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HISTOMORPHOMETRIC EVALUATION OF ORGANIC ENAMEL ELEMENTS IN ERUPTED BOVINE TEETH. PART I. ENAMEL TUFTS

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Abstract

The aim of the study was to compare erupted permanent bovine teeth of two types involved in the process of chewing in respect to the localisation and histomorphometric characteristics of enamel tufts. The research material comprised 240 fully erupted premolars and molars from the maxillae and mandibles of 27 heads of cattle from the Polish Black-and-White breed. Overall, 1,986 specimens of bovine teeth were analysed using a Nikon Eclipse E600 microscope with *Micro Image v4.0* software. Enamel tufts were relatively numerous in the enamel of smooth surfaces – on average from 5.6 to 6.4 per cross-section of tooth crown. The average length of the enamel tufts expressed by means of a median was smaller for premolars (89.3 μm) than for molars (123.9 μm). The analysis of the value of Spearman's rank correlation coefficient revealed that no relationship existed between the length and width of tufts in molars (r_s =0.26), and a low-level relationship was noted (r_s =0.45) in premolars. On the basis of the Mann-Whitney U test for the metric measurements, no significant differences were discovered for the width of enamel tufts (P=0.427), but significant differences were observed for the length of enamel tufts (P=0.032). The observed differences in the histological structure of the enamel of posterior bovine teeth in comparison to human teeth indicate that a certain degree of caution ought to be considered when using bovine teeth as a substitute for human teeth in *in vitro* trials.

Key words: bovine teeth, histomorphometry, organic elements, enamel tufts.

In recent years, there has been an increase in the number of publications relating to the use of bovine teeth in *in vitro* trials. This is most probably connected with the need for confirming the suitability of bovine teeth as substitutes for human teeth, since obtaining the latter for scientific research constitutes a considerable problem. However, the differences between the enamel of human and bovine teeth are still emphasised in many scientific studies (20).

Tooth enamel is a highly mineralised structure, which originates in the ectoderm and is synthesised by the specialised cells of the enamel organ. Ameloblasts, together with the secreted organic extracellular matrix, create the environment necessary for the process of mineralisation and crystal growth. In the course of enamel maturation, the majority of extracellular matrix proteins becomes degraded and is replaced primarily by calcium and phosphate ions, as a result of which tooth enamel is the hardest and the most highly mineralised tissue in the body (2, 6). Apart from mineral matter, enamel also contains some organic components. Enamel tufts are small organic elements, sometimes referred to as developmental changes, which are not able to be observed in clinical studies and are most frequently identified during *in vitro* experiments. Some authors suggest that enamel tufts play a role in better junction between enamel and dentine. Both components differ in structure and physical properties. However, no significant evidence has been found so far (1, 8).

Computer image analysis is commonly used in diagnostics. According to the universally accepted in the medical world opinion, it seems that *in vitro* trials, and particularly histomorphometric studies, still remain the most objective, with the available software enabling precise measurement, data archiving, as well as, longitudinal comparative analyses.

The aim of the research was to compare erupted permanent bovine teeth of two types involved in the process of chewing in respect to the localisation and histomorphometric characteristics of enamel tufts.

Material and Methods

The research material comprised erupted permanent bovine teeth from the maxillae and mandibles of 27 heads of cattle from the Polish Black-and-White breed (Fig. 1.a and 1.b). The heads of healthy, approximately four-year-old animals, after slaughter and examination by the relevant veterinary services, were transferred directly to the research laboratory. The biological material was collected, documented, and stored in accordance with the principles of bioethics (16, 19). Since bovine teeth required for study are 1st category production waste of special risk (animal production waste classified as 02 02 02), they cannot be consumed by humans (7, 17). Following the most recent legal regulations, due to their potential threat, *i.e.* active prions possibly present in special risk materials (SRMs), the materials used in this study should be and, thus, were utilised, in order to avoid the risk of their usage for any other purpose. There was no need to obtain the approval of the Bioethics Committee for this research; however, such an approval was obtained for the entire research project.

