VETERINARSKI ARHIV 78 (4), 331-342, 2008

Vulvar morphology and sympatry of *Haemonchus* species in naturally infected sheep and goats of Ogaden region, eastern Ethiopia

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KUMSA, B., A. TOLERA, R. ABEBE: Vulvar morphology and sympatry of *Haemonchus* species in naturally infected sheep and goats of Ogaden region, eastern Ethiopia. Vet. arhiv 78, 331-342, 2008.

ABSTRACT

A study was carried out to determine the type of vulvar process of 3187 and 2386 female *Haemonchus* worms recovered from naturally infected sheep and goats respectively, during the period from August 2003 to March 2004. In addition a total of 1159 adult male *Haemonchus* worms from sheep and 1285 from goats were subjected to a species identification study. The study revealed that out of the total female worms from sheep 49.5% linguiform, 28.5% knobbed and 23% smooth vulvar morph types were identified. Likewise, from goats 53.8% linguiform, 18. 5% knobbed and 27.6% smooth vulvar morph types were identified. Significant variations ($P<0.05$) were observed in proportions between the three major vulvar morph types in different months of the study period in both host species. Further sub-classification of the linguiform female worms from sheep revealed 27.2% linguiform A (LA), 14.8% linguiform B (LB), 5.3% linguiform C (LC) and 2.2% linguiform I (LI) subtypes. Similarly from goats 27.4 % LA, 17.5% LB, and 6.6% LC and 2.3% LI subtypes were identified. Within the linguiform vulvar flap types, the A subtype linguiform showed statistically significant ($P<0.05$) fluctuation during the months of study period in both host species. *Haemonchus* species identification based on morphometric parameters on spicules of 1159 adult male *Haemonchus* from sheep revealed 95.1% *H. contortus*, 3.5% *H. placei* and 1.5% *H. longistipes*, while out of 841 mature male *Haemonchus* from goats, 96.6% *H. contortus*, 2.9% *H. placei* and 0.5 % *H. longistipes* were identified. The study showed 57.9% *H. contortus* mono-species, 22.4% *H. contortus* and *H. placei* mixed infection, 7.9% *H. longistipes* and *H. placei* mixed infection and the rest 11.8% *H. contortus*, *H. placei* and *H. longistipes* triple infection of the examined sheep. Similarly in goats, 58.2% *H. contortus* mono-species, 38.2% *H. contortus* and *H. placei* mixed infection,

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ISSN 0372-5480
Printed in Croatia

3.6% *H. longistipes* and *H. placei* mixed infection and 0% triple *Haemonchus* species infection of the examined goats was observed. This finding revealed the coexistence and sympatry of two or three *Haemonchus* species in a single small ruminant host suggesting the occurrence of heterologous hosts for *Haemonchus* spp. which need to be taken into account in the control strategies against this parasite.

Key words: *Haemonchus* spp., Ogaden, small ruminants, vulvar morphology

Introduction

Diseases caused by gastrointestinal nematodes in domestic ruminants represent one of the major impediments to livestock production and cause enormous economic losses in a wide range of agroclimatic zones (WALLER, 1997). In Ethiopia, helminthosis is responsible for 25% mortality and 3.8% weight loss in highland sheep (BEKELE et al., 1992) and causes an estimated annual loss of about 700 million Ethiopian birr (HABTE-SILASSIE et al., 1991).

Haemonchus is regarded as one of the most prevalent and highly pathogenic, possesses the highest biotic potential, and has a prominent ability to develop resistance against most widely used anthelmintics and a unique survival strategy due to great biological and ecological plasticity. Hence compared to other gastrointestinal nematodes *Haemonchus* is the most important parasite of domestic ruminants especially in sheep and goats (BEKELE et al., 1992; PERRY et al., 1992; LE JAMBRE, 1995).

Two or sometimes three *Haemonchus* spp. are sympatric in many regions of the world especially in areas where sheep, goats, cattle and one humped dromedaries are reared together and share the same grazing pasture (JACQUIET et al., 1998; KASSAI, 1999).

