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Cardiac glands with a difference – scanning electron microscopy of the cardiac gland region in the stomach of the babirusa (Babyrousa babyrussa), domestic pig (Sus scrofa domestica), white-lipped peccary (Tayassu pecari) and Bennett’s wallaby (Macropus rufogriseus)

Abstract

The stomach of the babirusa differs from that of other pigs (Leus et al. 1999, Agungpriyono et al. 2000): it is larger and possesses a large diverticulum ventriculi, the gastric glands are confined to a small section at the end of the corpus ventriculi, the cardiac glands occupy a much larger surface area within the stomach (>70 % v. ~30 % in the domestic pig) and there are some variations in the distribution of endocrine cells. It was hypothesised that the babirusa is a non-ruminant foregut fermenting frugivore/concentrate selector (Leus et al. 1999). Scanning electron microscopy of very freshly fixed cardiac gland tissue from the stomachs of nine babirusa revealed that the surface of the whole of the cardiac gland region was characterised by a honeycomb pattern (Leus et al. 2002). At higher magnification the walls appeared to be almost entirely composed of bacteria. No histological study of the stomach of the babirusa or of those of other foregut fermenters with larger areas of cardiac glands has drawn attention to anything like this honeycomb structure. We therefore investigated fresh tissues from domestic pigs (Sus scrofa domestica), white-lipped peccaries (Tayassu pecari) and Bennett’s wallabies (Macropus rufogriseus). The ‘honey-comb’ appearance of the luminal surface of the cardiac gland region of the babirusa stomach was not found in the equivalent regions of the stomachs of the other species. The possibility remains that this structure is a feature unique to the babirusa. Possible hypotheses regarding its function include surface enlargement to increase attachment space and

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retention time of bacteria in a stomach without strong compartmentalisation and/or to increase the area for absorption of fermentation products. The explanation may be of direct consequence to the feeding requirements of babirusa in zoos, which in turn may be an important factor for the success of its conservation breeding program.

Keywords

histology, bacteria, forestomach, fermentation

1 Introduction

The stomach of the babirusa (Babyrousa babyrussae) differs from that of other pigs in several aspects (Leus et al. 1999, Agungpriyono et al. 2000): it is larger in size and possesses a voluminous diverticulum ventriculi; the cardiac glands are confined to a small section at the end of the corpus ventriculi; the cardiac glands occupy a significantly larger surface area within the stomach (> 70 % v. ~ 30 % in the domestic pig (Sus scrofa domestica)) (figure 1) and there are some variations in the distribution of endocrine cells (further studies are necessary to determine the significance of the latter). The pH in the lumen of the cardiac gland region was deemed suitable for the survival of the numerous microorganisms found therein. These organisms are likely to play a role in bacterial fermentation of plant structural compounds by means of enzymes which the mammalian host is unable to produce itself.

What little is known about the diet and digestion of babirusa in the wild and in captivity has been reviewed in Leus (1996), Leus (2000) and Leus et al. (in press). In the wild, babirusa appear to have a preference for fruits and seeds but to also consume leaves, grass, invertebrates and smaller vertebrates. They are less able to root with the nose in compact soil.

The above findings, together with a survey of the diets fed in captivity and the results of digestibility studies carried out on babirusa in zoos, strongly suggest that the babirusa is a non-ruminant foregut fermenting frugivore concentrate selector.

Previous studies of the stomach, which concentrated on the gross anatomical and light microscopic structure, largely made use of museum material or specimens not collected immediately after death (Langer 1973, 1988, Leus et al. 1999). More recently, we have been able to obtain stomach tissues from nine babirusa that were euthanised for veterinary reasons not related to the gastro-intestinal tract. This more immediate fixation of tissues permitted investigations of the gastric mucosa using scanning electron microscopy. The granular honey-comb pattern we found covering the cardiac gland region of the babirusa (Leus et al. 2001, 2002) prompted us to carry out additional comparative studies of fresh tissues from an initial limited selection of other animals that have larger areas of cardiac or mucogenic glands in their stomachs: the domestic pig, the white-lipped peccary (Tayassu pecari) and the Bennett's wallaby (Macropus rufogriseus) (figure 1).
Figure 1. Schematic diagrams of the stomachs of the babirusa (*Babyrussa babyrussa*) (from Leus et al. 1999), the domestic pig (*Sus scrofa domestica*), the white-lipped peccary (*Tayassu sp.*) and the Bennett's wallaby (*Macropus rufogriseus*) indicating the distribution of the cardiac gland epithelium. ([O = oesophagus; P = pylorus]

