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New sublinguiform vulvar flap of *Haemonchus* species in naturally infected domestic ruminants in Béja Abattoir, North Tunisia

Hafidh Akkari¹, Mohamed Gharbi², Sofia Awadi², Aziz Darghouth Mohamed², and Bersissa Kumsa^{3,4*}

¹Department of Applied Biotechnology, Higher Institute of Biotechnology of Béja, Béja, Tunisia

²Laboratoire de Parasitologie, École Nationale de Médecine Vétérinaire, Université de la Manouba, Sidi Thabet, Tunisia

³Department of Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia

⁴Aix Marseille Université, URMITE, Marseille, France

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ABSTRACT

The present study was conducted to determine the type of vulvar morphology in female *Haemonchus* worms collected from the abomasa of sheep, goats and cattle slaughtered for human consumption in Béja Abattoir in Tunisia with a Mediterranean type of climate. For the purpose, a total of 2450 female *Haemonchus* worms from abomasa of 363 sheep, 258 goats and 152 cattle were thoroughly examined for the types of their vulvar flap. Of the total 143 female *Haemonchus* worms from cattle 21.5% linguiform, 41.8% knobbed and 3.4% smooth vulvar flap types were recorded. Out of the total 645 female *Haemonchus* worms from abomasa of sheep, 42.5% linguiform, 36.3% knobbed and 21.2% smooth vulvar morph types were identified. In goats a total of 1662 female worms were differentiated into 27.7% linguiform, 65.8% knobbed and 6.4% smooth vulvar morph types. Further subclassification of 43 linguiform vulvar flap female *Haemonchus* worms from cattle revealed an overall proportion of 11.5% LA type, 26.3% LB type, 17.1% LC type, 0.8% LD type and 11.0% LI linguiform subtypes. Likewise, from sheep, of the total of 327 linguiform vulvar flap female worms, 31.6% LA type, 18% LB type, 26.3% LC type, 3.9% LD type and 3.5% LI linguiform subtypes were differentiated. From goats, of the total of 461 linguiform vulvar flap female worms, 48% LA type, 13.8% LB type, 21.6% LC type, 4.4% LD type and 12.3% LI linguiform subtypes were detected. The LB sublinguiform type had the highest overall proportion in cattle, whereas the LA sub linguiform type was the most predominant in both sheep and goats. An interesting finding of the current study is the documentation of a new type of sublinguiform vulvar flap type in female *Haemonchus* worms for the first time, from the Mediterranean type of climate in Tunisia.

*Corresponding author:

Dr. Bersissa Kumsa, Department of Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University, P.O.Box 34, Bishoftu, Ethiopia, Phone: +33 605 768 369; E-mail: bersissak@yahoo.com

To the best of our knowledge this is the first report of new vulvar morphology in female *Haemonchus* worms, designated as LD sublinguiform vulvar flap with three cuticular inflations. Further detailed studies are urgently needed on the prevalence, morphological, molecular and genetic characterization of *Haemonchus* spp. with different vulvar flap types in different agro-ecologies, animal species and breeds, and management systems in Tunisia and other countries.

Key words: female *Haemonchus*, knobbed, smooth, linguiform, new sublinguiform, vulvar flap

Introduction

Nematodes of the gastrointestinal tract of ruminants are responsible for huge economic losses in many countries of the world (WALLER, 1997). *Haemonchus* is one of the most prevalent and highly pathogenic abomasal nematodes of ruminants. It has the highest biotic potential, a prominent ability to develop resistance to the most widely used anthelmintics and a unique survival strategy, due to its great biological and ecological plasticity. Hence, compared with other gastrointestinal nematodes, *Haemonchus* is the most important parasite of domestic ruminants (KUMSA et al., 2008; HOBERG et al., 2010). Recent investigations reported an increase in the prevalence of this nematode in countries in temperate regions (JACKSON and COOP, 2000; HOSTE et al., 2002; WALLER et al., 2004; van DIJK et al., 2008) and infected flocks were even observed in latitudes above 65°N (LINDQVIST et al., 2001).

Previous investigators reported that the vulvar process of *Haemonchus* spp. worms varies both in shape and size (ROBERTS et al., 1954; ROSE, 1966; Le JAMBRE and WHITLOCK, 1968). Studying the vulvar morphology of female *Haemonchus* worms is useful to understand the biology of this nematode. In addition, the vulvar flap is considered as the marker of ecological adaptation (ROSE, 1966; JACQUIET et al., 1995) and has great taxonomic importance. TOD (1965) suggested that vulvar morphology is the reflection of true bionomic differences and manifests some genetic factors necessary to establish and develop inside hosts.