The vulvar process of *Haemonchus* spp. worms varies both in shape and size (ROBERTS et al., 1954; ROSE, 1966; 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 (ROSE, 1966; JACQUIET et al., 1995) and possess great taxonomic importance. TOD (1965) indicated that vulvar morphology is the manifestation of some genetic factors necessary to establish and develop inside hosts.

In spite of all this, there is paucity of information about the vulvar morphology of female *Haemonchus* worms in sheep and goats in Ethiopia. Also no study is available on the existence of heterologous *Hemonchus* spp. infections in sheep and goats in semiarid areas such as the Ogaden region in eastern Ethiopia. Therefore, the purpose of this study was to determine the type of vulvar process of female *Haemonchus* worms in naturally infected sheep and goats and to assess the degree of heterologus species of *Haemonchus* in naturally infected sheep and goats of the study area based on spicule morphometrics.

Materials and methods

Study area. The study was conducted in Ogaden located at 9^0 20' N in eastern Ethiopia. Ogaden is one of the semiarid parts of the country. The climate of Ogaden is

tropical with two rainfall periods per year. The main rainy season occurs from July to September while the shorter season occurs from March to May. The area received from 250 to 600 mm annual rainfall during the study period. The average annual maximum mean temperature was 35 °C while the minimum was 25 °C. The average altitude of the region is 1200 meters above sea level (ANONYM., 2003). The area is covered by 38% grassland and 54% of the region consists of bush, shrubs and bush and shrubs type of vegetation (ANONYM., 1993).

Study animals. Abomasa of 104 Ogaden sheep and 68 goats collected on monthly basis from August 2003 through March 2004 from Elfora export abattoir based at Debre Zeit were used to study the vulvar morphology of female worms. In addition, adult male *Haemonchus* worms from a total of 76 sheep and 55 goats were used for identification of *Haemonchus* species based on spicule morphometrics. In the semiarid region of Ogaden in eastern Ethiopia, sheep, goats, zebu cattle and one humped dromedaries share the same grazing pasture and farming is of the extensive and traditional type.

Sample collection and study design. Recovery of *Haemonchus* worms from abomasa of study sheep and goats were performed according the classical procedures described by KASSAI (1999). During the study period a total of 3187 female *Haemonchus* worms from sheep and 2386 from goats were examined under stereomicroscope to determine the type of cuticular process in the region of the vulva. From 30 to 100 female *Haemonchus* worms per abomasum were examined for type of cuticular process in the region of vulva as described by LE JAMBRE and WHITLOCK (1968). When less than 100 female worms were recovered all were examined microscopically for differentiation into linguiform, knobbed and smooth forms as recommended by DAS and WHITLOCK (1960) and described by TOD (1965). The majority of worms were studied as fresh samples, whereas some were studied as preserved samples in 70% ethanol alcohol.

Randomly selected 15 adult male *Haemonchus* worms per abomasum were used for the species identification study. The tails of male worms were cut before the bursa and stained with lactophenol blue for clear examination of the spicules under a microscope as indicated by JACQUIET et al. (1997). Out of the total abomasa collected from sheep and goats, only 76 from sheep and 55 from goats harbored a sufficient number of adult male *Haemonchus* worms for the species identification study

Vulvar morphology. The vulvar process of female *Haemonchus* spp. was classified under stereomicroscope into linguiform (with a supra vulvar flap), knobbed (with knob like vulvar process) 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).

Haemonchus spp. *identification.* Identification of *Haemonchus* species was performed by employing a rapid and easy method described by JACQUIET et al. (1997). The method allows identification of all *Haemonchus* species and is helpful in the study of sympatric *Haemonchus* spp. in naturally infected hosts. The method utilizes a discrimination function (DF) that combines three different parameters on spicules: total length (TL), distance from the hook to the tip of the right spicule (THr) and distance from the hook to the tip of the left spicule (THl) were measured using calibrated ocular micrometer under a microscope.

