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Current epidemiological and molecular patterns of haemonchosis in Cairo and Giza governorates, Egypt

N.M. Hassan[1], M.A. Helal[1], B.S. Elsawy[1], E.E. El Shanawany[1], N.M. Abu El-Ezz[1], H.A. Shalaby[1], A.H. Elnamaky[1], O.M. Kandil[1], M.S. Mohamed[1], H.M. Desouky[2] and E.B. Ata[1]

[1]Parasitology and Animal Diseases Department, [2]Animal Reproduction and Artificial Insemination Department, Veterinary Research Institute, National Research Centre, Dokki, Egypt

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Correspondence:
N.M. Hassan
nohamhassan555@yahoo.com

Abstract

This study explored current epidemiological and molecular patterns of *Haemonchus* species infecting sheep, particularly in Cairo and Giza governorates, Egypt. Mass screening of haemonchosis was implemented by detecting IgG using ELISA among living sheep reared by smallholder shepherds and via abomasal inspection for sheep at slaughterhouses. Molecular characterization of the adult *Haemonchus* worms was done through the Nuclear internal transcribed spacer-2 gene (ITS-2) and the mitochondrial cytochrome oxidase-1 gene (COX-1). The results indicated that haemonchosis was a seriously common parasitic infection among sheep, where the prevalence reached 70% for the living sheep and 37.1% for the slaughtered ones. A strong immune response in the abomasal mucosa was detected, with diffuse, multifocal infiltration of lymphocytes, plasma cells, macrophages, and eosinophils. Risk factors significantly linked to the high incidence were being 2 years or less, in autumn, of imported Sudanese breeds and living in a colder climate. The molecular screening revealed 100% identity of the tested worms as *H. contortus*, and no *H. placei* had existed. Based on the phylogenetic analysis, the Egyptian *Haemonchus* isolates of COX-1 sequences showed identity percent ranging from 94.85–99.37%, with previously GenBank recorded isolates from Egypt and Nigeria. However, the isolates of ITS-2 sequences from Egyptian *Haemonchus* revealed an identity percent of 98.29-99.78%, with those obtained from a small ruminant in Tanzania and a giraffe in Florida, USA. The current data highlighted the considerable risk of haemonchosis among sheep and revealed the importance of updating the epidemiological and molecular information to achieve a suitable preventive and control strategy.

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Introduction

Sheep production is one of the crucial livestock sectors worldwide (1,2). In Egypt, sheep are the most common small ruminants, with an estimated number of about 2.972 million heads raised for meat, milk, and wool. Most sheep breeds are indigenous, e.g., Rahmani, Ossimi, and Barki (3,4). Gastrointestinal nematodes (GINs) are actual threats to the health and productivity of livestock in temperate and tropical regions all over the world (5,6). Haemonchosis is among sheep's most serious parasitic diseases (7,8), caused mainly by *Haemonchus contortus* living in the abomasum. Being the most fertile GIN, it can lay up to 10,000 eggs per worm per day, which pass through feces and increase the likelihood of a widespread infection on pasture. In addition, it can suck the blood at a rate of 0.03 milliliters per worm per day, which

results in anemia and protein loss (9,10). Furthermore, its migration and feeding activity results in significant inflammatory alterations to the stomach tissue, which may lower the infected animals' capability to digest food and absorb nitrogen, organic matter, and energy in the diseased animals (11-13). Therefore, the infection among sheep caused substantial financial losses due to the increased susceptibility of animals to other infections, morbidities, cost of treatment, and high mortalities (14). Haemonchus placei is more adapted to cattle, but infections with species *H. contortus* and *H. placei* may occasionally occur in sheep and goats (15). *Haemonchus contortus* resistance to most anthelmintic medications is rapidly growing, causing significant health and economic problems (16). In addition, it is recognized by a high rate of gene flow across populations, conferring an opportunity to disseminate genes and awarding resistance to anthelmintics (17). Excessive dependence on synthetic drugs and improper dosing are among the main reasons for the emergence of anthelmintic resistance to all classes of anti-parasitic chemotherapeutics in ruminants (16). Multiple studies have recognized the negative effect of anthelmintic resistance on farming animals all over the world: in Europe (18), Africa (19), Asia (20), and the Americas (21,22). So, periodical assessment of the severity of the infection is essential due to the continuous variability of environmental and geographical parameters, population growth, and living conditions that change a parasite population's genetic makeup (23). Studying epidemiological patterns of *H. contortus* has been globally reported by Yin *et al.* (17) in China; Mannan *et al.* (24) in Bangladesh; Ndosi *et al.* (25) in Tanzania; Das *et al.* (26) in India; and Regassa *et al.* (6) in Ethiopia. Successful mass screening of parasitic infections among populations mostly relies upon coprological examination, postmortem investigation, and serological techniques using ELISA (27,28). However, they might have low sensitivity and cannot differentiate the circulating species/genotypes (29). The reliable identification of the helminths at the species level, the study of the phylogenetic relationships between the species, the understanding of their epizootiology, and the tracing of origins are considered the cornerstones for maintaining treatment efficacy and establishing efficient, eco-friendly alternative treatment, preventive, and control programs (30,31). Therefore, molecular tools were developed to detect the species specificity and intra-specific differences related to the geographical distribution (25). Mitochondrial DNA (mtDNA) has a higher substitution rate than nuclear DNA, so it is suitable for detecting differences between closely related ones (32). The internal transcribed spacer is the most conserved region, exhibiting high interspecific sequence divergence and intraspecific sequence homogeneity. *Haemonchus* species have two internal transcribed spacers, ITS-1 and ITS-2, known as the best markers for genetic studies at the species level (26).

