HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

الجمعية السعودية لعلوم الحياة
SAUDI BIOLOGICAL SOCIETY

Original article

Comparison of the first time detected *Oesophagostomum asperum* with *Oesophagostomum columbianum* in sheep and goats in Bangladesh based on the trinity: Morphology, morphometry and genetic diversity

Nusrat Nowrin Shohana, Anita Rani Dey, Sharmin Aqter Rony, Shirin Akter, Bimal Chandra Karmakar, Mohammad Zahangir Alam *

Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ARTICLE INFO

Keywords:
Oesophagostomum
ITS2
COX1
Morphology
Morphometry
Phylogeny

ABSTRACT

Oesophagostomum spp. (Family: Chabertiidae) is keeping a low profile in terms of severity in Bangladesh while maintaining economic loss through disguise within sheep and goats. The study was performed to identify prevalence, confirmation of species through morphology and morphometry followed by phylogeny using *ITS2* and *COX1* genes. In total 384 slaughterhouse-sourced small and large intestines were pooled from Mymensingh, Kishoreganj, Netrokona, Sherpur and Tangail districts of Mymensingh division. Followed by isolation, *O. columbianum* and *O. asperum* were identified following their key morphological features. Notably, *O. asperum* was first time detected in Bangladesh. The overall prevalence of *Oesophagostomum* spp. was found 60.93%. The prevalence of *O. columbianum* (64.95%) was almost double than that of *O. asperum* (35.04%). Among several characters, only the distance between anus to tail tip showed a significant morphological disparity in female. The Neighbor-joining (NJ) phylogenic trees based on *ITS2* and *COX1* genes confirmed the study species. The first time identified *O. asperum* along with morphometry and phylogeny will add value to the fact that nematodes are invisibly present with high prevalence in this country. This study will help to draw specific attention to command a practical control strategy for intervening in economic loss.

1. Introduction

Oesophagostomum or nodular worm is a bursate nematode parasite of large intestine belonging to the family Chabertiidae, a helminth with euryxenous host range starting from ruminants, apes, domestic and wild pigs, monkeys to human (Acevedo-Ramírez et al., 2019; Krief et al., 2010; Legesse and Erko, 2005; McCarthy and Moore, 2000; Stewart et al., 2013; Tariq et al., 2008). Among different *Oesophagostomum* species, *Oesophagostomum columbianum*, *O. asperum* and O. *venulosum* are the most dominant species in small ruminants around the world (Roeber et al., 2011). A plethora of literatures documented on the prevalence of *Oesophagostomum* spp. globally including in Central Mexico (4.5 % in sheep), Ethiopia (58.7 % in sheep and 50.8 % in goats), India (82.9 % in goats and 55.4 % in sheep), Cameron (90 % in both species), China (82.2 % in sheep) and Myanmar (Acevedo-Rami rez et al., 2019; Choubisa and Jaroli, 2013; Hou et al., 2022; Negasi et al., 2012; Ntonifor et al., 2013; Win et al., 2020). In Bangladesh, the reported prevalence of GI nematode infection ranged from 63 to 89.8 % and 63–92 % in sheep and goats, respectively (Chakrabortty et al., 2023; Mazid et al., 2006; Mohanta et al., 2007).

Oesophagostomum are geo-helminths and the suitable climatic ambience of Bangladesh paved their way towards high biotic potential with rapid establishment rate (Kusiluka and Kambarage, 1996; Mohanta et al., 2007). Food or soil contaminated with infective larvae (L3) is the principal route of transmission (Sweeny et al., 2012). Due to browsing feeding habit (top browser), goats are more prone to infection than sheep as larvae of this species are positively hydrotropic and available at the tip of grass-blade (Soulsby, 1982). Adult *Oesophagostomum* causes anemia and rapid weight loss while larvae infection induces black-green diarrhea with mucus and occasionally blood (Zhao et al., 2014).

Diagnosis of *Oesophagostomum* spp. observing only morphological criteria is not supportive enough as almost all nematodes show uniform outer view. However, application of former criterion along with morphometry and PCR-based tools devise solid and doubtless evidence

* Corresponding author.
E-mail addresses: shohana.vpar@bau.edu.bd (N.N. Shohana), anitadey@bau.edu.bd (A.R. Dey), s.a.rony@bau.edu.bd (S.A. Rony), shirin.akter@bau.edu.bd (S. Akter), bimal.231105901@bau.edu.bd (B.C. Karmakar), mzalam@bau.edu.bd (M.Z. Alam).

https://doi.org/10.1016/j.sjbs.2024.103980
Received 23 January 2024; Received in revised form 7 March 2024; Accepted 8 March 2024
Available online 11 March 2024

for specific identification (Dorris et al., 1999; Nath et al., 2021; Wang et al., 2013).

Nowadays, the identification of parasite species and their phylogram analysis are validated through genetic characterization (Cerutti et al., 2010). Besides, several studies accepted that genes of both ribosomal and mitochondrial DNA provide practical knowledge regarding diagnostic probes or species identification markers (Jex et al., 2008; Nath et al., 2021).

As a specific vaccine against *Oesophagostomum* spp. is yet to be ascertained and commercialized, implementation of effective management strategies is the best solution for now. But, in return, these strategies demand accurate identification of hosts and parasites to the species level (Chilton, 2004). Only a few studies detected *O. columbianum* in Bangladesh. Still, there is a paucity of research works mentioning the morphological, morphometrical and molecular identification of adult *O. asperum*. In this study, morphology, morphometry and genetic diversity of adult *O. columbianum* and *O. asperum* had been explored along with differentiation between these two species based on the trinity.

2. Methodology

2.1. Sampling area and sampling period

A slaughter-house based experiment was performed in Mymensingh, Kishoreganj, Netrokona, Sherpur and Tangail districts of Mymensingh in a year-long period from December 2021 to November 2022.

2.2. Sampling technique

A simple random sampling method was applied to study *Oesophagostomum* infection in Mymensingh. Sample requirement of this study was determined using the formula generated by Thrusfield (1995). Considering the prospective prevalence of 50 % (P = 0.50) (Chakrabortty et al., 2023) and a precision of 5 % (d = 0.05), 384 samples were obtained from the study areas of Mymensingh (Fig. 1) at 95 % (i.e. 1.96) confidence interval.

2.3. Collection of adult parasites

After collection of viscera particularly small and large intestines from sheep and goats, the samples were shifted into the laboratory and cut opened through long axis by giving longitudinal incision. The contents were processed for examination by simultaneous washing and sedimentation. Finally, the sediments were examined for intended nematode species for further processing.

2.4. Microscopic examination and identification of parasites

After isolation of adult parasites, they were washed multiple times with phosphate buffered saline (PBS) and subjected to morphological identification using lactophenol blue and identified according to keys and description given previously (Bowman, 2014; Gaddam et al., 2017; Singh, 2003; Soulsby, 1982,).

For morphometric analysis, adult nematodes were treated with warm glycerin alcohol to make them straight and convenient for measuring different body features using photomicroscope (Labomed, Los Angeles, USA). For male parasites, length of the spicules and gubernaculum were measured and calculation of the distances between the vulva to the anus, and the anus to the tip of the tail were performed for female. However, measurement of total body length, body width and esophageal length were also done.

