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Molecular Detection of Sheep *Haemonchus contortus* in Al-Diwaniyah Province/Iraq

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Abstract

Background: *Haemonchus contortus* infection is common in sheep in most parts of Iraq. The idea of this study is to accurately determine the infection of these nematode in sheep using a molecular method in Al-Diwaniyah province, central Iraq.

Methods: Two hundred forty (240) fecal samples were collected randomly and then microscopically tested for Trichostronglide eggs. The DNA was extracted from the eggs of the positive samples, then the PCR amplification of 2ITS-rDNA region was accomplished, and the successed amplified samples were subjected to gene sequence analysis.

Results: Out of the 240 examined samples, 58 (24.16%) contained Trichostronglide eggs, and after DNA extraction, 16 (27.58%) succeeded in amplifying the ITS2 region. Only 11 (68.75%) of which were successful in the sequence analysis to confirm the presence of the *Haemonchus contortus* parasite in central Iraq.

Conclusion: *Haemonchus contortus* is an endemic parasite that infects sheep in the study area.

Keywords: *Haemonchus contortus*, sheep, PCR, ITS2-rDNA, AL-Diwaniyah, Iraq.

Introduction

Internal parasites represented an important cause of disease and lose of production in small ruminants (Zajac, 2006). Gastrointestinal nematodes are recognized as a major constraint to both small and large-scale small ruminant production in developing countries (Martinez-Gonnalez, et al., 1998). These could be harmful to the health different regions of infected animals and causes economic losses due to mortalities, reduce weight gain and other production losses (Sissay, et al., 2007)*Haemonchus contortus* is a trichostrongylate (also known as barber pole worm) nematode that lives in other ruminants' abomasum of sheep, goat sand and has a direct life cycle (Bowman, 2009). In seriously infected animals, it causes extreme anaemia and death (Okaiyeto, et al., 2010) The depression of weight gain of the infected animals may be duo to loss of appetite, and effect on the productive and physiological parameters of the ruminants, especially sheep and goat (Bisset et al., 2001) , Due to inflammatory changes during the larval and adult phases of the abomasum wall cells (Beriajaya and Copeman, 2006) , Several epidemiological studies on the infection of gastrointestinal nematodes (GIN) have been carried out in various agro-ecological regions of the world to demonstrate the seasonal trend of haemonchosis ,(Agyei, 2003; Ng'ang'a et al., 2004).

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Due to most of the previous studies in Al-Diwaniyah province to detect *Haemonchus contortus* worms were limited to microscopic examinations, and for the purpose of confirming infection with this parasite, this study was conducted.

Materials and Methods

Fecal samples (5 grams) were collected from 240 sheep, of both sex and different ages, during the period which extended from the first of September 2019 to end of February 2020. All samples were transported with ice bags to the laboratory of parasitology in veterinary college/ University of Al-Qadisiyah for examination.

Flotation method with sheather's solution was used to detect the eggs of Trichostrongylidae in collected samples. DNA was extracted from eggs of fecal positive samples using gSYNC™ DNA Extraction Kit, Geneaid Biotech Ltd. ,USA, according to manufacturer's instruction(Accuprep,K-3036,Bioneer, Korea)and tissue genomic DNA by using the DNA extraction kit (DNA tissue isolation kit (AQXTDI, Quantiphore ,Turkye). About 200µ of fecal sample was applied to the bead tube and the put on ice . SDE1 buffer 300 µl and proteinase K 20µl were added. Then mix the vortex for 5 minutes at full pressure. The mixture was incubated for 20 minutes at 60 ˚C, and every vortex was incubated. Followed by 3 times freezing ,thawing . added 100 µl of the SDE2 buffer and the vortex . while the was mixture was incubated for 5 minutes on ice. Centrifugated for 5 minutes at 18000 xg. 200 µl of SDE3 buffer added. He mixed the vortex well and incubated the mixture for 2 minutes at room temperature. 250 µl of SDE4 buffer and 250 µl of absolute ethanol were added and mixed by vortex. The suspension was incubated in the lysis buffer at 60 °C for20 minutes. The samples were then extracted with phenol-chloroform-isoamyl alcohol and DNA was precipitated with an equivalent amount of isopropanol and 1 ml of 100% ethanol. With 750 µl of 70 percent ethanol, the pellet was washed, dried and eluted in 100 µl of TE buffer and stored until PCR amplification at −20 ˚C.

