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Morphological and molecular perspectives on *Haemonchus contortus* of goats: A comprehensive review

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Abstract

The barber's pole worm, or *Haemonchus contortus*, is a serious gastrointestinal parasite that affects domestic goats or *Capra hircus*, leading to severe economic losses in the livestock industry. This study aims to provide a detailed analysis of the morphological and molecular characteristics to better understand its biology, pathogenicity and potential control strategies. *Haemonchus contortus* is found in the abomasum of *Capra hircus*. *Haemonchus contortus* is one of the most important which sucks the blood and cause anemia and hypoproteinaemia. The production and well-being of *Capra hircus* are seriously threatened by the parasitic worm *Haemonchus contortus*. The study focused on key diagnostic features, including the buccal cavity structure, spicule length in males, vulvar morphology in females and the overall size and shape of the worms. High-resolution pictures using scanning electron microscopy (SEM) helped with precise identification and discrimination from other co-infection nematode species by displaying the complex surface features. Phylogenetic analysis was conducted to determine genetic diversity, population structure and evolutionary relationships. DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing of particular genetic markers are all part of molecular characterisation. *H. contortus* serves as a useful model for parasitic nematode biodiscovery research because it is a significant member of the order Strongylida.

Keywords: Anthelmintic resistance, β -tubulin gene, *Capra hircus*, Haemonchosis, *Haemonchus contortus*, ITS markers, morphology, morphometrics, molecular characterization, ITS markers, phylogenetic analysis

1. Introduction

Livestock farming is the familiar practice in rural India, about 70% population of India depends on agriculture for their source of income ^[1]. The domestic goat is one of the preliminary animals domesticated by humans ^[2]. The first livestock to be domesticated were goats and goat farming is firstly contributed for the agriculture ^[3]. Goats are belonging into herbivorous animals that are infected by many diseases. Because of gastrointestinal parasitic infection goats suffering from weaken their health which cause hurdles in the livestock production. Gastrointestinal parasites also decreased the disease resistance capacity that results into higher mortality of livestock ^[4]. One of the best groups of organisms on the planet is the nematode. Nematodes are free living and parasitic, they create the health and economic obstacles across the globe ^[40]. Infestations of gastrointestinal nematodes cause domestic goats to have decreased fertility, decreased work capability, decreased food intake, and decreased weight growth ^[5]. The origination of *Haemonchus* species is connected to the collection of antelopes in Africa at the time of late tertiary ^[6]. One of the main harmful gastrointestinal nematodes of domestic goats (*Capra hircus*) is *Haemonchus contortus* ^[7]. An intestinal worm that feeds on blood, *Haemonchus contortus*, inhibits the *Capra hircus*'s abomasums ^[8]. *Haemonchus contortus* commonly called as Barber ole worm ^[9] and stomach worm or wire worm ^[10]. *Haemonchus contortus* belonging into hematophagus parasites because of their blood sucking property ^[11]. *Haemonchus contortus* cause severe disease disease which is called as Haemonchosis in *Capra hircus* ^[12].

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The *Haemonchus contortus*-infected *Capra hircus* suffers from anemia, appetite loss, and gastrointestinal tract protein and iron loss, which finally leads to death [13]. The parasite *Haemonchus contortus* are yellow in colour. Their body is slender and filiform. Male possess tapering body towards the anterior end female having tapering body at the both ends [14]. Body length of male *Haemonchus contortus* is 10-20mm, whereas the body length of female is 20-30mm. Female *Haemonchus contortus* shows appearance of a barber pole because of twisting the white ovaries surrounding the red blood-filled gut. The female *Haemonchus contortus* is larger in size than male. Female *Haemonchus contortus* can be differentiated from the male *Haemonchus contortus* on the basis of their body length, number of cuticular ridges and vulval flap morphology. The tooth of the *Haemonchus contortus* presents in the poorly developed oral cavity, which is assist to perforate the gastric mucosa and suck the blood [15]. *Haemonchus contortus* males were distinguished by their developed copulatory bursa, which included a Y-shaped dorsal ray and an asymmetric dorsal lobe. Cuticle is present on both the male and female *Haemonchus contortus*. Three distinct layers of collagen and other secreted substances make

up the cuticle. Cuticles shield the worms in the host's digestive tract when they are within the host [16]. Specifically the female is recognized by their barber pole appearance [17]. *Haemonchus contortus* has separate sexes, hence it is dioecious and go through the obligate sexual reproduction [18]. Adult *Haemonchus contortus* live for three to six months. *Haemonchus contortus* completes its life cycle in the first five stages, from eggs to adults. Fecal waste secreted by the host contains eggs. The eggs hatch and become the larvae when there are sufficient soil bacteria for the larvae to flourish. L1, L2, and L3 larvae are in the juvenile stage, growing independently of their host and feeding on soil bacteria. The host is infected by the L3 larvae. L3 larvae transform into L4 larvae once they have entered the host's body. L4 larvae adhere to *Capra hircus* abomasum and draw blood from them. The adult *Haemonchus contortus* was then formed from L4 larvae. In a single day, the mature female lays 10,000 eggs, which are then transferred to the host through their waste [20]. Anemia, pale mucous membranes, submandibular edema, and sudden death are signs of a *Haemonchus contortus* infection [21].

