ANIMAL PRODUCTION

Filiform papillae of Holstein heifer tongues and their relationship with *Rhipicephalus (Boophilus) microplus* infestation

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ABSTRACT. Mammals' self-cleaning behavior helps to reduce infestations of ectoparasites, especially ticks. We investigated the relationship between measurements of the filiform papillae of heifer tongues and tick infestation. To assess the papillae, biopsies were performed on the anterior surface of the tongues of 15 Holstein heifers. The samples were processed for evaluation by scanning electron microscopy. Statistical analysis was performed through a completely randomized design. The parameters studied were submitted to analysis of variance to determine the effect of infestation on the length and base width of the papillae, distance between bases and between tips, and number of papillae per unit area. Heifers with greater numbers of papillae (16.68 ± 3.30 cm^2) and shorter filiform papillae (1.28 ± 0.35mm) had fewer ticks (P < 0.01) than heifers with fewer papillae (15.46 ± 1.91 cm^2) and longer lengths (1.45 ± 0.39mm). We concluded that the morphology of the filiform papillae varies between cattle of the same breed and that the length and the number of papillae per unit area are related to tick infestation.

Keywords: Cattle; morphology; grooming; self-cleaning; tick, tongue.

Introduction

The tongue of animals is an extensible muscular organ that performs functions of food gripping, chewing, and swallowing. It also provides the sense of taste and performs mechanical body self-cleaning (Kilinc et al., 2010; Eurell et al., 2006; Benetti et al., 2009). The surface of the tongue contains several types of papillae, with different distributions and functions (Benetti et al., 2009). Most mammals, including cattle, have four types of papillae: filiform, fungiform, foliate, and vallate (Dyce et al., 2004).

The filiform papillae on the tongue provide a suitably rough surface for food manipulation and grinding (Svejda & Skach, 1975). They are distributed throughout the body and tip of the tongue, but are mainly found in the anterior third (Benetti et al., 2009). According to Shao et al. (2010), the filiform papillae have the mechanical functions of food apprehension and self-cleaning, besides protecting the fungiform papillae and cleaning the interdental spaces of the lower jaw.

A summary of published papers that have provided measurements of cattle filiform papillae is presented in Table 1.

Table 1. Cattle filiform papillae measurements.

Breed/sex	Location	Base diameter mm	Length mm	No. of papillae cm^2	Author(s)
Angus cows	USA	0.5	1.5 - 2	$\cong 16$	Steflik et al., 1983
Holstein cows	Spain	0.45	1.5	-	Cabello et al., 1988
5-year-old male Zavot (Simmental x native cattle)	Turkey	-	1.8	-	Sari et al., 2010
Bos taurus (275 ± 25 kg)	Tibet (China)	0.2 – 0.5	0.4 – 2.0	40 ± 6	Shao et al., 2010
European steers 2-year-old	Brazil	0.6	1.8	20.9 ± 0.7	Veríssimo et al., 2015
Nelore steers 2-year-old	Brazil	0.6	2.2	25.2 ± 1.9	Veríssimo et al. 2015

$\cong$ approximately.

The damage caused to cattle by the tick *Rhipicephalus (Boophilus) microplus* has been well documented and is associated with poor performance and high cost of control as well as mortality among susceptible cattle (Veríssimo, 1993). The effects of this tick cause yearly losses of billions of dollars to the Brazilian cattle industry, according to estimates from Grisi et al. (2014).

Self-cleaning is an important behavioral activity among both wild and domestic animals by decreasing the levels of parasite infestation (Mooring et al., 1996; Mooring & Hart, 1997; Eckstein & Hart, 2000; Akinyi et al., 2013). Indeed, self-cleaning is largely responsible for the resistance to cattle ticks, mostly by the tongue, as noted by Riek (1956), Snowball (1956), Bennett (1969), and Kemp et al. (1976) in studies of the head movements of infested cattle. In turn, Koudstaal et al. (1978) confirmed that cattle lick themselves towards the places where the larvae are attached, to remove ticks.

Ungulates' use of their tongues has been extensively studied and reported (Mooring et al, 1996; Hart, 2000). Furthermore, a study on self-cleaning among cats showed that this behavior removes fleas (Eckstein & Hart, 2000). However, these studies have not linked the morphology of the tongue with parasite infestation.

