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ORIGINAL ARTICLE

Vulvar flap morphology of *Haemonchus contortus* in naturally infected slaughtered goats in Northern area of Bangladesh

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Abstract

Background: *Haemonchus contortus* is regarded as one of the most prevalent and highly pathogenic parasite of ruminants. Vulvar flap morphology of female *Haemonchus* worms is useful to understand the biology of this nematode. Traits of vulvar morphology are considered as markers of ecological adaptation. A study was carried out to determine the types of vulvar flap present in female *Haemonchus* worms in naturally infected slaughtered goats in Northern area of Bangladesh.

Methods: Abomasa of 520 goats (260 from Rajshahi and 260 from Noagon District) collected from January, 2013 through December, 2013 from different slaughter houses of northern area of Rajshahi, Bangladesh. Female *Haemonchus* worms were collected under a dissecting microscope and then washed under tap water to remove adhered feed residues from their body. All *Haemonchus* worms from each animal were preserved in 70% ethanol inside individually labeled universal bottles, until they were examined for the types of their vulvar flap. The statistical package SPSS 19 for Windows was used for data analysis. Parameters such as major vulvar morphotypes of *Haemonchus* spp were compared by Chi-square test in both study areas.

Results: The major vulvar morph types was knobbed like (46.1%) followed by linguiform (33.5%) and smooth (20.4%). Out of 260 adult female *Haemonchus* worms collected from goats of Rajshahi district, 43.0% linguiform, 21.1% smooth and 35.8% knobbed vulvar morphs were identified. In Noagon district, 23.8% linguiform, 19.6% smooth and 56.5% knobbed vulvar morphs were observed. Linguiform type vulvar morphs were predominant in Rajshahi whereas knobbed type in Noagon district. Statistically significant ($P<0.001$) fluctuation was observed among three major vulvar flap morph types in the study areas. Sub-linguiform B (LB) was most common (96.6%) compared to Sub-linguiform A (LA); 3.4% type vulvar flap. There were no Sub-linguiform C (LC) and Sub-linguiform D (LD) types vulvar flap of *Haemonchus* spp detected in the study area.

Conclusions: The study showed polymorphism in vulvar morphology of female *Haemonchus* spp of goats in selected area of Bangladesh. The morphological characters of female *Haemonchus* spp can help in the identification of type of species that occurs in study area.

Key words: Vulvar morphology, linguiform subtypes, *Haemonchus contortus*, Bangladesh.

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Introduction

Parasitic gastroenteritis caused by *Haemonchus contortus* is a major cause of economic losses in the livestock industry because it impairs weight gain and increases mortality in cattle and small ruminants, especially in tropical and subtropical areas. There have been many reports of anthelmintic resistance, including resistance to *Haemonchus* species, in small ruminants (Almeida *et al*., 2010). High prevalence of *Haemonchus* was reported by Hoste *et al.,* (2002); Bersissa and Abebe (2006); Githigia *et al*. (2005); Wang *et al.* (2006) who reported higher prevalence of *Haemonchus* over other abomasal nematodes and this might be due to various factors like its ability to produce large number of eggs (Getachew *et al.,* 2007) and this allows *Haemonchus* spp an advantage over other parasites in that it can easily contaminate grazing areas or its ability to survive adverse climatic conditions through hypobiosis (Waller *et al*., 2004). *Haemonchus* spp uses various extrinsic and intrinsic factors for survival and hence development in host (Getachew *et al.,* 2007). Hence compared to other gastrointestinal nematodes Haemonchus is the most important parasite of domestic ruminants especially in sheep and goats (Bekele *et al*., 1992; Le Jambre, 1995; Perry *et al*., 2002; Kumsa *et al*., 2008; Hoberg *et al*., 2010). In fresh specimens the most obvious feature in females is that the white egg filled uterus winding spirally around the blood filled intestine-giving rise to the so-called barber's pole effect. The vulva is located about a quarter body length from the tail and may or may not be guarded by variously shaped cuticular inflations (vulvar flaps). The form of the vulvar flap may range from an extreme linguiform shape to a knob shape or a complete absence (Linguiform, knobbed or smooth). The prevalence of these various vulvar flap configuration varies among species and subspecies (Soulsby, 1986; Urquhart *et al*., 1996). The vulvar flap of *Haemonchus* spp varies both in shape and size (Le Jambre and Whitlock, 1968). Study of vulvar morphology of female Haemonchus worms helps to understand the biology, considered as the marker of ecological adaptation (Jacquiet *et al*., 1995) and possess great taxonomic importance. Several author studied the vulvar morphology of female *Haemonchus* spp in different parts of the world (Gelaye and Wossene (2003); Bersissa and Abebe (2006); Rahman and Hamid (2007); Thomas *et al.* (2007); Gharamah *et al*. 2012). Nabila *et al*. (2014) examined 300 female Haemonchus worms from abomasa of native goats for the types of their vulvar flap and revealed that 23% linguiform, 50.0% knobbed and 18% smooth vulvar flap types.