Controlling haemonchosis is necessary for recouping losses since it adversely affects the small ruminant industry worldwide, including Egypt. To implement reliable parasite control, it is necessary to update a thorough understanding of the current epidemiological status impacting the spreading of disease. Despite numerous studies having been conducted on *H. contortus* infection in Egypt, there is a paucity of available data about its prevalence, molecular screening, and phylogeny in Cairo and Giza governorates. Therefore, this study aims to provide reliable epidemiological data regarding the current status of ovine haemonchosis, which may be instrumental in managing anthelmintic resistance, halting the spread of infection, and developing effective preventive and control strategies.

Materials and methods

Ethical approval

Ethical permission for the use of animals was approved by institutional guidelines of the National Research Centre's Animal Research Committee (13050425-1).

Studying area

The study was conducted in Giza governorate (29° 16′ N 29° 40′E/29.26° N 29.67°E), with geographical coordinates, latitude, and longitude of 30.0130557 and 31.2088526, and Cairo governorate (30°2′40″N 31°14′9″E), with geographical coordinates, latitude, and longitude of 30.0444196 and 31.2357116, in Egypt.

Study design, animals, and sampling

Thorough clinical inspection was fulfilled; sheep with signs of weakness, weight loss, inappetence, anemia, mucous membranes, rough coat, soft feces, or diarrhea were selected. The study was conducted on two groups of sheep; the first group included live sheep that were not exposed to approved treatment and were reared by small-scale smallholder shepherds, and the second one included sheep slaughtered at the abattoirs that were subjected to previous treatment using commercial local anthelmintics before slaughtering in their rearing farms. Data collected from the owners of slaughtered animals had reported the use of albendazole 1 mL 2.5%/ 5 kg BWt as a single dose for parasitic infection during rearing at 6- 6-month intervals. At the same time, there was a misuse of anti-parasitic chemotherapeutics among the live sheep in the form of inadequate dosing and a total absence of veterinary care in some cases. Moreover, sheep were housed in groups in well-ventilated, small farms. Fresh, clean tap water was available at all times. Sheep were fed on roughage stored either as hay or low-moisture, green, vegetative chopped food, and forage. A mass screening of haemonchosis using ELISA was carried out by collecting three hundred blood samples from the live sheep. A total of nine hundred thirty-two sheep were sampled at Elbasatine, Nahia, and Elmonieb abattoirs to study risk factors

associated with high incidence and abomasa histopathological alterations during the period extended from March 2023 to February 2024. The abattoirs were visited monthly for *Haemonchus* detection in the abomasa and collection of positive blood samples for *Haemonchus* (33). Before slaughtering, age, breed, sex, season, and climate were recorded at sampling time. The climate was divided according to the medium temperature of the different seasons of the study areas; the hot season includes Summer and Spring with an average temperature of about 93 F / 34 C, while the cooler one includes winter and autumn with an average temperature of about 77 F / 25. The number of inspected sheep could be categorized according to each criterion as follows: 582 and 350 for more than 2 years and less than 2 years, 500, 300, and 132 for Barki, Ossimi, and Sudanese breeds, 207 and 725 for female and male, 280, 240, 257, and 155 during Spring, Summer, Autumn, and Winter, and 520 and 412 in hot and cold climates, respectively.

Adult worms' recovery

Each abomasum was collected and delivered in a separate labeled plastic container in a cool box to the laboratory in the Parasitology and Animal Diseases Department, Veterinary Research Institute, National Research Centre. Abomas were opened from the greater curvature with careful removal of the ingesta. The worms of *Hamonchus* spp. were recovered from abomasa (33) and thoroughly washed using 0.15 M phosphate-buffered saline (PBS) at pH 7.2 and then kept at -20ºC in PBS until used for the preparation of crude antigen (34). The male worms were collected separately and kept in 70% ethyl alcohol at -20ºC until DNA extraction was performed (25).

Histopathological inspection

After the infected abomasa was inspected, 97 heavily infected abomasa out of 346 were handled. Tissue samples were taken from the fundic region of the abomasum, fixed in 10% neutral buffered formalin, dehydrated, cleared, and embedded in paraffin wax. Tissue sections at 4 µm were stained with H&E staining and examined microscopically for histopathological changes (35).

Sera preparation

The serum samples were collected after centrifugation, then aliquoted and stored at −20 °C till being utilized. Blood samples from one-day-old newborn lambs were collected and used to prepare negative sera. Positive sera for *Haemonchus* were prepared from the blood samples collected from the sheep that were confirmed to be infected after slaughtering at the abattoir.

Antigen

Crude somatic antigen was prepared from collected *Haemonchus* worms according to the procedure of Hassan *et al.* (36). The supernatant soluble antigen extract was assembled, and the protein content was measured by Peterson (37). The antigen was aliquoted and kept at -20 °C till further use. The antigen was aliquoted and kept at -20 °C till further use.