2.5. DNA extraction and PCR amplification

The microscopically identified adult parasites were considered for DNA extraction followed by preservation of DNA at −20 °C. The *ITS2* region of ribosomal DNA (rDNA) and *COX1* region of mitochondrial

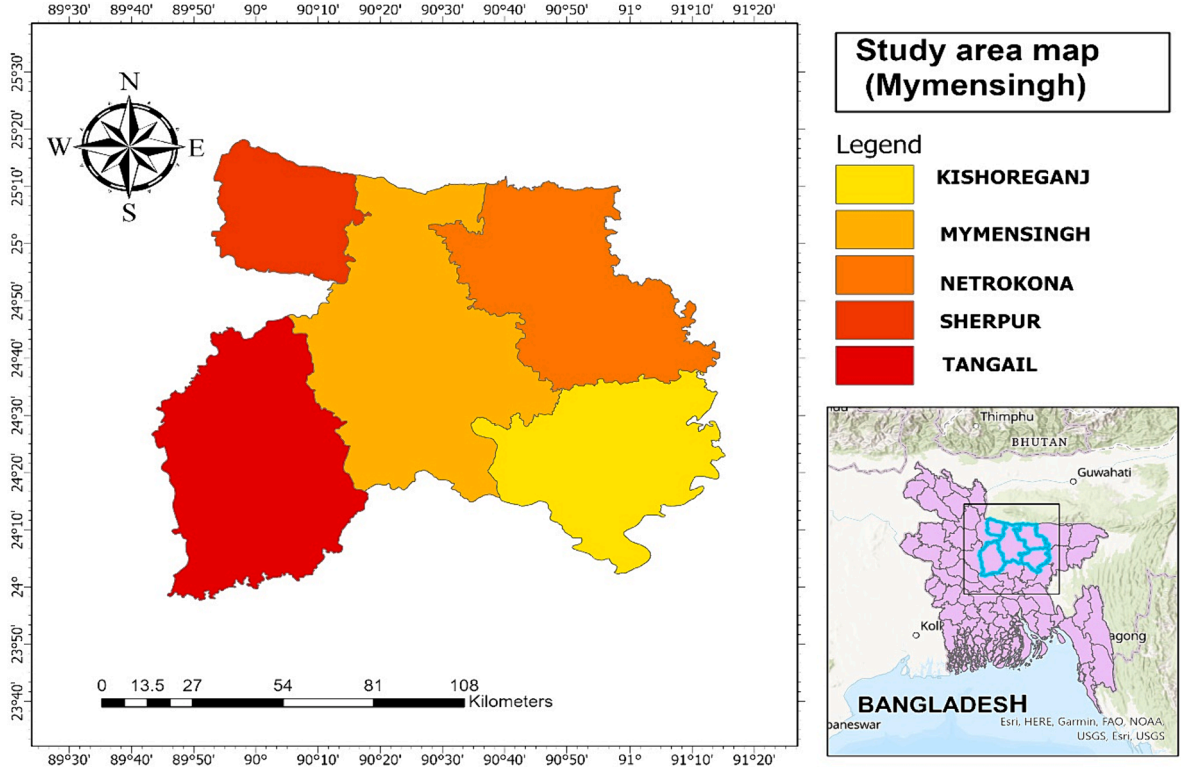

Fig. 1. Map showing the study area. Sheep and goat viscera were collected from several slaughter houses of Kishoreganj, Mymensingh, Netrokona, Sherpur and Tangail districts of Mymensingh division.

DNA (mtDNA) were selected and amplified using the primer sets, NC1 and NC2 for *ITS2* gene (350 bp) and Nemat F and Nemat R for *COX1* gene (720 bp) (Stevenson et al., 1995; Bowles et al., 1992). For both primer pairs, final reaction volume (25 µl) and quantity of primers (1 µl) were exactly the same. PCR conditions for both ITS2 and *COX1* genes are presented in Table 1. Extracted DNA was analyzed and examined through a gel documentation system (1.5 % agarose gel).

2.6. Sequencing of DNA

The positive PCR products showing clear single band in appropriate size were considered for sequencing by applying suitable primers. To single out the exact species of recovered sequences, the reference sequences were searched out through BLAST program, all of them were aligned and edited using MEGA software (Tamura et al., 2013). The aligned studied sequences were submitted in GenBank database with the accession number of LC777640-LC777643, LC777649-LC777650, LC778201-LC778206 and LC778212-LC778221.

2.7. Genetic data analysis

The genotypes, nucleotide diversity (Nd), haplotypes and haplotype diversity (Hd) of each gene were computed applying the DnaSP v6 program (Rozas, 2009). Pairwise comparisons (%) were performed between the studied genotypes and previously published sequences applying the program Bio-Edit (Hall, 1999). Genetic evaluation was conducted by adopting NJ method grounded on the Tamura-Nei model (Tamura et al., 2013). Besides, for NJ tree, bootstrap support and other contexts were adopted from the values available in MEGA (Tamura et al., 2013) along with a 50 % threshold level. In addition, a total of 29 sequences were retrieved from GenBank (**Supplementary file 1**).

2.8. Statistical analysis

Prevalence was calculated using GraphPad Prism software (Agresti and Coull, 1998). An independent sample *t*-test was executed to compare several morphometric characters of *O. asperum* and *O. columbianum* using SPSS version 25 (Statistical Package for the Social Sciences).

3. Results

3.1. Prevalence of Oesophagostomum in sheep and goats from Mymensingh

In this study, out of total 384 slaughtered viscera (small and large intestine), 60.93 % (234/384) samples manifested *Oesophagostomum* spp. infections in sheep (82/234) and goats (152/234). *O. columbianum* identified in 51 % goat and 27.28 % sheep, respectively. Also, *O. asperum* showed 32.89 % prevalence in goat and 17.45 % prevalence in sheep. However, irrespective of host species, the prevalence of infection was higher in *O. columbianum* (64.95 %) compared to *O. asperum* (35.04 %) (Fig. 2).

Table 1
Primers and their sequences used in the study.

Target Genes	Primers	Amplicon size	References
ITS2	NC1 (Forward): ACGTCTGGTTCAGGGTTGTT NC2 (Reverse): TTAGTTTCTTTTCCTCCGCT	350 bp	Stevenson et al., 1995
COX1	Nemat F: TTTTTTGGGCATCCTGAGGTTTAT Nemat R: TAAAGAAAGAACATAATGAAAATG	720 bp	Bowles et al., 1992

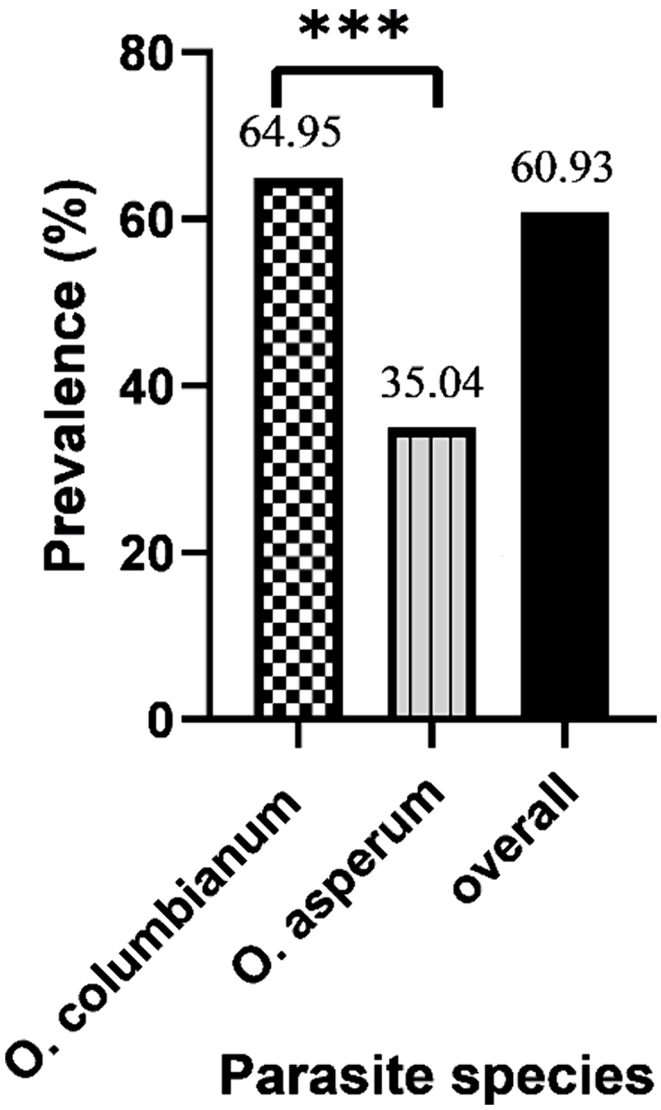

Fig. 2. Prevalence of *Oesophagostomum* species in Mymensingh. The bar diagram indicates significant difference in the prevalence between *O. columbianum* and *O. asperum*. ***, $p < 0.0001$.

