

How to Cite:

Al- Fatlawi, Z. A. A. (2022). Prevalence and molecular identification of heamunchus spp in camel in Al najaf province, Iraq. *International Journal of Health Sciences*, 6(S7), 4248–4251.
<https://doi.org/10.53730/ijhs.v6nS7.12796>

Prevalence and molecular identification of heamunchus spp in camel in Al najaf province, Iraq

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Abstract---The current study were showed through the period from January 2022 to July 2022, the total of examined samples 60 abomasumswere taken from slaughtered camels in Al Najaf province used to identify the species of Haemonchus Longistipes that infected camel and causing"Barber Pole Worm" disease of Trichostrongyloidea Haemonchus spp,particularly in tropical and subtropical areas causes anemia, emaciation and declined milk production.Gene extraction was done from samples and ITS2 ribosomal DNA was used as a gene marker for rapid PCR for DNA amplification, 30sample rapid PCR products were sent to local labrotary for DNA detection and results confirmed Haemonchus Longistipes with 35% infestation rate from total samples was sent, other wise this prevalence confirmed the results with geographical harmony results after identic with gen bank similarity.

Keywords---Identification, Haemonchus Longistipes., Camels, rapidPCR, Al Najaf.,Iraq.

Introduction

Structural morphological used to identify Haemonchus by cuticle,spicule length, levee paradigms and other structures (Behnam et.al.,2015) in camles mostly in tropical and subtropical areas causesing anemia, emaciation and decreased milk production (Nabavi et al., 2011; Alessandro, 2011. DNA is was used for molecular detection of nematodethrough markers (Heise et.al.,1999). Nematode infestations , high detailed ways are require to isolate Haemonchus spp, Behnam et al., (2015) . gastrointestinal nematodes was detrmine by ITS2-ribosomal DNA in ruminants and Samson-Himmelstjerna et. al. (2001) several Trichostrongylide nematodes (Michelle et.al.,2014). We report prevalence of Haemonchus species isolated from camels' abomasum by rapid PCR.

Materials and Methods

Specimens were directly collected from abomasum of camels' after slaughter total different age and sex of 60 camels, specimens collected 30 worms, examined for spicule length and right/left spicule barber length in male worms, nematodes was washed by phosphate saline solution and stored until used, adults crinkled by sterile forceps and putted in Eppendorf tube with 100 PSS extraction buffer and gently mixed at 55°C. Genomic DNA was extracted from each worm were using tissue isolation kit of DNA (ABM, Canada) as described by, manufacturer (Chilton et.al.2005). the samples isolated after were incubated 2 hours for DNA extraction and at 95°C for 5 minutes for deactivation of extraction proteinase-k., electrophoresis agarose were applied to confirmed the individual and exactitude of the DNA extracted. Fig (A) refer to rapid PCR technology and (B). DNA chilled and used as Templates for PCR

Genomic DNA were amplified by rapid PCR by used specific primers 5'-GAA GCG CGA TAC GCT TGAGC3' (for-REVERSE) and 5'-GGC AAA TAT GTC CCA CGT GC3' (for ward) for ITS2 region. Depend on the designed available of nuclide sequancethe primers done by data from ITS2 in GenBank (accession no. HQ844231.1). rapid PCR 100 µl were containing 1×PCR buffer, 2 mM MgCl₂, 2 mM dNTPs, 0.4 µmol of each primer, 2.5 unite/µl Taq DNA poly-merase (Cinnagen) and 1 µl DNA sample in an automated thermocycler (Biorad-Italia). The PCR was performed under the following conditions: 5 minutes of denaturation at 94°C rapid

