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Original Article

Prevalence, Haematological and Molecular Studies of *Haemonchus contortus* Isolated from Goat at AL-Muthanna Province, Iraq

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Abstract

Parasitic infections, especially helminthes infections of the gastrointestinal tract due to the latent symptoms, play a vital role in the growth and efficiency of ruminants. The current research was performed to determine the prevalence of haemonchosis in goats and the effect of some risk factors, such as age, sex, and months on the infection rate. Also, our study includes investigating the haematological and biochemical changes in the haemonchosis-infected goat, then utilizing the PCR technique to confirm the *H. contortus* diagnosis in goats. The result of the epidemiological study revealed that only 73 out of 693 examined goats were positive to infect with *Haemonchus spp*. at an infection rate of 10.53%. The incidence of Haemonchosis was related to climatic conditions, with the highest (23.07 %) and lowest (4.34 %) percentages occurring in October and June, respectively. Furthermore, the highest (14.01 %) and lowest (4.76 %) infection percentages were recorded in goats aged > 5 years and 9 months to 2 years old, respectively. According to sex, infection percentages were (14.24%) and (7.02 %) for females and males, respectively. Haematological and biochemical parameters results revealed that infected goats suffered from a gradual decline in Hb concentration, pocket cell volume, total erythrocyte count, total leucocyte count, lymphocyte, neutrophil, total serum protein and albumin, while eosinophil count was increased significantly. Also, serum enzymes ALP, ALT, and AST showed significant increases in infected goats. The result of PCR showed that specific primers (HcI-F, HcI-R) successfully amplify the ITS-2 rDNA gene with 295bp -long fragment to *H. controtus*. Due to the effect of age, sex and season on *H. contortus* infection, it is crucial to have control and prevention programs and treatment schedules in the herd.

Keywords: Prevalence, Haematological, Molecular, Haemonchus contortus in Goat

1. Introduction

Goats are primarily raised for meat and milk, and hair is in secondary importance of production and; that had a population of about 1.3 million in 1999 (1) is distributed throughout Iraq, with 12.5, 44.2, and 43.3% in the southern, central, and northern parts of the country, respectively (1).

Goats are susceptible to external and internal parasites like domesticated animals. Minimal research has been conducted in Iraq to investigate the various pathogenic, infectious agents and diseases, such as parasites, that restrict the growth and productivity of goats, unlike dairy cattle and sheep. Helminthes infections are one of the most critical diseases limiting the production of

small ruminants in many parts of the world (2). Besides, gastrointestinal nematodes are recognized as a major constraint to small and large-scale ruminant production due to the significant economic losses in developing countries caused by increased susceptibility of animals to other infections, morbidities, and mortalities, especially in heavily parasitized animals and in young animals (3). Furthermore, infected animals lead to organ condemnations, increased veterinary treatment costs, weight loss, reduced milk production and weak work capacity, reproductive capacity, decreased food intake and decreased animal growth rates, as well as increased treatment and management costs (4, 5). Among the gastrointestinal helminthes that endanger the survival and productivity of cattle, sheep, and goats is a gastrointestinal nematode infection known as Haemonchosis caused by *Heamonchuscontortus*, also known as the "barber's pole worm", which lives in the abomasum of sheep, goats and other ruminants, has a natural life cycle (6). *Haemonchus spp*. is an abomasal parasite that can cause significant mortality and economic losses in goats (2). The most common parasite is found in sheep and goats worldwide (7).

Haemonchosis is an economic disease that causes rapid death, depletion and severe anemia in their hosts. Clinical signs and complications depend on the parasite species and the infection intensity. In goats, Clinical sign range from subclinical weight loss to lethal pathologies such as anemia, diarrhea, and severe protein loss (2). Anemia is the most common pathological lesion caused by *H.contortus* infection; as a result, both adult and fourth-stage larvae suck blood, and migration of adult and larvae causes haemorrhages into the abomasum. Moreover, the average blood loss due to H. contortus infection is 0.03 ml/parasite/ day, and thus, blood loss leads to decreased body weight, reduced feed intake, and wool growth and also, maybe affect the productive and physiological parameters of sheep and goat (8). Several epidemiological, haematological, and enzymatic assay studies on haemonchosis infection have been conducted in various parts of the world (9). The objective of the present study was to determine the prevalence rate and the influence of seasonal variation, age and gender on the prevalence of haemonchosis. Also, study the hematological and biochemical changes in the infected goat with haemonchosis. Finally, the molecular diagnostic PCR technique is used to detect and diagnose *H. contortus* in the goats.