3.2. Morphological features of o. Asperum

Adult male and female *O. asperum* were dioecious and twinning in color (milky-white) with medium body size. Males were analogously shorter than the female worms. The adult parasite had a typical straight body with much broader anterior end, depicting as a hood and transversely striated cuticle. Buccal capsule was divided into two elements, external and internal corona radiata along with a well-developed and inflated cervical vesicle. A constriction separated the cervical groove from the rest of the body. And further down near the end of the groove, there was a depression, called the excretory canal. In the case of esophagus, they showed typical club-shaped pattern which can be characterized by initial tapering behind the esophageal duct followed by progressive inflation posteriorly (Fig. 3**A**).

Male had a convex-shaped bursa with bursal rays. The medio and posterio-lateral rays were fused proximally, and discernible from the latero-ventral ray. Dorsal rays had 2 short lateral branches along with externo-dorsal ray and spicule. Spicules were found paired and equal (Fig. 3**B, 3D**).

In female, tail was suddenly tapered from vagina to the tip of the tail. The constriction marked off a conical terminal posterior end where

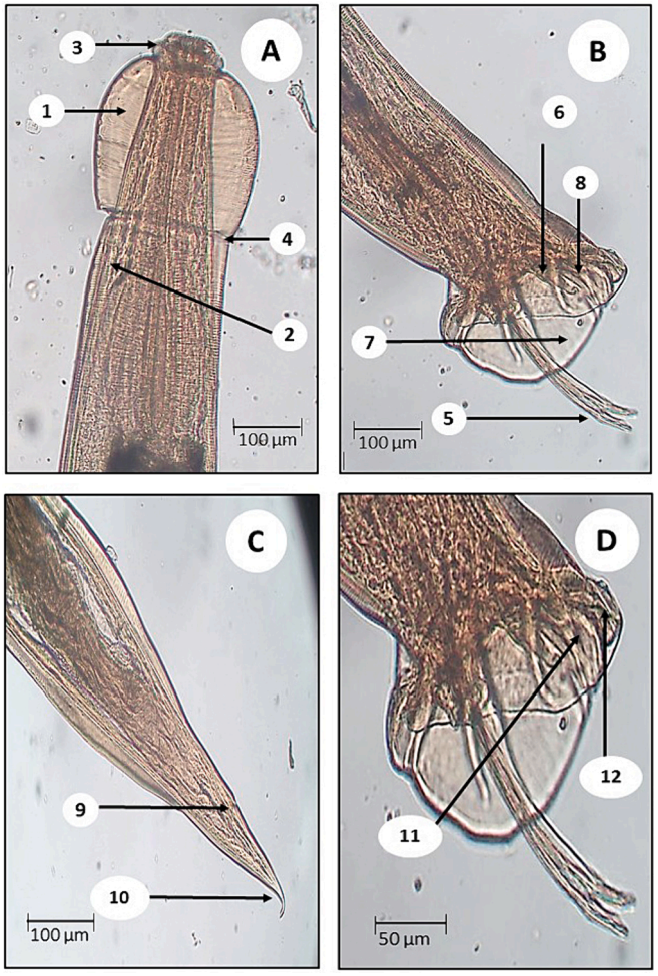

Fig. 3. Microscopic identification of *O. asperum* collected from sheep and goat viscera from Mymensingh. A) Anterior part (both male and female): 1- cervical vesicle, 2- excretory pore, 3- mouth collar, 4- cervical groove; B and D) Posterior part of male: 5- spicule, 6- ventral ray, 7- dorsal lobe of bursa, 8- lateroventral ray, 11- mediolateral ray, 12- posterior-lateral ray; C) Posterior part of female: 9- anus, 10- tail.

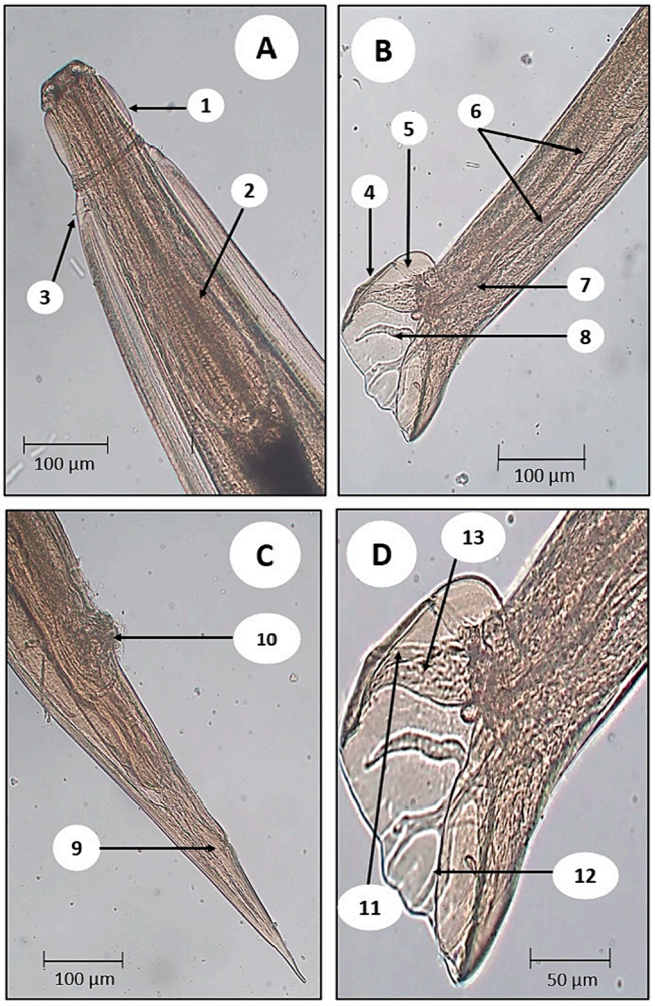

Fig. 4. Microscopic identification of *O. columbianum* collected from sheep and goat viscera from Mymensingh. A) Anterior part (both male and female): 1- cervical vesicle, 2- esophagus, 3- cervical papillae; B and D) Posterior part of male: 4- bursa, 5- ventral ray, 6- spicules, 7- gubernaculum, 8- externo-dorsal ray, 11- mediolateral ray, 12- dorsal ray, 13- posterio-lateral ray; C) Posterior part of female: 9- anus, 10- pars-ejectrix.

vulva and less prominent anus were located. However, in this nematode, vagina was long and opened into relatively anteriorly placed ovejector (Fig. 3**C**).

3.3. Morphological features of o. Columbianum

Despite being originated from the same genus, *O. columbianum* had some morphological disparity with *O. asperum*. At the anterior end, well-developed striated lateral alae, cephalic vesicle and cervical papillae (Fig. 4**A**) are those three striking features which display the discreteness of this species. Leaf-like projections known as external and internal corona radiata wrap the margin of small buccal capsule. On top of that, hook-like curvature of the upper portion of the body was also visible. However, inspite of housing several unique characters, inflated cervical vesicle and excretory canal were little to nowhere to be found.

In male, *O. columbianum* endorsed morphological uniformity with *O. asperum* in parameters like bell-shaped bursa, dorsal rays, lateral rays and spicule (Fig. 4**B, 4D**).

In female, the tail was gradually diminished along with distinct kidney-shaped pars-ejectrix (Fig. 4**C**).

3.4. Morphometric measurements of o. Asperum and o. Columbianum

A total of 200 adult parasites (50 male and 50 female for each species) were undergone through morphometry. Except for the length of spicules and gubernaculum for male and distance from vulva to anus for female; numerous morphometric features were common for both sexes. Measurements have been shown in Table 2. *Oesophagostomum columbianum* evidently stated morphometric deviation from *O. asperum*. Females displayed longer distances ranging 0.24–1.04 mm from vulva and

Table 2
Morphometric measurements of *Oesophagostomum* species.