However, information is not available on the vulvar morphology of female *Haemonchus* worms from domestic ruminants in Tunisia. Therefore, the purpose of this study was to determine the type of vulvar process of female *Haemonchus* worms collected from naturally infected sheep, goats and cattle slaughtered at Béja Abattoir in Tunisia, with a Mediterranean type of climate.

Material and methods

Study area. The survey was conducted on naturally infected sheep, goats and cattle slaughtered for human consumption at Béja Abattoir in the town of Béja, which is located in North West Tunisia, 100 km to the west of Tunis (the capital of Tunisia). Geographically, the area lies at 36°73' N latitude and 9°18' E longitude. The area has a

mean annual rainfall ranging between 350 and 1000 mm, a mean temperature of 18 °C and an average altitude of 222 m above sea level.

Study animals. During the study period the abomasa of 363 sheep, 258 goats and 152 cattle were collected on a monthly basis from January to June 2010 from Béja Abattoir and used to study the vulvar morphology of female worms. In northern Tunisia, sheep, goats and cattle share the same grazing pasture and farming is of an extensive and traditional type. Even though it is difficult to determine precisely the exact geographical origin of the study animals, most animals came from areas surrounding Béja town.

Worm recovery. A standard worm recovery procedure previously described by 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 then each was 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). All worms from each animal were preserved in 70% ethanol inside individually labeled universal bottles, until they were examined for the types of their vulvar process.

Vulvar type determination. Prior to vulvar type determination, each female *Haemonchus* worm was cleared in lactophenol blue as a temporary mount on a glass slide. Then, under the microscope, the female *Haemonchus* worms collected from each animal were classified according to their vulvar type as: linguiform (with a supravulvar flap), knobbed females (with knob-like vulvar process) and smooth females (without any vulvar process) (ROSE, 1966; JACQUIET et al., 1995). Furthermore, each linguiform female *Haemonchus* worm was classified into sublinguiform types (A, B, C and I) as described by Le JAMBRE and WHITLOCK (1968). Linguiform A has one cuticular inflation, linguiform B has no cuticular inflations, linguiform C has two cuticular inflations, while in linguiform I, the cuticular inflation arises from the linguiform process.

Interestingly, in this study a new type of sublinguiform female *Haemonchus* worms was encountered for the first time with three cuticular inflations. We designated this new sublinguiform female *Haemonchus* worm as linguiform D.

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 the monthly proportions of the major vulvar morphotypes and the subtypes of the linguiform morphotypes, for the studied months were all compared by ANOVA in both host species. A P value of less than 0.05 was considered as an indicator of significant differences among the compared parameters. Mean, confidence interval, percentage value, standard deviation and error were all employed when appropriate, to compare and describe the studied variables.

Results

The examination of vulvar cuticular process of 143 female *Haemonchus* worms from cattle showed the presence of 21.5% linguiform, 41.8% knobbed and 3.4% smooth vulvar types (Table 1). The knobbed vulvar flap was the most predominant type in cattle.

Similarly, out of the total 645 female *Haemonchus* worms from the abomasa of sheep subjected to the vulvar morphology study, 42.5% linguiform, 36.3% knobbed and 21.2% smooth vulvar types were identified (Table 1). The linguiform vulvar flap was encountered as the most predominant type in sheep hosts of the study area

In goats, a total of 1662 female worms were differentiated into: 27.7% linguiform, 65.8% knobbed and 6.4% smooth vulvar types (Table 1). The knobbed vulvar flap was the most predominant type in goat hosts of the study area.

Statistically significant ($P > 0.05$) fluctuation was not observed in the proportions of major vulvar flap types between the different months of the study period in all host species. Moreover, the proportions of these three major vulvar flap types did not show any statistically significant ($P > 0.05$) difference between sheep and cattle hosts.

Further sub-classification of 43 linguiform vulvar flap female *Haemonchus* worms from cattle revealed an overall proportion of 11.5% LA type, 26.3% LB type, 17.1% LC type, 0.8% LD type and 11.0% LI linguiforms subtypes (Table 2). The LB subtype was the dominant linguiform in cattle.

Likewise, from sheep, of the total of 327 linguiform vulvar flap female worms, 31.6% LA type, 18% LB type, 26.3% LC type, 3.9% LD type and 3.5% LI linguiform subtypes were differentiated (Table 2). The LA subtype was the dominant linguiform in sheep.

