69.1 % for camels. Additionally, testing in the Yabello district, southern Ethiopian rangelands, revealed that 73.8 % of the camels had discharged protozoan oocysts or helminth eggs in their faces (Duguma *et al.*, 2014). While Birhanu *et al.* (2014) (Birhanu *et al.*, 2014) noted a proportion of 55.5 % in different pastoral areas of Ethiopian. In Iran, the prevalence rate of gastrointestinal helminthes was 52 % that reported in Bactrian camels (Tajik *et al.*, 2011), mostly indicated by the presence of helminthe eggs. As well as in Riyadh, Saudi Arabia, 19 camels were reported to have internal parasites, which are reported to be positive in 59.6 % of cases (Al-Megrin, 2015). An infection rate of gastrointestinal parasites of 48.26 % in camels in Algeria was revealed by microscopic examination, which was recorded by (Bouragba *et al.*, 2020). Additionally, 50.3 % of camels had gastro-intestinal parasites in Somalia (Ibrahim *et al.*, 2016). In Egypt, 60 % of camels had parasitic infections (El-Khabaz *et al.*, 2019).

Protozoal infection in this study was 29 %, which was less than recorded by Hasan *et al.* (2021), in northern Iraq. While similar to the study of Bouragba *et al.* (2020), who reported that protozoal infection in Algerian camels was 17.02 % .

The current study found that camels had a 15 % cestode infection rate, which is consistent with the Pakistani reported incidence (22.5 %). However, it disagrees with the rate identified in camels in Somalia (6.0 %) (Ibrahim *et al.*, 2016; Mahfooz *et al.*, 2006) (Mahfooz *et al.*, 2006).

In this investigation, similar to rate in Pakistanian and Somalian camels, 2 % of camels were found to be infested by trematodes (Anwar & Hayat, 1998; Ibrahim *et al.*, 2016).

In the present research, the percentage of *Trichostrongylidae* spp. that detected by microscopical examination was 33 %, which is

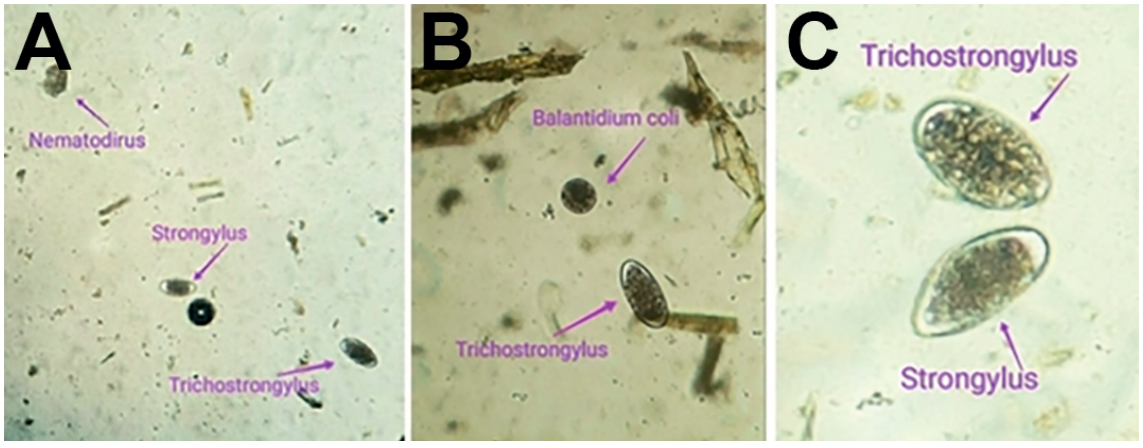


Fig. 5. (A) Eggs of *Strongyloide, Trichostrongylus*, and *Nematoodirus* (X10); (B) eggs of *Balantidium coli* and *Trichostrongylus* (X10); (C) *Strongyloide* and *Trichostrongylus* (X40).

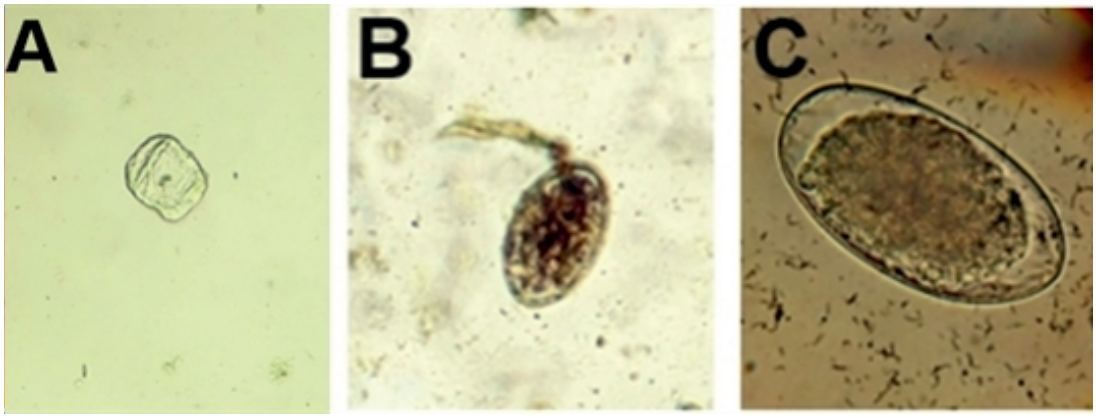


Fig. 6. Eggs of (A) *Monezia benedeni* (X10)*;* (B) *Paramphistomum cervi*, and *Haemonchus* spp.X40.

consistent with the results achieved by (Anwar & Hayat, 1998). Furthermore, the percentage of *Trichostrongylus* spp. was corresponded to that reported for camels in Egypt (38.8 %) (El-Khabaz *et al.*, 2019). Additionally, our performance percentage is lower than that recorded in province of Nineveh in Iraq (49 %) (Hasan *et al.*, 2021), but higher than India (10.71 %) (Rewatkar *et al.*, 2009). Moreover, the proportion of infestation of camels in China is 98.1 % (Guowu *et al.*, 2020). While 79.67 % and 75 % in southern and eastern Ethiopia, respectively (Bekele, 2002).