Indirect-Enzyme-linked immunosorbent assay

Indirect ELISA was utilized to assign antibodies against haemonchosis in sheep. Before use, checkerboard titrations assigned the working dilutions of the conjugate, antigen, and test sera. ELISA was performed according to Hassan *et al.* (34). The cutoff point of optical density values was assigned, as reported by Hegazi *et al.* (38).

DNA extraction and amplification

The genomic DNA extraction was carried out for the individual male *Haemonchus* adult worms according to the instruction manual of the GeneJET Purification Kit (Thermo Fisher Scientific®). Each sample was eluted in 40 µL of Tris-EDTA (TE) elution buffer. The total DNA concentration and the purity were measured spectrophotometrically, and the DNA was preserved at -20 °C for further processing. For differentiation between *H. contortus* and *H. placei*, a set of species-specific primer pairs was used, including HpL BotuF and HpL BotuR for *H. placei.* While Hcon BotuF1 and Hcon BotuR2 were utilized for *H. contortus* detection, table 1 (39). For confirmatory characterization and phylogenetic analysis, different primers, including *Haemonchus* JB3 and *Haemonchus* JB4.5 (based on the mitochondrial cox-1 gene) and Haemonchus NC2 and *Haemonchus* NC5 (based on the ITS-2 gene), were used (Table 1) (25). The mixture for each reaction was 2x master mix buffer, 10 pmol from each sense and antisense primer specific for each reaction, 100 ng from the extracted DNA, and nuclease-free water to complete the reaction. PCR products were visualized by electrophoresis in stained 1.5% agarose gels.

Sequencing and phylogenetic analysis

Samples of the amplified cox1 and ITS-2 genes were purified from the gel using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific®). The DNA was measured, and part was tested on gel while the rest was sent for sequencing. The obtained sequences were analyzed and aligned using the free online tool BioEdit 7.2 (https://bioedit.software.informer.com/7.2/). The free Blast tool of the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to detect regions similar to the biological sequences. The evolutionary history was done using the Maximum Likelihood method based on the Tamura Nei model (40). The phylogenetic analysis of the COX-1 gene involved 26 nucleotide sequences. At the same time, analysis of the ITS-2 gene involved 22 nucleotide sequences, including *Babesia vogeli* regions as an out-group. All positions containing gaps and missing data were removed. Evolutionary analyses were carried out using MEGA7 (41). The confidence level of the tree was assessed by bootstrapping using 1000 replicates.

Table 1: List of primers utilized in the current study

Parasite	Primer name	Sequences (5′- 3′)	Annealing (°C)	Size (bp)	Reference
H. placei	HpL BotuF HpL BotuR	CCAGACCCGAGACTCGCC CTGAAGGTAATGTCAAAATTTCT	58.5	459	(40)
H. contortus	Hcon BotuF1 Hcon BotuR2	TGTCGAACACGAAACTCGTC TGTGTCTCTACCGCCCGAGT	59	260	(40)
COX1	Haemonchus JB3 Haemonchus JB4.5	TTTTTTGGGCATCCTGAGGTTTAT TAAAGAAAGAACATAATGAAAATG	55	317	(25)
ITS2	Haemonchus NC2 Haemonchus NC5	TTA GTT TCT TTT CCT CCG CT GTAGGTGAACCTGCGGAAGGATCATT	58	520	(25)

V-Statistical analysis

The prevalence was presented as a percentage of the number of animals infected in the total number of animals examined. Data were summarized by descriptive statistics for the overall prevalence in sheep. A Chi-square test was utilized to investigate the monthly and seasonal infection rates. A Chi-square test was performed to assess the association of haemonchosis with age, sex, breed of tested sheep, and season and climate at the time of the sample collection. Variables were significant at $P \leq 0.05$. ORs (odds ratios) were reported to compare risks. All statistics were analyzed, and graphs were plotted using GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA, USA).

Results

Prevalence of haemonchosis

Detection of circulating anti-*Hamonchus* IgG antibodies revealed that (210/300) 70% of the live sheep was seropositive (Figure 1).

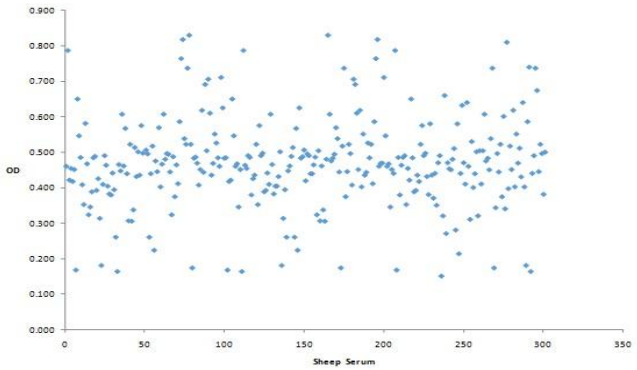

Figure 1: Sero-prevalence of haemonchosis is detected by detecting circulating IgG antibodies in live sheep reared in Cairo and Giza Governorate, Egypt, using indirect ELISA. The cutoff value for seropositivity was 0.43.

