

A Brief Study of Morphology of *Haemonchus contortus* and its Hematophagous Behaviour

¹Irfan-ur-Rauf Tak, ²Shoaib Ali Dar, ¹Jehangir Shafi Dar,
¹B.A. Ganai, ¹M.Z. Chishti and ³Fayaz Ahmad

¹Centre of Research for Development, University of Kashmir, Srinagar - 190 006

²Department of Zoology, Punjabi University Patiala

³Department of Zoology, University of Kashmir, Srinagar-190 006, Kashmir

Abstract: *Haemonchus contortus* (*H. contortus*) is a blood sucking intestinal helminth that lives in the abomasum of small ruminants worldwide. This parasite can be devastating to producers as it causes decreased production levels due to clinical signs such as anaemia, edema and death. The abomasae of sheep in which this parasite resides were collected from abattoirs of various districts and were then carried to laboratory for screening. It is cylindrical and has a striking reddish appearance due to its blood-feeding habit. The female is longer (18 to 30 mm) than the male (10 to 20 mm). The males possess a relatively developed copulatory bursa, with an asymmetric dorsal lobe and a Y-shape dorsal ray. They both possess a cuticle, with three different layers (made of collagen and other secreted compounds), which has a principal function to protect the worm while it is in the digestive system of the hosts. The spicules are 460-506 µm long, each provided with a small barb near its extremity. The vulva of the female is usually covered by a linguiform process (vulva flap) which is usually large and very prominent. Hematological studies revealed significant decrease in hemoglobin concentration, packed cell volume and total erythrocyte count in infected sheep whereas total leukocyte count showed significant increase in the infected ones. The present study will try to open a new window into the study of *H. contortus* which will be of great help in laying down the foundation of understanding the epidemiology and various aspects of other nematodes in the Kashmir Valley and will certainly be of potential significance in planning and grazing management and other prophylactic strategies for ruminants in different seasons of Kashmir Valley.

Key words: *Haemonchus contortus* • Helminths • Nematodes • Anthelmintics • PBS

INTRODUCTION

The state of Jammu and Kashmir is strategically located on the northern most part of India. It is geographically located between 32° 17' and 36° 58' northern latitude and 37°26' and 80° 30' eastern longitudes. Its snow-capped mountains, sprawling valleys, rivers, springs, plains and plateaus makes it a classic example of perfect tourist place. Ruminants are of considerable economic importance because ruminant rearing has been a major source of income especially to the marginal farmers and labourers of the country [1]. Domestic ruminants such as sheep, goats *etc.* are among those animals which were first tamed by man.

Archaeological evidence suggests that sheep were being raised for wool production as long as 4000 B.C (before Christ) [2]. Helminth parasitism of food and dairy animals cause significant economic losses throughout the world. The infection may be responsible for morbidity and mortality in many species but infection of ruminants particularly sheep is of much importance to livestock producers [3]. Although a number of helminth parasites affect ruminants but *H. contortus* [4] is of paramount importance as it affects its hosts in a number of ways and often results in their death. Until today very little work has been carried out on ruminant parasitism particularly those of *H. contortus* and its possible control in Kashmir Valley. Extensive work has being carried on the

anthelmintic resistance shown by the different kinds of parasites and antiparasitic efficacy of herbs and other plants [5, 6, 7]. Infections with *H. contortus* are major causes of economic losses in small ruminant husbandry [8]. Researchers worldwide have been studying new strategies and novel approaches to the control of *H. contortus* in hopes to alleviate the current dependency on anthelmintics that are becoming less efficacious [9].

MATERIALS AND METHODS

Naturally infected guts were obtained from slaughtered sheep on the day of slaughter from local slaughterhouses (Figure a-b) and guts were examined thoroughly especially the abomasums part and *H. contortus* was collected and placed in petridish containing 0.05M PBS (pH 7.4) for initial washing to remove host material and allow regurgitation of gut contents (Figure 1 and 2). The parasite was stored in collection vials containing PBS and the length and width was measured. *H. contortus* was identified based on standard body lengths of adult parasite: *H. contortus*: female (18 to 30 mm), male (10 to 20 mm) [10]. The parasite was processed for whole mount preparation and was prepared by following steps:

Fixation: The parasite was washed in normal saline to free it from mucus and fixed in hot (bubbling) 70% alcohol.

Preservation: The fixed parasite was then preserved in 70% alcohol to which 5% glycerine was added.

Clearing: After preservation, the parasite was cleared in order to remove its cuticle. The parasite was cleared in lactophenol for about $\frac{1}{2}$ - 2 hours. Lactophenol was preferred over glycerin since its action is comparatively faster.