From goats, of the total of 461 linguiform vulvar flap female worms 48% LA type, 13.8% LB type, 21.6% LC type, 4.4% LD type and 12.3% LI linguiform subtypes were detected (Table 2). The LA subtype was the dominant linguiform in goats.

The LB sublinguiform type was found in the highest overall proportion in cattle, whereas the LA sublinguiform type was the most predominant in both sheep and goats. In all domestic ruminant hosts in the study area, both LI and LD sublinguiform types were found in the lowest proportions.

An interesting finding of the current study was the documentation of a new type of sublinguiform vulvar flap type in female *Haemonchus* worms collected from sheep, goats and cattle hosts, for the first time from the Mediterranean type of climate in Tunisia. To the best of our knowledge this is the first report of new vulvar morphology in female *Haemonchus* worms, designated as a LD sublinguiform vulvar flap with three cuticular inflations (Fig 1). Photographs of all the vulvar flap types of female *Haemonchus* worms from cattle, sheep and goats slaughtered at Béja Abattoir identified during the study period are depicted in Fig 1.

Table 1. Monthly percentage value of vulvar flap types of female *Haemonchus* spp. in sheep, goats and cattle slaughtered in Béja Abattoir in Tunisia

Host	Vulvar morphology	January	February	March	April	May	June	Overall
Sheep (n = 645)	Linguiform	0	60.6	50.4	44.8	49.3	50	42.5
	Knobbed	33.3	25.7	41	48.5	44.1	25	36.3
	Smooth	66.7	13.6	8.6	6.7	6.5	25	21.2
Goats (n = 1662)	Linguiform	16.7	34.8	23.6	26.6	28.6	36.0	27.7
	Knobbed	73.8	59.4	70.7	66.5	65.1	59.5	65.8
	Smooth	9.5	5.7	5.7	6.9	6.2	4.4	6.4
Cattle (n = 143)	Linguiform	0	30.2	0	46.7	27.3	25	21.5
	Knobbed	0	53.5	0	53.3	68.8	75	41.8
	Smooth	0	16.3	0	0	3.9	0	3.7

Table 2. Monthly percentage value of linguiform subtype vulvar flaps of female *Haemonchus* spp in sheep, goats and cattle slaughtered in Béja Abattoir in Tunisia

Month	Cattle (n = 43)						Goats (n = 461)						Sheep (n = 327)					
	LD	LI	LC	LB	LA	LA	LD	LI	LC	LB	LA	LD	LI	LC	LB	LA		
January	0	0	0	0	0	0	14.3	28.6	14.3	0	42.8	0	0	0	0	0		
February	0	23.1	0	46.1	30.8	0	0	9.8	9.8	39.3	41	8.7	8.7	22.5	27.5	32.5		
March	0	0	0	0	0	2.4	6.6	29.3	9.6	52.1	5.2	5.2	8.2	38.0	8.9	39.5		
April	0	28.7	42.8	14.3	14.3	2	4	32	10	52	4.1	4.1	31.5	10.9	49.3	49.3		
May	4.8	14.3	9.5	47.6	23.8	4.6	10.3	19.5	16.09	50.6	5.3	5.3	15.8	10.5	68.4	68.4		
June	0	0	50	50	0	3.4	14.6	24.7	7.9	49.4	0	0	50	50	0	0		
Overall	0.79	11.0	17.1	26.3	11.5	4.4	12.3	21.6	13.8	48	3.9	3.5	26.3	18	31.6	31.6		

LA = one cuticular inflations; LB = without cuticular inflation; LC = two cuticular inflations; LD = three cuticular inflations; LI = the cuticular inflation arises from the linguiform processes.

Table 3. Prevalence of major vulvar flap types and linguiform subtypes of female *Haemonchus* spp. in sheep, goats and cattle slaughtered at Béja Abattoir in Tunisia

Species	Linguiform	Knobbed	Smooth	LA	LB	LC	LI	LD
Sheep	43/363 (11.8)	52/363 (14.3)	21/363 (0.6)	30/363 (8.3)	20/363 (0.5)	21/363 (0.6)	13/363 (3.5)	10/363 (2.7)
Goats	61/258 (23.6)	77/258 (29.8)	29/258 (11.2)	46/258 (17.8)	24/258 (9.3)	33/258 (12.8)	17/258 (6.6)	10/258 (3.9)
Cattle	6/152 (4)	8/152 (5.3)	2/152 (1.3)	3/152 (2)	5/152 (3.3)	4/152 (2.6)	3/152 (2)	1/152 (0.6)