The prevalence of haemonchosis was determined by inspecting the abomasa of the slaughtered sheep over the period extending from March 2023 to February 2024 at the Elbasatine, Nahia, and Elmonieb abattoirs, which was (346/932) 37.1%. Monthly prevalence of *Haemonchus* spp. revealed that sheep were highly infected in October at (45/77) 58.4%, while the infection rate was the lowest in February at (8/50) 16% ($\chi^2 = 50.606$ and *p-value* < 0.001), as demonstrated in figure 2. Moreover, the prevalence was the highest in autumn (135/257), 52.2% ($\chi2=9.342$ and *P*< 0.0001), while Spring recorded the lowest infectivity (81/280), 28.9% (Table 2). The prevalence was prominent in the colder climate, 43.9% ($\chi^2=1.895$ and P-value < 0.0001), in contrast to the hotter one, 31.7%.

The age of animals played a significant role in the infection of haemonchosis in the sheep. The results showed that the infection rate of young sheep (less than 2 years) was 49.4%, which was more than the older ones (more than 2 years) at 29.7% ($\chi^2=4.569$ and *P*<Value 0.0001), respectively. Meanwhile, the sex of the animals had no significant effect on the spread of infection among sheep (Table 2). It was found that the infection rate varied according to the breed of sheep. The prevalence of heamonchosis among breeds of Barki, Ossimi, and imported Sudanese sheep was (149/500) 29.85%, (119/300) 39.6%, and (78/132) 59%, respectively. The imported Sudanese sheep ($\chi2 = 10.093$ and P-value < 0.001) were the most affected by *Haemonchus* spp., while Barki was the least affected. The association between haemonchosis infection and risk factors related to age, sex, season, and sheep breed is illustrated in table 2 and figure 3. The odds ratio and 95% CI were calculated. It was found that young age (OR: 1.4559-95% CI: 1.1398 to 1.8596), autumn season (OR: 2.4334-95% CI: 1.8136 to 3.2650), colder climate (OR: 1.7782-95% CI: 1.3575 to 2.3292), and imported Sudanese breed (OR: 1.7619-95% CI: 1.2338 to 2.5160) are considered risk factors for infection of haemonchosis.

Abomasal macroscopic findings

Figure 4 illustrates the macroscopic examination of the infected abomasa. The examination revealed the presence of small blood clots and mucus in the ingested food. *Haemonchus* worms were found to be partially embedded in the mucosal membrane and partially submerged in the ingested food. The mucosal membrane showed edema and scattered white focal nodules. It was noted that mucosal folds thickened in the presence of petechial hemorrhages.

Table 2: The strength of the association between studied risk factors and infection status according to abomasal examination.

Variable		Total/ infected	Prevalence %	χ 2	OR	(95%CI)	*p*- Value
Age	<2 years	350/173	49.4	4.569	1.4559	1.1398 to 1.8596	0.0026
	>2 years	582/173	29.7		0.5728	0.4328 to 0.7581	0.0001
Gender	Male	725/268	36.9	0.013	0.9699	0.7050 to 1.3343	0.8509
	Female	207/78	37.6		1.0427	0.7577 to 1.4349	0.7972
Climate	Hot	520/165	31.7	1.895	0.5624	0.4293 to 0.7366	*P* < 0.0001
	Cold	412/181	43.9		1.7782	1.3575 to 2.3292	*P* < 0.0001
Breed	Barki	500/149	29.8	10.093	1.1771	0.8763 to 1.5811	0.2788
	Ossimi	300/119	39.6		2.0966	1.5449 to 2.8453	*P* < 0.0001
	Sudanese	132/78	59		1.7619	1.2338 to 2.5160	0.0018
Season	Spring	280/81	28.9	9.342	0.5944	0.4395 to 0.8039	0.0007
	Summer	240/84	35		0.8837	0.6504 to 1.2007	0.4293
	Autumn	257/135	52.2		2.4334	1.8136 to 3.2650	*P* < 0.0001
	Winter	155/46	29.6		0.6710	0.4618 to 0.9750	0.0364

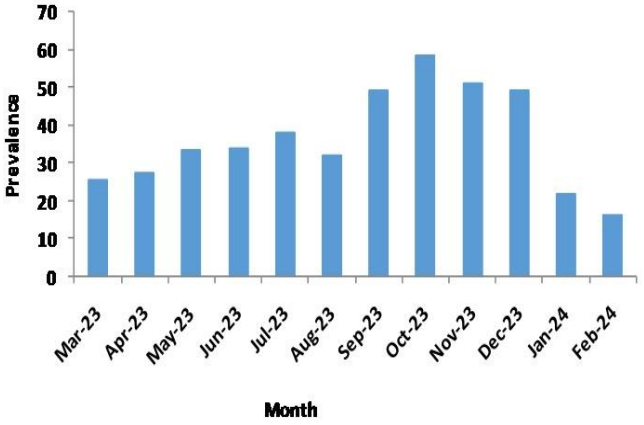

Figure 2: Monthly prevalence of *Haemonchus* spp. among sheep through abomasal inspection.