Mounting: The parasite was finally mounted in Kaiser's glycerin jelly for permanent mount preparation.

Collection and Examination of Blood Samples: The fresh faecal samples were collected from the rectum of sheep in suitable containers like screw-capped wide mouthed glass bottles. After that concentraion was done in order to seperate the parasitic objects from the bulk of the material. For this purpose floatation technique was employed. The method was found to be very useful in the examination of *H. contortus* infection. Light infection was invariably detected by this method. This method is



Fig 1: Slaughtering of sheep



Fig 2: Abomasum of sheep

based on the principle that lighter eggs float onto the surface of saturated solution and the eggs are easily skimmed out of the surface film. The most commonly used suspending media were:

- Saturated solution of common salt
- Zinc sulphate 32% solution
- Saturated sugar solution
- Saturated solution of sodium nitrate

After that blood samples were collected from sheep having infection with the help of syringe in the vials containing EDTA and were carried to laboratory in a ice-cabinet for further analysis. Blood smears were prepared from the fresh blood (i.e. EDTA free blood) for the differential leukocyte count. Serum was collected from the blood by centrifugation at 3000rpm for 10-15 minutes.

Hematological Parameters

Estimation of Haemoglobin Concentration: The haemoglobin was estimated by Cyanomethemoglobin method [11]. In this method, Ferricyanide present in the Drabkins solution converts ferrous (Fe^{2+}) iron of

haemoglobin to the ferric (Fe³⁺) state to form methemoglobin. Methemoglobin reacts with potassium cyanide to form Cyanomethemoglobin. The colour developed was measured spectrophotometrically at 540 nm [12, 13].

$$\text{Hb (g/100ml)} = \frac{\text{A540 test sample} \times 15.06 \times (\text{Standard concentration as stamped on the vial} \times 0.251)}{\text{A540 standard}}$$

Total Erythrocyte Count: An improved Neubaus chamber was used for counting RBC. The Hayem's dilution fluid which was used had following composition: Mercuric chloride (HgCl₂)-0.5gm; Sodium Chloride (NaCl)-1.0gm; Sodium sulphate (Na₂SO₄)-5.0gm; Distilled water (H₂O)-200ml. Blood was drawn upto the 0.5 mark in the RBC Pipette. The tip of the pipette was cleared and RBC dilution fluid was drawn up to 101 mark. The resulting solution was shaken for 3 minutes. The first few drops of the solution were discarded and then chamber was loaded by one or two drops of blood solution. RBCs were counted as follows

$$\text{RBC count} = \frac{\text{Number of cells counted} \times \text{dilution factor} \times \text{depth of chamber}}{\text{Area counted}}$$

Total Leukocyte Count: A white cell count (TLC) estimates the total number of white cells in a cubic millimeter of blood. This was done in the same manner as the RBC count was done. For leukocyte classification the nomenclature of England and Bain [14] was followed. Turk's WBC dilution fluid was used which had the following composition: Glacial acetic acid (CH₃COOH)-1.5 ml; 1% Aqueous solution of Gentian violet-1.0ml; Distilled water-100ml. This fluid contains two things, weak acid which lyse the RBC cells and stain which gives colour to the nucleus of WBC. Neubaur's haemocytometer was used for counting leucocytes. The blood was sucked up in the WBC Pipettes upto the 0.5 mark and then WBC dilution fluid was drawn upto the 11 mark of pipette. Solution was mixed gently and bubbling was avoided. The Neubaur's chamber was charged by the resulting mixture. The cells were counted under 40x objective lens.

$$\text{TLC} = \frac{\text{Cells counted} \times \text{blood dilution} \times \text{chamber depth}}{\text{Area of chamber}}$$

$$\text{TLC} = \frac{\text{Cells counted} \times 20 \times 10}{4}$$

TLC/cu.mm.= Cells counted x 50

RESULTS AND DISCUSSION

Looking at the importance of GI nematodes of small ruminant animals all over the world, the members of the Strongylida order, the superfamily Trichostrongyloidea are worth mentioning. These nematodes are divided into genus such as *Ostertagia* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Cooperia* spp. and *Haemonchus* spp. [15]. *H. contortus* is one of the major health and economic issues in the agricultural industry [16].

