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Prevalence of gastrointestinal parasites and phenotypic and genotypic assessment of albendazole resistance in *Haemonchus contortus* isolates from naturally infected sheep

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Abstract

This study explored albendazole resistance in *Haemonchus contortus* among naturally infected Egyptian sheep in Giza province. Three hundred sheep raised in a private farm were employed over a period extending from June 2024 to November 2024. Coprological examination was applied to whole animals. A fecal egg count reduction test was performed on two groups of heavily infected sheep: a control group and a treated group receiving 5 mg/kg albendazole 2.5%. Egg hatching inhibition test was conducted, using ten concentrations, 0.012 to 6.25 µg/ml of 2.5% albendazole. Allele-specific PCR was used to genotype H. contortus adults using an amplified fragment of the β-tubulin isotype 1 gene containing residue 200. The results revealed that 57.7% of sheep were infected with gastrointestinal parasites, with 80%, 45%, and 8% infected with *Eimeria* spp., Strongyles, and *Strongyloides papillosus*, respectively. The most prevalent Strongyle was *Haemonchus* spp. 65%, followed by *Trichostrongylus* spp. 25% and *Ostertagia spp.* 10%. The fecal egg count reduction was less than 95% at 3, 7, 15, and 30 days post-treatment: 90%, 87.46%, 83%, and 70%, respectively. The LC_{50} was higher than 0.1 µg/mL for albendazole. The genotypic results showed that heterozygous alleles were present in 54% of the samples. In comparison, homozygous susceptible alleles were detected in 18 isolates (46%), and no homozygous resistant alleles were found. The susceptible (S) allele frequency was 73%, whereas the resistant (R) allele frequency was 27%. This study emphasized the importance of periodic monitoring of albendazole resistance and a better understanding of its molecular mechanisms to maintain drug efficacy.

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Introduction

Sheep are among the most precious food animals essential for maintaining a country's food security worldwide (1,2). Haemonchosis represents a significant pathogenic parasitic threat to livestock, particularly in temperate and subtropical regions (3-5). The infection is primarily caused by the common stomach Trichostrongylid hematophagous nematode, Haemonchus contortus, leading to substantial financial losses estimated at tens of billions of dollars annually (6-9). This parasite's blood-sucking behavior causes significant blood loss, leading to anemia and hypoproteinemia. Furthermore, the infection diminishes digestive capacity, affecting energy and nutrient absorption. In severe cases of disease among young animals, sudden mortalities may occur (10). The primary method of controlling haemonchosis is through the use of chemotherapy with anthelmintics (11). The effectiveness of the drug may be significantly reduced, and the parasite may develop resistance to anthelmintics due to the widespread

and improper use of antiparasitic compounds (12). Anthelmintic resistance (AR) is recognized as a heritable trait that facilitates the transmission of anthelmintic insensitivity to subsequent generations of the worm population. The use of anthelmintics, the cost of assessing the effects of antiparasitic chemotherapeutics, and the high costs of employing the new generations of anthelmintics all lead to direct production losses in the form of subclinical sickness and severe symptoms (13,14). On a global scale, the AR has significant economic impacts on various animals, particularly small ruminants (15,16). Additionally, it poses adverse environmental and food safety effects (17). Multiple studies have emerged to identify factors related to AR and the optimal approaches to its management (18). Around the world, livestock producers in low-income nations rely on benzimidazoles, particularly albendazole. Whether or not a parasite infection is present, they commonly use it as part of antiparasitic preventive treatments (19,20). Albendazole works by binding to beta-tubulin and inhibiting microtubule synthesis, disrupting cell division and the parasite's overall metabolism (21). Isotype 1 "β-tubulin" gene mutations, including single-nucleotide polymorphisms (SNPs) at codons 167, 198, or 200, are associated with benimidazole resistance. Furthermore, it has been discovered that H. contortus can enhance drug biotransformation in helminths, which is one of the mechanisms of drug resistance (22). The primary methods for tracking albendazole susceptibility and/or resistance are fecal egg count reduction tests (FECRTs), egg hatch assays (EHAs), and polymerase chain reaction (23-26). Albendazole is widely used as an antiparasitic among Egyptian farmers in rural agricultural areas and on smallholder farms. This investigation is driven by the recent and ongoing increase in the prevalence of Haemonchus spp. among livestock in Egypt over the past few years, despite the continued use of chemotherapeutics (6,27,28).

In Giza province, Egypt, no previous study has focused on resistance to albendazole. This study aimed to investigate the current prevalence of haemonchosis in Egyptian sheep and to monitor the resistance of H. contortus to albendazole through phenotypic and genotypic analysis.

Materials and methods

Ethical approval

Ethical permission for the use of animals was approved under the National Research Centre's Animal Research Committee, Institutional Guidelines, number 13050425.