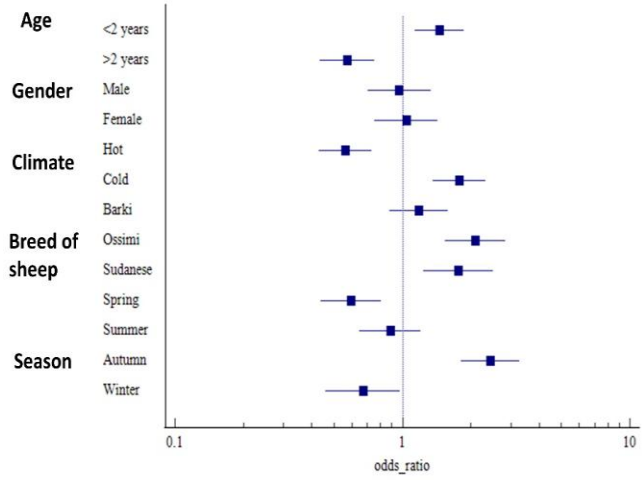

Figure 3: Prevalence rates and adjusted odds ratios (OR) with 95% confidence intervals (CIs) for infection with haemonchosis.

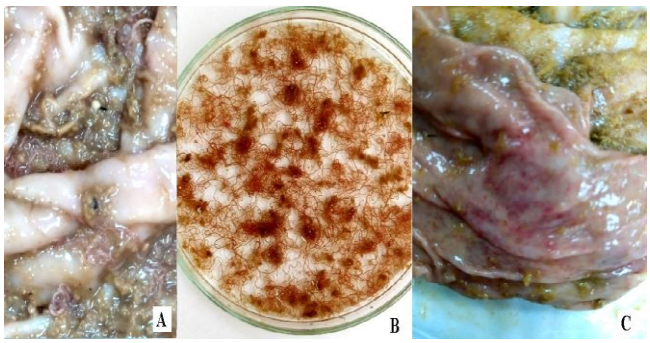

Figure 4: A. Abomasa of heavily infected sheep with haemonchosis contained hundreds of *Haemonchus* worms mixed with food ingesta. B. Collected *Haemonchus* worms. C. Abomasa showed a hemorrhagic, red, spotty mucous membrane due to hamonchosis.

Histopathological alterations

The microscopical examination of the fundus part of the abomasum is illustrated in figure 5. The examination revealed severe necrosis and desquamation of mucosal surface epithelium lining into the lumen associated with necrosis of gastric pits and neck. The epithelium lining of the abomasal glands showed degenerative and necrotic changes. In some areas, the destructed and atrophied glands were replaced by masses of inflammatory cells. Moreover, there was diffuse and massive multifocal inflammatory cell infiltration associated with interstitial edema in the lamina propria of abomasal mucosa, mainly in the deeper part, and the infiltrates were predominantly composed of mononuclear cells (lymphocytes, plasma cells, and macrophages) and eosinophils. In some cases, marked thickening of abomasal mucosa due to hyperplasia of dilated gastric glands was seen. In addition, mild to moderate mucosal and submucosal edema was observed. The blood vessels and lymphatics appeared relatively dilated.

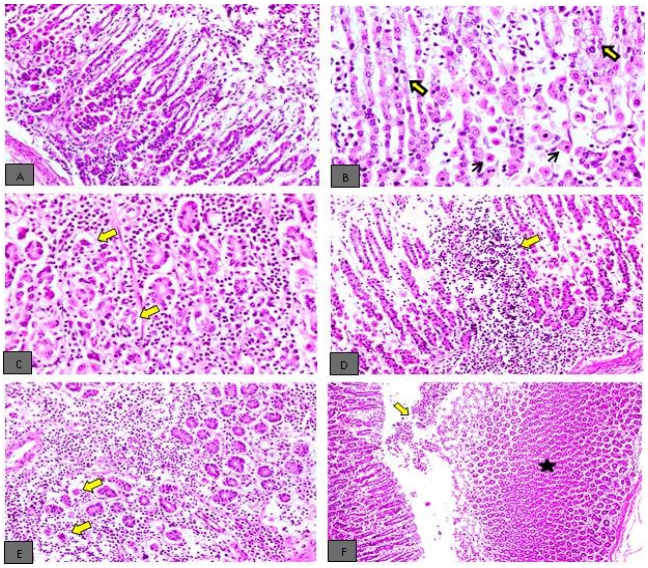

Figure 5: Abomasum infected with *H. contortus* (A): showing severe necrosis and sloughing of surface epithelium lining and gastric pits and neck into the lumen, in addition to degenerative and necrotic changes of the epithelium lining of gastric glands (H&E, X100). (B): Mucous degenerative and necrotic changes of epithelium lining of gastric glands at the neck and body parts, moderate inflammatory cell infiltration. Note parietal cells with profoundly eosinophilic cytoplasm and pyknotic nuclei (black arrows) and vacuolation of mucous cells (yellow arrows) (H&E, X200). (C): showing degenerative and necrotic changes of epithelium lining of gastric glands (yellow arrows) at the base part associated with heavy peri-glandular aggregations of mononuclear inflammatory cells and eosinophils in the deeper part of the lamina propria of the mucosa, with replacement of necrotic gastric glands by inflammatory cells (H&E, X200). (D): showing intensive focal aggregations of mononuclear inflammatory cells and eosinophils (yellow arrows), as well as interstitial edema in between the degenerated gastric glands and deeper part of the lamina propria of the mucosa ((H&E, X100). (E): showing massive diffuse infiltration of mononuclear inflammatory cells and eosinophils in the deeper part of the lamina propria of the mucosa, interstitial edema, and replacement of degenerated and atrophied gastric glands (yellow arrows) by masses of inflammatory cells (H&E, X100). (F): showing prominent hyperplasia of gastric glands (star), necrosis, and desquamation of surface epithelium lining into the lumen (yellow arrows). (H&E, X40).

