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Histomorphometric study of the prenatal development of the Circumvallate Papillae of one-humped camel (*Camelus dromedarius*)

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Abstract
This study aimed at investigating the prenatal development of camel circumvallates papillae using standard histomorphometric methods. In the experiment, fifteen *Camelus dromedarius* foetuses obtained from Sokoto metropolitan abattoir at different gestational ages were used for the study. The fetuses were weighed and grouped according to their gestational ages, which were estimated using their crown-vertebral-rump length. From the fifteen (15) samples used for the study, five (5) belongs to the first trimester, five (5) from the second trimester and five (5) belongs to the third trimester. A total of ten (10) foetuses were males and five (5) were females. Grossly, in all the stages of development, the tongues were observed to be elongated, with flat surfaces and rounded at the apices. At first trimester, the tongues were seen as smooth muscle mass, with almost uniform width and thickness throughout the length. They were uniformly pinkish, no pigmentation and no visual evidence of lingual papillae. At second trimester, the tongues were observed to have taken the normal shape of an adult tongue with variable size and shape of lingual papillae; tapering rostrally from the root to the apex. Biometrically, the weight of the foetuses were found to be 0.18±0.05 to 21.70±7.28 kg from the first trimester to second trimester, the crown – vertebral – rump – length were found to be 15.75±4.42 to 94.00±2.83 cm from the first trimester to the third trimester, weight of the head were found to be 25.05±15.17 to 1120.00±14.14 g and weight of the tongues were found to be 0.79±0.22 to 116.25±11.4 9g from first trimester to third trimester. Histological observations showed that the circumvallate papillae were generally lined with keratinised stratified squamous epithelium, which was composed by basal, spinosum, granulosum and corneum layers. Lingual glands are located in the deeper parts of the papillae. The surface epithelium was less keratinised compared to that of the surrounding surface except the peripheral parts of the papilla. Dermal interdigitations of variable sizes into the epidermis were observed along the whole surface. Few taste buds were observed along the medial papillary wall epithelium of the small-sized papillae.

Key words: Histomorphometry, Camel, Circumvallate, Papillae, Prenatal development.

1. Introduction
The one-humped Dromedary (*Camelus dromedarius*) and the two-humped Bactrian (*Camelus bactrianus*) camels are among the largest mammalian species, adapted to the desert where thorny plants with rough and hard stems grow and with its high temperatures and extreme desiccation [2, 3]. The camel's mouth is very sturdy and is developed to maintain efficient feeding of these plants and is rubbery so that thorns and branches won't damage it [2, 4].

Most available reports on the morphological features of the camel tongue [1, 3, 5, 9, 10, 12, 13, 15, 17, 18, 21, 2] were entirely concerned with structures of adult, thus there is a paucity of information about the prenatal development of camel circumvallate papillae in this area. In this research, gross changes, morphometric changes and histological differentiation involved in the development of the circumvallate papillae in one-humped camel will be described. The study will also add to the existing information on the morphometric analysis in camels. Therefore, this investigation was carried out to reveal and evaluate the pattern of the circumvallate papillae differentiation during prenatal life in one humped camel (*Camelus dromedarius)*.

2. Materials and methods

The study was carried out on 15 foetuses of the one-humped camel collected from the metropolitan abattoir, Sokoto at different gestational ages. The collected foetuses were then taken to the Veterinary Anatomy laboratory of Usmanu Danfodiyo University; where the weight and age of the fetus were determined. The foetal body weight was measured using electrical (digital) weighing balance for the smaller foetuses and compression spring balance (AT-1422), size C-1, sensitivity of 20 kg X 50 g in Kilogram for the bigger foetuses. The approximate age of the foetuses was estimated by using the formula adopted by Bello *et al.,* [3] using Crown Vertebral Rump Length (CVRL). Based on this, samples were divided into 1st trimester, 2nd trimester and 3rd trimester.