Study design and animals

This study was conducted on 300 sheep of both sexes, aged 6 months to 2 years, over 6 months from June 2024 to November 2024. The animals were raised by a small livestock holder. They were found to be separated into 4 groups of 75 animals each at a private farm in Giza province, Egypt (29° 16′ N 29° 40′E/29.26° N 29.67°E). Sheep under the experiment followed the same feeding and treatment regimes. They were provided with a balanced ration, green forages, hay, and free access to clean tap water. The farm owner had agreed to participate in the experiment. The main complaint from the farm owner was the low weight gain. The farm did not engage in a well-established de-worming routine, but they were subjected to oral de-worming every 6 months as a prophylactic treatment, with no comprehensive veterinary care. The most commonly utilized anthelmintic was albendazole 2.5%. No anthelmintics were given to sheep for the three months preceding the experiment.

Coprological examination

Whole sheep were parasitologically examined using the flotation concentration technique with a salt-saturated solution to detect gastrointestinal parasites according to Urquhart *et al.* (29) as follows: Five to ten grams of feces per animal were collected by rectal palpation using gloves. Each sample was placed in a labeled plastic container with a lid and sent to the laboratory in a cool box for additional processing. The degree of infection was determined by fecal egg count using the McMaster technique in strongyle-infected sheep. According to Rizwan (30), a low infection was defined as 100 to 800 eggs per gram (EPG), a moderate infection as 801 to 1200 EPG, and a high infection as more than 1200 EPG. Fecal culture was performed separately for each positive sample, as described by Radostits (31). The most prevalent Strongyle worm was identified by counting one hundred collected larvae. The counting was done in five replicates. Morphological identification of nematode larvae relied on investigation of the caudal and cranial extremities (32).

Fecal egg count reduction test (FECRT)

The FECRT was done according to Kaplan *et al.* (33). Based on the fecal egg count and larval identification, sixty out of the three hundred sheep, heavily infected with *H. contortus*, were selected. They were divided into two separate groups: an untreated control group (n = 30) and a treated group (n = 30). The treated one received an oral dose of 5 mg/kg of Albendazole Suspension®, 2.5% (Pharma Swede-Egypt). We selected this commercial brand of albendazole because we found that livestock owners have widely used it throughout the study area for a long time. Fecal egg count was carried out for each sheep at 3, 7, 15, and 30 days post-treatment. The FECRT was estimated as described in (34,35). The efficacy was assessed using the following formula, according to Várady et al. (36): FECRT% = (Pretreatment - Post-treatment)/Pretreatment*100. Accordingly, if the reduction in fecal egg count in the treated group is less than 95% compared with the untreated control, the worms are considered albendazole-resistant (33).

Egg hatch inhibition test

An egg hatch inhibition test was done on each group of sheep at the farm (Group I: VI). Strongyle eggs, predominantly *H. contortus*, were collected from fecal samples according to Coles and Jackson (36). Fresh fecal samples were collected, pooled, homogenized with a glass rod, sieved through 250, 100, and 25 µm sieves using a salt-saturated solution, and centrifuged at 1500 rpm for 20 min. The suspended eggs were washed several times with distilled water to remove excess salt. Following the methods of von Samson-Himmelstjerna (37), the fresh eggs were incubated for 48h at 10 different concentrations (0.012 to 6.25 µg/ml) of albendazole® Pharma Swede 2.5%, Egypt. To prepare the stock solution, 400 µL of albendazole 2.5% was diluted in 9.6 mL of DMSO 20%. Albendazole concentrations were determined by 2-fold serial dilutions of the stock solution in distilled water in a 24-well plate. The control wells received only the diluents. Accordingly, 100 fresh eggs in 20 µL of DW were added per well. Each albendazole concentration was tested in triplicate. Then, the plate was tightly covered to prevent drying and kept at 27ºC for 48 h; after that, 10 µL of Lugol's iodine per well was added to prevent egg development. Well examinations were performed under an inverted microscope (Olympus, CKX53, Japan) at 100× magnification. The unhatched eggs and 1st-stage larvae per well were recorded. Thereafter, the lethal concentration 50 of albendazole (LC_{50}) that prevented 50% of the eggs from hatching was determined. Eggs with LC50 values of 0.1 µg/mL in the four sheep groups were deemed to have resistance to albendazole according to Coles and Bauer (33).

DNA extraction

Three heavily infected sheep with *H. contortus* were euthanized. *Haemonchus* worms were collected from the abomasum, and the male worms were thoroughly washed with PBS (pH 7.2) and identified according to Urquhart et al. (29). The male worms were selected and preserved in 70% ethanol at -20ºC until use. Total genomic DNA was extracted from adult males using the GeneJET Purification Kit (Thermo Fisher Scientific) according to the instruction manual. The quality and concentration of the obtained DNA were evaluated spectrophotometrically before being preserved at -20°C for further processing.