Morphology of *Haemonchus Contortus*: It is cylindrical and has a striking reddish appearance due to its blood-feeding habit. The female is longer (18 to 30 mm) than the male (10 to 20 mm). Their red and white appearance is due to their white ovaries winding around the blood-filled intestines and is at the origin of its common name "barber-pole" worm. The males possess a relatively developed copulatory bursa, with an asymmetric dorsal lobe and a Y-shape dorsal ray. They both possess a cuticle, with three different layers (made of collagen and other secreted compounds), which has a principal function to protect the worm while it is in the digestive system of the hosts. The spicules are 460-506 µm long, each provided with a small barb near its extremity. The vulva of the female is usually covered by a linguiform process (vulva flap) which is usually large and very prominent, but may be reduced to a small knob like structure in some specimens (Pmg. 1-4).

Hematological Study

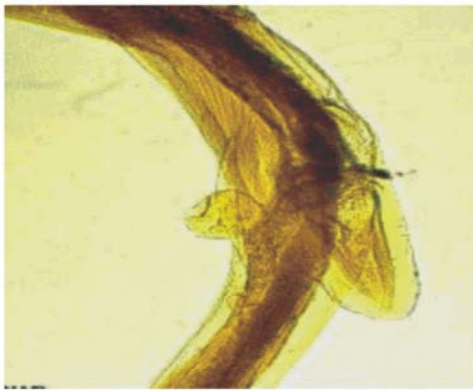
Hemoglobin: Uninfected sheep showed slight decrease in Hb concentration in summer as compared to spring and winter. This may be due to increase in ambient



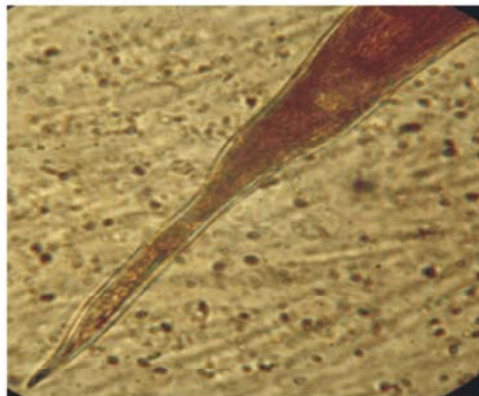
Pmg 1: Male *H. contortus* (Anterior end)



Pmg 2: Male *H. contortus* (Posterior end)



Pmg 3: Female *H. contortus* (Vulval region)



Pmg 4: Female *H. contortus* (Posterior end)

temperature and also due to depression of thyroid secretion which is associated with decreased erythropoiesis. The results are in accordance with [17-20], who reported the decrease in haemoglobin concentration in summer as compared to spring and winter. Infected sheep however showed significant decrease in hemoglobin concentration and the effect was most prominent in summer season. This can be due to the acute loss of blood by sucking activity of *H. contortus* which sucks about 0.05ml blood/worm/day. The results are in

accordance with [21-23] who reported decrease in hemoglobin concentration in sheep experimentally infected with *H. contortus*.

Total RBC Count: Similarly in case of total RBC count uninfected sheep showed slight decrease in summer as compared to other seasons. However the RBC count showed a slight increase in spring which may be due to the nutritional status of the sheep and the overall climatic conditions during the spring season. The results are in accordance with [17- 20] who reported decrease in RBC count in summer as compared to spring and winter. Infected sheep however showed significant decrease in total RBC count in all the seasons and here also it may be attributed to the acute loss of blood by sucking activity of *H. contortus* which sucks about 0.05ml blood/worm/day. The results are in accordance with [21, 22, 24] who reported decrease in RBC count in nematode infected sheep.

WBC Count: Uninfected sheep showed reduction in the WBC count in the summer season while as infected sheep showed significant increase in WBC count and the effect was most prominent in summer season and less in winter. The reason in case of uninfected sheep could be due to the physiological responses to hot climate which include decrease in food take and expansion of plasma volume resulting in haemodilution while as in case of infected ones it may be due to the immune response of body against the parasites as a means of self defense. In the uninfected sheep similar results have been reported by [25, 26]. The results of the infected ones are in accordance with those of [21, 27-29] who reported increase in WBC count in nematode infected sheep.

CONCLUSION

It should be concluded that *H. contortus* represents a severe health problem in small ruminant production system and its consequences can be extensive ranging from reduced productivity to mortality. Infection with *H. contortus* causes severe pathogenic effects in host animals. So modern diagnostic techniques need to be incorporated to estimate the degree of infection. It could also be concluded that changes in the hematological parameters in sheep infected with *Haemonchus* reflects a severe veterinary problem and causes pathological conditions like anemia, weight loss, poor wool and milk production. The present observation may suggest in

planning chemotherapeutic and prophylactic strategies for the control of *H. contortus* in the region. With the early diagnosis of Haemonchosis in sheep, a treatment schedule could be designed to avoid more infection and animal losses on the farm level and in turn economical losses.

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