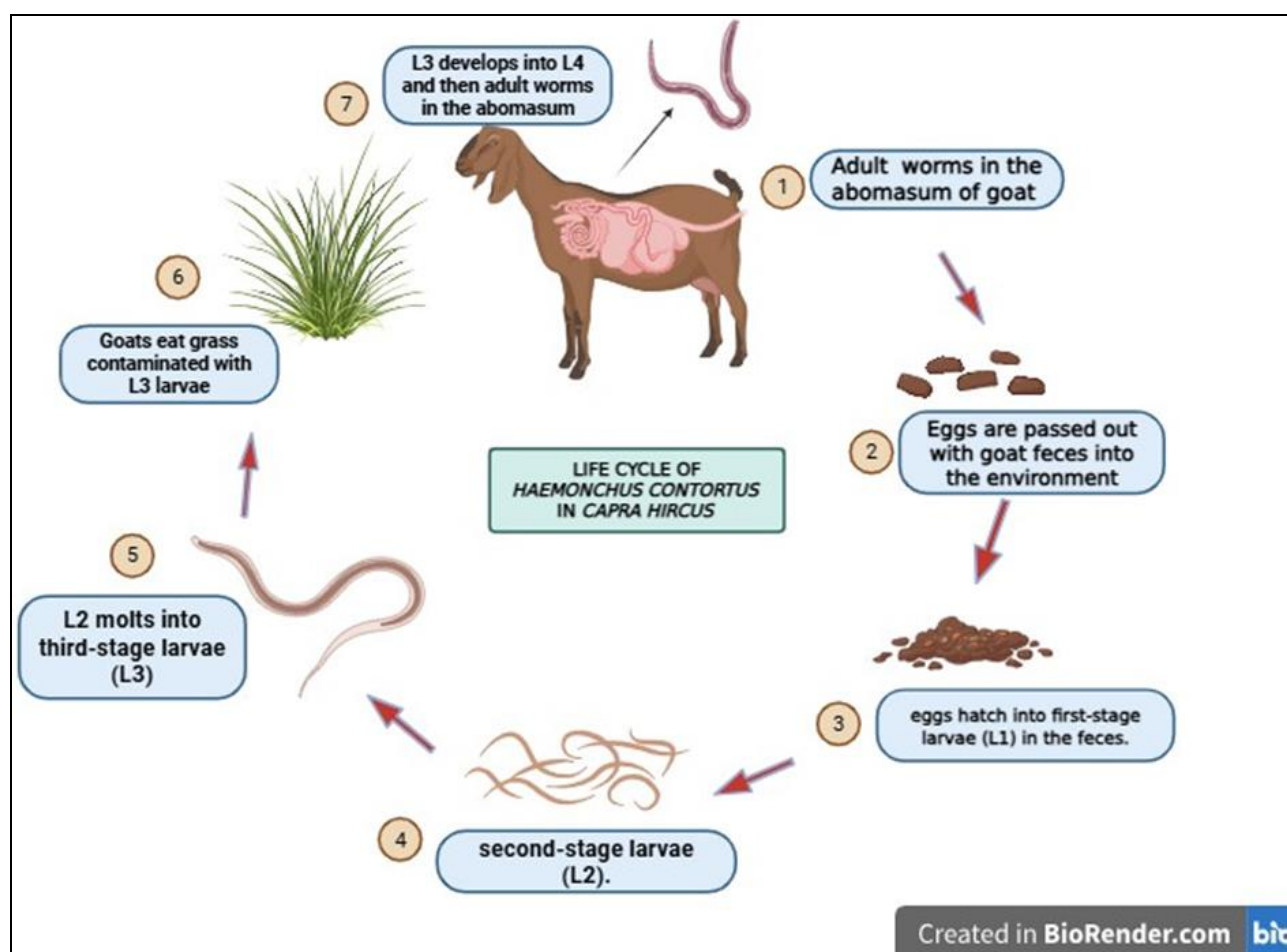


Fig 1: Life cycle of *Haemonchus contortus* in *Capra hircus*

Molecular characterisation is the primary method used for phylogenetic analysis and nematode species validation. It is useful to comprehend the genetic variety of *Haemonchus contortus* both within and between the species in order to identify transition patterns, track the spread of drug-resistant alleles, and create efficient control measures. The nuclear ribosomal DNA's internal transcribed spacer (ITS) 2 region is a crucial marker due to its easy application, distinct conserved areas, sufficient number of rRNA clusters, and sufficient

variance to differentiate closely related species [22-23]. These parasitic worms' evolutionary success has a substantial influence on human health as well as financial losses in livestock productivity.