Allele-Specific Polymerase Chain Reaction (AS-PCR)

The albendazole resistance represented by the TAC single-nucleotide polymorphism (SNP) in codon F200Y was elucidated using the designed primers (Table 1) to carry out this reaction. Allele-specific PCR was performed with four primers: 3F, 4R, 5F, and 6R, whereas 3F and 4R were non-allele-specific primers, 5F was a resistant allele-specific primer, and 6R was a susceptible allele-specific primer according to Arafa *et al.* (38).

Table 1: Primers utilized in the current research experiment

ID	Sequence 5′–3′ (Tm °C)	Pair	Size	Target	Benzimidazole
1F	CGT TCT TCA GGA GGC AAG (53.2)	1F/2R	924	Common	N/A
2R	GCA GAC AGT GGA GCA AAA C (54.4)				
3F	AAA TAA GTC TCA CCA CCT GTA AAC (52.9)	3F/4R	294 bp	Common	N/A
4R	AGA CAT TGT GAC AGA CAC TTC (52.5)				
5F	GTA GAG AAC ACC GAT GAA ACATA (52.2)	5F/4R	193bp	F200Y	Resistant
6R	GAG CTT CGT TGT CAA TAC AGA (52.5)	3F/6R	145bp	F200Y	Susceptible

Briefly, two separate reactions were done. The first one was conducted in a volume of 25 µl. The reaction mixture was composed of 12.5 µl of 2x master mix, 2 µl of the 10 pmol working primers (1F and 2R), 50-100 ng of gDNA, and nuclease-free water to a final volume of 25 µl. The thermal program consisted of 1 cycle at 94°C for 8 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 20 s, and extension at 72°C for 1 min. While the final extension was at 72°C for 7 min. The second reaction obtained amplicon was used as a template in the following nested PCR utilizing the primers 3F, 4R, and 5F (F200Y BZ resistant) and 6R (F200Y BZ susceptible) using a thermal program of 1 thermal cycle at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 30 s, and annealing at 54°C for 30 s and extension at 72°C for 1 min (22). The obtained amplicons were electrophoresed in red safe-stained 1.75% agarose and visualized under UV light. BZ resistance was genotyped by AS-PCR in adult H. contortus based on band profiles. The genotypic and allelic frequencies were determined using the methodology of Pierce and ProQuest (39). The obtained amplicons were sent for sequencing to confirm the presence of the mutation.

Statistical analysis

The prevalence was presented as the percentage of infected animals among the total number of animals. Data were displayed by descriptive statistics for the overall prevalence among sheep. A chi-square test was utilized to investigate the most prevalent gastrointestinal parasites. The analysis of the data obtained from the fecal egg reduction test and egg hatching ability test was performed using the mean and standard error (SE). Statistical comparisons of the means across treatments were performed using one-way ANOVA in SPSS version 10. A *p*-value of 0.05 was assumed for

statistical significance. The LC_{50} of Albendazole was determined using Probit analysis (40) in SPSS version 20 for Windows.

Results

Coprological findings

The overall prevalence of gastrointestinal parasites infecting sheep was 57.7% (173/300). Eggs of *Haemonchus* spp. and *Strongyloides papillosus*, as well as *Eimeria* spp. Oocysts were detected in the fecal samples (Figure 1). The results showed that the prevalence of Strongyles among sheep was 45% (149/300); however, 80% (240/300) of sheep were infected with Eimeria spp., and 8% (24/300) with Strongyloides papillosus ($\chi^2 = 58.481$; p-value < 0.001). The fecal egg counts of the positive fecal samples for the Strongyle group showed that most (40%) were of moderate intensity, 35% were heavily infected, and 25% were of low infection intensity. Larval identification revealed that the most prevalent Strongyle was Haemonchus (65%), followed by Trichostrongylus spp. (25%) and Ostertagia spp. (10%) ($\chi^2 = 242.500$; P-value < 0.001).

Fecal egg count reduction test

In figure 1 and table 2, the fecal egg count reduction percentage following oral administration of 5 mg/kg albendazole is shown. Where the mean fecal egg count of sheep at 0 days was (1305±116.2 and 1485±178.5) and at 30 days post-treatment was (1185±188.4 and 445.5±53.5) for the control and the albendazole-treated groups, respectively. The FECR% reached (90%, 87.46%, 83%, and 70%) at (3, 7, 15, and 30) days post-treatment, respectively. The results indicated the emergence of albendazole resistance in Haemonchus among the inspected sheep.