The foetuses were then placed on a dorsal recumbency and a caudo-lateral skin incision was made at the intermandibular fossa, ventral to the base of the pharynx as described by Bello *et al.,* [3]. The organs were examined in situ and exteriorised. The length, weight, and width of each tongue were measured using a ruler and thread, weighing balance, and vernier callipers, respectively. The length was taken from the cranial pole (tip) to the caudal pole (root) along the longitudinal axis while the width was taken as the distance between the two lateral aspects of the tongue, measured at the apex or the tip, mid-length (body) and root with vernier calliper, metric ruler for bigger tongues and recorded in millimeter. The organs were weighed using an electronic weighing (Mettler® balance P1210, Mettler Instruments AG, Switzerland) with a sensitivity of 0.01 g. The data obtained were expressed as mean ± standard error of the mean (mean ± SEM).

1cm^2 thick of sample from each segment was collected and fixed in 10% formalin solution. After fixation was achieved, the tissue sample was processed for paraffin blocks preparation. The sections of 5 µm were subjected to haematoxylin and eosin for routine morphology [2]. The standard sections were examined under light microscope and micrographs taken using Sony digital camera (x5) with 12.1 mega pixel.

3. Results and Discussion

From the fifteen (15) samples used for the study, five (5) belongs to the first trimester, five (5) from the second trimester and five (5) belongs to the third trimester. A total of ten (10) foetuses were males and five (5) were females (Table 1). The tongue of camel was elongated and dorsoventrally flattened along its cranial two-thirds on the floor of the buccal cavity between the rami of the mandible from first trimester to third trimester with a rounded apex and a well-developed torus (Plate 1).

Grossly, in all the stages of development, the tongues were observed to be elongated, with flat surfaces and rounded at the apices (Plates I-III). At first trimester, the tongues were seen as smooth muscle mass, with almost uniform width and thickness throughout the length. They were uniformly pinkish, no pigmentation and no visual evidence of lingual papillae (Plate I). At second trimester, the tongues were observed to have taken the normal shape of an adult tongue; tapering rostrally from the root to the tip or apex. The tongues were pinkish with prominent lingual papillae as evident at the posterior one-third of dorsal part of the tongue and less evident at the remaining two-thirds (Plate II). At third trimester, the tongues had developed into the typical adult tongue, with prominent lingual papillae at the tip, laterals, dorsum and the root. The tongue is relatively darkish as compared to those of first and second trimesters (Plate III). There were numerous large circumvallate papillae arranged on each side closer to one another forming two lines almost parallel to the rim of lingual torus. The shape and size of the papillae revealed remarkable differences with advancement in gestation; they were not identical or symmetrical in the two lines in the same specimen. This finding is in agreement with the previous studies of Qayyum *et al.,* [23] on Llama; Erdunchaolu *et al.,* [10] and Peng *et al.,* [19] on Bacterian camel, Emura *et al.,*, [8] on sheep; abundant as in ruminants Qayyum and Beg, [22] and Prakash and Rao, [21]; Chamorro *et al*., [4] on Horse; Kumar *et al.,* [17] and Tadjalli and Pazhoomand, [25] on Sheep. Contrarily, single in mouse, rat and hamster by Kurtul and Atalgin, [18] and Iwasaki *et al.,* [12].

Plate I: Photograph showing camel fetus tongue at second trimester with clearly evident and differentiated lingual papillae X 75

Plate II: Photograph showing camel fetus tongue at second trimester with clearly evident and differentiated lingual papillae X 75

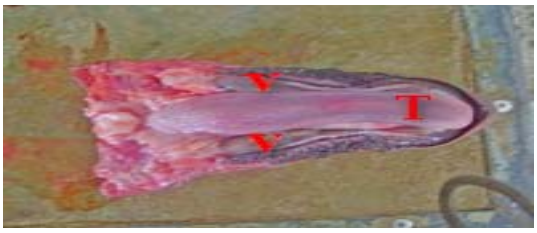

Plate III: Photograph showing camel fetus tongue at third trimester with clearly evident and differentiated lingual papillae X 75

Biometrically, the weight of the foetuses were found to be 0.18±0.05 to 21.70±7.28 kg from the first trimester to second trimester, the crown – vertebral – rump – length were found to be 15.75±4.42 to 94.00±2.83 cm from the first trimester to the third trimester, weight of the head were found to be 25.05±15.17 to 1120.00±14.14g and weight of the tongues were found to be 0.79±0.22 to 116.25±11.49g from first trimester to third trimester (Table 1 and 2). The result shows that with the advancement of gestation the morphometric data were increasing progressively. This is in accordance with the finding of Qayyum *et al.,* [22] on Llama; Erdunchaolu *et al.,* [10] and Peng *et al.,* [20] on Bacterian camel, Emura *et al.*,,[8] on sheep; Chamorro et al., [4] on Horse; Kumar *et al.,* [17] and Bello *et al.,*[2, 3] on camel digestive tract.

