Research Article

Isolation, Identification, and Molecular Characterization of *Haemonchus contortus* in Slaughtered Small Ruminants in the Chattogram Metropolitan Area, Bangladesh

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Abstract | *Haemonchus contortus* is a highly destructive parasitic nematode in small ruminants that causes significant economic losses to sheep and goat breeding operations worldwide. To combat this problem and minimize economic damage, effective preventative measures should be aimed at high-risk populations and a thorough understanding of the epidemiology is crucial. This study aimed to improve the understanding of the molecular epidemiology of *Haemonchus contortus* in sheep and goat carcasses in the Chattogram Metropolitan area in Bangladesh. A sample of 400 abomasa (150 from sheep and 250 from goats) was collected from five different slaughterhouses in the Chattogram Metropolitan area and analyzed using morphological and microscopic methods. The Polymerase Chain Reaction (PCR) was then employed to amplify the sec internal transcribed spacer (ITS-2) of the nuclear ribosomal DNA and the mitochondrial nicotinamide dehydrogenase subunit 4 gene (nad4) to identify *H. contortus*, followed by direct sequencing. Out of the 400 samples tested, 186 (46.5%) were positive for *H. contortus*. The parasite was found in 51.3% of sheep and 43.6% of goats. Through the sequencing analysis, 45 nad4 genotypes and 10 ITS-2 genotypes were discovered. The prevalence of *Haemonchus contortus* isolated was not influenced by demographic factors such as age, gender, breed, and seasons in the study. Our results highlight the state of our knowledge on the spread of *H. contortus* parasite among small ruminants in Chattogram slaughterhouses.

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Introduction

Haemonchus contortus is a prevalent blood-sucking parasite among other gastrointestinal nematodes in small ruminants (O'Connor *et al.*, 2006). This parasite inhabits the abomasum of the host and causes haemonchosis, leading to significant economic losses from decreased meat, milk, wool, and

leather production, as well as from clinical symptoms of blood loss (Kumsa *et al*., 2008). *Haemonchus contortus* can cause anemia, edema, and in severe cases, death in affected animals, particularly during hot and humid summer months (Easwaran *et al*., 2009). In some regions, other species such as *H. placei* and *H. longistipes* of *Haemonchus* can coexist in shared pastures used by all ruminants (Brasil *et al*., 2012; Jacquiet *et al*., 1998). Each worm is estimated to suck approximately 0.05 mL of blood daily and waste 0.1 mL of blood due to leakage (Soulsby, 1982; Urquhart *et al*., 2000). The parasite has a remarkable ability to survive due to its ecological adaptability (Troell *et al*., 2006).

Bangladesh is the fourth largest producer of goats (26.9 million) and sheep (3.8 million) in Asia, and the livestock sector contributes 1.85% to the country's gross domestic product (DLS, 2023). Livestock is crucial for reducing poverty and generating income in Bangladesh. The majority of these animals are owned by nomads who are able to roam freely, while small farms are mostly found in big cities. This results in increased contact between different animal species during pasture and irrigation (Hussain *et al*., 2014; Shen *et al*., 2017). Despite this, parasitic infections remain a major challenge in livestock management. Effective control of *H. contortus* infections requires the development of appropriate management strategies. However, overreliance on anthelmintics has led to widespread drug resistance in *H. contortus* populations, making the development of effective control strategies more challenging (Kaplan, 2004). Molecular identification of *H. contortus* species is crucial for implementing effective control strategies. In general, *H. contortus* has a complex genetic structure, and there are different strains and species of this nematode. Different species or strains of *H. contortus* may have varying susceptibility to environmental factors, such as temperature or humidity (Kaplan, 2004). Targeted control measures can be implemented based on this information to prevent or manage *H. contortus* infections. Morphological identification of Haemonchus contortus using microscopy has long been a reliable method for detecting and distinguishing the parasite. However, Molecular techniques can differentiate between closely related species or strains of parasites that may have varying levels of pathogenicity. This information is crucial for designing targeted control strategies that are specific to the particular species or strain present in a given region.