Egg hatch inhibition test

The mean unhatched egg count and egg hatch inhibition percentage varied with albendazole concentration across fecal samples from different sheep groups (Table 3). At controls, the mean number of unhatched eggs was 21±3.4%, where egg hatch inhibition % was zero. The highest hatch inhibition percentage was observed at an albendazole concentration of 6.25 µg/mL (Figure 2). In contrast, it reached 93.6%, 90.2%, 78.4%, and 82.2% for groups I, II, III, and IV, respectively. Meanwhile, the lowest hatch inhibition % was observed at a concentration of 0.012 µg/ml (Figure 2), whereas it was 11.3, 17.7, 13.0, and 8.8% for groups I, II, III, and IV, respectively. By decreasing the albendazole concentration, a significant decrease in mean unhatched egg count and the mean egg hatch inhibition percent ($P \leq 0.0001$) was observed. The LC50 value was 0.161, 0.132, 0.351, and 0.247 µg/ml for albendazole in groups I, II, III, and IV, respectively. The inspected group in which the LC_{50} value for albendazole was more than 0.1 µg/ml was deemed to carry albendazole-resistant strains of *H. contortus*.

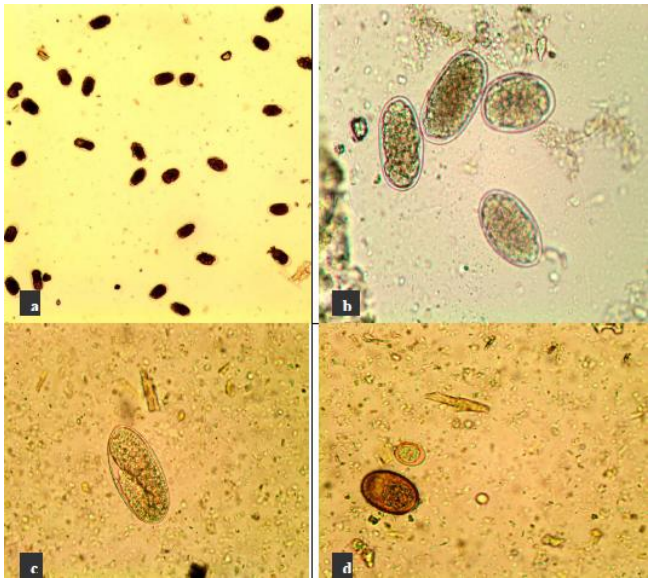

Figure 1: Shows the gastrointestinal parasitic eggs and oocytes detected in the infected sheep under the study, where figure (1-a) and (1-b) showed *H. contortus* eggs collected from heavily infected sheep at 4x and 20x, respectively, (1-c) showed *Strongyloides papilosus* egg at 20x, and (1-d) showed *Eimeria* spp. Oocysts at 20x.

Table 2: Variable means fecal egg count and fecal egg count reduction percent between the naturally infected sheep in the control and albendazole-treated groups

Mean FEC	Control group	Albendazole-treated group
Pre-treatment 0 days	1305±116.2[a]	1485±178.5 [a]
Post-treatment 3 days	1255±177.2 [a]	148.5±17.8 [c]
FECR%	3.8%	90%
Pos- treatment 7 days	1360±119.4 [a]	186.1±22.3[c]
FECR%	-4.2%	87.46%
Post-treatment 15 days	1655±173.5 [a]	252.4±30.3[bc]
FECR%	-26.8%	83%
Post-treatment 30 days	1185±188.4 [a]	445.5±53.5[b]
FECR%	9.1%	70%

Allele-Specific Polymerase Chain Reaction (AS-PCR)

The AS-PCR technique used in the present study was beneficial for characterizing and genotyping worm populations by amplifying an amplified fragment of the β-tubulin isotype 1 gene containing residue 200, which is involved in BZ resistance (Figure 3). Nested PCR was performed to amplify an inner portion of the β-tubulin gene using 1F/2R primers, producing a PCR product of approximately 924 bp (Figure 4). Allele-specific PCR was performed with four primers: 3F, 4R, 5F, and 6R, whereas 3F and 4R were non-allele-specific primers, 5F was a resistant allele-specific primer, and 6R was a susceptible

allele-specific primer. The PCR master mix was prepared in two separate tubes, each containing 2 allele-nonspecific primers and one allele-specific primer, so each reaction generated two fragments: one allele-nonspecific and one allele-specific. The specific observed bands were the susceptible allele-specific gene at 145 bp, the resistant allele-specific gene at 193 bp, and the non-allele-specific gene at 294 bp (Figures 3-5). A standard, unspecific band, along with the susceptible band, was observed in the homozygous (SS) susceptible allele (Figure 4). The homozygous resistant allele (RR) showed the resistant band in addition to the non-allele-specific band. Still, all three bands were heterozygous (RS) (Figure 5). The chromatogram of the sequenced (RS) sample showed no point mutation (TTC/TAC) at the F200Y locus of the partial β-tubulin isotype 1 gene (Figure 6). According to the genotypic results, 21 out of 39 isolates (54%) were heterozygous for susceptible alleles. In comparison, 18 isolates (46%) were homozygous for susceptible alleles, and no isolates were homozygous for resistant alleles. The susceptible (S) allele frequency was 73%, whereas the resistant (R) allele frequency was 27% in the isolates.