2. Materials and Methods

2.1. Study Area and Sample Collection

This study was carried out on 693 faecal samples from local goats collected from villages located in Alkider, Rumathia and Samawah in Muthanna province, south of Iraq, from October 2020 to July 2021. Five grams of feces per animal were collected directly from the rectum of both sexes (male 356, female 337). The goats were divided into three age groups: 9 months to 2 years, 3 to 4 years, and > 5 years. Samples were applied in plastic containers separately with a lid, and the data pertaining to the age, gender and feces consistency were recorded and transported in a cool box to the laboratory of parasitology in the veterinary college/ University of Al-Muthanna for examination.

2.2. Laboratory Examination of Faecal Samples

Faecal samples collected from all goats were examined daily using the direct smear and floatation technique with sheather's solution to detect *Haemonchus spp*. eggs according to (10). The eggs of *H. contortus* were identified as described earlier (11).

2.3. Hematological and Biochemical Examination

Ten ml blood samples were drawn from the jugular vein of each examined goat by using disposable syringes (5 ml EDTA tube and 5 ml without EDTA tube). Serum was obtained according to the method previously described (12) and stored at -20 °C in Eppendorf tubes for subsequent biochemical tests, including total serum protein (TSP), alanine transaminase (ALT), aspartate transaminase (AST), Alkaline Phosphatase (ALP) and Albumin (ALB) have been measured according to standard procedures using

automatic analyzer Tehnicon RA-1000 (Tehnicon Instruments, Tarrytown, NY, USA) with commercial kits Randox (Randox Laboratories LTD, Crumlin, Antrim, UK). While, the EDTA blood samples were used in estimated (Hb), packed cell volume (PCV %), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC %) based on the method of Singh (12).

2.4. DNA Extraction and PCR Assay

The DNA Stool Mini Kit /QIAGEN / Germany was used to isolate genomic DNA from eggs isolated from faecal samples of a goat infected with *Haemonchus spp*. DNA integrity was analyzed on 1% agarose gel electrophoresis containing 0.05 % ethidium bromide. The PCR reaction was performed to distinguish *H. contortus* using specific primer HcI-F (5'-CTC GTC TGG TTC AGG GTT-3') and reverse primer HcI-R (5'-GTA ACC TCG CTG AGC TCA-3'), which amplified the ITS-2 rDNA gene with 295bp -long fragment according to the method by Yin, Gasser (13). The PCR program was performed at 94C° for 5 min, followed by 35 cycles of 94C°for 45sec, 58C° for 45sec and 72 °C for 45 sec; this was followed by a final extension step at 72 °C for 3 min. All results of the PCR were analyzed after the amplification stage. Five µl from amplification samples were directly loaded in a 1.5% agarose gel electrophoresis, and a UV transilluminator visualized the products.

2.5. Statistical Analysis

The data of this study were analyzed according to the program of analysis variance by using SPSS software statistical program (SPSS for windows ver.17.00). The difference in mean values was considered significant at ($P \leq 0.05$).

3. Results

The results of the parasitological examination in the present study showed that the eggs of *Haemonchus spp*. are found in faecal samples of goats, as shown in figure 1. The result of the epidemiological study revealed that only 73 out of 693 examined goats were positive to infect with *Haemonchus spp.* based on the floatation technique during the study period from October 2020 to July 2021 at an infection rate of (10.53%) as shown in table 1. The percentage of infection with *Haemonchus spp*. was associated with specific seasons. Moreover, the current study found that the highest percentage of *Haemonchus spp.* infection was 23.07% in October. While the lowest percentage of infection occurs in June (4.34%), as shown in table 1 ($P \leq 0.05$).