Molecular tools provide valuable information including determining the source of infection, tracking transmission routes, and identifying potential risk factors. This knowledge helps in developing effective control measures that target specific stages or sources of transmission (Yin *et al*., 2013). It provides insights into the prevalence, distribution, and changes in parasite populations over time, enabling the assessment of control program effectiveness and the identification of areas requiring intensified interventions. In Bangladesh, there is a lack of information about parasitism in slaughterhouses. A recent study found the presence of *Haemonchus contortus* in goats at local slaughterhouses in Sylhet (Nath *et al*., 2021).

Molecular identification of *H. contortus* can provide valuable information on the transmission dynamics and epidemiology of the parasite, which can inform the design of targeted control strategies (Sargison, 2011). Moreover, identifying the species and strains of *H. contortus* present in a particular area can help establish baseline data for monitoring changes in the prevalence and distribution of the parasite over time, allowing for early detection of new outbreaks (Besier *et al*., 2016). Conducting molecular identification on *Haemonchus contortus* is crucial for evaluating effective control strategies and understanding the epidemiology of the parasite. The current study focuses on identifying *H. contortus* in small ruminants in slaughterhouses and examining the factors related to infection such as age, sex, breed, host, body condition, seasons and regions.

Materials and Methods

Study area and specimen collection

This study was conducted on goats and sheep that were raised in different parts of Bangladesh and slaughtered in the abattoirs of the Chattogram Metropolitan area. Samples of the abomasa were collected from five different abattoirs located in Jhautala, Firingebazar, Pahartali, Colonethat, and Halishahar on a weekly basis from January to December 2015. Data on the demographic factors (age, sex, breed, host, body condition, seasons, and regions) was recorded for each abattoir through questionnaires during the time of sampling. A total of 250 goats and 150 sheep were sampled, with adult worms being extracted from the

abomasa following the protocol described by Iqbal (Iqbal *et al*., 1993) and (MAFF, 1986) with slight modifications. In brief, adult worms were recovered from the abomasa of sheep and goats obtained from the slaughterhouse. After slaughtering, the abomasum was removed from the other stomach parts and ligated at both ends. After that, the abomasum was transported directly to the Chittagong Veterinary and Animal Sciences University's Department of Parasitology laboratory for more research. It was dissected along its greater curvature, and the contents were poured into a glass beaker. The mixture was then put through a series of washings, sedimentations, and decants until the supernatant was sufficiently clear to make worm collecting simple. Both the abomasum and its contents were carefully examined, and individual adult male worms were collected and identified by microscopic examination of spicules, according to MAFF (1986). They were then preserved in 70% ethanol and stored at -20°C until DNA extraction was performed. To reduce the possibility of inaccurate DNA amplification from female worm eggs, only mature male worms were employed.

Specimen isolation and microscopic examination

The collected parasite samples were washed with normal saline and put into sterile petridishes for examination. The worms were visually inspected before being studied under a microscope for species identification, following the process outlined by Soulsby (1982). In brief, the worms were placed in a glycerine alcohol solution (90 mL 70% ethanol and 10 mL glacial glycerine) for 24 hr until they become transparent, then mounted with glycerin jelly (10 g gelatin, 500 mL glycerine, 10 g phenol, and 60 mL distilled water). Observations and measurements were conducted under a light microscope (Olympus BX-53, Tokyo, Japan) with an ocular micrometer. Out of the 400 collected samples, only 186 (109 goats and 77 sheep) that showed positive results under the microscope were subjected to a conventional polymerase chain reaction (PCR) for further confirmation.

DNA extraction and PCR amplification of specimens

Total genomic DNA was extracted from adult worms using G-spin™ Total DNA Extraction Kit (REF-17045, iNtRON Biotechnology, Korea) according to the manufacturer's instruction, which was then stored at -20°C. To confirm the species of *Haemonchus*, a conventional PCR was conducted to amplify 321 bp of the ITS-2 of the nuclear rDNA using the forward primer NC1-F (5'-ACGTCTGGTTCAGGGTTGTT-3') and the reverse primer NC1-R (5'-TTAGTTTCT TTTCCTCCGCT-3'), as described previously by Stevenson *et al*. (1995) and Akkari *et al*. (2013), and the 800 bp of *nad*4 gene was also amplified using Primer1-F (5'-GGATTTGGTCAGCAAATTGAA-3') and Primer2-R(5'-GCCTGCAAATGAATTAACA-3'), (Yin *et al*., 2013).