In all, laboratory experiments were conducted on 240 fully erupted teeth, 120 premolars, and 120 molars. The specimens were prepared within 24 h after slaughter, when the photographic documentation had been completed. The teeth were cut in the mesio-distal direction, or along the bucco-lingual plane (Fig. 1.c). Each tooth was cut along its long axis using a low speed Isomet saw (Buehler) with a 0.1 mm thick diamond blade cooled with distilled water. From each bovine tooth 4 to 15 sections were obtained, each approximately 0.4 mm thick. The sections were placed individually in distilled water in marked sterile containers, which were shaken for 1 min in an ultrasonic bath.

Overall, 993 intact sections of bovine teeth were obtained. Both sides of the cross-sections were analysed, which meant that there was a total of 1,986 specimens. The tooth sections were assessed using a Nikon Eclipse E600 microscope with *Micro Image v4.0* software. The localisation of enamel tufts was initially determined on unstained sections, and subsequently after staining them with haematoxylin and eosin (H.E.) or by Mallory method. The tufts were counted, and their distribution, as well as, their frequency of occurrence at the enamel-dentine junction in the occlusal surface projection, or in the smooth side-surface projection was determined. The results of these measurements were then described and subjected to statistical analysis. Spearman's rank correlation coefficient, as well as, the Mann-Whitney *U* test were used in order to detect significant differences between the two studied functional groups in respect to the feature analysed (14).

Results

Depending on the location of the enamel, various shapes of enamel prism cross-sections were observed. For each section the presence of enamel tufts in the enamel at the enamel-dentine junction (EDJ) was confirmed (Figs 2.a and 2.b). No differences were observed in respect to the numbers and the metric values of the tufts specified for unstained and stained specimens. In many images, the enamel tufts displayed diverse measurements with structures similar to pinnate or palmate vascular systems of leaves, which had come into contact with the sheaths of neighbouring enamel rods. These elements were relatively numerous in the enamel of smooth surfaces – on average from 5.6 to 6.4 per cross-section of tooth crown (Table 1). On average, the number of enamel tufts was generally higher in molars than in premolars but no statistically significant differences (P>0.05) were noted (Table 2).

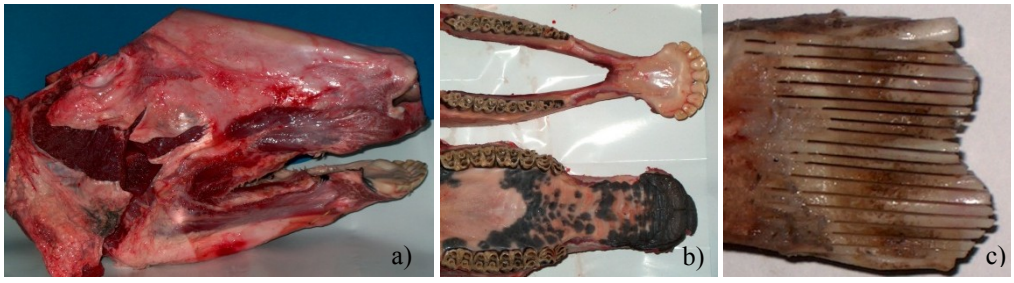


Fig. 1: Pictures: a) a bovine head; b) the maxilla and mandible of a 4-year-old cow; c) an incised tooth crown

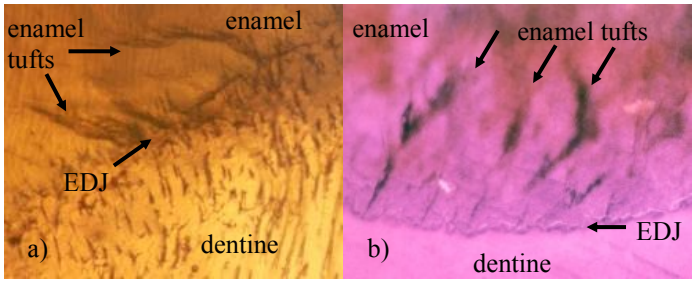


Fig. 2: Visible enamel tufts on a specimen of a) distal surface of a bovine upper left premolar (200×), stained according to the Mallory technique; b) occlusal surface of a bovine lower left molar (200×), H.E.