Table 1: The mean CVRL, sex and mean weight of the foetuses at various gestational ages.

Trimester	Mean CVRL (cm±SEM)	Sex	Sex	Mean Weight (kg ± SEM)
		Male	Female	
1st	15.75±4.42	3	2	0.18±0.05
2nd	44.75±10.78	3	2	2.37±1.43
3rd	94.00±2.83	4	1	21.70±7.28
	Total	10	5	

Tale 2: The morphometrical parameters of the tongue at the various gestational ages.

Trimester	First	Second	Third
LENGTH (cm±SEM)	**2.05±0.67**	**8.65±1.66**	**14.9±0.42**
WIDTH (mm±SEM)			
Tip	1.3±0.20	9.50±4.60	18.50±3.20
Body	3.00±1.40	12.30±3.40	25.00±0.70
Root	4.30±2.30	14.50±3.90	31.50±1.10
Thickness (mm±SEM)			
Tip	0.07±0.01	4.29±0.53	5.50±0.15
Body	0.28±0.16	5.87±1.43	10.78±0.37
Root	1.56±1.04	9.16±2.90	16.35±0.63

Histological observations showed that the tongue has three layers: tunica mucosa, tunica submucosa and tunica muscularis. The three layers were seen right from the first trimester, as shown in the Figure 1. The development of lingual papillae was found to be in succession; from the stages of ordinary epithelial cell linings with undifferentiated protrusions (papillae) and developing slightly into differentiated papillae, then finally to the developed papillae, with continuous maturity at post-natal stage. These were seen with advancement in gestational age from first trimester through second trimester and to third trimester. Specialization of the lingual muscles also followed the same trend of development, from immature muscle fibres to mature differentiated muscle. At the first trimester, the three layers were clearly seen with slight differentiation of the epithelia into the formation of the lingual papillae (Fig. 1-4). At second trimester, there was development of the three layers, with differentiation of the epithelial cells in the forming the papillae, with clear evidence of the differentiation among the papillae and the muscles of the tongue (Fig. 5-8). At third trimester, the three layers were more differentiated with developed epithelial cells into differentiated lingual papillae. The maturity of the tongue can be said to continue after birth, with the formation of non-keratinised stratified squamous epithelium and the further differentiation of the muscle which were clearly evident in one-year old camel not seen at third trimester (Fig 13-16).

This is in accordance with the finding of Qayyum *et al.,* [22] on Llama; Erdunchaolu *et al.,* [10] and Peng *et al.,* [19] on Bacterian camel, Emura *et al.,* [7] on sheep; Chamorro *et al*., [4] on Horse; Kumar *et al.,* [17].

Histological observations showed that the circumvallate papillae were generally lined with keratinised stratified squamous epithelium, which was composed by basal, spinosum, granulosum and corneum layers (Fig. 1). Lingual glands located in the deeper parts of the papillae, and they opened into the annular groove. The surface epithelium was less keratinised compared to that of the surrounding annular pad except the peripheral parts of the papilla. The above findings coincide with those reported in Bacterian camel by Qayyum *et al.,* [23] and Erdunchaolu *et al.,* [10], on Horse by Chamorro *et al.,* [4]; on cattle by Karadag *et al.,* [13] and on deer by Adnyane *et al.*,[1]. In addition, we also observed taste buds on both sides of the secondary groove and within the epithelial lining of deep sinuses in the lateral papillary wall, which has not been reported before. In contrast, Doughbag [5] and Salehi *et al.* [24] found taste buds on the dorsal surface of the circumvallate papillae of the embryonic tongue. Dermal inter-digitations of variable sizes into the epidermis were observed along the whole surface. Few taste buds were observed along the medial papillary wall epithelium of the small-sized papillae. In the typical papillae, several taste buds with prominent taste pores were found along the whole length of the medial papillary wall epithelium at second trimester. However, they were concentrated in the deeper parts of the medial wall at third trimester (Fig. 2 and 3). The taste buds were spindle or columnar-shaped with variability in diameter.