A 50 µL reaction was used in each PCR amplification and it consisted of 25 µL of master mix (which included DNA polymerase, buffer, and four types of nucleotides in certain quantities, as well as MgCl2), 5 µL of DNA sample (with a concentration of over 30 ng/µL), 2.5 µL of forward primer (10 pmol/µL), 2.5 µL of reverse primer (10 pmol/µL), and 15 µL of distilled water. The PCR reaction for the amplification of 321 bp of ITS-2 involved an initial heating at 95°C for 2 min, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 7 min. The nad4 gene amplification started with an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 secs, annealing at 55°C for 30 secs, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The amplified fragments were separated through 1.5% gel electrophoresis, stained with ethidium bromide, and the bands were visualized on a UV transilluminator. The positive PCR products were then purified using a Favor Prep™ PCR Clean-Up Mini Kit and 40 µL of elution buffer was used to retrieve the pure DNA.

Sequencing and phylogenetic analysis

The purified DNA amplicons of ITS-2 (10) and nad4 (45) gene samples from the PCR were directly sequenced in both directions with the primers used for the PCR by the ABI 3100 Genetic Analyzer and the BigDye™ Terminator v.3.1 Sequencing Kit on an automated sequencer (ABI3730XL, Applied Biosystems, USA). DNA sequences were assembled with Codoncode Aligner version 7.1.1 software (CodonCode Corporation). Nucleotide sequences were aligned using Clustal W (Larkin *et al*., 2007). The obtained sequences of the ITS-2 and nad4 genes were compared with the published reference sequences of *H. contortus* deposited in GenBank using NCBI BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Evolutionary analysis and phylogeny were conducted using the ITS-2 and nad4 gene in MEGA 7.0, with

the reliability of the tree confirmed through bootstrap analysis with 1000 replicates (Kumar *et al.*, 2016). The phylogenetic tree was constructed using the Tamura 3-Parameter model (Tamura, 1992), taking into account the host and country, with an appropriate outgroup included in the analysis.

Statistical analysis

The data collected was analyzed statistically to determine the correlation between the prevalence and sampling of animals. The data was recorded in a Microsoft Excel 2010 spreadsheet and analyzed using STATA version 11.0 software (Stata Corporation, 2009; website URL: http://www.stata.com). The Chi-square test was used to determine the significance of factors that are related to the infection. A multivariable logistic regression analysis was carried out to evaluate the impact of various variables on the results. The significance level was set at a 95% confidence interval and a P value of less than 0.05 (Schwartz, 1993).

Results and Discussion

Microscopic detection of H. contortus

We noticed that the female worm had a "barber's pole" appearance to the naked eye. The average length and width of male worms were 10.65 mm and 0.22 mm respectively, while the average length and width of female worms were 21.33 mm and 0.48 mm respectively (as shown in Figure 1a). The body of the worm appeared slender and tapered at the anterior end in males and both ends in females under the microscope (Figure 1b). Overall, 46.5% of the total population were positive. Among them, *H. contortus* was found in 51.3% (77/150) of Sheep and 43.6% (109/250) of goats of the populations we studied (Table 1). The positive cases of *H. contortus* observed under the microscope were 51.3%, 43.6%, 50.8%, 42.5%, 47.3%, 44.3%, 41.9, and 75.0% in sheep, goat, young, adult, male, female, healthy, and anemic groups of animals respectively (as seen in Table 1).