According to PCR analysis, the positive value of *Trichostrongylus* is high. It is 45.5 % (60 out of 300). Our results are consistent with Pathak and Chhabra (2010) who reported 26 % for *Trichostrongylus axei* and 65.2 % for *Trichostrongylus colubriformis* in camels using the PCR-RFLP technique. In another study, 54.44 % were found positive for *Trichostrongylus* in PCR analysis (El-Alfy *et al.*, 2019; Bakooie Katrimi *et al.*, 2022). Additionally, 31.45 % of goats in Bangladesh tested positive for Trichostrongylus (Ahmed *et al.,* 2022). In Iran, 1.8 % of Trichostrongylus isolates were recorded in the abomasum and small intestine of sheep and goats (Ghatee *et al.*, 2020 ).

Also Pilarczyk *et al.* (2021) showed that *Trichostrongylus* eggs are also the most common pathogen infecting ruminants, followed by *Trichuris spp*. Additionally, Ashrafi *et al.* (2020) demonstrated that various of *Trichostrongylus* species, including *T. colubriformis*, *T. vitrinus*, and *T. orientalis* are zoonotic nematodes that are widely distributed among herbivores worldwide. The presence of genus such as *Nematodes*, *Fascioliosis* and *Moniezia* shows that camels are sensitive to various internal diseases (Locklear *et al.*, 2021). According to the findings of the research, the disease affected camels depending on the ecology of different organisms, environment, mixing with other animal species, and culture of human poulation (Osman, 2014). These findings emphasize the importance of camel diseases for animal and human health (Elmahallawy *et al.*, 2023; Thamsborg *et al.*, 2017 ).

## Conclusion

The present Study show that nematodes are the most common type of parasite, followed by *protozoan oocysts* and *tapeworms*. Many species such as *Trichostrongylus*, *nematodes*, *Fasciola* and

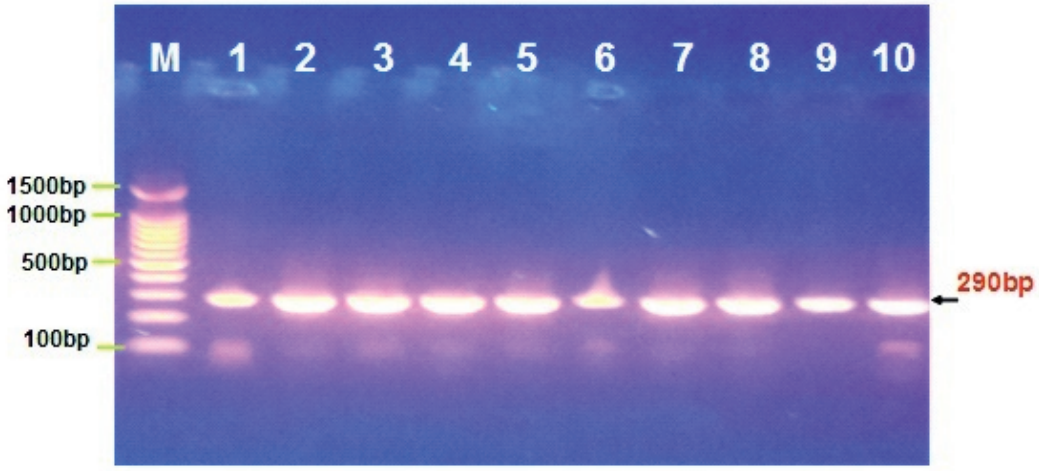


Fig. 7. Agarose gel electrophoresis that explains the PCR product analysis of *Trichostrongylus* spp. from camel fecal samples. Rows 1 through 10 are some positive samples for *Trichostrongylus* spp. at 290 bp result size, and M is the indication (1500 – 100 bp).

*Moniezia* have been found to cause disease in camels. In microscopic examination, *Trichostrongylus* spp. is the main species with a standard detection rate of 33 %. The 33 % of *Trichostrongylus* spp. samples were confirmed by PCR tests showed that the positive was percentage 45.4 % (30 out of 66 samples). These results highlight the importance of combating parasites in camels because they have significant clinical, public health and financial implication and all factors contribute to the overall health and well-being of camels and humans.

**Conflict of Interest**

Authors state no conflict of interest.

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**Data Availability Statement**

On request, the corresponding author will send you the information that was utilized to support the study's findings.

**Authors Contribution**

Hadeel Hadi Albayati, Alaa Mohammed Al Khafaji, and Amal kamel were involved in conceptualization; Hadeel Hadi Albayati and Alaa Mohammed Al Khafaji conducted methodology; Hassan Al-Karagoly conducted formal analysis, Alaa Mohammed Al Khafaji and Amal kamel, were in charge of investigation, data curation, and study validation, while Hadeel Hadi Albayati and Hassan Al-Karagoly worked on writing, reviewing. Hadeel Hadi Albayati and Amal kamel also managed the project. The final draft of the work was approved by all authors.

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