Parameters (millimeter)	*Oesophagostomum asperum*		*Oesophagostomum columbianum*	
	Male	Female	Male	Female
Total body length	7.60–10.0	9.8–13.48	6.0–10.0	9.0–14.4
Total body width	0.34–0.38	0.42–0.48	0.28–0.40	0.28–0.50
Length of the esophagus	0.62–0.64	0.60–0.68	0.60–0.70	0.60–0.76
Length of spicules	0.90–1.33	–	0.20–1.44	–
Length of the gubernaculum	0.07–0.12	–	0.07–0.16	–
Distance from vulva to anus	–	0.28–0.55	–	0.24–1.04
Distance from anus to tail tip	–	0.10–0.14	–	0.10–0.46

0.10–1.46 mm to tail tip depositing anus as the middle point within these two features. While numerous parameters complied similar ranges with *O. asperum*, length of spicules (0.20–1.44 mm) of *O. columbianum* showed variation in the sense of elongation (Table 2).

3.5. Genetic analysis of o. Columbianum and o. Asperum

3.5.1. Species identification and genotyping

For the identification of species, amplification of *ITS2* gene was followed by sequencing of 23 representative samples from different areas of Mymensingh. A 320 base pair consensus length was obtained for all the samples. From 23 *ITS2* sequences, 6 distinct genotypes were obtained. The sequence similarities were 98.1–99.6 % and 99.6–100 %, when contrasted with one another, or with 2 *ITS2* BLAST sequences (1 for each) of *O. columbianum* (JX188470.1) and *O. asperum* (KM200806.1), respectively. Following the comparison of 6 study genotypes with the one *ITS*2 sequence of *Oesophagostomum bifurcum* (HQ283349.1), the nucleotide identities ranged from 21.2 % to 26.2 % involving both study species (Table 3).

Four *ITS2* genotypes of *O. columbianum* were aligned with the reference sequence (JX188470.1) and nucleotide positions 68, 106, 121, 156, 157 and 238 displayed polymorphisms, each depicted as single nucleotide polymorphism (SNP). There were two transversions (G<->A) and four transitions (T<->C) among those 6 polymorphisms (Table 4). Besides, *O. columbianum* also showed the genetic divergence of 0.00343 (nucleotide diversity) and 0.8492 (genotype diversity). Alignment of 2 *ITS2* genotypes of *O. asperum* with the recovered GenBank sequence (KM200806.1) detected only a single nucleotide polymorphism (SNP) at nucleotide position 140 which was a transversion (G<->A). *ITS*2 sequences of *O. asperum* showed the nucleotide diversity of 0.00081 along with the genotype diversity of 0.261.

3.5.2. Phylogenetic analysis using ITS2 gene

Following the identification of species, the phylogram was created using 6 studied sequences of *O. columbianum* and *O. asperum* combining with 19 *ITS2* sequences of *Oesophagostomum* from different countries (**Supplementary file 1**) where *Haemonchus contortus* used as out-group (Fig. 5). The analysis separated the NJ phylogram into two main clusters depicting two study species. Cluster I was unique with the studied and reference sequences of *O. asperum* with strong nodal support (74 %). But cluster II divided into two sister clades where sister clade 1 clustered with the sequences of *O. columbianum* and sister clade 2 with the sequences of *O. bifurcum*. The unique clustering pattern of studied species with the reference sequences of *O. columbianum* and *O. asperum* confirming the species of the studied sequences.

3.5.3. COX1 gene: Genetic diversity

Total 25 *COX1* sequences of *O. columbianum* and *O. asperum* were analyzed. A 695 base pair gene fragment was considered for making a phylogenetic tree. From the 25 *COX1* amplicons, 16 distinct haplotypes were identified (6 from *O. columbianum* and 10 from *O. asperum*). For *O. columbianum* (6 isolates), the nucleotide diversity was 0.00534 and haplotype diversity was 0.8364. Besides, for 10 haplotypes of *O. asperum,* haplotype diversity was noted 0.9231 with nucleotide diversity, 0.0315.

Table 4
Nucleotide details and distribution of 4 genotypes from 9*O. columbianum* worms isolated from sheep and goats.

Genotypes	Nucleotide position					
	68	106	121	156	157	238
JX188470.1	T	G	C	T	G	T
OC1	.	.	.	C	A	C
OC2	C	.	T	C	A	C
OC3	C	.	.	C	A	C
OC4	.	A	.	C	A	C

OC, *O. columbianum;* Dot (.) represents similar position with JX188470.1

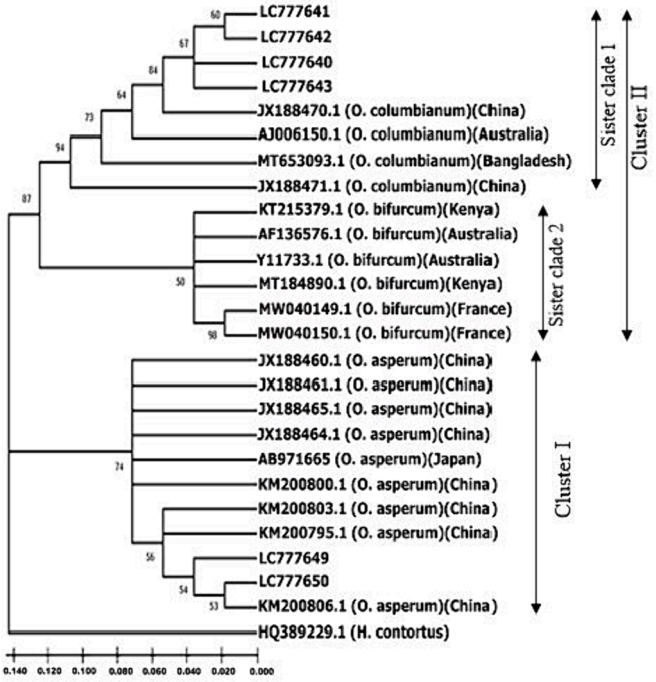

Fig. 5. The Neighbor-joining phylogenetic analysis was based on 25 nucleotide sequences of *ITS2* gene of *O. columbianum* and *O. asperum*. Among them nineteen *ITS2* sequences were retrieved from GenBank databases and applied for analysis with six studied sequences (Accession nos. LC777641-LC777643, LC777649-LC777650). Only > 50 % of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown. The sequence of *Haemonchus contortus* (Accession no. HQ389229.1) was used as out group.

Table 3
Pairwise identities (%) among 6 ITS2 genotypes of *O. columbianum* and *O. asperum* from Mymensingh using reference sequences of different *Oesophagostomum* spp. from GenBank.

Sample ID	1	2	3	4	5	6	7	8	9
1.OA1									
2.OA2	99.6								
3.OC1	79.9	80.2							
4.OC2	79.9	80.2	99.3						
5.OC3	79.6	79.9	99.6	99.6					
6.OC4	80.2	80.5	99.6	99.0	99.3				
7. O. asperum (KM200806.1)	99.6	100	80.2	80.2	79.9	80.5			
8.*O. columbianum* (JX188470.1)	80.2	80.5	98.7	98.1	98.4	98.4	80.5		
9.*O. bifurcum* (HQ283349.1)	26.2	26.2	21.6	22.2	21.9	21.2	26.2	22.5	

OC, *O. columbianum;* OA, *O.asperum;* Black boxes indicate 100%sequence similarity

3.5.4. COX1 gene: Phylogenetic analysis

All recovered sequences were harmonized with other available *COX1* reference sequences of both study species with 97 %-100 % similarity.

To genetically compare all recovered *COX1* sequences from Mymensingh district with foreign countries, a NJ phylogenic tree was constructed. For this, 16 studied haplotype data set of *O. columbianum* and *O. asperum* along with 7 sequences from China, 3 from Brazil and as out-group, *Haemonchus contortus* was employed (**Supplementary file 1**). Here, two crucial clusters were viewed by bootstrap analysis with 1000 replicates. The large group divided into two sister clades with strong bootstrap support (100 %). Sister clade 1 and 2 uniquely grouped with the studied and reference sequences. But the smaller group (Sister clade 3) of Chinese reference sequences did not grouped with the study sequences. The clade forming nature of studied sequences confirmed species identification. The isolates of *O. columbianum* from Brazil and China formed monophyletic clade with the studied isolates of *O. columbianum* (Accession no: LC778201- LC778206) from Bangladesh. Besides, the isolates of *O. asperum* from China formed cluster with Bangladeshi isolates (LC778212-LC778221). However, another small group is found occupying only Chinese isolates of *O. asperum*. In this tree, it is evident that, the isolates did not follow any specific relation in terms of host and geographical origin (Fig. 6).