The objectives of this work were to ascertain the morphology of the filiform papillae of Holstein heifers and determine the relationship between the morphology of the filiform papillae and infestation by the tick *Rhipicephalus (Boophilus) microplus*.

Materials and methods

This study was approved by the Ethics Committee on Animal Use of the Institute of Animal Science (protocol number 144/2011).

Fifteen Holstein heifers with mean age of 18 months were used. They were kept in confinement with access to a paddock that was naturally infested with *Rhipicephalus (Boophilus) microplus*, located at the Zootechnical Institute in Nova Odessa, state of São Paulo, Brazil. They were fed with Tifton hay (13% crude protein (CP) and concentrate based on corn and soybean meal (14% CP). Minerals and water were offered ad libitum.

Biopsies

The biopsies were performed on samples obtained with disposable punch measuring 6 mm in diameter, in the center of the anterior third of the tongue, 3 cm from the tip, to evaluate the filiform papillae. This was chosen because the filiform papillae were larger. The animals were intravenously sedated with 2% xylazine (analgesic and muscle relaxant) at a dose of 1 mL per 100 kg of body weight, followed by application of anesthetic ointment (lidocaine hydrochloride, 2.0 g) at the biopsy site. After the tissue removal procedure, triamcinolone acetonide ointment, an anti-inflammatory agent specific to the oral region, was applied (1.0 mg g^{-1}).

Scanning electron microscopy (SEM)

Immediately after biopsy sample collection, the material was immersed in saline solution and taken to the laboratory, where it was scrubbed to remove impurities and was dipped in Karnovsky's solution (Karnovsky, 1965) (2.5% glutaraldehyde and 2.5% formaldehyde, in 0.05 M sodium cacodylate buffer at pH 7.2, with 0.001 M $CaCl_2$). The material was then stored in a refrigerator until the biopsy procedure. This was done under an LEO VP-435 scanning electron microscope at the Electron Microscopy Support Center for Applied Agricultural Research (NAP/MEPA) of the Luiz de Queiroz Agricultural College (ESALQ), University of São Paulo (USP).

In the laboratory, the samples were dehydrated in solutions with increasing concentrations of acetone (30, 50, 70, 90 and 100%). The samples were left in these solutions for approximately 15 minutes (30% and 50%), 30 minutes (70%) and 10 minutes (90% and 100%). They were then washed three times (10 minutes each). After this process, the samples were moistened in a solution of 100% acetone, critical-point dried (Baltec CPD 030 apparatus), attached to stubs, metalized with gold (Baltec SCD 050 sputter apparatus), and kept in a container with silica gel.

Inside the microscope, all the stubs were turned to view the papillae in the caudal direction, since they were originally on the dorsal surface of the tongue. The filiform papillae were then measured in terms of their length, base width, and distance between bases and between tips, with the aid of the tool for measurements between two points available in the SEM software (Figure 1).

The number of papillae per cm^2 was calculated based on the number of papillae contained in the biopsy.
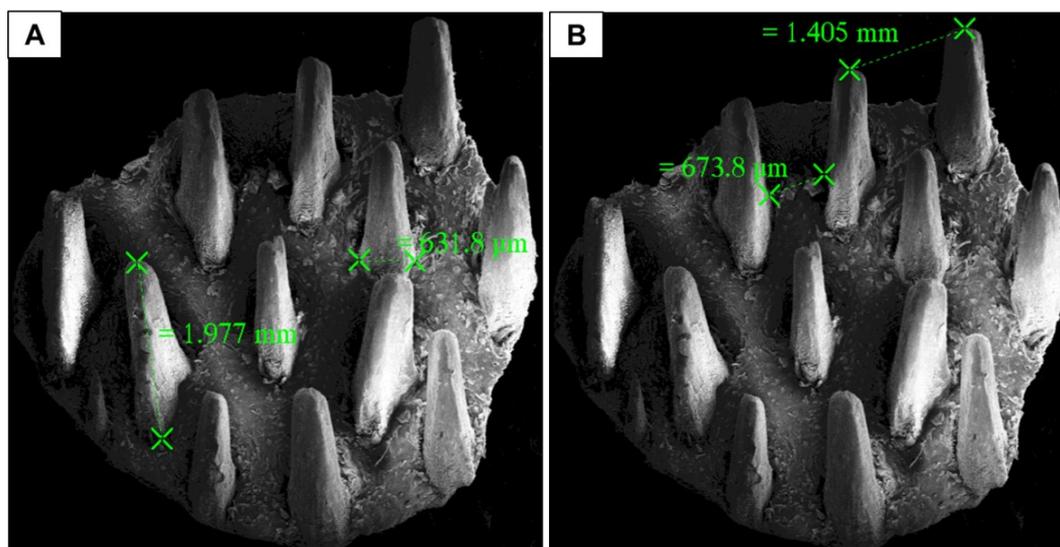
Figure 1. Scanning electron microscopy showing filiform papillae of a Holstein heifer tongue. A: Measurement of the length and base width of the papillae; B: Linear distance between bases and tips of the papillae.