Data analysis. Microsoft excel was used to store all the data and summarize simple statistics. The statistical package SPSS 11.5 for Windows was used for data analysis. Parameters such as monthly proportions of the major vulvar morphotypes, the subtypes of the linguiform morhpotypes, heterologous *Haemonchus* spp.and different spp. of *Haemonchus* for the studied months were all compared by ANOVA in both host species. A P value of less than 0.05 was considered as indicator of significant difference among compared parameters. Mean, confidence interval, percentage value, standard deviation and error were all employed when appropriate to compare and describe the studied variables.

Results

Vulvar morphology. Examination of the cuticular process of 3187 female *Haemonchus* worms from sheep revealed the presence of 49.9% linguiform, 28.5% knobbed and 23.0% smooth vulvar morphs types (Table 1). Similarly from goats a total of 2386 female worms were differentiated into 53.8% linguiform, 18.4% knobbed and 27.6% smooth vulvar morph types (Table 1). The linguiform vulvar flap was encountered as the most predominant form of morph type in both sheep and goat hosts of the study area. The proportions of the three major vulvar morph types were not significantly ($P>0.05$) different between the sheep and goat hosts. There was no significant ($P>0.05$) fluctuation in the proportions of major vulvar morph types during the different months of the study period in both host species.

Further classification of 1580 linguiform vulvar flap female *Haemonchus* worms from sheep revealed overall proportions of 27.2% LA type, 14.8% LB type, 5.3% LC type and 2.2% LI linguiforms subtypes (Table 2). Likewise from goats, out of the total of 1285 linguiform vulvar flap female worms 27.3 % were categorized into LA type, 17.5% LB type, 6.6% LC type and 2.3% LI linguiform sub types were differentiated (Table 2). In both host species a statistically significant ($P<0.05$) variation was observed in different months of the study period among the different linguiform sub morphotypes.

The A sub type linguiform appeared as the dominant linguiform sub type in both host species followed by B, C and I sub types of linguform vulval morphs. The vulvar morph types of female *Haemonchus* worms from sheep and goats of Ogaden encountered during the study period are presented in Figs 1-6.

Table 1. Monthly percentage value of the major vulvar morphs of female *Haemonchus* spp.

Host	Vulvar Morphs	VIII	IX	X	XI	XII	I	II	III	Average
Sheep (n = 3187)	Linguiform	63.1	60.0	69.2	40.0	60.0	41.5	29.4	32.7	49.5
	Knobbed	19.5	27.2	22.2	43.1	14.5	18.1	35.7	39.7	28.5
	Smooth	17.3	12.7	8.5	16.8	25.5	40.4	34.9	27.7	23.0
Goat (n = 2386)	Linguiform	ND	ND	59.2	66.5	53.8	59.0	37.6	46.7	53.8
	Knobbed	ND	ND	20.7	20.7	15.1	20.2	20.1	13.7	18.4
	Smooth	ND	ND	20.0	12.7	31.0	20.7	41.4	39.7	27.6

ND = Not done; n = number of worms; VIII - III = indicates months from August to March

Table 2. Monthly percentage value of subtypes of the linguiform vulvar flap type in sheep and goats

	Sheep (104)				Goat (68)			
Month	LA%	LB%	LC%	LI%	LA%	LB%	LC%	LI%
August	33.2	15.1	5.4	9.4	ND	ND	ND	ND
September	29.7	20.2	10.0	0.0	ND	ND	ND	ND
October	35.7	23.0	9.7	0.7	45.0	10.7	2.2	1.2
November	20.9	18.4	0.8	0.0	41.5	17.2	5.2	2.5
December	37.5	11.2	9.5	1.7	25.4	15.9	8.7	3.8
January	21.4	14.8	2.8	2.5	19.5	27.0	10.7	1.7
February	18.5	7.3	2.3	1.3	20.2	11.6	4.5	1.2
March	20.3	8.3	2.3	1.8	12.5	22.7	8.2	3.2
Average	27.2	14.8	5.3	2.2	27.3	17.5	6.6	2.3

ND = Not done; 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

Table 3. Proportion of *Haemonchus* spp. in relation to worm poulation and number of hosts

Species of Host	Sheep	Goat
N° of animal examined	76	55
N° of worms examined	1159	841
Average No of worms examined	15.2	15.3
H. contortus		
% of worms	95.1	96.5
% of animals infected	100	100
H. placei		
% of worms	3.4	3.0
% of animals infected	28.9	34.5
H. longistipes		
% of worms	1.5	0.5
% of animals infected	14.5	5.4

Table 4. Percentage of mono and mixed species of *Haemonchus* infections in sheep and goats.