4. Discussion

Oesophagostomum, a nodular worm inhabiting in small and large intestine of cattle, sheep, pig and primates as a serious pathogen and causing severe interference in absorption of nutrients, movement of bowel and digestion (Soulsby, 1982). This parasite is prevalent world-wide but more commonly in tropical and subtropical areas (Nath et al., 2014; Ntonifor et al., 2013; Win et al., 2020).

The frequency of *Oesophagostomum* worm encountered in this experiment (60.93 %) is higher but not as much as Mazid et al., (2006) (89.89 %) and Nath et al., (2014) (92 %) documented. Besides, Islam et al., (2017) (15.9 %), Rahman et al., (2017) (10.8 %), Hassan et al., (2011) (10.9–12.9 %) and Dagnachew et al., (2011) (37.6 %) reported comparatively lower frequency. Both of the study species belong to the same genus as well as share common location and food resources which might lead to 'interspecies competition'. Higher prevalence of *O. columbianum* may be an indication of triumph over *O. asperum*. Besides parasite species, there is also variation in prevalence between two host species. As a top browser, goats are more prone to infection (Soulsby, 1982) and in sheep, presence of putative quantitative trait loci (QTL) responsible for resistance against internal nematode infection is already reported (Dominik et al., 2010). Besides, lack of sufficient available data concerning prevalence of only *O. asperum* in Bangladesh pairing with smaller and scattered study areas might be the reasons behind the variation in prevalence. However, the noticeable contrasts amid the present and previous findings may be as a result of variation in location, climate, sample size, nature and population size of studied animals together with methods of examinations.

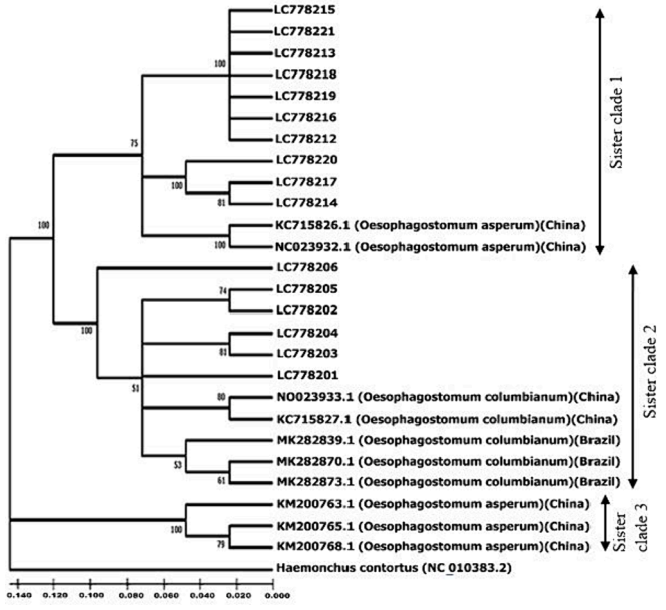

Fig. 6. The Neighbor-joining phylogenetic analysis was based on 26 nucleotide sequences of *COX1* gene of *O. columbianum* and *O. asperum*. Only > 50 % of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) was represented in the dendogram. Ten *COX1* sequences were retrieved from GenBank databases and used for analysis with sixteen studied sequences (Accession nos. LC778201-LC778206, LC778212-LC778221). The sequence of *Haemonchus contortus* (Accession no. NC_010383.2) was used as out group.

In morphometry of male *O. asperum*, total body length and esophageal length were found 7.60–10.0 and 0.62–0.64 mm, respectively which are relatively shorter than the features described by Soulsby (1982). Body width, length of the gubernaculum and spicules were recorded 0.34–0.38, 0.07–0.12 and 0.90–1.33 mm, sequentially. In female, only total body width of the study *O. asperum* (0.42–0.48 mm) were broader than the features illustrated by Soulsby (1982). As per our knowledge, still today, there is scarcity of articles mentioning measurements of other morphometric features of adult *O. asperum*, namely, esophageal length, distance between vulva to anus and anus to tip of the tail, length of the gubernaculum and spicules.

All adult male and female *O. columbianum* were pointed out by observing their morphological characters marking both anterior and posterior ends which displayed analogy with the specimens described previously (Gaddam et al., 2017; Nath et al., 2021). Interestingly, anterior end was identical irrespective of sex of the parasites. Even if there was presence of well-developed lateral alae, cephalic vesicle was merely present in the current study. Nath et al., (2021) agreed with the statement while Goodey (1924) and Soota (1981) outlined highly developed cephalic vesicle. *Oesophagostomum columbianum* was occupied with two pear-shaped cervical papillae just behind the constriction. All of these observations are compatible with the description of Nath et al., (2021) and Zhao et al., (2014). Yadav and Tendon (1992) addressed bending of the anterior end like a hook which also represent one of the findings of the present study. Descriptions of these inquiry namely, moderate sized worm with sexual dimorphism, females being larger than the male, straight body with tapering at both ends are also in agreement with Gaddam et al., (2017) and Nath et al., (2021). Striated cuticle with full-length lateral alae matched with the characteristics reported by Goodey (1924), Nath et al., (2021) and Zhao et al., (2014). The Scanning Electron Microscopy findings of Yadav and Tandon (1992), Gaddam et al., (2017) and Soulsby (1982) supported the present study inferences. In male *O. columbianum*, present study reported dorsal

This study announced with certainty the morphological elements of *O. asperum* describing dioecious nature and sexual dimorphism are in agreement with Nath et al., (2021). Unlike *O. columbianum*, *O. asperum* depicted inflated cervical vesicle which mimics the explanation of Gaddam et al., (2017) and Singh (2003). However, there was absence of lateral alae in this study which is in coincidence with the statement of Soulsby (1982). Yadav and Tandon (1992) illustrated similar features of this study regarding mouth collar and cervical groove. In male *O. asperum*, the statement of this study explaining developed bursa along with lateral and dorsal rays expressed similarity with Gaddam et al., (2017) and Singh (2003). Spicules were equal in length just like Soulsby (1982) detected. In female, Gaddam et al., (2017) and Singh (2003) reported that the tail tapered abruptly with shorter distance between vulva and tail tip which is coherent with the present study findings. In addition, the length and position of vagina endorse the descriptions of Singh (2003).

lobe with several ventral and lateral rays. All of these are in coincidence with the findings of Gaddam et al., (2017), Singh (2003) and Soulsby (1982). Spicules were equal and alate which are identical to the statement of Singh (2003) and Soulsby (1982). In females, anus appeared as a fissure and tail was tapered following gradual reduction manner with slightly prominent vulva. Vagina was shorter which opened posteriorly in a kidney-like structure, Pars-ejectrix which are coherent with Khanmohammadi et al., (2013) and Soulsby (1982).

Unlike anterior end, during morphometry, lower part of *O. columbianum* showed more versatility and represented several elements appropriate not only for identification but also to sort sexuality. In male, length of the body was found relatively shorter than the reports previously published by Goodey (1924), Nath et al., (2021) and Soota et al., (1981). Yet, total body width and length of gubernaculum were in harmony with Goodey (1924) and Nath et al., (2021). However, longer esophageal (0.60–0.70 mm) and spicule length (0.07–0.16 mm) were outlined in multiple literatures (Nath et al., 2021; Ransom, 1911; Soota et al., 1981) than those in the present study. In female *O. columbianum*, higher range of body length (12–18 mm) had been documented in former experiments (Goodey, 1924; Nath et al., 2021; Soota et. al., 1981) compared to this research work (9.0–14.4 mm). Interestingly, total body width and length of the esophagus were also coherent with the results of Nath et al., (2021) and Soota et al., (1981). Besides, distance between vulva to anus and anus to tip of the tail supported the statements of the previously published data (Goodey, 1924; Nath et al., 2021). Both for morphology and morphometry, inspite of having same origin, due to genetic changes over time, variation (based on physical and reproductive parts) may exist between species within the same genus.