H. contortus is a type of ruminant gastrointestinal nematode that typically resides in the real stomach. Out of all the *Haemonchus* parasites, *H. contortus* is considered the most common and pathogenic species (Troell *et al.*, 2006). In order to establish successful control measures, it is crucial to have a complete understanding of the molecular epidemiology of the parasite. During the investigation, *H. contortus* was found in 46.5% of the small ruminants. Compared to previous reports from other countries, such as 55.5% in Benin (Attindehou *et al.*, 2012), 78% in China's Heilongjiang region (Wang *et al.*, 2006), and 80.2% in Ethiopia (Fentahun and Luke, 2012), the infection rate in Bangladesh is relatively low. In the study area, sheep were more infected (51.3%) than goats (43.6%). This supports the findings of Fentahun and Luke (2012) who found that sheep had a higher prevalence rate (81.2%) than goats (73.5%). Factors such as close grazing, which increases exposure risk, poor hygiene, and contaminated wool in the perineal region, can all contribute to a higher prevalence of *H. contortus* in sheep.

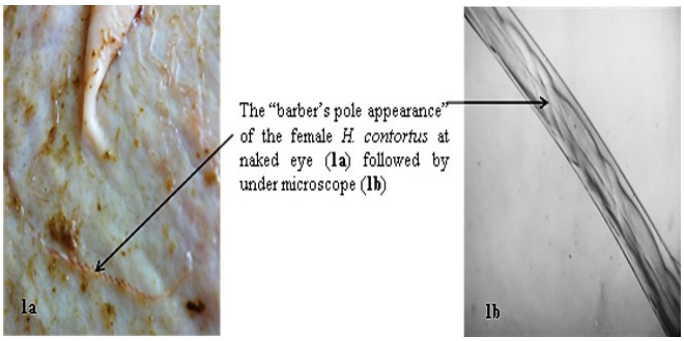

Figure 1: *The "barber's pole appearance" of the female Haemonchus contortus was shown at the naked eye (1a) and under the microscope (1b).*

Table 1: *Detection of Haemonchus contortus by microscopic and PCR.*

Variable	Categories (Total No.)	Microscopic observation		PCR technique	
		No. infected	Percentage	No. infected	Percentage
Host	Sheep (150)	77	51.3	77	51.3
	Goat (250)	109	43.6	109	43.6
Age	Young (191)	97	50.8	97	50.8
	Adult (209)	89	42.5	89	42.5
Sex	Male (285)	135	47.3	135	47.3
	Female (115)	51	44.3	51	44.3
Body condition	Healthy (344)	144	41.9	144	41.9
	Anaemic (56)	42	75.0	42	75.0
Total		186	46.5	186	46.5

Identification of H. contortus by PCR

Out of 400 sheep and goats, 186 (46.5%) were found to be infected with *H. contortus* using PCR (Table 1). The amplicon length was determined by comparing it to known lengths of 321 bp for ITS-2 and 800 bp for nad4 gene on a 1.5% gel electrophoresis under UV light (Figure 2). The infection rate varied depending on the sample location, but in all slaughterhouses

except SH-1, sheep had a higher infection rate than goats (Table 2). Sheep proved to be more susceptible to *H. contortus* with 51.3% (77/150) positive results, while goats had 43.8% (109/250) positive results (Table 1). The infection rate slightly differed when comparing age, sex, and health status, but there was no significant difference in the prevalence of *H. contortus* between young and adult animals, sex, or sheep and goat breeds (Table 3). However, the body condition of the animals (healthy or anaemic) greatly impacted the prevalence of *H. contortus*, with higher rates in anaemic sheep (70.8%) and goats (78.1%) (Table 3). No significant seasonal influence was observed (P = 0.543).

The prevalence in the coastal area was slightly higher at 58.6% compared to the hilly area (45.5%) and the plain area (35.3%). The rainy season posed a higher risk compared to other seasons, with a recorded prevalence of 69.9% during August, September, and October. In contrast, the prevalence was 45.5% during the summer months of April, May, and June. The lowest prevalence of 24% was observed in winter, specifically December, January, and February (Table 3). However, no significant associations ($p > 0.05$) were found between the prevalence of haemonchosis and both the environmental seasons and regions.