2 Materials and Methods

Stomachs from nine babirusa in five zoological collections were used for this study. Details of the animals and the fixation materials employed are contained in Leus et al. (2002). Stomachs from three domestic pigs were obtained fresh from the slaughter house. Tissues from two white-lipped peccaries and five Bennett's wallabies were obtained from zoological gardens during autopsy immediately after death. All of these animals were euthanised for reasons not related to the gastrointestinal system. Samples were initially fixed either in Bouin's fluid or 3% gluteraldehyde. Those fixed in the former were kept in the fluid for 24 h only, after which they were stored in 70% alcohol. For scanning electron microscopy all specimens were placed in a solution of 3% gluteraldehyde in 0.1 M sodium cacodylate buffer (pH = 7.3) overnight and then immersed in 2% guanidine hydrochloride and 2% tannic acid for a second night (Murakami et al. 1977). They were subsequently post fixed in 2% osmium tetroxide in distilled water for 8 hours. Dehydration in graded acetones was followed by critical point drying using carbon dioxide (Cohen 1979). After they were mounted on aluminium stubs, the specimens were
sputter coated with 20 μm gold/palladium (Echlin 1975) and viewed in a Phillips 505 scanning electron microscope.

3 Results

The luminal cardiac gland region of the babirusa was typically characterised by a honey-comb pattern (figure 2). The walls of the honey-comb had a very granular appearance at low magnification. At higher magnification these walls appeared to be almost entirely composed of a bacterial microflora. The glandular tissue of the mucosa was situated underneath the honey-comb layer.

Figure 2. Scanning electron micrograph of the cardiac gland region of the stomach of the babirusa (Babyrussa babyrussa). The surface of the “honey-comb cells” has a granular appearance. The glandular epithelium underlies the honey-comb layer. Scale bar = 1 mm.

In the domestic pig the cardiac gland region had the appearance shown in figure 3. The mucosal surface could be seen partially covered with mucus, ingesta and bacteria. The openings to the gastric pits appeared as round dark depressions on the luminal surface.

Figure 3. Scanning electron micrograph of the cardiac gland region of the stomach of the domestic pig (Sus scrofa domestica) Scale bar = 1 mm.

Figure 4. Scanning electron micrograph of the cardiac gland region of the stomach of the white-lipped peccary (Tayassu pecari) Scale bar = 1 mm.

Figure 5. Scanning electron micrograph of the cardiac gland region of the stomach of the Bennett’s wallaby (Macropus rufogriseus) Scale bar = 1 mm.
The cardiac gland region of the peccary was covered in an amorphous layer of mucus. The underlying luminal surface of the epithelium was dimpled in appearance, thereby reflecting the location of the gastric pits and grooves (figure 4).

The mucosal surface of the cardiac gland region in the stomach of the Bennett’s wallaby is illustrated in figure 5. The openings to the gastric pits appeared as dark invaginations between ridges on the luminal surface.

4 Discussion

The ‘honey-comb’ appearance of the luminal surface of the cardiac gland region of the babirusa stomach was not found in the equivalent regions of the stomachs of the domestic pig, white-lipped peccary or the Bennett’s wallaby.

In the babirusa, light microscopy and electron microscopy have shown that the ‘honey-comb’ has a cellular infrastructure built on top of the epithelial ridges between the cardiac pits (Leus et al. 2002). This structure is very delicate, which may explain why it was not detected in previous studies of babirusa stomachs collected 90 minutes or longer after death (Leus et al. 1999).

Two questions can be posed: 1) is the honey-comb structure a feature unique to the babirusa, 2) what may be the function of this structure? A literature review revealed no reference to a microscopic honey-comb structure on the surface of the cardiac gland area of other wild pigs and non-ruminant forestomach fermenters. To eliminate the possibility that this was due to the fragile structure having been lost due to tissue autolysis, or the method of fixation or processing of the tissues, fresh stomachs of the domestic pig, white-lipped peccary and the Bennett’s wallaby were also investigated.