LA sublinguiform type, ×40

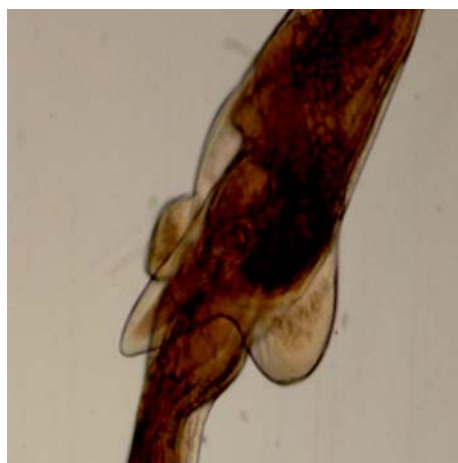


LB sublinguiform type sublinguiform type, ×40

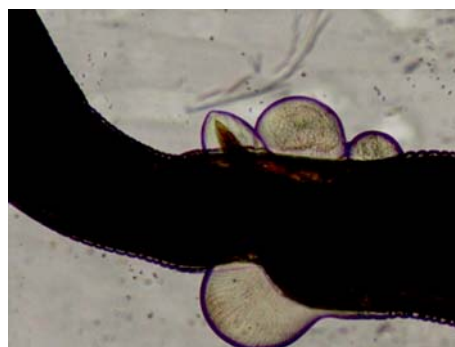


LC sublinguiform type, ×40

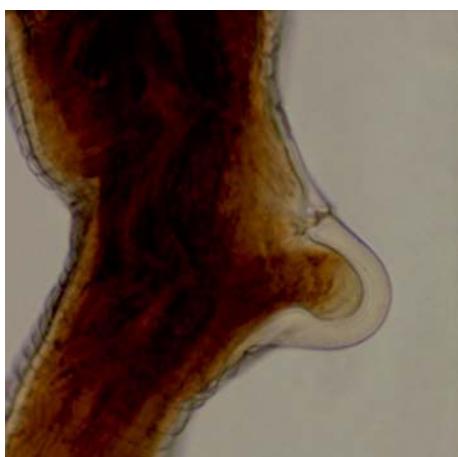
Fig. 1. The different vulvar flap types of female *Haemonchus* in sheep, goats and cattle slaughtered at Béja Abattoir in Tunisia



LI sublinguiform type, ×40



LD sublinguiform type, ×40



Knobbed flap type, ×40



Smooth flap type, ×40

Fig. 1. The different vulvar flap types of female *Haemonchus* in sheep, goats and cattle slaughtered at Béja Abattoir in Tunisia (continued)

The highest percentages of 5.3% (8/152) knobbed type and 3.3% (5/152) LB sublinguiform type were recorded from a total of 152 abomasa of cattle (Table 3). In sheep, the highest percentages of 14.3% (52/363) knobbed vulvar flap type and 8.3% (30/363) LB type flaps were encountered in the examined abomasa. In goat abomasa, 29.8% (77/258) knobbed vulvar type and 17.8% (30/258) LA highest percentages were recorded (Table 3).

Interestingly, the new type of vulvar flap LD (sublinguiform D) was collected from the abomasa of all the examined species of domestic ruminants in a low proportion of 0.6% (1/152) in cattle, 2.7% (10/363) sheep and 3.9% (10/258) goats (Table 3).

Discussion

The finding of the predominance of knobbed vulvar flap types in both cattle and goats in the current study is in line with the previous reports by JACQUIET et al. (1995 and 1998), RAHMAN and HAMID (2007) and GHARAMAH et al. (2012). However, this observation is in contrast to the previous works of ROBERTS et al. (1954), TOD (1965), GELAYE and WOSSENE (2003) and THOMAS et al. (2007) who reported the predominance of linguiform vulvar morphotypes in small ruminants. This difference is most probably attributed to the variation in the agro-ecology, animal management and genetics of parasites among the various studies. The observation of the predominance of the linguiform vulvar flap types in *Haemonchus* spp. from sheep coincides with the investigations by the aforementioned authors.

No statistically significant 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 findings reported by ROBERTS et al. (1954), TOD (1965), Le JAMBRE and WHITLOCK (1968), THOMAS et al. (2007) and KUMSA et al. (2008).