Table 2 also shows the prevalence rates and differences between age groups and sexes. In table 2, females had the highest percentage of infection with *Haemonchus spp*. at 14.24%. In contrast, males had a lower percentage of infection with *Haemonchus spp*. of 7.02%. Furthermore, this table revealed the highest percentage of *Haemonchus spp*. infection in the age group > 5 years of 14.01%. However, the current study found that goats aged 9 months to 2 years old had a lower percentage of infection, 4.76 % ($P \leq 0.05$).

The present study showed that the duration of hematologic changes was significantly different between infected and non-infected controls. The data indicated that the goats infected by Haemonchosis have a gradual decline in Hb concentration, pocket cell volume (PCV) and total erythrocyte count compared to healthy control animals. Furthermore, total leucocyte count, lymphocyte and neutrophil were significantly decreased ($P<0.05$) in infected goats post-infection. However, eosinophil count showed a significant increase ($P<0.05$) in the infected animals. In addition, Basophil count showed non-significant changes during the period post-infection compared to control, as shown in the table 3.

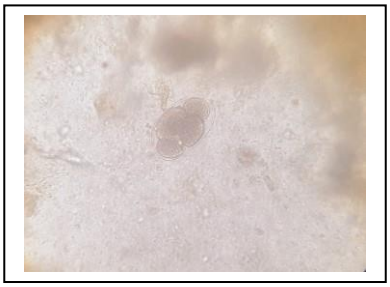

Figure 1. Eggs of Haemonchusspp unstained isolated from infected goat (40X)

Table 1. The percentage of Infection goats with *Haemonchus spp.* according to months or years

Months of year	No. of Goat examined	No. of infected Goat	Percentage %
October 2020	78	18	23.07%
November	73	13	17.80%
December	67	10	14.92%
January 2021	74	7	9.45%
February	67	4	6.25%
March	63	5	7.93%
April	70	4	5.71%
May	65	5	7.69%
June	69	3	4.34%
July	67	4	5.97%
Total	693	73	10.53%

Table 2. The percentage of Infection goats with *Haemonchus spp*. according to age and sex

	Age			Male			Female		
	Inspected	Infected	%	Inspected	Infected	%	Inspected	Infected	%
9monthes -2 years	168	8	4.76	112	2	1.78	56	6	10.71
3-4 years	211	21	9.95	124	8	6.45	87	13	14.94
> 5 years	314	44	14.01	120	15	12.5	194	29	14.94
Total	693	73	10.53	356	25	7.02	337	48	14.24

Table 3. The Changes of some hematological parameters due to Haemonchosisin goat

Haematological parameters	Groups	Mean± SE	*P*-Value
HB(gm/dl)	Control Infected	8.811±0.014* 7.226±0.086	0.024
PCV (%)	Control Infected	33.489±0.052* 26.761±0.232	0.005
TEC($\times 10^6/\mu L$)	Control Infected	21.434±0.041* 13.599±0.145	0.005
TLC	Control Infected	12.049±0.055* 6.831±0.130	0.044
Lymphocyte%	Control Infected	7.555±0.075* 4.180±0.071	0.217
Basophil (%)	Control Infected	0.113±0.012 NS 0.103±0.001	0.091
Neutrophil (%)	Control Infected	4.681±0.024* 1.872±0.014	0.040
Eosinophil (%)	Control Infected	0.111±0.003 0.195±0.003*	0.940
No. of animals	Control	20	NS=Non significant *= $P<0.005$

In addition, the biochemical results in table 4 showed a significant decrease (P<0.05) in total serum protein and albumin during the infection goat period. In contrast, active serum enzymes ALP, ALT, and AST showed significant increases (P<0.05) compared to healthy control animals, as shown in table 4.

The 73 faecal samples that were positive for haemonchosis by flotation technique were classified as *Haemonchus spp*. and were examined using PCR to confirm the diagnosis specie. In addition, the results of the present study showed that total DNA was successfully extracted and purified from eggs by use of a specific DNA extraction kit from QagainCompany. The whole genomic DNA was loaded on the agarose gel electrophoresis to check the quality and purity of the extracted DNA band. The PCR technique was done to diagnose and distinguish *H. controtus* in the infected goats. The result of PCR revealed that specific primers (HcI-F, HcI-R) successfully amplify the ITS-2 rDNA gene with 295bp-long fragment to *H. controtus*, as shown in figure 2. Also, the PCR result showed that 54(73.97%) out of 73 goats were positive for *H. controtus*.