Information on the gross and microscopic anatomy of the stomach of the closest relatives of the babirusa, the other wild pigs, is rare (Macdonald 1991, Leus and Macdonald 1997), but suggests that there is little difference with the anatomy of the stomach of the domestic pig. Scanning electron micrographs of the luminal surface of the cardiac gland region of the stomachs of the domestic pigs investigated as part of this study, revealed that the surface and gastric pits are lined by epithelial cells as described in the literature, without the additional overlying honey-comb infrastructure seen in the babirusa. Further studies are required to show if among the Suidae, the honey-comb structure is a feature unique to the babirusa. In any case, the proportion of the stomach surface covered by cardiac glands is much smaller in the stomach of the domestic pig (about 30%) compared to that of the babirusa (> 70%) (Leus et al. 1999). Furthermore, what little fermentation may take place in the cardiac gland region of the stomach of the domestic pig is of minor importance in the digestive processes of this species (Keys and DeBarthe 1974).

In contrast, forestomach fermentation has been shown to be an important aspect of diet digestion in the closest relatives of the pigs, the peccaries (Tayassuidae) (Shively et al. 1985; Lochmiller et al. 1989). In the peccary stomach, fermentation takes place in the gastric pouch and its two blind sacs,
lined by a combination of cornified epithelium and cardiac glands (Langer 1978, 1979, Shively et al. 1985, Lochmiller et al. 1989). Nevertheless, scanning electron micrographs of the cardiac gland region failed to reveal the typical honey-comb layer which was so apparent in the babirusa.

The presence of significant areas of cardiac or other mucogenic glands in regions of the stomach where fermentation takes place is not unique to the babirusa or the peccaries. It also occurs in the Bradypodidae and Megalonychidae (sloths) and the Camelidae (Langer 1988, Stevens and Hume 1995). However, only two groups of animals share with the babirusa the possession of an enlarged region of the stomach lined almost exclusively with cardiac glands: the colobine monkeys (colobus monkeys and langurs) and macropod marsupials (kangaroos and wallabies) (Kunh 1968, Gemmel 1977, Langer 1988, Stevens and Hume 1995). For both these groups, forestomach fermentation is a crucial step in the digestive process (Moir 1965, Bauchop and Martucci 1968, Ohwaki et al. 1974, Bauchop 1978, Kay and Davies 1994).

In the macropod marsupial stomach, fermentation takes place in the forestomach (itself composed of a saciform and a tubiform region) lined with stratified squamous epithelium and cardiac glands (Langer et al. 1980, Langer 1988, Stevens and Hume 1995). The present study did not detect a honey-comb layer overlying the surface epithelium of the cardiac gland region of the stomach of the Bennett's wallaby. The Bennett's wallaby was selected for this initial stage of the comparative research because it shows a large amount of cardiac glands in the stomach and because the species occurs in captivity in large numbers in Europe. This made it more likely that fresh tissue from euthanised animals would become available within a short frame of time. However, further research is needed to investigate the absence or presence of the honeycomb structure in the stomachs of other kangaroos and wallabies, specifically those with relatively less grass and more grass roots, leaves and fruits in their diet, e.g. Macropus eugilis or Thylagale sp. Also the stomachs of the colobine monkeys remain to be investigated.

At present the possibility remains that the honey-comb layer in the cardiac gland region of the babirusa stomach is a feature unique to this species. Until further studies are carried out we can only speculate on the function and significance of this structure. The honey-comb layer is potentially a unique adaptation of the babirusa for surface enlargement in order to increase attachment space and retention time of bacteria in a stomach without strong compartmentalisation. At the same time it may increase the area for absorption of fermentation products. All this may be of direct consequence for the feeding requirements of babirusa in zoological gardens, which in turn, may be a key factor for the success of its conservation breeding program.

5 Conclusions

1. The bacteria-filled ‘honey-comb’ layer at the luminal surface of the cardiac gland region of the babirusa stomach was not found in the equivalent
regions of the stomachs of the domestic pig, White-lipped peccary or the Bennett's wallaby.

2. The possibility remains that the honey-comb layer in the cardiac gland region of the babirusa stomach is a feature unique to this species.

3. Further studies are needed to investigate the function of this structure and the possible consequences for the feeding requirements of babirusa in captivity.

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References