Internal transcribed spacer 2 (ITS2) .The ribosomal DNA The rear area was intensified by the primers forward (NC1: 5-ACGTCTGGTTCAGG- GTTGTT-3) and reverse (NC2: 5-TTAGTTTCTTTTCCTCC GCT-3),(Chilton NB,2004). A final reaction volume of 20 µl containing 15 µl of a PCR mix containing 1.5 U Taq DNA polymerase was performed in the PCR reactions. (2x Master Mix RED MyGene. Bioneer. Korea). 10 pmol of each primer, and 3 µl of DNA sample. The PCR program was an initial denaturation Period for 5 min at 95˚C, followed by 35 cycles of 95˚C for 30 sec (denaturation), 50˚C for 30 sec (annealing),and 72˚C for 30 sec (extension), followed by a final extension 5 min at 72˚C.A 1.5% agarose gel was the PCR products using a UV Transilluminator .

Results

Out of 240 microscopically examined fecal samples, 58 (24.16%) were revealed Trichostrongylidae eggs positive. Of the 58 samples, only 16 (27.58%) were successful in amplifying the of ribosomal DNA internal transcribed spacer 2 (ITS2) region by using the NC1 and NC2 primers yielded bands with the size of 320 bp (Figure 1).

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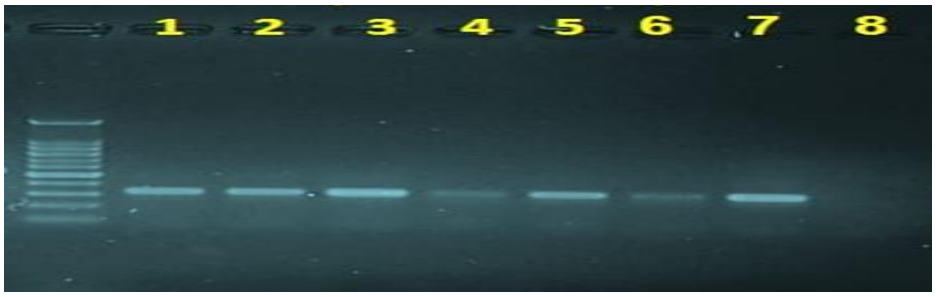
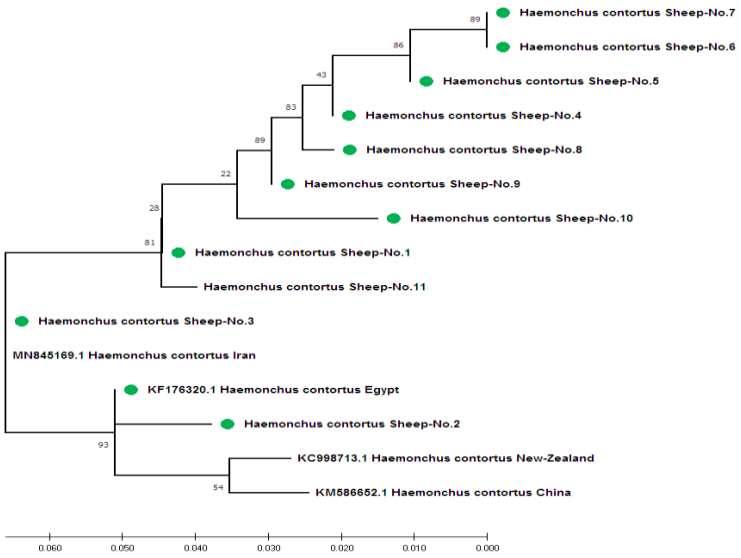


Figure(1): PCR product of positive samples (1-7) with 328bp, and negative one (8), electrophoresis in 1.5% agarose gel.