Table 3: Mean unhatched egg count and hatch inhibition percent of *H. contortus* eggs at different concentrations of albendazole for different sheep groups.

Drug concentration µg/ml	Group I		Group II		Group III		Group IV		F-value	P-value
	Mean un- hatched eggs%	Hatch Inhibition %	Mean un- hatched eggs%	Hatch Inhibition %	Mean un- hatched eggs%	Hatch Inhibition %	Mean un- hatched eggs%	Hatch Inhibition %		
6.25	95 ± 2.8^{aA}	93.6	92.3 ± 3.9^{abA}	90.2	83.0 ± 1.5^{bA}	78.4	86.0 ± 2.0^{abA}	82.2	4.033	0.051
3.125	88.3 ± 1.6^{aAB}	85.2	85.3 ± 3.1^{aAB}	81.4	77.0 ± 1.5^{bA}	58.2	82.6 ± 1.4^{abA}	78.0	5.350	0.026
1.56	83.3 ± 1.6^{aBC}	78.9	77.3 ± 1.6^{aBC}	71.3	67.0 ± 3.0^{bB}	55.2	77 ± 1.62^{aB}	70.8	9.702	0.005
0.78	79.0 ± 2.0^{aBC}	73.4	71.6 ± 1.6^{bCD}	64.1	64.6 ± 3.1^{bB}	52.3	70.0 ± 1.1^{bC}	62.0	7.553	0.010
0.39	74.6 ± 3.1^{aC}	67.9	67.6 ± 2.6^{abCDE}	59.0	62.3 ± 3.9^{bB}	50.2	66 ± 1.0^{abCD}	56.9	3.177	0.085
0.195	63.6 ± 2.4^{aD}	54.0	65.3 ± 2.3^{aDE}	56.1	60.6 ± 4.6^{aBC}	39.6	63 ± 1.0^{aD}	53.1	0.444	0.728
0.097	55 ± 5.7^{aD}	43.0	61 ± 2.3^{aEF}	50.6	52.3 ± 2.9^{aCD}	33.3	60.6 ± 0.67^{aD}	50.2	1.541	0.277
0.048	45 ± 2.8^{abE}	30.3	54 ± 3.7^{aFG}	41.7	47 ± 2.7^{abDE}	27.4	43.7 ± 1.8^{bE}	28.6	2.537	0.130
0.024	41.7 ± 1.6^{bE}	26.1	50.3 ± 3.1^{aG}	37.1	42.6 ± 2.8^{abE}	26.1	38.3 ± 1.6^{bF}	21.9	4.329	0.043
0.012	30 ± 5^{aF}	11.3	35 ± 5^{aH}	17.7	31.3 ± 0.67^{aF}	13.0	28 ± 1.0^{aG}	8.8	0.676	0.0591
Control	21 ± 3.4^{F}	0	21 ± 3.4^{I}	0	21 ± 3.4^{G}	0	21 ± 3.4^{H}	0		
F -value	58.4		44.2		39.9		159.6			
P- value	0.000		0.000		0.000		0.000			

Capital letters: means that within the same column, different letters are significantly different at ($P < 0.01$). Small letters: imply that within the same row, different letters are significantly different at ($P < 0.01$).

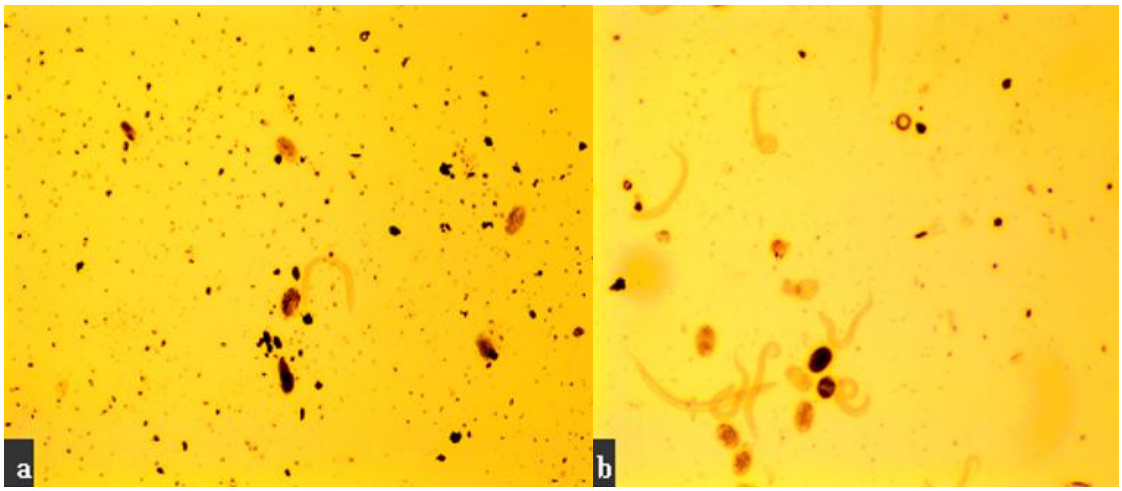

Figure 2: (2-a) showed a high count of unhatched *H. contortus* eggs when incubated with albendazole at a concentration of 6.25 µg/ml. In contrast, (2-b) showed a high count of 1st stage larvae following incubation of *H. contortus* eggs with albendazole at a concentration of 0.012 µg /ml.