Type of worm community	Sheep (n = 76)	Goat (n = 55)
H. contortus	57.9%	58.2%
H. placei	0%	0%
H. longistipes	0%	0%
H . contortus + *H. placei*	22.4%	38.2%
H. contortus + *H. longistipes*	7.9%	3.6%
H. contortus + *H. placei* + *H. longistipes*	11.8%	0%

Haemonchus species. Spicules morphometrics study of 1159 male worms from sheep disclosed the presence of 1102 (95.1%) *H. contortus*, 46 (3.4%) *H. placei* and 17 (1.5%) *H. longistipes*. Likewise out of 841 adult male Haemonchus from goats 812 (96.5%) *H. contortus*, 25 (3.0%) *H. placei* and 4 (0.5%) *H. longistipes* were identified (Table 3). The proportions of the three *Haemonchus* species were not significantly ($P>0.05$) different between the sheep and goat hosts. *H. contortus* was identified as the most predominant species in both sheep and goats. Even though the contribution to the total species composition is small *H. placei* infected 28.9% sheep and 34.5% goats whereas *H. longistipes* infected 14.5% sheep and 5.4% goats (Table 3).

Fig. 1. Linguiform A

Fig. 2. Linguiform B

Fig. 3. Linguiform C

Fig. 4. Linguiform I

Figs. 1.-4. The different vulvar morph types female *Haemonchus* in sheep and goats of Ogaden region

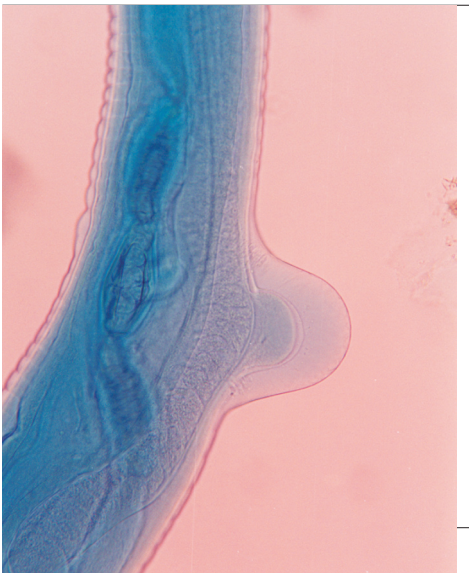

Fig. 5. Knobbed vulvar morph type

Fig. 6. Smooth vulvar morph

Figs. 5.and 6. The different vulvar morph types female *Haemonchus* in sheep and goats of Ogaden region

The study showed *H. contortus* mono species infection in 57.9% sheep and 58.2% goats as a predominant type of infection in both hosts (Table 4). Mono species *H. placei* and *H. longistipes* infection was never observed in both sheep and goats hosts. Whereas 22.4% sheep and 38.2% goats were with *H. contortus* and *H. placei* mixed species infection, 7.9% sheep and 3.6% goats were with *H. contortus* + *H. longistipes* mixed species infection and 11.8% sheep were with *H. contortus, H. placei* and *H. longistipes* triple mixed species infection, but triple *Haemonchus* spp. infection was not recorded in goats of the study area (Table 4). No association was observed between study months and type of *Haemonchus* spp. combinations during the study period.

Discussion

The results of the current study revealed the predominance of the linguiform vulvar morph types in both sheep and goats of the study area. This observation is in line with the earlier studies of ROBERTS et al. (1954), TOD (1965), JACQUIET et al. (1995, 1998), GELAYE and WOSSENE (2003) and THOMAS et al. (2007). In both host species, there was no significant ($P>0.05$) difference in the proportions of the three major vulvar morph types (linguiform, knobbed and smooth) between the different months of the study

period. Absence of monthly fluctuations in the proportion of the major vulvar morphs was reported by several previous investigators (ROBERTS et al., 1954; TOD, 1965; LE JAMBRE and WHITLOCK, 1968; JACQUIET et al., 1995; GELAYE and WOSSENE, 2003; THOMAS et al., 2007).