Molecular identification of *Haemonchus* spp.

The molecular identification of the obtained worms using 2 different sets of specific primers for *H. contortus* and *H. placei* for each DNA sample extract revealed that all the adult males were classified under the *H. contortus* species, and no *H. placei* worms were identified.

Phylogenetic analysis

The obtained *H. contortus* was identified by partially amplifying the COX-1 gene and the ITS-2 region. The obtained sequences of the COX-1 gene were uploaded to the Gene Bank with the accession numbers (PP590303, PP590304, PP590305, PP590306), while those of the ITS region were (PP590638, PP590639, PP590640, and PP590641). Phylogenetic analysis based on the COX-1 gene revealed that the locally obtained isolates were related to each other and classified under the same clade or cluster of the *H. contortus* species. The nucleotide identities between the *H. contortus* species were revealed by a percentage composition of 94.85–99.37%. The obtained isolates were closely related to those previously detected in Egypt (AB682706. and AB682707.1) and those recently isolated in Nigeria during 2021 (OL435361.1 and OL435444.1), as shown in figure 6. On the same side, analysis based on the ITS-2 confirmed the previously obtained results that our detected isolates were *H. contortus*, as the identity percentage with the previously recorded ones was 98.29-99.78%. The obtained isolates were closely related to those obtained from a small ruminant in Tanzania during 2021 (OK181226.1) and those obtained from a giraffe in Florida, USA (EU086389.1), as illustrated in figure 7.

Figure 6: The phylogenetic analysis of the COX-1 gene created by MEGA 7 software. The black circles indicate the sequences obtained in this study.

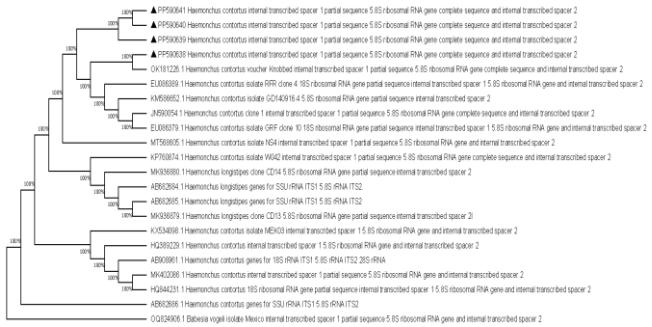

Figure 7: The phylogenetic analysis of the ITS-2 gene created by MEGA 7 software. The black triangles indicate the sequences obtained in the current study.

Discussion

Haemonchosis is one of the threats to sheep health and production worldwide (16). The study revealed that *H. contortus* infection is widespread among sheep across Egypt, particularly in Giza and Cairo governorates. The results showed a high seroprevalence of haemonchosis, 70% among the living sheep reared by the shepherds. These results are similar to those obtained by Mir *et al.* (42) in India, who found that 72.22% of sheep were seropositive using crude somatic antigens. Moreover, Hassan *et al.* (43) in Beni Suef, Egypt, revealed 64.48% seropositivity for *H. contortus* using larval antigen. While Gowda (27) in India, it was observed that 58.66% of the migratory sheep were seropositive for *H. contortus* using somatic antigen. These variations might be correlated to the type of antigen and its preparation method, the immunity level of animals, and the number of screened sheep. Moreover, the results disclosed that the prevalence of haemonchosis was 37.1% (346/932) through abomasal examination of the slaughtered sheep at the abattoirs. Variable incidences of *Haemonchus* infection were recorded at slaughterhouses by Abdo *et al.* (44) in Ethiopia at 69.6%, Mannan *et al.* (24) in Bangladesh at 51.3%, and Regassa *et al.* (6) in Ethiopia at 34.40%. Geographical differences, management care, and prevention and control measures between countries might impact the prevalence of diseases among animals. The current study showed a variable incidence of haemonchosis in both live sheep that were not subject to adequate authorized treatments or care and the slaughtered sheep that had been provided with treatment regimes. This might indicate the danger of the misuse of anthelmintics and the possibility of the emergence of anthelmintic resistance among worm populations and decreasing drug efficacy (45,46).

It is evident that the infection of haemonchosis significantly increased during the autumn season at 52.2%, where it reached 58.4% in October. This was reflected in the infectivity during the colder climate in Cairo and Giza Governorates, where the infection reached the highest level compared to the summer season (p-value <0.001). These results might coincide with those Bentounsi and Cabaret (47) reported, who mentioned that mild or cool semiarid climatic conditions are the most favorable for strongyles infection. Similarly, Mushonga *et al.* (48) noticed the high prevalence of *H. contortus* among sheep in October. Meanwhile, Brik *et al.* (49) found that the highest infection rate was during Spring (36.36%), followed by autumn (32.37%), while a low rate of 2.7% was observed in the summer. It was stated that the prevalence of different nematode species was related to the change in climate at different ecologies (50-52).