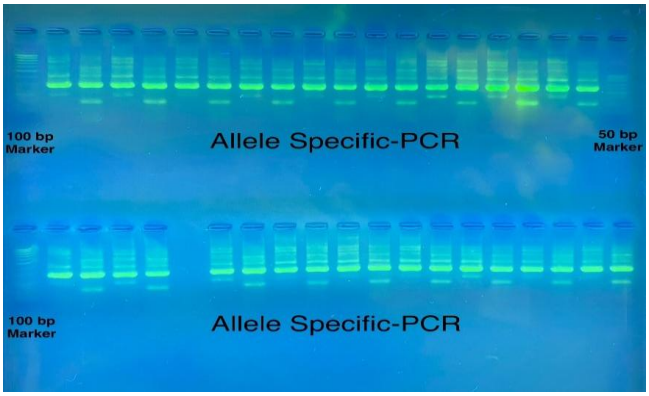

Figure 3: Allele Specific PCR (AS-PCR) of adult male *H. contortus* of β-tubulin isotype 1 gene showing the genotyping patterns.

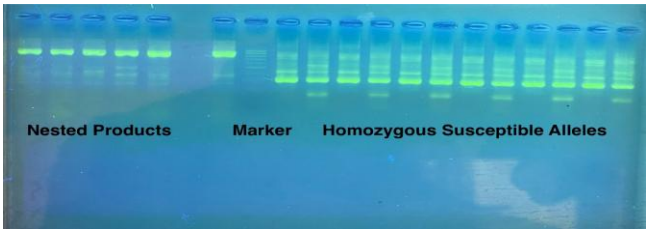

Figure 4: Nested PCR amplification of adult male *H. contortus* of β-tubulin isotype 1 gene showing the amplified PCR fragment (924 bp) and Allele Specific PCR (AS-PCR) pattern of homozygous susceptible allele (294 bp and 145 bp).

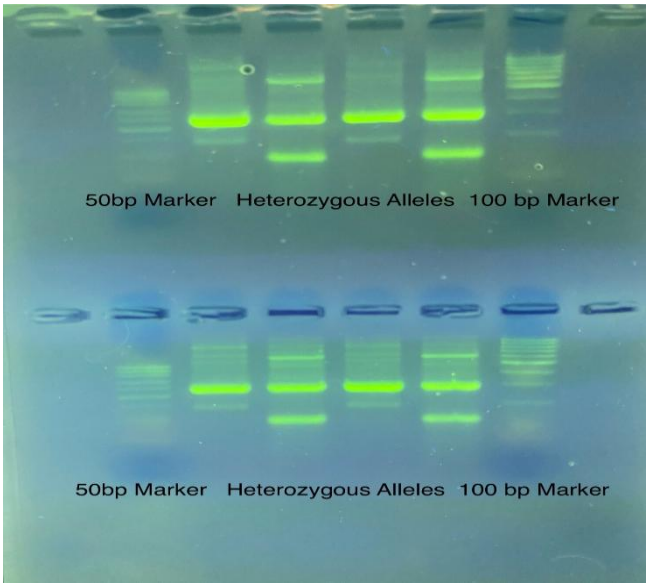

Figure 5: Allele Specific PCR (AS-PCR) of adult male *H. contortus* of β-tubulin isotype 1 gene showing the heterozygous allele (294 bp and 193 bp).

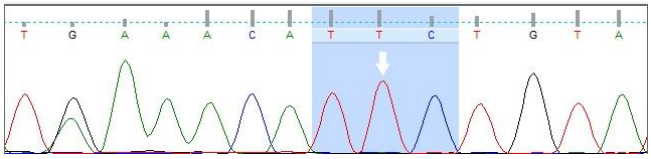

Figure 6: Nucleotide sequence alignment of PCR products of the partial β-Tubulin isotype 1 gene shows the chromatogram of the sequenced (RS) sample with a T-A point mutation marked by the white arrow at the F200Y locus in the sequence.