Unfortunately, there are several articles where both of the species were studied for morphological analysis without involving any morphometrical comparison between them which justifies lack of referencing data regarding the difference between adult *O. asperum* and *O. columbianum* in case of morphometry (Gaddam et al., 2017; Zhao et al., 2013; Zhao et al., 2014).

When the study sequences were compared with reference sequences from China, Japan and Australia from BLAST search, sequence identities ranged from 97 to 100 % (NCBI, 2022). Distinguished morphological characters of these two study species is proved by very high intra specific variation (19.5–20.1 %) between them which is supported by Hu et al., (2014) and Li et al., (2016). Furthermore, slightly higher sequence diversity has also been reported by Jia et al., (2014) and Zhao et al., (2014). In this research work, 0.7–1 % and 0.4 % variation in sequence identities were observed in *ITS*2 sequences among *O. columbianum* and *O. asperum* isolates, respectively. The value of disparity exactly coincides with the variation of reference sequences from China and Brazil (Bacelar et al., 2022; Li et al., 2016; Zhao et al., 2014). However, divergence (0–1.5 %) was also reported among *O. asperum* isolates from Shaanxi province and Hunan province, China (Li et al., 2016; Yu et al., 2012). Among 23 sequences of *ITS*2 gene, we determined 6 unique genotypes. Of them, recovered 4 isolates of *O. columbianum* outnumbered the previously reported articles including a single isolate from Bangladesh and two isolates from China (Nath et al., 2021; Zhao et al., 2013). Among 6 isolates, 2 of them belong to *O. asperum* but the number varied in several studies experimented with this species including Makouloutou et al., (2014), Yu et al., (2012) and Zhao et al., (2013).

Among several mtDNA, cytochrome oxidase subunit 1 (*COX1*) gene was more suitable genetic markers than that of nuclear rDNA due to maternal origin and higher substitution rates, thus making it proficient to differentiate among closely connected groups (Anderson et al., 1998; Liu et al., 2012; Troell et al., 2006). Here, the *COX1* gene was adopted to ascertain the presence and level of genetic diversity of *O. columbianum* and *O. asperum* in Mymensingh. Comparatively lower nucleotide diversity (0.009) has been reported in China (Li et al., 2013) than that of *O. columbianum* (0.00534) and *O. asperum* (0.0315) of this study. This variation of nucleotide diversity may be due to scarce availability of relevant sequences in GenBank. Here, 25 *COX1* sequences from Mymensingh denoted 16 unique haplotypes with high haplotype diversity (Hd) of both species (*O. columbianum*: 0.8364 and *O. asperum*: 0.9231). These significant values are supported by Li et al., (2013) who found nine haplotypes for p*COX1* gene (Hd = 0.81) and fifteen haplotypes for pnad1 gene (Hd = 0.939). High degrees of haplotype diversity have also been documented in other nematode species including *Haemonchus contortus* isolates originated from Bangladesh (77 haplotypes), Thailand (122 haplotypes) and Pakistan (73 haplotypes) (Dey et al., 2019; Hussain et al., 2014; Pitaksakulrat et al., 2021). Disparity of genetic diversity may resurface when a species undergoes mutations and genetic differentiation over an extended period of time since their time of origin.

As a nematode, *Oesophagostomum* spp. is difficult to identify and differentiate according to their morphological features. To serve the purposes of detection and distinction, the revolutionary molecular technique has evolved and is accepted worldwide (Yu et al., 2012). The nuclear (*ITS2*) and mitochondrial (*COX1*) genes play an effective role in performing accurate genetic analysis of the parasites. The *ITS2* gene of rDNA is an important marker in identifying helminths (Králová-Hromadová et al. 2012; Luton et al., 1992; Stevenson et al., 1995; Zhao et al., 2014) because of its easy amplification, enough conserved regions and large variation to discriminate the inter and intra species (Ghobashy and Taeleb, 2015). In the study, within the phylogenetic tree, *O. columbianum* and *O. asperum* formed distinct monophyletic clusters with sequences from several countries mostly China, Japan and Australia. Bootstrap support (87 % and 74 %) of *O. columbianum* and *O. asperum* expressed noteworthy harmony with Yu et al., (2012) and Zhao et al., (2014).

Besides *ITS2*, mtDNA is also practiced as a marker because of its greater variation rate and uniparental inheritance (Liu et al., 2012; Nath et al., 2021). The phylogeny of *COX1* followed the same pattern of *ITS2*. *COX1* gene sequences also formed a distinctive group of both study parasites and GenBank references of *O. columbianum* and *O. asperum* with strong bootstrap value (100 %). This finding is consistent with Zhao et al., (2013). Besides, Bangladeshi isolates of *Haemonchus contortus* and *Mecistocirrus digitatus* also formed cluster with China and Japan in dendograms made with *ITS2* and *nad4* genes (Dey et al., 2019; Parvin et al., 2021). In these phylogenic trees, the reason behind considering sequences from China, Japan and Australia as a sister clade by study sequences might be owing to importation of a great quantity of animals and by products from those countries.

5. Conclusions

Oesophagostomum is highly prevalent and *O. columbianum* is more common compared to *O. asperum* in Mymensingh, Bangladesh. Morphology of both species showed distinct morphological features especially in cephalic vesicle and excretory canal at the anterior end as well as shape of spicule in male and visibility of anus in female at the posterior part. In morphometry, female features of *O. columbianum* showed larger distance ranges than those in *O. asperum*. Among all the morphometric features of *O. asperum* female, only the distance between anus to tail tip showed significant difference with *O. columbianum* female. The phylogenetic analysis of the sequence data belonging to both *ITS*2 and *COX1* genes confirmed two distinct species as *O. columbianum* and *O. asperum* while *O. asperum* was first time detected in Bangladesh.

Author's contribution

NNS received the thesis grant. NNS and MZA were involved in designing the present study and collection of data. NNS, BCK and MZA performed laboratory experiments. NNS and ARD conducted data analyses. NNS, SA and MZA interpreted the data. NNS, SAR and MZA wrote the draft of the manuscript. All authors checked and approved the final manuscript.

Ethical approval

The present study was approved by Animal Welfare and

Experimentation Ethics Committee (AWEEC) of Bangladesh Agricultural University, Mymensingh, Bangladesh (AWEEC/BAU/2021 (49)).

CRediT authorship contribution statement

Nusrat Nowrin Shohana: Conceptualization, Funding acquisition, Methodology, Writing – original draft, Writing – review and editing, Formal analysis, Software. **Anita Rani Dey:** Writing – review and editing, Visualization, Validation, Software. **Sharmin Aqter Rony:** Methodology, Supervision, Validation. **Shirin Akter:** Methodology, Writing – review and editing, Supervision. **Bimal Chandra Karmakar:** Data curation. **Mohammad Zahangir Alam:** Conceptualization, Funding acquisition, Visualization, Investigation, Supervision, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors greatly acknowledge Bangladesh Agricultural University for funding the research project (2020/957/BAU) and Ministry of Science and Technology, The Government of Bangladesh for National Science and Technology grant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2024.103980.

References

Acevedo-Ramírez, P.M.D.C., Hallal-Calleros, C., Flores-Pérez, I., Alba-Hurtado, F., Mendoza-Garfías, M.B., Del Campo, N.C., Barajas, R., 2019. Anthelmintic effect and tissue alterations induced in vitro by hydrolysable tannins on the adult stage of the gastrointestinal nematode *Haemonchus contortus*. Vet. Parasitol. 266, 1–6.

Agresti, A., Coull, B.A., 1998. Approximate is better than "exact" for interval estimation of binomial proportions. Am. Stat. 52, 119–126.

Anderson, T.J., Blouin, M.S., Beech, R.N., 1998. Population biology of parasitic nematodes: applications of genetic markers. Adv. Parasitol. 41, 219–283.