Materials and Methods

We used information from a bibliographic search for this review. We used Google Scholar to get data. We used the following keywords to search the data: Haemonchosis,

Haemonchus contortus, *Capra hircus*, morphology, morphometrics, molecular characterization, ITS markers, β -tubulin gene, phylogenetic analysis and anthelmintic resistance. Literature review was done by internet access and visiting institutional library. The research papers and articles were studied from NCBI, Pub-Med, INFLIB-NET, DOAJ, UDL, DOAB, Digital Library of India, Open-J-Gate and Google Scholar. During this period, studied existing thesis related to *Haemonchus contortus* on Shodhganga. Number of research papers, research articles, reference books related to nematodes was collected from the institutional library and bibliographic survey. The techniques and knowledge required for understanding the molecular characterization of *Haemonchus contortus* were acquired through participation in workshops and seminars on molecular techniques.

Results and Discussion

Bibliographic research served as the foundation for this survey's findings. The study of nematodes first began in the 17th century. In 1906, B. H. Ransom studied the life history of *Haemonchus contortus*, they described about the feces of infested animal contains egg which was hatch from embryo [24]. Dixon R. W. (1911) worked on *Haemonchus contortus* of sheep and goat, they reported that the, for egg hatching above 40°F temperature is required. When the temperature goes below 40°F then eggs remain dormant. The unhatched eggs are killed by soon freezing or drying [25]. Veglia F. (1916) carried out experiment on larvae of *Haemonchus contortus*, they were seen that, the larvae which was exposed to the maximum temperature they were died. The larvae which were survive longer that are protected by grass from the direct sunlight [26]. Margaret E. Tod (1965) studied on morphology of *Haemonchus contortus* from different geographic regions, they found that high percentage of female *Haemonchus contortus* affected to goats in Orissa, India [27]. Peter B. Daskalov (1969) determined the genetical polymorphism in female *Haemonchus contortus* and distinguished that female *Haemonchus contortus* on the basis of three morphological characters which consisting linguiform flap, knoblike projection and smooth bodied females [28]. Author Sood M. L. (1978) studied that the oesophagus has typically triradiate and the lumen is lined by cuticle. Brush border present at the intestinal epithelium. They also investigated that the glycogen and lipids were stored in intestinal epithelium. Their biochemical study revealed that the brush border consists enzymes which were used for extracellular digestion and absorption of nutrients [19]. Use of benzimidazoles (anthelmintic drug) higher than recommended and frequent use of benzimidazole resulted into development of benzimidazole resistance in *Haemonchus contortus* that was investigated by Van Aken D(1989) [29]. According to Eva-Maria Bennet's (1981) biochemical analysis of *Haemonchus contortus*, parasites that were able to withstand benzimidazole had a reduced rate of uptake of glucose from the *in vitro* medium [30].

The molecular characterisation of β -tubulin genes found in *Haemonchus contortus* populations resistant to benzimidazole was the subject of a 1993 study by author Marcel S. G. Benzimidazole drug used as anthelmintic drug to control parasites, benzimidazole drug interact with β -tubulin. Author investigated the resistivity and hypersensitivity of benzimidazole drug due to mutation occurred in the β -tubulin gene [31]. Three *Haemonchus* species-*Haemonchus contortus*, *Haemonchus placei*, and *Haemonchus longistipes*- were the subject of genetic characterisation research by Jacquet

P.(1994). According to their findings, the *Haemonchus contortus* was the most genetically and morphologically diverse of the two species [32]. Rajat Garg (2009) According to their studies, three classes of anthelmintics-benzimidazoles, imidazoles, and ivermectin are being used in India to treat haemonchosis. Frequent dosing, high stocking rates, high pasture contamination with infectious larvae, and grazing land shrinkage as a result of extensive industrialization all contribute to an increase in benzimidazole-resistant strains of *Haemonchus contortus* [33]. Variability in the ITS-1 sequences of *Haemonchus contortus*, which is isolated from goats in the Indian state of West Bengal, was studied by Bandyopadhyay S. (2011). Through molecular characterisation, they contrasted the internal transcribed spacer 1 (ITS-1) gene region's nucleotide sequence variations. By analyzing SSCP (Single Stranded Conformation Polymorphism) during ITS-1 amplification, they discovered three different conformations in both male and female parasites. Two single nucleotide polymorphisms (SNPs) were found in the male parasite at positions 106 and 107, and in the female parasite at position 157 [34].