Table 2: *Prevalence of Haemonchus contortus in different slaughterhouse (SH) at Chattogram.*

Location	Slaughter-house (SH)	Host	No. of samples	No. of *H. contortus* positive	(%)
Jhautala	SH-1	Sheep	30	11	36.6
		Goat	50	23	46.0
Firingeba-zar	SH-2	Sheep	30	16	53.3
		Goat	50	21	42.0
Pahartali	SH-3	Sheep	30	15	50.0
		Goat	50	24	48.0
Colonethat	SH-4	Sheep	30	12	40.0
		Goat	50	19	38.0
Halishahar	SH-5	Sheep	30	23	76.6
		Goat	50	22	44.0
		Total	400	186	46.5

Table 3: *Prevalence of Haemonchur contortus based on demographic characteristics (age, sex, breed, body condition, and seasonal influence).*

Variable	Host	Group	No. examined	No. infected	Percentage (%)	P value
Age	Sheep	Young (< 1year)	38	26	68.4	0.100
		Adult (> 1year)	112	51	45.5	
	Goat	Young (< 1year)	153	71	46.4	0.079
		Adult (> 1year)	97	38	39.1	
Sex	Sheep	Male	103	54	52.4	0.584
		Female	47	23	48.9	
	Goat	Male	182	81	44.5	0.682
		Female	68	28	41.1	
Breed	Sheep	Local	150	77	51.3	0.158
	Goat	Black Bengal	174	82	47.1	
		Jamunapari	49	18	36.7	
		Saanen	27	9	33.3	
Body condition*	Sheep	Healthy	126	60	47.6	0.03
		Anaemic	24	17	70.8	
	Goat	Healthy	218	84	38.5	0.03
		Anaemic	32	25	78.1	
Season		Winter	133	32	24.0	
		Rainy	133	93	69.9	0.543
		Summer	134	61	45.5	
Region		Coastal	133	78	58.6	
		Plain	133	47	35.3	0.664
		Hilly	134	61	45.5	

**Significant risk factor.*

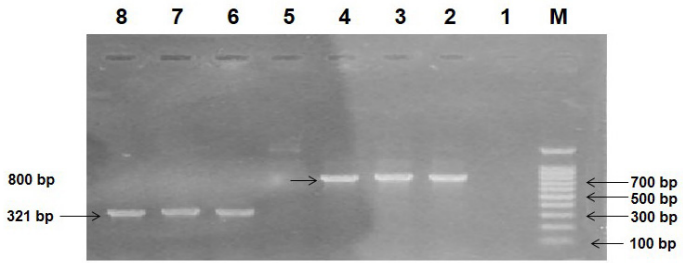

Figure 2: *The 1.5% of gel electrophoresis displays amplicons produced by conventional PCR. Lane 1= Negative control, Lane 2 = Positive control (nad4), Lane 3-4 = Suspected sample, Lane 5 = Negative control, Lane 6= Positive control (ITS-2), Lane 7-8 = Suspected sample. M = DNA Marker. The lengths of the individual amplicons were compared with the known lengths of 321 bp for ITS-2 and 800 bp for the nad4 gene in the UV light chamber.*

According to the results of the study, the prevalence of *H. contortus* was higher in young sheep (68.4%) and goats (46.4%) compared to adult sheep (45.5%) and goats (39.1%). Qamar *et al.* (2009) also found a similar pattern, with a slightly higher prevalence in young (39.9%) compared to adult (33.2%) small ruminants. This pattern has been reported in other studies as well (Gamble and Zajec, 1992; Colditz *et al.*, 1996; Magona and Musisi, 2002). The higher prevalence in young animals is attributed to their lower level of immunity compared to adults who have built immunity through gradual exposure to these parasites. In this study, male sheep had a higher estimated prevalence of *H. contortus* compared to female sheep (52.4%), although this relationship was not statistically significant, unlike in some prior studies where female hosts had higher prevalence (Maqsood *et al.*, 1996; Gauly *et al.*, 2006). A study conducted in Benin (Attindehou *et al.*, 2012) found almost identical occurrence of haemonchosis in both sexes (55.4% and 55.6%).