Bacelar, P.A.A., Monteiro, K.J.L., Calegar, D.A., Santos, J.P., Coronato-Nunes, B., Carvalho-Costa, F.A., 2022. Cytochrome c oxidase subunit 1 gene reveals species composition and phylogenetic relationships of *Oesophagostomum* spp. infecting pigs in northeastern Brazil. Rev. Bras. Parasitol. Vet. 31, e018421.

Bowles, J., Blair, D., McManus, D.P., 1992. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol. Biochem. Parasitol. 54, 165–173.

Bowman, D.D., 2014. Georgis' parasitology for veterinarians-e-book, 10th ed. Elsevier Health Sciences.

Cerutti, M.C., Citterio, C.V., Bazzocchi, C., Epis, S., D'Amelio, S., Lanfranchi, P., 2010. Genetic variability of Haemonchus contortus (Nematoda: Trichostrongyloidea) in alpine ruminant host species. J. Helminthol. 84, 276–283.

Chakrabortty, M., Shohana, N.N., Begum, N., Dey, A.R., Rony, S.A., Akter, S., Alam, M.Z., 2023. Diversity and prevalence of gastrointestinal parasites of Black Bengal goats in Natore. Bangladesh. J. Adv. Vet. Anim. Res. 10, 80–87.

Chilton, N.B., 2004. The use of nuclear ribosomal DNA markers for the identification of bursate nematodes (order Strongylida) and for the diagnosis of infections. Anim. Health Res. Rev. 5, 173–187.

Choubisa, S., Jaroli, V., 2013. Gastrointestinal parasitic infection in diverse species of domestic ruminants inhabiting tribal rural areas of southern Rajasthan. India. J. Parasit. Dis. 37, 271–275.

Dagnachew, S., Amamute, A., Jemberu, W.T., 2011. Epidemiology of gastrointestinal helminthiasis of small ruminants in selected sites of North Gondar zone, Northwest Ethiopia. Ethiop. Vet. J. 15, 57–68.

Dey, A.R., Zhang, Z., Begum, N., Alim, M.A., Hu, M., Alam, M.Z., 2019. Genetic diversity patterns of *Haemonchus contortus* isolated from sheep and goats in Bangladesh. Infect. Genet. Evol. 68, 177–184.

Dominik, S., Hunt, P.W., Mcnally, J., Murrell, A., Hall, A., Purvis, I.W., 2010. Detection of quantitative trait loci for internal parasite resistance in sheep. I. Linkage analysis in a Romney×Merino sheep backcross population. Parasitology 137, 1275–1282.

Dorris, M., Ley, P.D., Blaxter, M.L., 1999. Molecular analysis of nematode diversity and the evolution of parasitism. Parasitol. Today 15, 188–193.

Gaddam, R., Murthy, G.S.S., Kommu, S., 2017. Occurrence of *Oesophagostomum* species in slaughtered sheep in area of Hyderabad, Telangana State. J. Parasit. Dis. 41, 809–813.

Ghobashy, M.A., Taeleb, A.A., 2015. Molecular characterization of Raillietina (R.) spp. Ortlepp, 1938 (Cestoda: Cyclophyllidea: Davaineidae) infecting domestic and wild birds (Columba livia and Columba livia domestica). World J. Zool. 10 (2), 136–141.

Goodey, T., 1924. Oesophagostome of goats, sheep, and cattle. J. Helminthol. 3, 97–110.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series [London]: Information Retrieval Limited c1979-c2000 95-98.

Hassan, M.M., Hoque, M.A., Islam, S.K.M.A., Khan, S.A., Roy, K., Banu, Q., 2011. A prevalence of parasites in Black Bengal goats in Chittagong. Bangladesh. Int. J. Livest. Prod. 2, 40–44.

Hou, B., Yong, R., Wuen, J., Zhang, Y., Buyin, B., Subu, D., Zha, H., Li, H., Hasi, S., 2022. Positivity rate investigation and anthelmintic resistance analysis of gastrointestinal nematodes in sheep and cattle in ordos China. Animals 12, 891.

Hussain, T., Periasamy, K., Nadeem, A., Babar, M.E., Pichler, R., Diallo, A., 2014. Sympatric species distribution, genetic diversity and population structure of *Haemonchus* isolates from domestic ruminants in Pakistan. Vet. Parasitol. 206, 188–199.

Islam, M.S., Hossain, M.S., Dey, A.R., Alim, M.A., Akter, S., Alam, M.Z., 2017. Epidemiology of gastrointestinal parasites of small ruminants in Mymensingh. Bangladesh J. Adv. Vet. Anim. Res. 4, 356–362.

Jex, A.R., Hu, M., Littlewood, D.T.J., Waeschenbach, A., Gasser, R.B., 2008. Using 454 technology for long-PCR based sequencing of the complete mitochondrial genome from single Haemonchus contortus (Nematoda). BMC Genomics 9, 11.

Jia, Y.Q., Zhao, G.H., Hu, B., Cheng, W.Y., Du, S.Z., Bian, Q.Q., Ma, X.T., Yu, S.K., 2014. Genetic variability in the three mitochondrial genes among *Oesophagostomum asperum* isolates from different regions in Shaanxi and Hunan Provinces. China. Mitochondrial DNA. 25, 298–302.

Khanmohammadi, M., Halajian, A., Ganji, S., 2013. First scanning electron microscope observation on adult Oesophagostomum venulosum (rudolphi, 1809) (nematoda: strongylida, chabertiidae). Veterinarija Ir Zootechnika. 62, 56–61.

Králová-Hromadová, I., Bazsalovicsová, E., Oros, M., Scholz, T., 2012. Sequence structure and intragenomic variability of ribosomal ITS2 in monozoic tapeworms of the genus Khawia (Cestoda: Caryophyllidea), parasites of cyprinid fish. Parasitol. Res. 111, 1621–1627.

Krief, S., Vermeulen, B., Lafosse, S., Kasenene, J.M., NieguITSila, A., Berthelemy, M., L'Hostis, M., Bain, O., Guillot, J., 2010. Nodular worm infection in wild chimpanzees in western Uganda: a risk for human health? PLOS Negl. Trop. Dis. 4, e630.

Kusiluka, L., Kambarage, D., 1996. Diseases of small ruminants: a handbook. In, Common Diseases of sheep and goats in Sub-Saharan Africa. edn. VETAID, Scotland Kuznetsov D, Kuznetsova N 2007: Sequences of the second internal transcribed spacer of ribosomal DNA for three species of *Trichostrongylus* (Nematoda: Trichostrongylidae from sheep in Russia. Helminthologia. 44, 43-46.

Legesse, M., Erko, B., 2005. "Prevalence of intestinal parasites among school children in a rural area close to the southeast of Lake Langano, Ethiopia".Ethiop. J. Health Dev. 18, 117–120.

Li, F., Hu, T., Duan, N., Li, W., Teng, Q., Li, H., Cheng, T.Y., 2016. Sequence variation in two mitochondrial DNA regions and internal transcribed spacer among isolates of the nematode *Oesophagostomum asperum* originating from goats in Hunan Province. China. J. Helminthol. 90, 1–6.

Li, J.Y., Liu, G.H., Wang, Y., Song, H.Q., Lin, R.Q., Zou, F.C., Liu, W., Xu, M.J., Zhu, X.Q., 2013. Sequence variation in three mitochondrial DNA genes among isolates of *Ascaridia galli* originating from Guangdong, Hunan and Yunnan provinces. China. J. Helminthol. 87, 371–375.

Liu, G.H., Li, B., Li, J.Y., Song, H.Q., Lin, R.Q., Cai, X.Q., Zou, F.C., Yan, H.K., Yuan, Z.G., Zhou, D.H., Zhu, X.Q., 2012. Genetic variation among *Clonorchis sinensis* isolates from different geographic regions in China revealed by sequence analyses of four mitochondrial genes. J. Helminthol. 86, 479–484.

Luton, K., Walker, D., Blair, D., 1992. Comparisons of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). Mol. Biochem. Parasitol. 56 (2), 323–327.