In the present study, within the linguiform vulvar flap morphotypes a clearly defined monthly variation was observed, where the A subtype linguiform showed significant ($P<0.05$) fluctuations in different months of the study period, while all the other linguiform subtypes showed no significant ($P>0.05$) fluctuation during months of the study period. This is most probably suggestive of the lower ability of the A subtype linguiform in coping with the dry months of the study period as compared to the B subtype linguiform as described by LE JAMBRE and WHITLOCK (1968). The A subtype linguiform was also observed to be the dominant contributor of the linguiform morphotype composition. This finding is in contrast to the findings of THOMAS et al. (2007) who reported the predominance of the C subtype linguiform morph type in small ruminants of Hawassa. This is most probably attributed to the differences in the climates of the two study sites and the smaller size per abomasum in the previous study. The study showed widespread and common polymorphism of vulvar morphology of female *Haemonchus* worms in sheep and goats of the Ogaden region. Many investigators indicated that vulvar polymorphism has advantages such as increasing the ability to use a wider range of available habitats and it is 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).

Species identification of individual male worms from naturally infected sheep and goat hosts indicated the presence of cross infection, circulation, coexistence and sympatry of different *Haemonchus* spp. among heterlogous hosts, such as sheep, goats, zebu cattle and dromedaries, all of which share common grazing pasture under field conditions in the Ogaden region, favoring the transmission of infection among them. The finding of the predominance of *H. contortus* in both sheep and goats hosts indicates that *H. contortus* is well adapted and preferentially infects small ruminants which are highly susceptible and the primary host of this species compared to both *H. placei* of cattle and *H. longistipes* of camels. This observation is in agreement with previous investigations of LICHTENFELS et al. (1994), AMARANTE et al. (1997), JACQUIET et al. (1995, and 1998), GELAYE and WOSSENE (2003) and ACHI et al. (2003).

The study also showed that both *H. placei* and *H. longistipes* could infect small ruminants but at a lower rate than the principal species. This suggests the occurrence of cross infection and the wide range of host species for *Haemonchus* spp. of domestic ruminants. This wide range of small ruminant hosts for *H. placei* and *H. longistipes* is advantageous as they are maintained in a greater number of susceptible hosts that ingest

infective larvae while sharing common grazing pasture. This finding is in line with the work of JACQUIET et al. (1998) and ACHI et al. (2003) who suggested that the wide range of susceptible hosts could be a survival strategy for these species in Africa.

In the study, mono species *H. contortus* infection was encountered as the most predominant form of infection in both host species. In contrast there was no mono species infection with both *H. placei* and *H. longistipes*. The association of *H. contortus* and *H. placei* was moderate (22.4% in sheep and 38.2% in goats) whereas *H. contortus* and *H. longistipes* was at a low level of association. On the other hand *H. contortus, H. placei* and *H. longistipes* triple species association was only observed in sheep. This finding is in line with the work of ACHI et al. (2003) who reported that the presence of cross infection and circulation of species of the genus *Haemonchus* among species of hosts sharing the same grazing pastures. This also suggests that *Haemonchus* spp. associations are common in the abomasa of naturally infected domestic ruminants. The significance of different species of hosts as a source of infection for other susceptible species of animals should be considered while designing management and control strategies against haemonchosis of domestic ruminants of an area.

In conclusion, the study showed that sheep and goats of the study area were infected by similar types of *Haemonchus* spp. and also harbour a similar pattern and distribution of vulvar morphotypes of female *Haemonchus* worms. The investigation of the vulvar morphology of female *Haemonchus* spp. revealed the predominance of linguiform vulvar morph types, whereas the study of *Haemonchus* spp. identification revealed the predominance of *H. contortus* in both sheep and goats. Further investigation of the biology and significance of vulvar morphology of *Haemonchus* spp. and species identification in all species of domestic ruminants in all seasons in different agroecology and managements need to be pursued.