Furthermore, the local sheep breed (51.3%) and the Black Bengal goat breed (47.1%) were more susceptible to *H. contortus* than the Jamunapari (36.7%) and Saanen (33.3%) sheep breeds. In Bangladesh, the prevalence of haemonchosis was investigated using coproscopic examination and found to be higher in Black Bengal goats (57.1%) and other crossbred goats (55.8%), as reported by Nuruzzaman *et al.* (2012). However, in this study, none of the host-specific characteristics such as age, sex, or breed had a significant ($p > 0.05$) impact on the prevalence of *H. contortus*, which may occur in small ruminants due to the shared pastures and similar risk of infection for all (Attindehou *et al.*, 2012). The body condition of the host was significantly associated with the parasitic status in this study. The prevalence of *H. contortus* was significantly higher in anemic sheep (70.8%) and goats (78.1%) compared to healthy sheep (47.6%) and goats (38.5%). In South Africa's scarce areas, goats were examined for clinical anemia caused by *Haemonchus* parasites and found to be a health-threatening worm (Vatta *et al.*, 2002). These findings were supported by Attindehou *et al.* (2012), who found that haemonchosis was present in 90.7% of anemic small ruminants and 36% of non-anemic small ruminants.

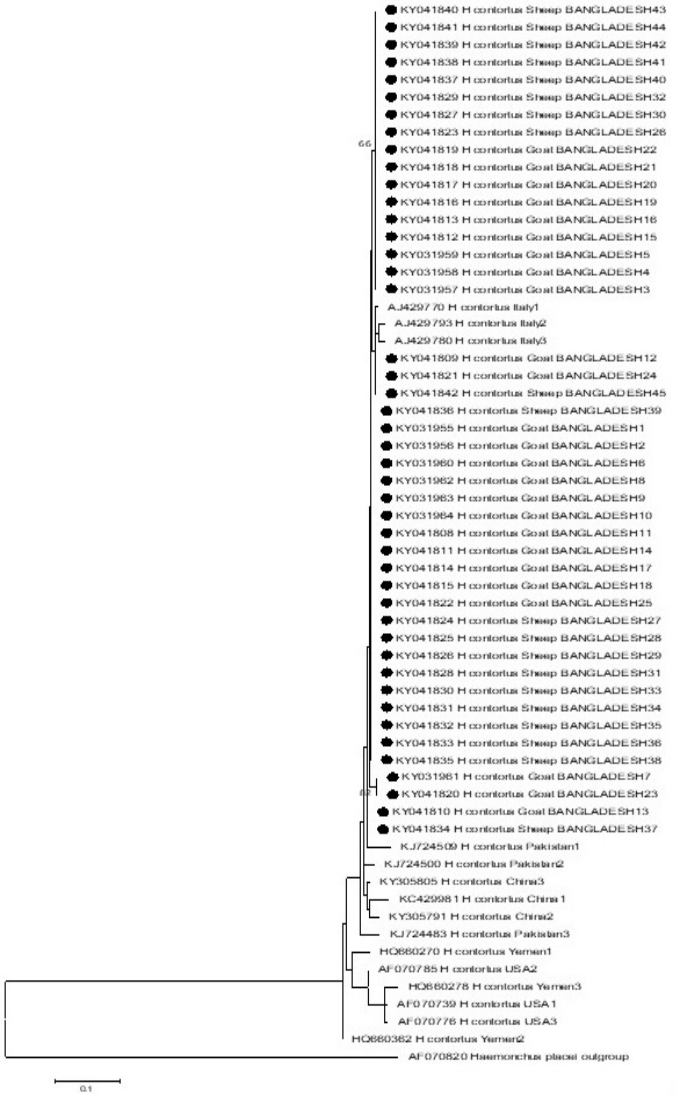

Figure 3: *A phylogenetic tree was constructed based on partial sequences of 5 nad4 genes for Haemonchus contortus. The tree was generated without nucleotide gaps using a Maximum Likelihood analysis with 1000 replicates based on the Tamura 3-parameter model. Only bootstrap values greater than 50% from 1000 replicates are displayed at the nodes. Positions containing gaps and missing data were removed, and other settings were set to default values in MEGA 7.0. H. placi (accession no. AF070820) was used as an outgroup. Black-filled circles represent the sequences generated in this study.*