Table 4. The Changes of some biochemical parameters due to Haemonchosis in goats

Biochemical parameters	Groups	Mean± SE	*P*-Value
ALP (IU/l)	Control Infected	182.280 ±0.204 219.021±0.586*	0.028
AST (IU/l)	Control Infected	128.537±0.144 188.180±0.480*	0.032
ALT (IU/l)	Control Infected	29.310±0.266 37.756±0.164*	0.045
Albumin (gm/dl)	Control Infected	5.025±0.031* 2.466±0.149	0.000
TSP (gm/dl)	Control Infected	8.777±0.042* 6.321±0.116	0.002
No. of animals	Control Infected	20 20	*= P<0.005

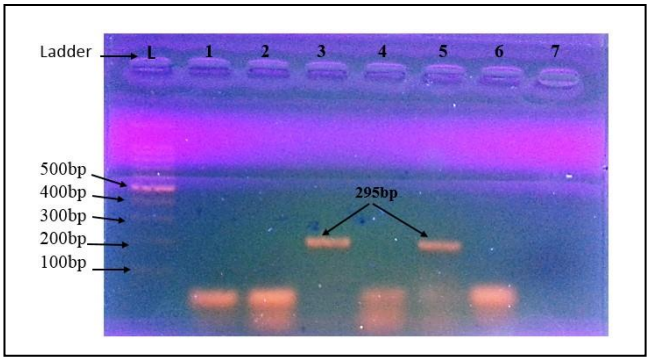

Figure 2. Agarose gel electrophoresis of amplified DNA of *H. controtus* by using primers (HcI-F, HcI-R) for amplifying the ITS-2 rDNA gene, Lane L, DNA ladder, Lanes 3,5 positive to 295 bp

4. Discussion

Haemonchosis is a severe health problem in terms of prevalence and pathogenicity; often, small ruminants, particularly sheep and goats, decline production due to high morbidity, mortality and cost of treatment and control programs (14). A few studies explained the influence of sex, age and seasons on the prevalence of Haemonchosis in goats. The result of the current study showed that the overall infection rate among 693 examined goats was 73(10.53%) in Iraq. These results agreed with previously published works (12, 15) and disagreement with several previous studies (5), which reported that the percentage of infection was 68% and 77.7%. Respectively. The variation in prevalence results between this study and others could be due to differences in animal management, nutrition, natural resistance, treatment programs, and local geo-climatic factors (1, 11). In addition, this study found that the highest rate of Haemonchosis infection occurred in October (autumn), with a rate of 23.07 %. While the lower percentage of infection was recorded in June (summer) at an infection rate of 4.34%. These findings were consistent with those of several previous studies (4, 16), but they differed from those of (2), who found that the highest percentage of infection was in the summer at 22.22 %. The explanation of this discrepancy is attributed to the characteristics of the climate, characterized by a favourable temperature and rainfall in autumn, ensuring the development and survival of pre-parasitic stages and leading to increased availability of infectious larvae in the study area. Furthermore, moisture is important for developing and surviving infective nematode stages on pasture. Summer, in contrast, is a low-risk season for infection because the heat and lack of moisture destroy eggs and larvae. Infections on pastures only resume in the autumn and with the first rains (4, 6, 11). Furthermore, the present study showed that the percentage of infection with Haemonchosis in females (14.24 %) is higher than males7.02 %. These results agreed with many studies Qamar and Maqbool (14) and disagreement with previous findings (5, 6) that reported the highest percentage of infection in males than females. The different susceptibility of haemonchosis between males and females is possibly due to the number of females over males in the herd and the length of their presence in the pasture due to reproductive stress and decreased immune status (16). Also, the study found that the highest percentage of Heamonchosis infection occurs in the age group > 5 years, 14.01%. Whereas the lower percentage was shown in the age group 9 months to 2 years old at 4.76 %. The findings were in agreement with many previous studies (16, 17) and discrepancies with (14) which recorded the higher infection rate in animals below 9 months of age. The difference in the distribution of infection among age groups is attributed to several factors, including grazing habits, management practices and breeding systems of the examined animals, anthelmintic utilized, phase of infection during the examination, and detection method and laboratory techniques used.