Further sub-classification of 69 linguiform vulvar flap female *Haemonchus* spp from goats revealed an overall proportion of 63.77% LB type and 36.23% LC type. Tod (1965) indicated that vulvar morphology is the manifestation of some genetic factors necessary to establish and develop inside hosts. Traits of vulvar morphology are considered as markers of ecological adaptation. The variation of vulvar morphology indicates manifestation of genetic factors during worm establishment and development (Gharamah *et al*., 2011) and variations may be due to ecological difference (Bersissa and Abebe, 2006). It is also believed that vulvar flap morphology helps to understand and know more about the biology of Haemonchus species and determine the type of population that occurs in area (Le Jambre and Whitlock, 1968; Eysker and Ploeger, 2000). There is no published report on the vulvar morphology of female *Haemonchus* spp from any species of ruminants in Bangladesh. Therefore, the purpose of this study was to determine the type of vulvar flap of female *Haemonchus* spp in naturally infected goats that helps to distinguish *H. contortus* females from other species.

Materials and Methods

Study area: Rajshahi and Noagon districts were selected for this study.

Study animals: Abomasa of 520 goats (260 from Rajshahi and 260 from Noagon District) collected from January, 2013 through December, 2013 from different slaughter houses of northern area of Rajshahi, Bangladesh were used to study the vulvar morphology of female *Haemonchus contortus*.

Worm recovery: A standard procedure (Kumsa and Wossene; 2007) was employed for female *Haemonchus* worm recovery. Each abomasum was opened along its greater curvature and the contents were washed into a bucket and carefully examined for the presence of Haemonchus worms. Female Haemonchus worms were collected under a dissecting microscope and then washed under tap water to remove adhered feed residues from their body. Morphological identification was made as described by Taylor *et al*. (2007). Recovered worms from each animal were preserved in 70% ethanol inside individually labeled universal bottles, until they were examined for the types of their vulvar flap.

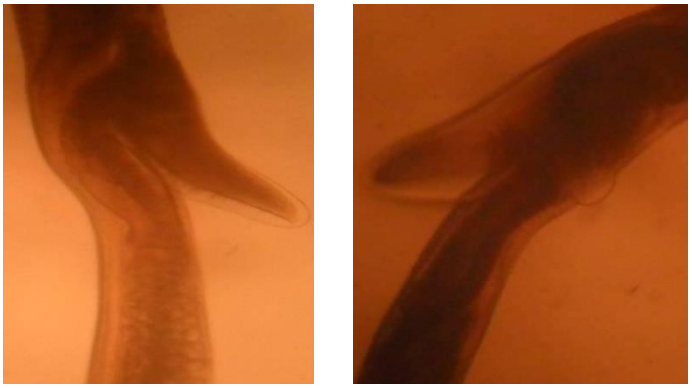
Vulvar morphology: Prior to vulvar flap type determination, each female Haemonchus worm was cleared in lactophenol blue as a temporary mount on a glass slide. Then each worm was examined under stereomicroscope to determine the type of cuticular process in the region of the vulva. The vulvar flap of female *Haemonchus* spp were classified under stereomicroscope into linguiform (with a supra vulvar flap), knobbed (with knoblike vulvar flap) or smooth (without any vulval process) vulvar morphotypes as described by Rose (1966) and Le Jambre and Whitlock (1968). All the linguiform morph types of female *Haemonchus* worms were further sub classified into linguiform A (with one cuticular inflation), linguiform B (without cuticular inflation), linguiform C (with two cuticular inflation) and linguiform I (the cuticular inflation arises from the linguiform process) as described by Le Jambre and Whitlock (1968).