Acknowledgements

The authors thank Dr. Esayas GELAYE for sharing his experience and constructive suggestions. We thank all the staff of the Elfora export abattoir and department of parasitology and pathology of faculty of veterinary medicine at Debre Zeit for their all support. The research was financially supported by the graduate and research program of Addis Ababa University.

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Received: 6 May 2007
Accepted: 2 July 2008

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SAŽETAK

Od kolovoza 2003. do ožujka 2004. istraživana je morfologija vulvarnih zalistaka u 3187 ženki oblića roda *Haemonchus* podrijetlom iz ovaca i 2386 ženki podrijetlom iz koza. Istraživanje je obuhvatilo i 1159 mužjaka podrijetlom iz ovaca i 1285 podrijetlom iz koza. Ustanovljeno je da je 49,5% ženki izdvojenih iz ovaca imalo jezikolike, 28,5% čvorolike i 23% glatke vulvarne zaliske. U 53,8% ženki parazita iz koza ustanovljeni su jezikoliki, 18,5% čvoroliki i 27,6% glatki vulvarni zalisci. Značajne razlike ($P<0,05$) između tri glavna morfološka tipa vulve pretraženih oblića iz ovaca i koza zabilježene su tijekom različitih mjeseci. Daljnja klasifikacija jezikolikih vulvarnih zalistaka pokazala je da izdvojene ženke iz ovaca posjeduju 27,2% jezikolikih zalistaka suptipa A, 14,8% jezikolikih zalistaka suptipa B, 5,3% jezikolikih zalistaka suptipa C i 2,2% jezikolikih zalistaka suptipa I. Slično je pronađeno i za obliće izdvojene iz koza pa je tako 27,4% ženki imalo jezikolike vulvarne zaliske suptipa A, 17,5% jezikolike vulvarne zaliske suptipa B, 6,6% jezikolike vulvarne zaliske suptipa C i 2,3% jezikolike vulvarne zaliske suptipa I. Unutar jezikolikog tipa, razlike među suptipovima su bile u korelaciji s mjesecom u kojem su istraživane. Morfološke pojedinosti u mužjaka temeljile su se na morfometrijskim svojstvima spikula i to na osnovi iscrpne analize 1159 primjeraka iz ovaca. Na osnovi različite morfologije ustanovljeno je 95,1% oblića vrste *Haemonchus contortus*, 3,5% vrste *Haemonchus placei* i 1,5% vrste *Haemonchus longistipes*. Od ukupno 841 mužjaka iz koza čak 96,6% pripadalo je vrsti *Haemonchus contortus*, 2,9% vrsti *Haemonchus placei* i 0,5 % vrsti *Haemonchus longistipes*. Invazija vrstom *Haemonchus contortus* dokazana je u 57,9% ovaca. U 22,4% ovaca dokazana je mješovita invazija vrstama *Haemonchus contortus* i *Haemonchus placei,* dok je u 7,9% ovaca dokazana mješovita invazija vrstama *Haemonchus longistipes* i *Haemonchus placei*. U preostalih 11,8% ovaca dokazana je mješovita invazija trima vrstama i to *Haemonchus contortus, Haemonchus placei* i *Haemonchus longistipes*. Slično je ustanovljeno i u koza. Tako je u 58,2% koza bila prisutna samo vrsta *Haemonchus contortus*. U 38,2% koza dokazana je mješovita invazija vrstama *Haemonchus contortus* i *Heamonchus placei* dok je u svega 3,6% koza dokazana mješovita invazija vrstama *Haemonchus longistipes* i *Heamonchus placei*. Ni u jedne koze nije dokazana mješovita invazija trima vrstama. Rezultati pokazuju da se u jednog domaćina mogu naći istodobno dvije ili tri vrste roda *Heamonchus* odnosno da postoji simpatrija što treba uzeti u obzir u kontroli tih parazita.

Ključne riječi: *Haemonchus* spp., Ogaden, mali preživači, morfologija vulve