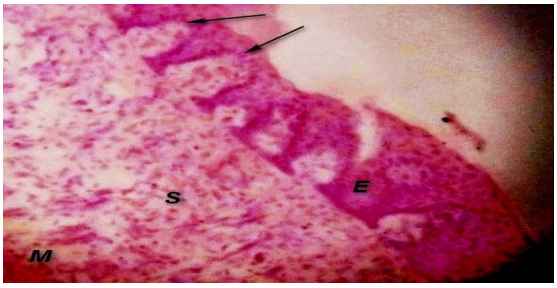

Fig 1: Photomicrograph of camel tongue (DORSAL) at first trimester showing Tunica mucosa (E), Tunica submucosa (S) and *Tunica muscularis* (M) with slight differentiation of the epithelia cells in the formation of papillae (black arrow) H&E x200

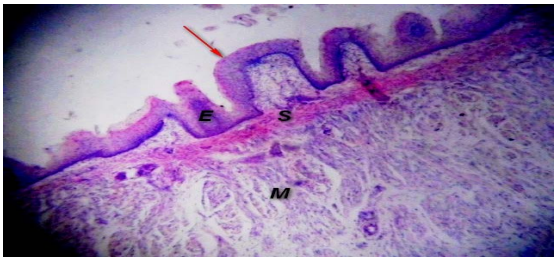

Fig 2: Photomicrograph of camel tongue (DORSAL) at second trimester showing Tunica mucosa (E), Tunica submucosa (S) and Tunica muscularis (M) with differentiation of the epithelial cells in the formation of papillae; Circumvallate red arrow) H&E x200

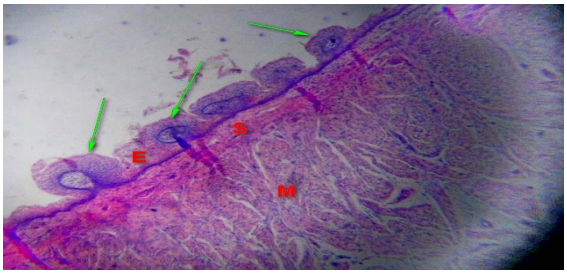

Fig 3: Photomicrograph of camel tongue (DORSAL) at third trimester showing Tunica mucosa (E), Tunica submucosa (S) and Tunica muscularis (M) with differentiation of the epithelial cells in the formation of papillae; Circumvallate (green arrow), H&E x200.

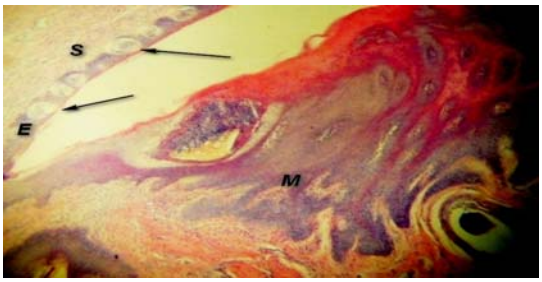

Fig 4: Photomicrograph of camel tongue (DORSAL) at third trimester showing Tunica mucosa (E), Tunica submucosa (S) and Tunica muscularis (M) with developed non-keretinised stratified squamous epithelium (black arrow), well developed taste buds and developed differentiated skeletal muscle (M) H&E x300

4. Conclusion

From the research work, we demonstrated the stages of development of circumvallate papillae with special interest in taste buds at different gestational ages of one humped camel. The histological differentiation of the dromedarian camel circumvallate papillae is similar to that of sheep, goat, camel, llama, and guanaco but differs from that of the rat, cat, dog and pig. Meanwhile, considering the histological features observed in the study, the dromedarian camel circumvallate papillae and taste buds may become fully matured and functional at post-natal age.

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