In the current study, within the linguiform vulvar flap type *Haemonchus* worms collected from sheep and goats, the LA type was encountered as the most predominant sublinguiform. This finding is in agreement with the previous work of JACQUIET et al., (1995) and KUMSA et al. (2008), who reported the predominance of the LA sublinguiform type in small ruminants. However, on the other hand, this finding contrasts with the previous report by THOMAS et al. (2007) who reported the predominance of the LC sublinguiform type in small ruminants in Hawassa in Ethiopia. This may probably be attributed to variations in agro-ecological conditions, environmental factors and the seasons of the surveys between the various study sites.

The present work showed the widespread occurrence of polymorphism in vulvar morphology of female *Haemonchus* worms of domestic ruminants in Tunisia. Several investigators have pointed out that vulvar polymorphism has taxonomic significance,

great biological advantages in increasing the ability of parasites to use a wider range of available habitats and hosts, and is also considered as a marker of ecological adaptation and helps to understand the biology of parasites (ROBERTS et al., 1954; DAS and WHITLOCK, 1960; TOD, 1965; ROSE, 1966; JACQUIET et al., 1995).

The report of a new type of sublinguiform vulvar flap, designated as LD for the first time in the current study, warrants the attention of professionals around the world to investigate further the role and importance of this structure in parasitic nematodes. We believe that it is most probably an indication of adaptation to this environment by *Haemonchus* population, occurring in the Mediterranean climate prevailing in North Africa. As no previous studies on the vulvar morphology of *Haemonchus* worms from any species of ruminants are available in Tunisia, it is possible that this new type of LD sublinguiform vulvar flap type has adapted to live in this particular type of environment.

Further detailed studies are urgently needed on the prevalence, epidemiology, morphological, molecular and genetic characterization of *Haemonchus* spp. with different vulvar flap types in different agro-ecologies, animal species and breeds and management systems in Tunisia and other countries.

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Novi sublingviformni vulvarni zalistak oblića *Haemonchus* sp. u prirodno
invadiranih domaćih preživača zaklanih u klaonici Béja u sjevernom Tunisu. *Vet.*
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SAŽETAK

Istraživanje je poduzeto kako bi se odredio morfološki tip ženki oblića roda *Haemonchus* sakupljenih iz sirišta ovaca, koza i goveda zaklanih u klaonici Béja na području Tunisa s mediteranskom klimom. Ukupno je bilo izdvojeno 2450 ženki oblića roda *Haemonchus* iz sirišta 363 ovce, 258 koza i 152 goveda. Nakon izdvajanja određivane su razlike u izgledu vulvarnog zaliska. Od ukupno 143 ženke izdvojene iz sirišta goveda 21,5% je imalo linguiformni tip zalistaka, 41,8% zalistak s izbočinom dok je samo 3,4% imalo glatke zaliske. Među oblicima (645) izdvojenima iz ovaca ustanovljeno je 42,5% s linguiformnim zaliskom, 36,3% sa zaliskom s izbočinom i 21,2% oblića s glatkim zaliskom. Mikroskopskim pregledom 1662 ženke izdvojene iz koza u njih 27,7% ustanovljeni su linguiformni zalisci, u 65,8% zalisci s izbočinom te u 6,4% glatki zalisci. Daljnjim svrstavanjem 43 ženke s linguiformnim zaliscima podrijetlom iz goveda pokazalo se da 11,5% pripada LA podtipu, 26,3% LB podtipu, 17,1% LC podtipu, 0,8% LD podtipu i 11,0% LI podtipu. Među 325 oblića s linguiformnim zaliscima izdvojenih iz ovaca 31,6% je bilo LA podtipa, 18% LB podtipa, 26,3% LC podtipa, 3,9% LD podtipa i 3,5% LI podtipa. Među 461 oblicem s linguiformnim zaliscima podrijetlom iz koza 48% bilo je LA tipa, 13,8% LB tipa, 21,6% LC tipa, 4,4% LD tipa i 12,3% LI podtipa. LB tip zaliska bio je najučestaliji u oblića izdvojenih iz goveda dok je LA bio najčešći tip zaliska u oblića izdvojenih iz ovaca i koza. Ovo je prvi nalaz novog tipa sublingviformnih vulvarnih zalistaka u ženki oblića roda *Haemonchus* na području Tunisa s mediteranskom klimom. Taj zanimljiv novi nalaz sublingviformnog vulvarnog zaliska s tri kutikularna udubljenja nazvan je LD tip.

Ključne riječi: *Haemonchus* sp., ženka, linguiformni zalistak, novi sublingviformni zalistak, vulvarni zalistak