Data analysis: Microsoft excel was used to store all the data and summarize simple statistics. The statistical package SPSS 19 for Windows was used for data analysis. The variation among major vulvar morphotypes of female *Haemonchus* spp were compared by Chi-square test in both study area. A P-value of less than 0.05 was considered as indicator of significant difference among compared parameters.

Results and Discussion

Out of total 520 female *Haemonchus* spp 33.5%, 20.4% and 46.1%, respectively were linguiform, smooth and knobbed major vulvar morph types (Table 1; Photograph 1). Statistically significant ($P<0.001$) fluctuation was observed among three major vulvar flap morph types in the study area. The finding of the predominance of knobbed vulvar flap types in goats in the current study is in line with the previous reports of other authors (Jacquiet *et al.* 1995 and 1998; Rahman and Hamid, 2007; Gharamah *et al.*, 2012; Akkeri *et al.*, 2013; Nabila *et al*., 2014). However, this observation is in contrast to some previous reports (Gharamah *et al.,* 2011; Thomas *et al.,* 2007; Gelaye and Wossene, 2003; Tod, 1965; Roberts *et al.,* 1954) where the linguiform type of vulvar morph was predominant. No statistically significant ($P>0.05$) difference was noticed in the proportions of the three major vulvar flap types (linguiform, knobbed and smooth) collected from domestic ruminants between the different months of the study period. This observation corroborates the earlier other findings Roberts *et al.*1954; Tod, 1965; Le Jambre and Whitlock, 1968; Thomas *et al.,* 2007). The vulvar flap of *Haemonchus* spp. worms varies both in shape and size (Roberts *et al.,* 1954; Le Jambre and Whitlock, 1968). About 43.0%, 21.1% and 35.8% of total 260 adult female *H. contortus* from Rajshahi district were rspectively linguiform, smooth and knobbed type of vulvar morphs. Whereas in Noagon district, knobbed type (56.5%) were dominant (Table 2). We observed the widespread occurrence of polymorphism in vulvar morphology of female *H. contortus* of goats in Bangladesh. The study showed widespread and common polymorphism of vulvar morphology of female *Haemonchus* spp in goats of the Northern region of Bangladesh. Many investigators indicated that vulvar polymorphism has some advantages like increasing the ability to use a wider range of available habitats and it is a marker of ecological adaptation, has great taxonomic significance and is important to understand the biology of these parasites (Roberts *et al*., 1954; Das and Whitlock 1960; Tod, 1965; Rose, 1966; Jacquiet *et al*., 1995). This difference of major vulvar morph types of female *Haemonchus* spp is most probably attributed to the variation in the sample size, examination procedure, agro-ecology, animal management and genetics of parasites among the various studies.

Photograph 1. Linguiform (left), Knobbed (middle) and Smooth (right) type of vulvar flap of *Haemonchus contortus* with light microscope (10X)



Photograph 2: LB Sublinguiform (left), LA Sublinguiform (right) type of *Haemonchus contortus* with light microscope (10X)

Table 1. Overall prevalence of major vulvar morph types of adult female *Haemonchus contortus* (n=520)

Major vulvar morph types of *Haemonchus* *contortus*	**No.of female *Haemonchus* *contortus* (n=)**	**%**	**Chi square Value**	**Significance level**
Linguiform	174	33.5	22.288	0.000 (***)
Smooth	106	20.4		
Knobbed	240	46.1		

n= Number of observation, **=Significant at 0.1% level (P<0.001).

Table 2. Prevalence of major vulvar morphs of female *Haemonchus contortus* in both study area

Study area	Major Vulvar morph type of *Haemonchus contortus*	No. of female *Haemonchus contortus*	%	Chi-square value	P-value
Rajshahi (n=260)	Linguiform	112	43.0	25.236	0.000 (***)
	Smooth	55	21.1		
	Knobbed	93	35.8		
Noagon (n=260)	Linguiform	62	23.8	27.563	0.000 (***)
	Smooth	51	19.6		
	Knobbed	147	56.5		

n= Number of observation, ***=Significant at 0.1% level (P<0.001).