Evaluation of tick infestation

Before the biopsy samples were collected (done in a month favorable for the free-living stages of *R. microplus*: high heat and humidity), four counts of the natural infestation were made at weekly intervals to determine the number of female specimens of *Rhipicephalus (Boophilus) microplus* larger than 4.5 mm that were present on the entire body surface. The mean number of ticks on each animal was calculated for the period, and the overall mean number of ticks on all the animals (33 ticks) was used as a parameter to separate the animals into two groups: low infestation (mean number of ticks below the overall mean) and high infestation (mean number of ticks above the overall mean).

Statistical analyses

The design was randomized and the data were analyzed with the statistical analysis software SPSS 12.0. One-way analysis of variance (ANOVA) was used to determine whether there were differences among the heifers in relation to the mean length, base width, and distances between bases and tips of the papillae, and to determine the effect of tick infestation on these parameters. Because the data had normal distribution, Pearson's correlation coefficient between the measurements was calculated.

Results

The results of the measurements of the filiform papillae of Holstein heifers and the mean number of ticks found on each of them are shown in Table 2. Table 3 reports the ANOVA results, which showed that tick infestation had significant effects on the length of the papillae and the number of papillae per.

All the measurements of the biopsy samples differed between the heifers, especially regarding the mean length of the papillae. The highest mean value observed was 1.97 mm, and the lowest was 0.94 mm. The same occurred in relation to the base width, which ranged from 0.43 to 0.92 mm. The variations in the distances between the bases (0.50 to 0.87 mm) and between the tips (1.03 to 1.55 mm) were lower among them.

Animals with smaller papilla length ($P < 0.01$), smaller base width ($P > 0.05$), and greater number of papillae per unit area ($P < 0.01$) had lower tick infestations (Table 3).

The correlation coefficients between the measurements of the filiform papillae are found in Table 4. The papilla length was not significantly correlation with any of the other variables. The correlation between the papilla width and the distance between tips was positive and significant. The distance between the bases also had a strong positive correlation with the distance between the tips. Finally, the number of papillae per unit area was negatively correlated with all variables, but this was only significant in relation to the width and the distance between the papillae.

Table 2. Number of filiform papillae per cm^2, mean number of ticks, and estimated means and standard deviations of length, base width, distance between bases and between tips of the filiform papillae of each animal.