On the other hand, gastrointestinal nematodes reduce appetite and food digestibility, as well as the diversion of nutrients from production sites to tissue repair, resulting in changes in hematological and biochemical markers (18). The results of hematological studies have shown that goats infected with Haemonchosis have significant changes in hematological parameters, such as a decrease in Hb concentration, pocket cell volume, and total erythrocyte count, which may cause anemia in infected goats. These results were consistent with many previous studies (9, 19). The adult *H. contortus* may cause these changes in hematological parameters and can suck and leakage of blood from the site of attachment. Moreover, the decreasing hematological parameters in infected goats may be attributed to the bleeding of the abomasum due to the injuries (3).

Furthermore, this study found that total leucocyte count, lymphocyte and neutrophil was noticeably decreased in infected goats compared to non-infected animals. The results of this study are similar to (9, 19). These differences can be attributed to increased sequestration of white blood cells to the abomasum

where the worm is localized or may be due to the worm releasing substance stimulating the migration of neutrophil and lymphocyte cells from circulation or indicating immunodepression (19). In contrast, the results showed that eosinophil count significantly increased the infected animals. These findings are similar to that recorded by Terefe, Yacob (20). This increase may be due to cell phagocytic activity that digests suspended particles and parasite residues due to the cellular immune response (19, 21). In addition, the biochemical results in the present study showed a significant decrease in total serum protein and albumin during the infection goat period. These results were consistent with several previous studies (1, 19). These changes may be due to decreased food intake and protein loss in the gut during haemonchosis infection, which is responsible for protein-losing enteropathy.

Moreover, Blood loss, hemorrhagic gastritis, and protein leakage into the gastric lumen disrupted intercellular unions and increased gut permeability, resulting in decreased protein concentrations. Also, severe infection with *H. contortus* produces abomasum haemorrhage that leads to loss of albumin from the blood vessels. In contrast, the study showed significant increases in the activity of serum enzymes ALP, ALT, and AST in *H. contortus*-infected goats compared to healthy control animals. These results were in agreement with many studies (21). This elevation can be attributed to the damage of abomasal mucosa by *H. contortus* larvae that attacked the muscular layer of the abomasal mucosa, which leads to peptic ulcer and ulcerative colitis, to be responsible for enzyme release from affected mucosa. Also, variations in serum enzyme ALT and AST may be due to fourth-stage larvae approaching adulthood and with their well-equipped buccal cavity-causing traumatic damage to the lining of abomasal mucosa.

On the other hand, the most common and sensitive approach has recently been applied polymerase chain reaction (PCR) to detect and distinguish *H. contortus* in the infected goats (22). PCR results showed that specific primers (HcI-F, HcI-R) successfully amplify the ITS-2 rDNA gene with 295bp -long fragment to *H. controtus*. Also, the PCR result revealed that 54 (73.97%) out of 73 goats were positive for *H. controtus*. This result was consistent with (10, 23). The use of this technique in the detection and differentiation of *H. controtus* in the current study is attributed to the findings of previous studies, which have shown that diagnosing parasite species based on their morphological characteristics alone may give imprecise results for accurate identification due to marked variations within and between species; as a result, molecular techniques PCR have been used (24). In addition, the choice of these primer sets in the current study can be attributed to the consistency and stability of the ribosomal DNA gene's second internal transcribed spacer (ITS2) region, making them a prominent target for species variation. Furthermore, gene sequences from these locations have been developed for molecular phylogenetic studies (24).

Authors' Contribution

Study concept and design: H. J. J.
Acquisition of data: M. M. M. A.
Analysis and interpretation of data: N. A. A.
Drafting of the manuscript: Z. K. A.
Critical revision of the manuscript for important intellectual content: S. M. A.
Statistical analysis: A. N. K.
Administrative, technical, and material support: H. J. J.

Ethics

The study protocol of the study was approved by the ethics committee of the Al Muthanna University, Samawah, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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