The prevalence of haemonchosis varied among different regions, with the highest recorded in the coastal region (58.6%), followed by the hilly region (45.5%) and the plain region (35.3%). The environmental conditions, such as relative humidity and ambient temperature, in the coastal areas may provide suitable conditions for the hatching of gravid eggs, resulting in the presence of infective larvae. Animals become infected when they ingest grasses containing these larvae during grazing. In this study, the burden of haemonchosis exhibited significant seasonal variation. The infection intensity was higher during the rainy season (69.9%), followed by the summer season (45.5%) and the winter season (24%). These findings align with a study by Attindehou *et al*. (2012), which reported a higher prevalence during the rainy season (79.4%) compared to the dry season (36%). Adequate rainfall and moisture during the rainy season may enhance larval development and survival on the grazing lands, resulting in an increased occurrence of haemonchosis. Conversely, the winter season, characterized by suboptimal temperature and moisture, may induce hypobiosis, leading to a decreased number of hatched gravid eggs. These factors likely contribute to the lower prevalence of infection during the dry season.

Sequence and phylogenetic analyses

A total of 45 nad4 (25 goats and 20 sheep) and 10 ITS-2 samples with positive results were selected for sequencing analysis. Samples with high-quality DNA were preferred for sequencing to minimize errors and improve accuracy. We successfully obtained 800-bp nad4 and 321-bp ITS-2 nuclear rDNA sequences through sequencing and alignment analysis. The genetic analysis revealed 45 different nad4 genotypes among the *H. contortus* sequences and found that these sequences showed 98.5-100% identity with reference sequences in the GenBank database. Then constructed the phylogenetic tree by using the Maximum Likelihood (ML) method with identified 45 nad4 gene sequences (Figure 3). The tree was constructed using three sequences from each nation (China, Pakistan, Yemen, Italy, and the United States) and five randomly selected sequences from the study. The ML method, combined with bootstrap analysis using 1000 replicates, revealed major clades with over 50% nodal support, using *Haemonchus placei* (AF070820) as the outgroup. Most isolates from the same continent clustered together, but one Yemeni isolate merged with US isolates, and the Bangladeshi isolates formed a group with a nearly identical Chinese isolate. The Pakistani and Chinese isolates also formed the same cluster with strong nodal support.

A 321-bp product of the *H. contortus* ITS-2 gene was obtained and ten genotypes were identified with a similarity range of 99.8-100% among the sequences analyzed. A phylogenetic tree was constructed using 25 randomly selected ITS-2 gene sequences and the Maximum Likelihood (ML) method (Figure 4). Five sequences were randomly selected from each nation (Bangladesh, China, Pakistan, India, and Thailand). The dendrogram showed that the *H. contortus* haplotypes were randomly spread among haplotypes from different countries, with significant nodal support of 66%. No unique relationships were found in the analysis of country phylogenetics. The representative sequences from this study were submitted to the DNA Data Bank of Japan and the European Nucleotide Archive (ENA), with the accession numbers: H. contortus nad4, KY031955-KY031964, KY041808-KY041842; H. contortus ITS-2, KU558755-KU558759.

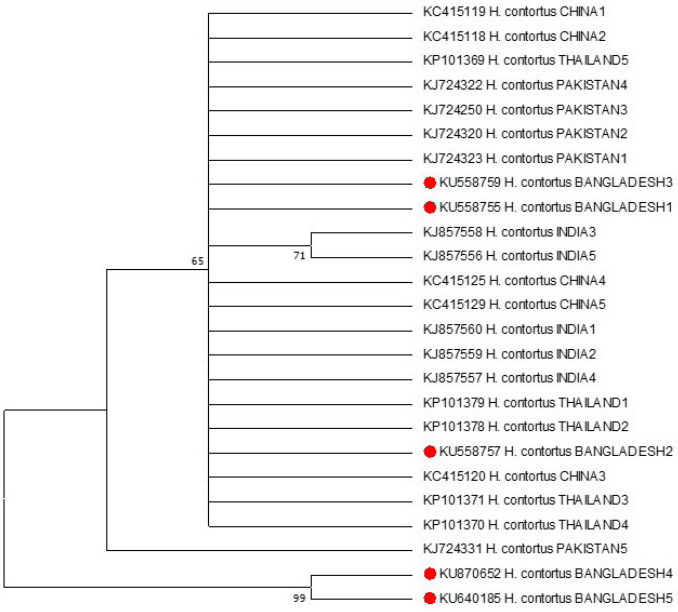

Figure 4: *Phylogenetic tree was constructed based on partial sequences of ITS-2 genes for Haemonchus contortus using MEGA 7.0 and the maximum-likelihood method with Tamura 3-parameter model. The topology was supported by 1000 replications. Bootstrap values lower than 50 were omitted. Red-filled circles represent the sequences generated in this study.*