Discussion

Haemonchosis is a widespread pathogenic health hazard for various livestock (4,27,41). It is mainly caused by the H. contortus parasite, which has become resistant to the most commercially authorized antinematodal chemotherapeutics (42,43). The current study showed that sheep were heavily infected with GIPs (57.7%), whereas 45% were infected with strongyles. *H. contortus* was the most prevalent GIN (65%) among sheep. These results might agree with those obtained by Mengist (44) (67.57%) and Abdo *et al.* (45) (69.6%). Otherwise, lower prevalence of haemonchosis had been reported by Brik (46) (23.92%) and Ahmad (47) (25.55%). These variations might be explained by differences in climatic conditions and management factors in the research region, as well as by differences in sample size and animal immunity levels.

Albendazole, a broad-spectrum anthelmintic in the benzimidazole class, is widely used in Egypt due to its affordability and ease of use. Animal owners typically purchase the drug and use it as a preventative measure without a veterinarian's advice. Consequently, drug resistance may result from inappropriate use of this medication, such as obtaining too few or too many doses, as well as selection of benzimidazole-resistant alleles or gene mutation that confers drug resistance (48,49). Survivors of treatment carrying resistant alleles can pass on their genes to their offspring. Early detection of this issue could prevent the extensive spread of resistance alleles within the helminth population, even though their prevalence remains low (11,50). Various phenotyping methods have been utilized as drug resistance diagnostics (51). In the current study, the FECRT revealed the development of albendazole resistance among the *H. contortus* population infecting the examined sheep. In contrast, the FECR% reached 90%, 87.46%, 83%, and 70% at 3, 7, 15, and 30 days post-treatment, respectively. These results agreed with the FECR% reported by Meenakshisundaram (52) (86.50% and 84.47%), Mohammedsalih (53), Aboelhadid (22) (86.68%), and Shehab and Hassan (54) (84%). Moreover, the current egg-hatching inhibition test showed that the hatchability inhibition percentage for the different examined sheep groups ranged from 82.2% to 93.6% and 8.8% to 11.3% at

the highest and lowest albendazole concentrations (6.35 µg/ml and 0.012 µg/ml, respectively). Additionally, the estimated LC_{50} appeared to be greater than 0.1 µg/ml. This indicated the development of resistance to albendazole (33). Similarly, Meenakshisundaram and Anna (52) reported an LC50 of 0.299 µg/mL for albendazole against *H. contortus* eggs at sheep farms. Furthermore, Kalu and Ebuk (24) reported LC50 values of 0.11-0.19 µg/ml for gastrointestinal Trichostrongyloid nematodes in goats. It was clear that increasing the concentration of albendazole increased the percentage of hatching inhibition, and vice versa. Therefore, these might be due to the hydrophobic properties and high lipid solubility of albendazole; in addition, its ability to penetrate the eggshell and its high affinity for "â-tubulin" (55).

The parasite *H. contortus* is one of the significant members of the family *Trichostrongylidae* regarding anthelmintic resistance. This is not only due to this species's ability to develop resistance to all major drug classes but also to its significant economic importance worldwide. They exhibited resistance to benzimidazoles (BZs), imidazothiazoles, macrocyclic lactones, and the amino-acetonitrile derivative class. This species is a good model for studying resistance because of its fecundity, relatively large size of the adult worms, and the ease of establishing and maintaining large infections. This resistance threatens the sustainability of small ruminant productivity worldwide (56).

The diagnosis of benzimidazole (BZ) resistance is mainly limited to conventional *in vitro* and *in vivo* methods, which are protracted and laborious and only sensitive to highly prevalent resistant worms in a population (57). The molecular tools have been developed to provide early, high-accuracy, high-sensitivity detection of BZ resistance, even in a single worm or larva (58-60). In BZ-resistant worms, the benzimidazole target molecule, β-tubulin, undergoes genetic polymorphism, so the drug can no longer recognize or bind to the target and thus becomes ineffective (37). The resistance is characterized by a higher frequency of individuals in a population who can tolerate benzimidazole doses. This trait is heritable to subsequent generations. Resistance to benzimidazole in *Trichostrongylid* nematodes is considered primarily due to the F200Y single-nucleotide polymorphism (SNP) in the isotype-1 of the β-tubulin gene (60). However, the F167Y mutation has also been linked to resistance (61), and E198A, another SNP, has been involved in resistance in some isolates (62). Once one of these mutations is identified in a worm, the affinity of β-tubulin for benzimidazole is reduced, leading to resistance. Molecular methods were developed to monitor and measure the frequency of resistance alleles at three SNPs in the isotype-1 β-tubulin gene, particularly codon 200 (37).