Further classification of 174 linguiform vulvar flap female *Haemonchus*s spp from goats revealed overall 3.4% as LA and 96.6% as LB linguiform subtypes. This variation was statistically significant (Table 3; Photograph 2). This finding is in line with the previous reports by Nabila *et al*. (2014) who stated the predominance of the LB sub-linguiform type in goats. Whereas, this finding is in contrast with the some previous works (Jacquiet *et al.,* 1995, Kumsa *et al*., 2008; Akkari *et al*., 2013). Probably the LA type have lower ability in coping with this climatic condition of Bangladesh than the LB sublinguiform type. Our results indicate an adaptation of *Haemonchus contortus* population to the tropical climate prevailing in northern area of Bangladesh. This may probably be attributed to variations in agro-ecological conditions, environmental factors and the seasons of the surveys between the various study sites.

Table 3. Prevalence of linguiform subtypes of vulvar flap in female *Haemonchus contortus* (n=174)

Linguiform subtypes of *Haemonchus* *contortus*	**No. of female *Haemonchus* *contortus***	**% of identified female *Haemonchus* *contortus***	**Chi-square value**	**Sig. level**
LA	6	3.4	37.29	0.000 (***)
LB	168	96.6		
LC	-	-		
LD	-	-		
LI	-	-		

n=Number of observations, '-' = Not found; LA = with only one cuticular inflations; LB = with out cuticular inflation; LC =with two cuticular inflations; LI = the cuticular inflation arises from the linguiform processes and LD = sublinguiform vulvar flap with three cuticular inflations, ***Significant at 0.1% level (P<0.001).

Conclusion

The study showed polymorphism in vulvar morphology of female *Haemonchus* spp of goats in selected area of Bangladesh. The morphological characters of female *Haemonchus* spp can help in the identification of type of species that occurs in study area. Further investigations using advanced molecular techniques should be carried on genetic diversity and pathogenecity of *H. contortus* in special and other nematodes in general as drug resistance is another emerging challenge besides economic loss they made.

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References

1. Akkari H., Gharbi M, Awadi S., Mohamed AD., & Kumsa, B. New sub-linguiform vulvar flap of *Haemonchus* species in naturally infected domestic ruminants in Béja Abattoir, North Tunisia. Veterinarski arhiv. 2013; 83(3): 281-291.
2. Almeida FA, Garcia KCOD, Togerson PR, Amarante AFT. Multiple resistance to anthelmintics by *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep in Brazil. Parasitology International. 2010; 59(4): 622-625.
3. Bekele T, Woldeab T, Lahlou-Kassi A, Sherngton J. Factors affecting morbidity and mortality on farm and on station in Ethiopia highland sheep. Acta Tropica. 1992; 52: 99-109.
4. Bersissa K, Abebe W. Abomasal nematodes of small ruminants of Ogaden region, eastern Ethiopia: Prevalence Worm Burden and Species composition. Revue de Médecine Vétérinaire. 2006; 157:27-32.
5. Das KM, Whitlock JH. Subspeciation in *Haemonchus contortus* (Rudolphi, 1803) Nematoda, Trichostrongyloidae. Cornell Veterinarian. 1960; 50: 182-197.
6. Demissie T, Tesfaye D, Fekadu A, Asefa I. Study on abomasal nematodes of sheep and goats: Comparison and characterization of vulvar morphology of *Haemonchus* in Hawassa, Ethiopia. African Journal of