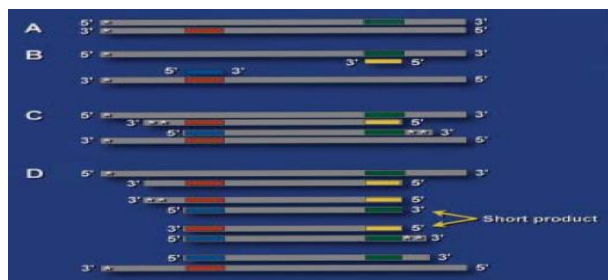


Fig (A) short products A, Double-stranded “target” B, Double-stranded DNA is denatured by increasing the temperature in the reaction C, Double-stranded DNA is produced in a 5'-3' alignment from the 3' end of the primers by using a thermostable DNA polymerase. “Shaggy-ended” double-stranded DNA results with new complementary strands containing primer-annealing sites for subsequent amplification reactions. D, In this second PCR cycle, matching DNA strands are synthesized for the 4 DNA strands that comprise the 2 double stranded DNA structures formed in C. Of the 8 single-stranded DNA strands, 2 are of a length equal to and including the distance between the primer-annealing sites. These short DNA products accumulate exponentially with subsequent PCR cycles.

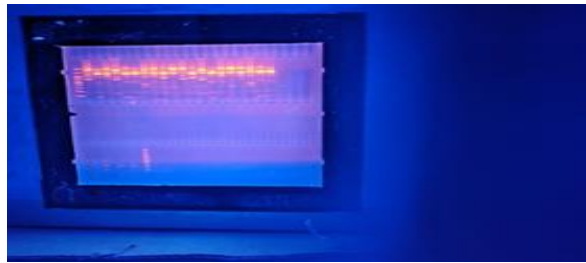


Fig (B) Genome electrophoresis for *Haemonchus* spp The application of Rapid PCR technology compared to organism culture to susceptibility testing: by direct detection in primary specimen; with viable organisms not required successful identification. Less severe specimen transportation requirements.

Results

Total infection rate of *Haemonchus* spp. method recorded the overall prevalence rate of haemonchosis in camels was 35% (21/60). On the other hand, molecular (Rapid PCR) analysis identified *Haemonchus longistipes*. Infection in positive specimens was identified microscopically also found positive. The prevalence of *Haemonchus* spp. determined by the two methods was significantly different ($P \leq 0.05$). PCR products ITS2-rDNA revealed a single fragment of 277 bp for all samples as shown in Fig (C).

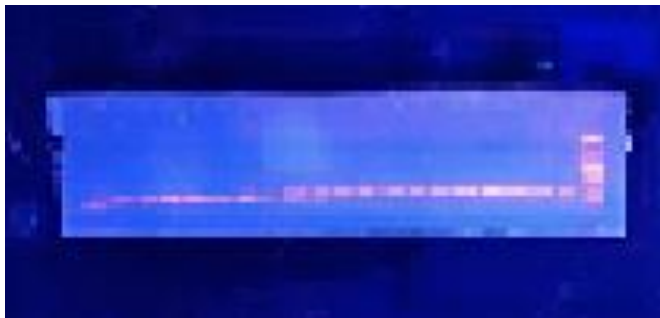


Fig (C) agarose gel confirming the presence of *Haemonchus longistipes* ribosomal DNA for *Haemonchus* isolates. The restriction fragments were analyzed using electrophoresis on 2% agarose gels, discolored and photographed upon trans-illumination. A evaluation of sequence in our article with GenBank presented 100% similarity *Haemonchus longistipes* from camels in Egypt and 99.4%, with camels from Japan and 97.7%, from Iran with accession numbers respectively. This results.

Discussion

The current study determines the prevalence of *Haemonchus longistipes* among camels in Al Najaf province. Abomasum samples collected from camels were shown by microscopy technique and rapid PCR. Although microscopy is cheaper to perform and only method to determine generally infestation, greatly prevalence of *Haemonchus longistipes* were verified by the rapid PCR in our study. *Haemonchus*

Longistipes is a extremely pathogenic abomasal nematode with also the greatest heterogeneity, identification of species could assist its control. (Samson et al., 2001). rapidPCR which explain worms variety and genotyping (Gasser et al., 2004a). Gasser et al., (2004), showed that genof DNA from the ITS gene would be specific trichostrongylids identification. otherwise the our results screened high degree of similarity were showed 100 % with Haemonchus longistipes isolated in the NCBI data base from Egypt, Japan and Iran 100%-99.4%-97.7% Our findings confirm those of Meshgi et al., (2015) who found that in camels from Iran H. longistipes was prevalent.

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