Eleven of the 16 samples were successful in gene sequence analysis, where they were subsequently subjected to bioinformatics analysis and the genetic tree mapping. It was proved through the results of the gene sequence that all the samples were representative of the *H. contortus* parasite, that which recorded in gene bank under accession numbers of : MW553283, MW553284, MW553285, MW553286, MW553287, MW553288, MW553289, MW553290, MW553291, MW553292 and MW553293. The phylogenetic tree analysis revealed that *Haemonchus contortns* identified ranged between 98.46%-100.00% with the Iranian, 98.39% - 99.61% with Egyptian, 97.12% - 98.82% with the New Zealand and 96.40% -98.68% with Chinese isolates (Figure 2).



Figure(2):Phylogenetic tree analysis based on ITS2 small subunit ribosomal RNA gene partial sequence in local *H. contortus* sheep isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *H. contortus* sheep isolates (No.1-No.11) were showed closed related to NCBI-BLAST *H.contortus* Iran and Egypt isolates at total genetic changes (0.060-020%).

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Discussion

Gastro-intestinal parasite (GIPs) infections considered the most important problem in the herds around the world especially in tropical and sub-tropical regions (Traorea *et al*., 2013). Emphasis that certain GIPs causes diarrhea, Anemia, loss of weight, recumbency, odema, anorexia, death in chronic cases due to blood sucking from abomasum of grazing ruminants such as *H. contortus* which known as barber-pole worm. The measurements of many of the differences between worm genera are very small, making it difficult to observe, that coincides with Van Wyk *et al*., (2004), and similarity through measurements and extensions .Here we depend on molecular study to confirm the infection with this nematode. The present stud showed 27.58% (16 /58) of molecular analyzed samples were as Trichostrongylidae nematodes which diagnosed in fecal of sheep by using conventional PCR assay when access to amplified of ITS-2 gene to produce a fragment of 321 bp from 58 positive samples examined by Microscopic.

This result was less than previously scored by both Fentahum and Luke ,(2012) in Ethiopia, Gana *et al*., (2015) in Nigeria and Hassan *et al*., (2017) in Sudan whereat they reported that the sheep infections with *H. contortus* were 81.2%, 85% and 77.7% respectively. The difference in result may be due to differences in studies conditions, numbers of samples or PCR technique conditions. According to molecular DNA sequencing technique which used to detect the sheep Trichostrongyloidae genera; only 11 samples gave a good sequence, and confirmed presence only one species which were *Haemonchus contortus* 100% (11/11) ,contrary to what was stated in the microscopic examination, where the examination there indicated the presence of four genera as mentioned in the results.

The positive *H. contortus* registered in the gene bank as an Iraqi isolate under the following accession numbers: MW553283, MW553284, MW553285, MW553286, MW553287, MW553288, MW553289, MW553290, MW553291, MW553292 and MW553293.

According to the phylogenetic tree analysis, it appeared that the Iraqi *H. contortus* isolates were 98.46% - 100.00% similar identity to the Iranian isolates, followed by the Egyptian isolates by 98.39% - 99.61%, then the New Zealand isolates with 97.12% - 98.82% and finally they were close to the Chinese isolates, at 96.40% -98.68%. The molecular diagnosis of *H. contortus* is important in order to understand the genetic structure and status of genetic variation of the parasite populations which has important suggestions for epidemiology and effective control of Haemonchosis in different areas and countries.

Conclusion

Among different genera in Trichostrongylidae, the *H. contortus* appeared the most prevalent nematode in study area, and the Polymerase chain reaction technique with gene sequence analysis is the more effective method in Trichostrongylidae nematodes determination.

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