The current findings verified that an animal's age was a significant factor in the spread of sheep haemonchosis. In contrast to the older sheep (>2 years), the infectivity of the younger sheep (<2 years) was noteworthy, reaching 49.4 and 29.7%, respectively. These findings concurred with those of Dagnachew *et al.* (53), Brik *et al.* (49), and Qamar *et al.* (54). That could be because younger sheep have less immunity than older sheep. In addition, it is possible that young sheep being fed on grasslands, particularly in their first year, exposed them to infections (55-58).

The current findings made it clear that the animals' sex had no discernible impact on the infection's ability to spread among sheep. This was consistent with the data obtained by Qamar *et al.* (54) and Razzaq *et al.* (59). The findings were in conflict with those of Fentahun and Luke (60), who showed a prevalence of 77.1% in males and 80.9% in females, as well as those of Brik *et al.* (49), who claimed that females are more prone to parasitism. The hormone variations between animals may impact an animal's susceptibility to parasites. Males are more vulnerable to infection than females because of androgen hormones. In contrast, females may be more resistant because of the effects of estrogen, according to research by Urquhart *et al.* (61). In contrast, Raza *et al.* (62) stated a higher prevalence in females due to lower resistance during reproductive events and unbalanced feed intake.

It was found that the occurrence of haemonchosis is significantly influenced by the breed of sheep. In this instance, the infection rates for Barki, Ossimi, and imported Sudanese sheep are 29.85, 39.6, and 59%, respectively. According to McManus *et al.* (63), a sheep's breed may impact its susceptibility to *Haemonchus* infection. This could be caused by genetic variables and the host's tolerance to infection in addition to the variety of *Haemonchus* spp. in the various breeds and nations.

Migration and development of *Haemonchus* in the lining mucosa of the abomasa and its feeding habits can cause severe histopathological changes. These damages enhanced the infiltration of immune cells into abomasal lining tissue (12). The present findings disclosed great damage to the infected abomasal mucosa, including diffuse and massive multifocal inflammatory cell infiltration associated with interstitial edema in the lamina propria of abomasal mucosa, mainly in the deeper part, and the infiltrates were predominantly composed of mononuclear cells (lymphocytes, plasma cells, and macrophages) and eosinophils; hyperplasia of dilated gastric glands; and dilatation of blood vessels and lymphatics. Besides, degenerative and necrotic changes in gastric glands were more notable. Similarly, Simpson (64) and Stear *et al.* (65) found that haemonchosis induced superficial damage to epithelial cells, hypertrophy of the mucosa, and edema, followed by the formation of lymphoid aggregates and the infiltration of inflammatory cells in response to tissue damage. This damage might lead to destructive dystrophy of the gastric mucosa of infected sheep, causing severe health illness, including malfunction of the digestive system, impaired growth, and weight loss.

While electron microscopy is a benchmark technique for confirming the worm, and parasitological examination is the

golden standard for identifying Haemonchus species, both procedures are time-consuming, labor-intensive, and occasionally insensitive (66). Nucleic acid amplification and sequencing are significantly more effective and valuable tools for identifying almost all infectious disease pathogens of veterinary concern (67-70).

The PCR technique can detect and discriminate *H. contortus* infections from those caused by other trichostrongylids (71). The primer set used in this study was characterized by high sensitivity and specificity to differentiate between *H. contortus* and *placei* (40). The obtained results confirmed the presence of *H. contortus* only. The same results were obtained in different studies, as this type was predominant in small ruminants, while the *H. placei* type was the most common in cattle (72). At the same time, Gareh *et al.* (15) exhibited the existence of *H. placei* and *H. longistipes* in goats in Assiut Governorate, Upper Egypt, Egypt. It is important to note the presence of a clear correlation between the grazing pasture with the management system and the infection type with the *Hemonchus* spp. as previously recorded (30).

For more confirmation of the obtained results, different sets of primers based on the ITS2 and COX-1 genes were used to partially amplify the corresponding genes. The alignment and the phylogenetic analysis of the obtained sequences confirmed the obtained *H. contortus* species. The homology between the obtained isolates was above 99.5%. Studies on the population genetics of *H. contortus* have revealed that this species has minimal genetic differentiation within continuous geographical regions and substantial population variance within populations, which is probably due to high gene flow caused by host movements (73,74). Nonetheless, evidence for the global gene flow has been reported, and these are related to the parasite's limited capacity for dissemination and the host's limited ability to move across continents (23). Molecular taxonomy techniques have been utilized for polymorphism research in recent years, and accordingly, the *H. contortus* and *H. placei* were more closely related (75). In a comparative analysis of the second internal transcribed spacer (ITS2) sites in *H. contortus* and *H. placei*, Stevenson *et al.* (76) found just three variations in the ITS2 nucleotide sequences. To summarize, the ITS region is appropriate for molecular diagnostic purposes of *Haemonchus* species and across a diverse range of trichostrongylid nematodes (77).