Agricultural Research. 2013;8(39): 4922-4927.
7. Eysker M, Ploeger HW. Value of present diagnostic methods for gastrointestinal tract nematodes infections in ruminants. In: Symposia of the British Society for Parasitology, UK, Cambridge University press , 2000;37:109-119.
8. Gelaye E, Wossene A. Small ruminant haemonchosis: morphological and prolificacy study in eastern Ethiopia. Bulletin of Animal Health and Production in Africa. 2000; 51: 67-73.
9. Getachew T, Dorchies P, Jacquiet P. Trends and challenges in the effective and sustainable control of 10. *Haemonchus contortus* in sheep. Parasite. 2007; 14 (1): 3-14. https://doi.org/10.1051/parasite/2007141003
11. Gharamah AA, Rahman WA, Nor SAM. Phenotypic differences of *Haemonchus contortus* from sheep and goats in the States of Perak and Kelantan, Peninsular Malaysia. Acta Parasitologica. 2012; 56: 412-417.
12. Gharamah AA, Rahman WA, Siti-Azizah MN. Morphological characterization of *Haemonchus contortus* in sheep (*Ovis aries*) and Goats (*Capra hircus*) from two Governorates in Yemen. World Journal of Zoology. 2011;6(3): 263-267.
13. Githigia S, Thamsborg S, Maingi N, Munyua W. The epidemiology of gastro-intestinal nematodes in goats in the low potential areas of Thica district, Kenya. Bulletin of Animal Health and Production in Africa. 2005;5:5-12.
14. Hoberg EP, Kumsa B, Pilitt PA, Abrams A. Synlophe structure in *Pseudomarshallagia elongata* (Nematoda: Trichostrongyloidea), abomasal parasites among Ethiopian ungulates, with consideration of other morphological attributes and differentiation within the Ostertagiinae. Journal of Parasitology. 2010;96: 401-411.
15. Hoste H, Chartier C, Le Frileux Y. Control of gastrointestinal parasitism with nematodes in dairy goats by treating the host category at risk. Veterinary Research. 2002;33: 531-545.
16. Jacquiet P, Humbert JF, Comes AM, Cabaret J. Thiam A, Cheikh D. Ecological, morphological, and genetic characterization of sympatric *Haemonchus* spp parasites of domestic ruminants in Mauritania. Parasitology. 1995;110: 483-492.
17. Jacquiet P, Cabaret J, Thiam E, Cheikh D. Host range and the maintenance of *Haemonchus* spp. in an adverse arid climate. International Journal of Parasitology. 1998;28: 253-261.
18. Kumsa B, Tolera A, Abebe R. Vulvar morphology and sympatry of *Haemonchus* species in naturally infected sheep and goats of Ogaden region, eastern Ethiopia. Veterinarski Arhiv. 2008;78: 331-342.
19. Le jambre LF, Whitlock JH. Seasonal fluctuation in linguiform morphs of *Haemonchus contortus cayugensis*. The Journal of Parasitology. 1968; 54: 827-830.
20. Nabila S, Jamila S, Al-Malki, FI Al-Omari. International Conference on Advances in Agricultural, Biological & Environmental Sciences (AABES-2014) Oct 15-16, 2014 Dubai (UAE).
21. Perry B, Randolph T, Mcdermott J, Ones SK, Tornton PK. Investing in Animal Health Research to alleviate Poverty, (International Livestock Research Institute (ILRI), Nairobi, Kenya, 2002;148.
22. Rahman WA, Hamid SA. Morphological characterization of *Haemonchus contortus* in goats (*Capra hircus*) and sheep (*Ovis aries*) in Penang, Malaysia. Tropical Biomedicine. 2007;24: 23-27.
23. Roberts FHS, Turner HN, Mckevett M. On the specific distinctness of the ovine and bovine strains of *Haemonchus contortus* (Rudolphi) Cobb (Nematoda: *Trichostrongylidae*). Australian Journal of Zoology. 1954; 2: 2753-2795.
24. Soulsby EJL. Helminths Arthropods and Protozoa of Domesticated Animals, 8th edition. Bailliere Tindall, London. United Kingdom. 1986.
25. Taylor M, Coop R, Wall R. Veterinary Parasitology 3rd ed. Blackwell publishing, Oxford. 2007.

26. Thomas N, Teshale S, Kumsa B. Abomasal nematodes of sheep and goats slaughtered in Awassa Ethiopia: Species composition, prevalence and vulvar morphology. Helminthologia. 2007;44: 70-75.
27. Tod EM. On the morphology of *Haemonchus contortus* (Rudolphi) Cobb (Nematoda: *Trichostrogylidae*) in sheep and cattle. Australian Journal of Zoology. 1965;13:773-781.
28. Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW. Veterinary Parasitology, 2nd Edition. Blackwell Science Ltd. London. 1996.
29. Waller PJ, Bernes G, Rudby-Martin L, Ljungstron BL, Rydzik A. The epidemiology of abomasal nematodes of sheep in Sweden with particular reference to over-winter survival strategies. Veterinary Parasitology. 2004; 122:207-220.
30. Wang CR, Qiu JH, Zhu XQ, Han XH, Ni HB, Zhao JP, Zhou QM, Zhang HW, Lun ZR. Survey of helminthes in adult sheep in Heilonggjiang province, peoples Republic of China. Veterinary Parasitology. 2006;140: 378-373.