In our study, we employed AS-PCR to genotype the Egyptian *H. contortus* population using an amplified fragment of the β-tubulin isotype 1 gene containing residue 200, which is frequently associated with BZ resistance worldwide. The current investigation found that the genotypic frequencies of the heterozygous RS allele (54%) and the homozygous susceptible SS allele (46%) were attributed to benzimidazole exposure or genetic transfer (22). Perhaps the most clinically relevant finding is that no homozygous resistant RR allele has been identified, suggesting that the Egyptian BZ resistance condition is currently in the intermediate phase of resistant gene selection. The development of resistance has three phases: the susceptibility phase, where the frequency of resistant individuals within the population is low; the intermediate phase, which develops when the exposure to a drug is continued and the frequency of heterozygous individuals among the population increases; and the final resistant phase, which develops due to the sustained selection pressure with the homozygous resistant individuals predominating within the population (63). Therefore, the selection of BZ resistance develops more slowly in Egypt than in other countries where individual treatment strategies are followed up with unknown generic drugs (64). These results agreed with those of Aboelhadid et al. (22) and Arafa et al. (38), who reported that the two forms were only observed in single male isolates and in pooled larvae of H. contortus from Egypt for the F200Y polymorphism in isotype 1 of the β-tubulin gene. Despite mutations in one of F200Y, F167Y, or E198A, which may afford resistance to BZ, testing of β-tubulin suggests that combinations of two or three of these genetic changes do not exist in the same β-tubulin isotype 1 allele, so multiple mutations at these codons in the same allele might be lethal (65). Another finding is that the susceptible allelic frequency was 46% higher than the resistant one, suggesting that Targeted Selective Treatment (TST) may be sufficient to slow the spread of BZ resistance in Egypt. Further studies are recommended to clarify the molecular mechanism of benzimidazole resistance in Egypt.

Conclusion

It was concluded that haemonchosis is the most prevalent gastrointestinal nematodiasis infecting Egyptian sheep. The study documented the development of resistance to albendazole among the *H. contortus* population naturally infected sheep in Giza province. This study emphasized the importance of periodic monitoring of albendazole resistance and a better understanding of its molecular mechanisms to maintain drug efficacy.

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Conflict of interest

The authors declare no competing interests.

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انتشار الطفيليات المعوية والتقييم الظاهري والجيني لمقاومة الألبيندازول في عزلات الهومونكس كونتورتس من الأغنام المصابة بشكل طبيعي

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الخلاصة

اكتشفت هذه الدراسة مقاومة ألبيندازول في الاغنام المصرية خمجة طبيعيا بديدان الهيمونكس كونتورتس بمحافظة الجيزة .استُخدمت ثلاثمائة من الأغنام المربأة في مزرعة خاصة وذلك خلال فترة امتدت من يونيو ٢٠٢٤ الى نوفمبر ٢٠٢٤. أُجري فحص البراز على جميع الحيوانات .أُجري اختبار اختزال عدد البيوض في البراز لمجموعتين من الأغنام الخمجة بشدة وهي مجموعة السيطرة وأخرى مُعالَجة بجرعة ٥ ملغم/كغم من ألبيندازول ٢,٥%. تم إجراء اختبار تثبيط فقس البيض باستخدام عشرة تركيزات (من ٠,٠١٢ إلى ٦,٢٥ ميكروغرام/مل) من ألبيندازول ٢,٥%. استُخدم تفاعل البوليميراز المتسلسل الخاص بالأليل لتحديد النمط الجيني لديدان الهومونكس كونتورتوس البالغة باستخدام جزء مُضخّم من جين بيتا توبيولين النمط ١ الذي يحتوي على البقايا ٢٠٠.وقد أظهرت النتائج أن ٥٧,٧% من الأغنام كانت خمجة بالطفيليات المعوية، منها ٨٠% و٤٥% و٨% مصابة بأنواع الأيميريا، والأسترونجيل، والأسترونجيلويدس بابيلوسوس على التوالي .وكانت أنواع الأسترونجيل الأكثر انتشارا هي الهومونكس (٦٥%)، تليها تريكوسترونجيلوس (٢٥%)، ثم أوستراتاجيا (١٠%). كان الاختزال في عدد البيوض في البراز أقل من ٩٥% :(٩٠%، ٨٧,٤٦%، ٨٣%، و٧٠%) بعد العلاج بـ (٣، ٧، ١٥، و٣٠ يوما)، على التوالي .كان التركيز المميت للنصف اعلى من ٠,١ ميكروغرام/مل من ألبيندازول .أظهرت النتائج الجينية وجود أليلات متغايرة الزيجوت في ٥٤% من العينات، بينما وُجدت أليلات متماثلة الزيجوت حساسة في ١٨ عزلة (٤٦%)، ولم يُعثر على أليلات مقاومة متماثلة الزيجوت .بلغ تواتر الأليلات الحساسة ٧٣%، بينما سُجلت تواتر الأليلات المقاومة في ٢٧% من العزلات . وأكدت هذه الدراسة على أهمية المتابعة الدورية لمقاومة الألبيندازول والفهم الأفضل لآليتها الجزيئية للحفاظ على فعالية الدواء.