Nematodes with direct life cycles and high infection rates, such as *Haemonchus*, combine to produce a huge effective population size and a wide genetic variety (78). The genetic diversity parameters between *Haemonchus* species may be attributable to variances in their prolificacy, prepatent period, host predominance, and evolutionary rate (79). Because the genome of mitochondrial DNA substitutes more frequently than that of nuclear DNA, variations between closely related individual parasites can be reconciled (80-82). Variations found in the ND4 gene (17,23,71,80,81) and COX-1 gene (72,82) were the basis for most genetic diversity studies for *Haemonchus* populations previously reported from different countries. The current investigations indicated a high rate of gene flow among *Haemonchus* parasites infecting livestock in Egypt with 100% homology between COX-1 sequences and clustering of haplotypes coming from the specific host. The results provided agreed with those of Brasil *et al.* (83), who found that populations of *Haemonchus* spp. had substantial rates of gene flow among Brazilian sheep. The demographic structure was further clarified by comparing the COX-1 haplotypes of *Haemonchus* isolates from Egypt with those from other countries. The *Haemonchus* isolates from Egypt (AB682706.1 and AB682707.1) and Nigeria (OL435361.1 and OL435444.1) had the highest level of identity with our isolates. The observed clustering pattern could be supported by the possibility of host movement across African regions or evolutionarily comparable ancestors in both countries.

Conclusion

The study concluded that *H. contortus* infection is prevalent among sheep across Egypt, particularly in Cairo and Giza Governorate. The high incidence of *H. contortus* is significantly associated with age, breed of sheep, season, and climate. The disease is considered a life threat, causing severe destructive damage and necrosis of the lining of the abomasal mucosa. Phylogenetic analysis reveals a close association with Nigeria, Tanzania, and USA *H. contortus* isolates. Strict preventive and control measures during the importing sheep breeds and accurate use of anthelmintic treatment should be considered to combat the disease.

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Conflict of interest

The authors declare no competing interests.

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الأنماط الوبائية والجزيئية الحالية لداء المنقوسات الدموية في الأغنام المصرية بمحافظتي القاهرة والجيزة، مصر

نوهى محمود فهمي حسن[١]، محمد عبد المغني هلال[١]، بسمة سعيد محمد الصاوي[١]، إيمان الدسوقي الشناواني[١]، نادية محمد طلعت أبو العز[١]، حاتم عبد الموجود شلبي[١]، أميرة حسن النمكي[١]، أمنية محمد قنديل[١]، منى سعيد محمود محمد[١]، حسن محمد دسوقي[٢] و عماد بشير عطا[١]

[١]قسم الطفيليات وأمراض الحيوان، [٢]قسم التكاثر الحيواني والتلقيح الاصطناعي، البحوث البيطرية، المركز القومي للبحوث، الدقي، مصر

الخلاصة

سعت هذه الدراسة إلى استكشاف الأنماط الوبائية والجزيئية الحالية لأنواع ديدان المنقوسات الدموية التي تصيب الأغنام خاصة في محافظتي القاهرة والجيزة، مصر. تم تنفيذ فحص شامل لداء المنقوسات الدموية من خلال الكشف عن الكلوبولين المناعي ج باستخدام اختبار تفاعل الأنزيم المناعي الممتز بين الأغنام الحية التي يربيها الرعاة أصحاب الحيازات الصغيرة، ومن خلال فحص المنفحة للأغنام في المسالخ. تم إجراء التوصيف الجزيئي لديدان المنقوسات الدموية البالغة من خلال الجين النووي الداخلي المنتسخ فاصل-٢ وجين الميتوكوندريا السيتوكروم أوكسيديز-١. أشارت النتائج إلى أن داء المنقوسات الدموية هو عدوى طفيلية شائعة بشكل خطير بين الأغنام حيث بلغت نسبة الإصابة به ٧٠% في الأغنام الحية و٣٧,١% في الأغنام المذبوحة. مع وجود تغيرات التهابية وتنكسية هائلة في بطانة الغدد المعدية. حيث تم الكشف عن استجابة مناعية قوية في الغشاء المخاطي للمعدة الحقيقية، مع تسلل منتشر ومتعدد البؤر للخلايا الليمفاوية وخلايا البلازما والبلاعم والحمضات. وقد تم اكتشاف أن عوامل الخطر التي ارتبطت بشكل كبير بارتفاع معدل الإصابة هي: كون الحيوانات تبلغ من العمر عامين أو أقل، وخلال فصل الخريف، وأن يكون من سلالة سودانية مستوردة وزيادة العدوى في المناخ الأكثر برودة. كشف الفحص الجزيئي عن هوية ١٠٠% للديدان التي تم اختبارها على أنها المنقوسات الدموية، ولم يكن هناك وجود الى *H. placei*. استنادا إلى التحليل التطوري، أظهرت عزلات المنقوسات الدموية المصرية من تسلسلات وجين الميتوكوندريا السيتوكروم أوكسيديز-١ أن نسبة الهوية تراوحت بين ٩٤,٨٥-٩٩,٣٧% مع العزلات المسجلة مسبقا في بنك الجينات من مصر ونيجيريا. ومع ذلك، كشفت عزلات تسلسل الجين النووي الداخلي المنتسخ فاصل-٢ من ديدان المنقوسات الدموية المصري عن هوية بنسبة ٩٨,٢٩-٩٩,٧٨% مع تلك التي تم الحصول عليها من مجتر صغير في تنزانيا وزرافة في فلوريدا، الولايات المتحدة الأمريكية. وتسلط البيانات الحالية الضوء على الخطر الكبير لمرض المنقوسات الدموية بين قطاع الأغنام في مصر، كما كشفت الدراسة عن أهمية تحديث المعلومات الوبائية والجزيئية لتحقيق استراتيجية الوقاية والسيطرة المناسبة.

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