Animals	Number of papillae cm^2	Mean number of ticks	Mean length (mm)	Base width (mm)	Distance between bases (mm)	Distance between tips (mm)
53	14.33	18.25	1.32 ± 0.18def	0.65 ± 0.08bcd	0.50 ± 0.10b	1.23 ± 0.25ab
54	16.99	37.75	1.97 ± 0.22a	0.60 ± 0.09cde	0.56 ± 0.16b	1.25 ± 0.32ab
617	14.01	40.75	1.89 ± 0.48ab	0.49 ± 0.08ef	0.87 ± 0.30a	1.50 ± 0.35a
620	15.57	43.25	1.55 ± 0.14cd	0.66 ± 0.09bcd	0.65 ± 0.09ab	1.41 ± 0.13ab
621	14.91	26.00	1.19 ± 0.12efgh	0.69 ± 0.10bcd	0.67 ± 0.15ab	1.55 ± 0.25a
622	12.13	37.25	1.29 ± 0.07defg	0.82 ± 0.07ab	0.53 ± 0.14b	1.37 ± 0.31ab
624	12.46	68.75	1.39 ± 0.18cde	0.74 ± 0.14bc	0.72 ± 0.18ab	1.36 ± 0.33ab
626	8.64	11.50	1.24 ± 0.12defgh	0.62 ± 0.06cde	0.73 ± 0.15ab	1.54 ± 0.18a
632	15.92	49.50	1.24 ± 0.14defg	0.57 ± 0.11d	0.65 ± 0.18ab	1.24 ± 0.28ab
633	21.23	30.50	0.95 ± 0.20gh	0.43 ± 0.09f	0.56 ± 0.11b	1.03 ± 0.14b
636	14.30	7.75	0.94 ± 0.23g	0.69 ± 0.10bcd	0.81 ± 0.17a	1.50 ± 0.29a
638	15.57	43.75	1.10 ± 0.12efgh	0.56 ± 0.06def	0.54 ± 0.07b	1.23 ± 0.24ab
639	11.58	47.50	1.02 ± 0.07fgh	0.92 ± 0.17a	0.72 ± 0.24ab	1.44 ± 0.21a
640	16.99	18.00	1.70 ± 0.21abc	0.68 ± 0.08bcd	0.86 ± 0.17a	1.55 ± 0.38a
644	17.15	15.00	1.65 ± 0.25b	0.61 ± 0.10cde	0.57 ± 0.06b	1.21 ± 0.31ab
Mean	14.78	33.03	1.36 ± 0.38	0.63 ± 0.15	0.67 ± 0.20	1.36 ± 0.31

Means followed by different letters in the column differ (P < 0.05, Tukey test).

Table 3. Dimensions of Holstein heifers' filiform papillae according to the level of tick infestation (below or above the average).

Level of tick infestation	Length (mm)	Base width (mm)	Distance between bases (mm)	Distance between tips (mm)	Number of papillae per cm^2
Below mean	1.28 ± 0.35 a	0.61 ± 0.13 a	0.67 ± 0.18 a	1.36 ± 0.33 a	16.68 ± 3.30 a
Above mean	1.45 ± 0.39 b	0.65 ± 0.17 a	0.67 ± 0.21 a	1.35 ± 0.29 a	15.46 ± 1.91 b

Means followed by different letters in the column differ (P <0.01)

Table 4. Pearson correlation between measurements of Holstein heifers' filiform papillae (L, length; W, width; Db, distance between bases; Dt, distance between tips) and number of papillae per unit area (number of papillae cm^2).

	L	W	Db	Dt
L	1	-	-	-
W	0.039	1	-	-
Db	0.088	0.119	1	-
Dt	0.040	0.247**	0.562**	1
N° papillae cm^2	-0.109	-0.465**	-0.161*	-0.368**

** Significant Pearson correlation (P < 0.001). * Significant Pearson correlation (P < 0.05).

Discussion

Filiform papillae have different sizes depending on their location on the tongue, but the greatest number of them and the largest ones are on the anterior third (Benetti et al., 2009; Shao et al., 2010). Their main roles are the mechanical functions of apprehension of food and grooming (Mooring et al., 1996; Mooring & Hart, 1997; Iwasaki, 2002; Dyce et al., 2004; Shao et al., 2010). The diameter of the base and number of papillae cm^2 in our study were similar to the parameters found by Steflik et al. (1983) in Angus cows. The diameter of the base was similar to that found by Veríssimo et al. (2015), but these authors found a larger number of papillae cm^2. The mean length of the papillae found by us (1.36 mm) was one of the lowest averages compared with those found by other authors (Steflik et al., 1983; Cabello et al., 1988; Sari et al., 2010, Veríssimo et al., 2015). The differences in the measurements observed by other authors can be attributed to differences in breed, origin and/or age of the animals, and also the specific tongue site where the measurements were made. The morphology of the papillae observed in this study was conical and convex, like a rose thorn, oriented in the flow direction, as found by Boshell et al. (1982), Steflik et al. (1983), Cabello et al. (1988), Dyce et al. (2004), Sari et al. (2010), and Veríssimo et al. (2015). This orientation of flow direction helps guide food to the esophagus, in preparation for swallowing.