Table 1
Specific values for the characteristics relating to the number of enamel tufts at the enamel-dentine junction per crown cross-section in the studied groups of permanent bovine teeth

Characteristics of erupted bovine teeth	Premolar teeth			Molar teeth		
	Smooth surfaces total	Occlusal surface projection	Total	Smooth surface total	Occlusal surface projection	Total
Minimum	2.4	0.0	2.4	1.9	0.0	1.9
Maximum	10.0	2.1	11.8	11.3	5.4	13.8
Arithmetic mean	5.6	0.6	6.3	6.4	1.4	7.7
Median	5.1	0.2	5.6	6.6	1.5	7.4
Standard deviation	1.52	0.66	1.89	2.45	1.31	2.91
Coefficient of variation (%)	41.28	118.99	39.25	39.72	92.61	34.14

Table 2
Specific values for the characteristics relating to the number of enamel tufts for smooth tooth surfaces per crown cross-section in the studied groups of permanent bovine teeth

Characteristics of erupted bovine teeth	Premolar teeth				Molar teeth			
	Mesial surface	Distal surface	Buccal surface	Lingual or palatal surface	Mesial surface	Distal surface	Buccal surface	Lingual or palatal surface
Minimum	1.8	2.0	0.8	1.0	1.6	1.8	1.8	2.0
Maximum	3.5	3.8	4.0	4.0	4.6	5.0	4.8	4.5
Arithmetic mean	2.7	2.7	2.7	2.8	3.2	3.3	3.2	3.2
Median	2.8	2.6	3.0	2.5	3.3	3.3	3.1	3.3
Standard deviation	0.63	0.54	1.29	0.97	0.88	1.15	1.04	0.92
Coefficient of variation (%)	23.64	19.66	48.81	35.40	27.68	35.11	32.85	28.46

Table 3
Specific characteristics relating to the measurement of the length and width of enamel tufts in the studied groups of permanent bovine teeth

Characteristics of enamel tufts in erupted bovine teeth	Length (µm)		Width (µm)	
	Premolar teeth	Molar teeth	Premolar teeth	Molar teeth
Minimum	28.4	37.3	9.1	12.7
Maximum	267.2	365.3	130.9	168.3
Arithmetic mean	107.5	147.8	57.1	73.5
Median	89.3	123.9	58.7	72.0
Standard deviation	60.39	89.42	31.98	41.34
Coefficient of variation (%)	56.19	60.51	56.04	56.27

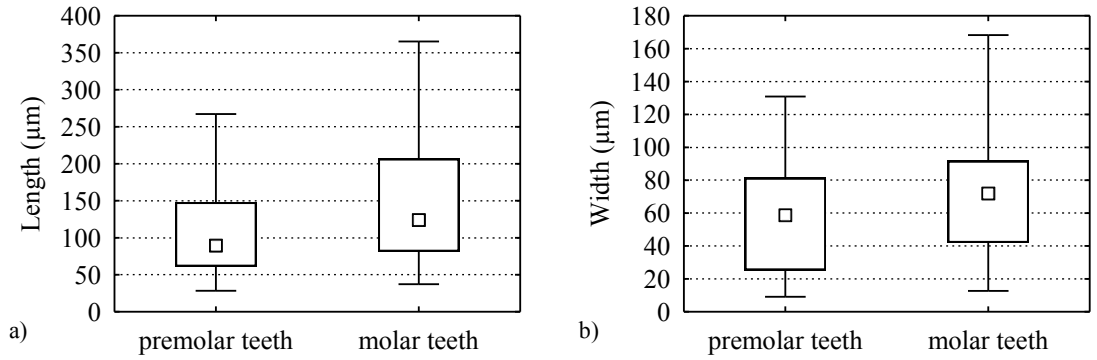


Fig. 3. Quantile box plots for a) length of enamel tufts, b) width of enamel tufts.

The measurements of the length and width of the enamel tufts were highly diverse, as the coefficients of variation ranged from 56.04% to 60.51%. The average length of the enamel tufts expressed by means of a median was smaller for premolars (89.3 μm) than for molars (123.9 μm). No substantial differences were observed when analysing the average width of the enamel tufts in the studied groups of teeth (Table 3, Fig. 3).