Chandra S. (2015) investigated benzimidazole resistance in goats from various geographical areas of the Indian state of Uttar Pradesh using the fecal egg count reduction test (FECRT) and allele specific PCR (AS-PCR). They claimed that *Haemonchus contortus* was the most common species in the research region based on fecal culture and PCR-RFLP on the β -tubulin isotype 1 gene. According to the FECRT results, they looked into the prevalence of benzimidazole resistance in every location. They found that 55-85% of *Haemonchus contortus* were homozygous resistant (rr), 10-21% were homozygous susceptible (ss), and 5-24% were heterozygous (rs) based on the AS-PCR data. Allele frequencies were found to be 67- 87.5% for the resistant allele (TTC) and 12.5-33% for the susceptible allele (TAC). Based on these findings, the author highlighted the state's concerning benzimidazole resistance situation [35]. The incidence and pathophysiology of *Haemonchus contortus* infection in goats were investigated by Dutta Biswajit (2017). They stated that the abomasum had ulcerative haemorrhagic areas where the parasites were attached. Under a microscope, they showed that the abomasum had large haemorrhages in the mucosa and submucosa. They saw mononuclear cells and eosinophils infiltrate [36]. The antioxidant and oxidant state of goats with naturally occurring *Haemonchus contortus* infections was assessed by Rashid S. (2019). Abdominal tissues from both infected and non-infected goats were used. Aspartate and alanine aminotransferases, acid and alkaline phosphatases, reduced glutathione, protein carbonyl, malondialdehyde, superoxide concentration, catalase, glutathione s-transferase, glutathione reductase, and glutathione peroxidase were all measured. Infected tissue showed increased levels of catalase, glutathione s-transferase, and glutathione reductase activity. They discovered that infected animals had significantly higher levels of aspartate, alanine amino transferases, acid phosphatases, and alkaline phosphatases. They claimed that the worm burden was the cause of all of these observations [37]. Cintli Martinez-Ortiz-de- Montellano (2021) used scanning electron microscopy to examine the vulval architecture of Mexican *Haemonchus contortus*. They used goats that were purposely infected to obtain 14 mature *Haemonchus contortus*. Type 1 (vulval flap) and Type 2 (epitygma) were the two types of vulval structures they

described. According to their findings, Type 1 featured one or more knobs positioned in various locations around the vulva, as well as vulval flaps of varying sizes. Variations in the epiptygma were part of the Type 2. An intraspecific polymorphism in vulval structures was proposed by the study^[17]. According to Aman Dev Moudgil (2022), the most suitable genetic markers are needed for the molecular identification and study of genetic diversity within and between species. Molecular markers include mitochondrial NADH dehydrogenase subunit 4, species-specific rDNA internal transcribed spacer 2 (ITS-2), and the 28S-18S rRNA intergenic spacer^[38]. *Haemonchus contortus* was recognized and distinguished using morphological and molecular characteristics by Kumar N. and Das B. (2023). They took 726 *Haemonchus contortus* worms from 61 goats, 581 of which were female and 145 of which were male. According to their findings, female worms were 20±0.09 mm long while male worms were roughly 12±0.06 mm long. They used PCR and electrophoresis to align the 165 bp internal transcribed spacer 1 (ITS-1) or 256 bp ITS 2 plus nucleotide sequences. They discovered that their isolates shared >95% and >94% homology with *Haemonchus contortus*'s previously published ITS 1 and ITS 2 Plus sequences, respectively^[15]. Vishal Bhagat (2024) used AS-PCR to examine the prevalence status and identify benzimidazole resistance in *Haemonchus contortus* goats from Maharashtra's Marathwada area. They found that *Haemonchus contortus* was present in 21.21% of the 264 goat abomasa they collected. Three genotypes (RR, SR, and SS) were found in the 168 adults *Haemonchus contortus* worms after a thorough examination. They determined that the allele with great resistance was present. They stated that the genotypes associated with resistance at codon 200 of the β -tubulin isotype 1 gene were found in the Indian state of Maharashtra in goats called *Haemonchus contortus*. According to the author, data from investigations is crucial for comprehending benzimidazole resistance and can be used to coordinate government, veterinary, and farmer actions. rising after 2015^[39]. Goats are more prone to infection than sheep, according to author Wei's (2025) research, and infection rates started to rise after 2015^[42].

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