Makouloutou, P., Nguema, P.P., Fujita, S., Takenoshita, Y., Hasegawa, H., Yanagida, T., Sato, H., 2014. Prevalence and genetic diversity of *oesophagostomum stephanostomum* in wild lowland gorillas at moukalaba-doudou national park. Gabon. Helminthologia. 51, 83–93.

Mazid, M.A., Bhattacharjee, J., Begum, N., Rahman, M., 2006. Helminth parasites of the digestive system of sheep in Mymensingh. Bangladesh. Bangl. J. Vet. Med. 4, 117–122.

McCarthy, J.S., Moore, T.A., 2000. Emerging helminth zoonoses. Int. J. Parasitol. 30, 1351–1360.

Mohanta, U.K., Anisuzzaman Farjana, T., Das, P.M., Majumder, S., Mondal, M.M.H., 2007. Prevalence, population dynamics and pathological effects of intestinal helminths in black bengal goats. Bangl. J. Vet. Med. Anim. Health. 5, 63–69.

Nath, T.C., Bhuiyan, M.J.U., Mamun, M.A., Datta, R., Chowdhury, S.K., Hossain, M., Alam, M.S., 2014. Common infectious diseases of goats in chittagong district of Bangladesh. Int. J. Agric. Res. 1, 43–49.

Nath, T.C., Park, D.L.H., Choe, S.S., Ndosi, B.A., Kang, Y., Bia, M.M., Eamudomkarn, C., Mohanta, U.K., Islam, K.M., Bhuiyan, J.U., Jeon, H.K., 2021. Morphometrical and molecular characterization of oesophagostomum columbianum (Chabertiidae: Oesophagostominae) and haemonchus contortus (Trichostrongylidae: Haemonchinae) Isolated from Goat (Capra hircus) in Sylhet, Bangladesh. J Parasitol. Res., 8863283

NCBI *Nucleotide*. 2022. National Center for Biotechnology Information. online. Available from: https://www.ncbi.nlm.nih.gov .

Negasi, W., Bogale, B., Chanie, M., 2012. Prevalence, species composition and associated risk factors in and around mekelle town, Northern Ethiopia. Eur. J. Biol. Sci. 4, 91–95.
Ntonifor, H., Shei, S., Ndaleh, N., Mbunkur, G.N., 2013. Epidemiological studies of gastrointestinal parasitic infections in ruminants in Jakiri, Bui Division, North West Region of Cameroon. J. Vet. Med. Anim. Health. 5, 344–352.
Parvin, S., Dey, A.R., Akter, S., Anisuzzaman Talukder, M.H., Alam, M.Z., 2021. Molecular identification of *mecistocirrus digitatus* in cattle from mymensingh region of Bangladesh. J. Bangladesh Agril. Univ. 19 (1), 67–72.
Pitaksakulrat, O., Chaiyasaeng, M., Artchayasawat, A., Eamudomkarn, C., Boonmars, T., Kopolrat, K.Y., Prasopdee, S., Petney, T.N., Blair, D., Sithithawon, P., 2021. Genetic diversity and population structure of *Haemonchus contortus* in goats from Thailand. Infect. Genet. Evol. 95, 105021.
Rahman, M.A., Labony, S.S., Dey, A.R., Alam, M.Z., 2017. An epidemiological investigation of gastrointestinal parasites of small ruminants in Tangail. Bangladesh. J. Bangladesh Agric. Univ. 15, 255–259.
Ransom, B.H., 1911. The nematodes parasitic in the alimentary tract of cattle, sheep and other ruminants, vol 71, US, Dept of Agric Bureau of Animal btd Bull.
Roeber, F., Jex, A.R., Campbell, A.J.D., Campbell, B.E., Anderson, G.A., Gasser, R.B., 2011. Evaluation and application of a molecular method to assess the composition of strongylid nematode populations in sheep with naturally acquired infections. Infect. Genet. Evol. 11, 849–854.
Rozas, J., 2009. DNA sequence polymorphism analysis using DnaSP. Bioinformatics for DNA Sequence Analysis. 537, 337–350.
Singh, K.S., 2003. Veterinary helminthology ICAR. New Delhi 354–366.
Soota, T.D., 1981. On some nematodes from the unnamed collection of the zoological survey of India, along with the description of a new species. Rec. Zool. Surv. India 79, 55–71.
Soulsby, E.J.L., 1982. Helminths, arthropods and protozoa of domesticated animals. Bailliere Tindall, London 7, 186–191.
Stevenson, L.A., Chilton, N.B., Gasser, R., 1995. Differentiation of Haemonchus placei from H contortus (Nematoda: Trichostrongylidae) by the Ribosomal DNA Second Internal Transcribed Spacer. Int. J. Parasitol. 25, 483–488.
Stewart, C.P., Iannotti, L., Dewey, K.G., Michaelsen, K.F., Onyango, A.W., 2013. Contextualising complementary feeding in a broader framework for stunting prevention. Matern. Child Nutr. 27–45.
Sweeny, J.P., Robertson, I.D., Ryan, U.M., Jacobson, C., Woodgate, R.G., 2012. Impacts of naturally acquired protozoa and strongylid nematode infections on growth and faecal attributes in lambs. Vet. Parasitol. 184, 298–308.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 60. Mol Biol. Evol. 30, 2725–2729.
Tariq, K., Chishti, M., Ahmad, F., Shawl, A.S., 2008. Epidemiology of gastrointestinal nematodes of sheep managed under traditional husbandry system in Kashmir valley. Vet. Parasitol. 158, 138–143.
Thrusfield, M., 1995. Veterinary Epidemiology, 2nd edn. Blackwell Science Ltd, Elsevier, Cambridge.
Troell, K., Engström, A., Morrison, D.A., Mattsson, J.G., Hoglund, J., 2006. Global patterns reveal strong population structure in *Haemonchus contortus*, a nematode parasite of domesticated ruminants. Int. J. Parasitol. 36, 1305–1316.
Wang, Y., Liu, G.H., Li, J.Y., Xu, M.J., Ye, Y.G., Zhou, D.H., Song, H.Q., Lin, R.Q., Zhu, X. Q., 2013. Genetic variability among *Trichuris ovis* isolates from different hosts in Guangdong Province, China revealed by sequences of three mitochondrial genes. Mitochondrial DNA 24, 50–54.
Win, S.Y., Win, M., Thwin, E.P., Htun, L.L., Hmoon, M.M., Chel, H.M., Thaw, Y.N., Nyein, C.S., Phyo, T.T., Thein, S.S., Khaing, Y., Than, A.A., Bawm, S., 2020. Occurrence of gastrointestinal parasites in small ruminants in the central part of Myanmar. J Parasitol. Res., 8826327
Yadav, A.K., Tandon, V., 1992. Stereoscan studies of two species of the genus Oesophagostomum (Nematoda, Chabertiidae). Acta Parasitol. 37 (3), 135–137.
Yu, S.K., Hu, B., Deng, Y., Li, H.M., Ren, W.X., Bian, Q.Q., Gao, M., Wang, X.Y., Cong, M. M., Song, J.K., Lin, Q., Xu, M.J., Zhao, G.H., 2012. Phylogenetic studies of Oesophagostomum asperum from goats based on sequences of internal transcribed spacers of ribosomal deoxyribonucleic acid (DNA). Afr. J. Microbiol. Res. 6, 3360–3365.
Zhao, G.H., Hu, B., Cheng, W.Y., Jia, Y.Q., Li, H.M., Yu, S.K., Liu, G.H., 2013. The complete mitochondrial genomes of *Oesophagostomum asperum* and *Oesophagostomum columbianum* in small ruminants. Infect. Genet. Evol 19, 205–211.
Zhao, G.H., Hu, B., Song, J.K., Jia, Y.Q., Li, H.M., Wang, C.R., Lin, Q., Xu, Q.X., Yu, S.K., Deng, Y., 2014. Characterization of *Oesophagostomum asperum* and *O. columbianum* by internal transcribed spacers of nuclear ribosomal DNA. J. Helminthol. 88, 74–81.