The evolutionary relationships between sheep and goats were observed using a phylogenetic tree (Figure 3) based on 5 nad4 haplotype sequences. There was no clear grouping between the two populations of

H. contortus, supporting the lack of host specificity reported by Gharamah *et al*. (2012). Further analysis (Figure 4) based on 5 ITS-2 sequences showed no distinct grouping of *H. contortus* among the host populations. Dey *et al*. (2019) reported the absence of phylogeographic structuring among *H. contortus* populations from sheep and goats in seven geographical areas in Bangladesh, suggesting that multiple hosts share common grazing pastures. Significant gene flow between populations may also result from animal movements (Shen *et al*., 2017). Thus, further research is needed to better understand the molecular properties of *H. contortus*. The tree revealed low genetic variation in the *H. contortus* population in Bangladesh, echoing the findings in Malaysia (Gharamah *et al*., 2012), where *H. contortus* isolated from Malaysia and Yemen formed distinct clades with strong gene flow and limited genetic variation. In conclusion, the study highlights the presence of *H. contortus* infection in slaughterhouse animals in Bangladesh.

The limitation of this study was the limited availability of high-quality samples for selection in sequencing. Furthermore, we preferred samples with high-quality DNA that were free of contaminants and had a high yield for sequencing. The cost of sequencing also influenced sample selection, as sequencing, all available samples may have been too expensive.

Regarding the economic impact of Haemonchosis, the disease can lead to reduced productivity in livestock, including decreased growth rates, decreased milk production, and even death in severe cases. This can have significant economic implications for the agricultural sector in Bangladesh where livestock farming is an important source of income and food. In Bangladesh, where agriculture and livestock farming are vital to the economy and the livelihoods of many people, the economic impact of haemonchosis could be substantial. Infected animals may require medical treatment, which can incur costs for farmers in terms of veterinary services and medications. Additionally, reduced productivity and potential losses due to mortality can affect the income and food security of farmers who rely on their livestock.

Conclusions and Recommendations

It may be concluded that the total prevalence of *H. contortus* in small ruminants in the research area was alarmingly high, but the causes for this high incidence could not be determined in this investigation due to the lack of significant relationship among the majority of parameters studied. During the study of the nad4 gene, only minor genetic variants were found. This study recovered DNA sequences of *H. contortus* from a slaughterhouse in Chattogram, Bangladesh for the first time and could serve as a starting point for further investigations aimed at developing control strategies for *H. contortus* in the country. A farm-based extended study may be necessary in the future to uncover other suspected causes.

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Novelty Statement

This study presents a unique exploration into the prevalence and genetic diversity of Haemonchus contortus, a parasitic nematode that affects small ruminants in the Chattogram Metropolitan area. This research contributes to the understanding of parasitic infections in this region, shedding light on the specific strains of Haemonchus contortus present and their potential impact on livestock health. Furthermore, it lays the foundation for the development of targeted control and prevention strategies, with potential implications for the livestock industry and overall agricultural sustainability in Bangladesh.

Author's Contribution

Muhammad Abdul Mannan: Conceptualization, methodology, data curation, software, formal analysis, writing-original draft.
Sharmin Chowdhury: Data curation, investigation, funding acquisition.
Md Abul Hashem: Formal analysis, writing, review and editing.

Md Hazzaz Bin Kabir: Formal analysis, software, writing, review and editing, Supervision.

Ethics statement

This study was approved by the ethical committee of Chattogram Veterinary and Animal Sciences University authority (ethical approval number was CVASU- PAPA: 0007.2014-15/02) and the Chattogram City Corporation, Bangladesh.

Conflict of interest

The authors have declared no conflict of interest.

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