Steflik et al. (1983) and Sari et al. (2010) described the histological and structural characteristics of the filiform papillae of *Bos taurus* and reported they were composed of columns of differentiated keratinized cells that were rich in tonofilaments. They concluded that the papillae were formed by a thick stratified squamous epithelium covering a core of connective tissue, thus resembling a mammalian hair bulb. The histology of

filiform papillae includes high quantities of keratohyalin granules, which may be associated with the mechanical function of these papillae on the tongue, according to Dyce et al. (2004).

Cabello et al. (1988) described the filiform papillae of cattle as rising abruptly from a surface covered with abundant scales, similar to our findings (Figure 1). We observed that the papillae became narrower towards their tips, so the distance between the tips was much greater than that between the bases (almost twice the distance).

Shao et al (2010) compared bovine tongues from *Bos taurus* and *Bos grunniens* (yak) and found that the filiform papillae gradually increased in size from the body to the tongue tip, in both species. Using scanning electron microscopy and optical microscopy, these authors observed that the filiform papillae were covered with continuous tough keratinized epithelium along 60% of their length and that the surface of the tip consisted of irregular scaly cornified epithelial cells that were serrated and jagged. According to the authors, this enhances the mechanical function of this type of papilla. They concluded that filiform papillae have mechanical functions of food apprehension and grooming in both species, while protecting the fungiform papillae and performing mechanical cleaning of the interdental spaces of the lower jaw. We also found that the papilla tip surfaces were irregular and bipartite in some animals (see the papilla at the center of the biopsy shown in Figure 1).

Veríssimo et al. (2015) described an association between the anatomy of the tongue and tick resistance in European (Holstein and Brown Swiss) and Nelore (Zebu) bulls. The Nelore filiform papillae located 3 cm from the tip were longer and more numerous than those of European animals' tongues, and they were more resistant to cattle ticks. The self-cleaning activity performed by the tongue is highly effective in removing ectoparasites. Among ungulates, grooming is an important factor in reducing tick infestation (Mooring et al., 1996; Mooring & Hart, 1997; Mooring & Samuel, 1998; Mooring et al., 2004), including infestation by *Rhipicephalus (Boophilus) microplus* (Riek, 1956; Snowball, 1956; Bennett, 1969; Kemp et al., 1976; Koudstaal et al., 1978). According to our results, animals with greater numbers of papillae per unit area have a rougher tongue than those with fewer papillae per unit area, and this improves the efficiency of self-cleaning. Regarding the association between longer papillae and the presence of a greater number of ticks, it is possible that longer papillae are more flexible, and thus fold more easily, resulting in less efficient self-cleaning efficiency. Such papillae would therefore not be as efficient as a stiff bristle brush for removing ticks from the hide. However, when comparing a breed of tick-resistant cattle (Nelore) with susceptible cattle breeds (European, Holstein, and Brown Swiss), Veríssimo et al. (2015) noted that the resistant breed had significantly larger papillae.

Due to the harm caused by ectoparasites and the high cost of controlling them borne by the Brazilian cattle industry, there is a pressing need to study this relationship with a greater focus on the mechanical action of filiform papillae in animals' self-cleaning process to remove ectoparasites. Histological and morphological studies to obtain greater details of the bovine tongue (organ size, muscles, presence of pigments, number, and size of filiform papillae are necessary to analyze the size, angle of curvature and keratinization of papillae, among other aspects, to determine correlations with the phenotype of resistance or susceptibility to ticks and other ectoparasites. Selection of cattle with greater self-cleaning efficiency will reduce the damage caused by infestations of ectoparasites, in addition to promoting sustainability, especially since this is a natural process not involving use of chemicals. The chemicals used for combating ticks generate residues in the animals, farm workers and the environment, thereby affecting both animal welfare and product quality.

Conclusion

The results of this study indicate that the morphology of the filiform papillae of Holstein heifer tongues varies between animals of the same breed, and the length and number of papillae per cm^2 are related to tick infestation.

Data availability

Does not applicable.

Acknowledgments

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