The analysis of the value of Spearman's rank correlation coefficient revealed that no relationship existed between the length and width of enamel tufts in erupted molars (r_s=0.26), and a low-level relationship was noted in premolars (r_s=0.45). On the basis of the Mann-Whitney *U* test for the metric measurements of enamel tufts, no significant differences were discovered for the width of enamel tufts (P=0.427), but significant differences were observed for the length of enamel tufts (P=0.032).

Discussion

This study has taken into account the experiences of other researchers and therefore *ex tempore* specimens were studied in order to safeguard against the destructive influence of chemical substances. The authors of other research often stated that both stained and unstained specimens were subjected to microscopic examination, without specifying the staining techniques (4, 13). The available literature does not provide any specific suggestions concerning the staining techniques, which appear to be the most useful in dental research: different staining techniques were used depending on the aims of the studies, and the material used in the research. For this reason, two staining techniques were selected for the study, H.E. and Mallory, which are highly rated for staining organic elements of bovine teeth (10).

The earlier research indicated that enamel tufts in human teeth were similar in shape to leaves or leaf fragments that penetrated between enamel rods (9). In the research enamel tufts with a pinnate or palmate vascular leaf system were more frequently observed and were less patulous than the tufts in human teeth. The number of tufts on smooth surfaces was relatively greater than in occlusal surface projections. The smooth surfaces had a similar number of tufts, whereas in occlusal surface projections there was a greater differentiation, with a relatively larger number of tufts per crown cross-section occurring in erupted molar teeth (from 1.9 to 13.8 per crown cross-section) than in premolars (from 2.4 to 11.8). The authors of the study have not been able to find any information in the literature relating to enamel tufts in bovine teeth, which would refer to their numbers and metrical values. In the bovine teeth examined in the course of the study, the number of enamel tufts was considerably lower, on average 2 to 8 times fewer tufts per crown cross-section, than in erupted human molar teeth. An examination of the enamel tuft structures in bovine teeth conducted by the authors of the study revealed that, just as in human teeth, they originate at the enamel-dentine junction but are not linked with dentinal tubules. Similarly to human teeth, the tufts in bovine teeth reveal a complex three-dimensional micro-structure. This multidimensional structure has been under intensive investigation for a number of years, but such a research focused only on human teeth (3, 8, 11, 12).

Because of a considerable diversification in the metric measures, the coefficient of variation and the quantile values were also calculated along with the arithmetic mean and the standard deviation. This made it possible to avoid over- or underestimating the average values in any analysis of the measurements due to the occurrence of extremely high or extremely low measurement values. Mehr *et al.* (9) observed a considerable diversification both in respect to the width and length of erupted human molars. A similar diversification of these parameters/values was observed in this study; however, their average values, measured by means of the arithmetic mean and the median, were considerably lower in the bovine teeth examined, though higher for molars than for premolars. The values of the median and the arithmetic mean were very similar in case of the width of enamel tufts in cattle. Bovine teeth contained tufts whose maximum lengths were approximately half the length of the tufts in human teeth, while the width of the tufts was similar for human and bovine teeth. In dentistry, just as in other areas of

medicine, before any clinical trials are initiated, for example on dental materials, it is necessary to ascertain whether the alternative biological substitute resembles human material. In the study the histological similarity of two functional groups of bovine teeth was evaluated, and the data obtained were compared with data relating to human teeth. It seems that expanding the pool of information in respect to the histomorphometry of tooth enamel can contribute towards a more definitive evaluation of the suitability of bovine teeth in further stages of experimental research (16, 19).

Considerable tooth diversity has been documented across 14 species of *Bovidae* such as *Chiroptra*, *Sirena*, *Cetacea etc*., which seems to indicate that it may be necessary to specify the breed of cattle, whose teeth are used in experimental research (5, 15). The premolar and molar bovine teeth used in the study belong to the selenodont type and were obtained from one breed of cattle. Although it must be accepted that the structure of posterior bovine teeth is different than the structure of human teeth, the mandibular incisors of bovine origin are quite frequently used as a substitute for human teeth in dental research (18).

In conclusion, a histomorphometric analysis of organic enamel elements in bovine teeth indicates the existence of considerable differences in the length of enamel tufts between premolars and molars. The observed differences in the histological structure of the enamel of posterior bovine teeth in comparison to human teeth indicate that a certain degree of caution ought to be taken when using bovine teeth as a substitute for human teeth in *in vitro* trials.

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