



Animal biology and pathology

# Light and scanning electron microscopy of the cardiac gland region of the stomach of the Babirusa (*Babyrousa babyrussa* – Suidae, Mammalia)

Kristin Leus<sup>a,\*</sup>, Alastair A. Macdonald<sup>b</sup>, Gordon Goodall<sup>b</sup>, David Veitch<sup>c</sup>, Steven Mitchell<sup>b</sup>, Luc Bauwens<sup>a</sup>

<sup>a</sup> Centre for Research and Conservation, Royal Zoological Society of Antwerp, Koningin Astridplein 26, 2018 Antwerp, Belgium

<sup>b</sup> Department of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Summerhall, Edinburgh EH9 1QH, Scotland, UK

<sup>c</sup> Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Summerhall, Edinburgh EH9 1QH, Scotland, UK

Received 12 August 2003; accepted after revision 2 July 2004

Available online 25 August 2004

Presented by Pierre Buser

## Abstract

Previous studies have indicated that the gross anatomical structure of the stomach of the babirusa (*Babyrousa babyrussa*) differs markedly from that of all other pigs. This light and scanning electron microscopic study revealed a previously unknown, microscopic structure characterised by a 'honeycomb' pattern at the luminal surface of the tunica mucosa. The walls of the 'honeycomb' were about 0.20–0.25 mm high and appeared almost entirely composed of various types of bacteria. Underneath the bacteria the walls were formed by thin tubes composed of non-glandular squamous epithelial-like cells, extending from the tops of the ridges between each glandular pit. There is as yet no evidence of a comparable structure in the stomach of any other pig, or to our knowledge any other forestomach-fermenting mammal. **To cite this article:** K. Leus et al., *C. R. Biologies* 327 (2004).

© 2004 Académie des sciences. Published by Elsevier SAS. All rights reserved.

## Résumé

**Examen en microscopie optique et à balayage de la région des glandes cardiales de l'estomac du babiroussa (*Babyrousa babyrussa* – Suidae, Mammalia).** De précédentes études avaient montré que la structure macroscopique de l'estomac du babiroussa (*Babyrousa babyrussa*) diffère remarquablement de celle de tous les autres Suidés. Cette étude en microscopie optique et à balayage a révélé une structure microscopique inconnue jusque-là, caractérisée par une disposition en nid d'abeille

\* Corresponding author.

E-mail addresses: [kristin.leus@zooantwerpen.be](mailto:kristin.leus@zooantwerpen.be) (K. Leus), [A.A.Macdonald@ed.ac.uk](mailto:A.A.Macdonald@ed.ac.uk) (A.A. Macdonald), [Gordon.Goodall@ed.ac.uk](mailto:Gordon.Goodall@ed.ac.uk) (G. Goodall), [David.T.Veitch@ed.ac.uk](mailto:David.T.Veitch@ed.ac.uk) (D. Veitch), [Steve.Mitchell@ed.ac.uk](mailto:Steve.Mitchell@ed.ac.uk) (S. Mitchell), [Luc.Bauwens@zooantwerpen.be](mailto:Luc.Bauwens@zooantwerpen.be) (L. Bauwens).

de la surface luminale de la tunique muqueuse. Les parois de cette structure ont une hauteur d'environ 0,20–0,25 mm et apparaissent presque entièrement composées de diverses bactéries. Sous les bactéries ces parois sont formées de tubes fins, constitués de cellules non glandulaires d'aspect malpighien, reliant les sommets des crêtes entre chaque fosse glandulaire. Il n'y a jusqu'ici pas d'évidence d'une structure comparable dans l'estomac d'aucun autre Suidé ou, à notre connaissance, d'aucun autre mammifère à fermentation gastrique. **Pour citer cet article : K. Leus et al., C. R. Biologies 327 (2004).**

© 2004 Académie des sciences. Published by Elsevier SAS. All rights reserved.

**Keywords:** histology; digestion; bacteria; forestomach fermentation; swine

**Mots-clés :** histologie ; digestion ; bactéries ; fermentation gastrique ; Suidés

## 1. Introduction

The babirusa, *Babirusa babirusa*, is a member of the pig family (Suidae) and endemic to the tropical forests of the Indonesian islands of Sulawesi, Buru and the Sula and Togian island groups [1,2]. The stomach of the babirusa differs from that of other pig species in several aspects [3,4]: it is of a much larger size; possesses a large diverticulum ventriculi; the gastric glands are confined to a small section at the end of the corpus ventriculi; the mucus producing cardiac glands occupy a much larger surface area within the stomach (> 70% vs ~ 30% in the domestic pig) and there are some differences in the distribution of endocrine cells. The pH measured in the lumen of the cardiac gland region was deemed suitable for the survival of the microorganisms found therein [3]. It was hypothesized that the babirusa is a non-ruminant foregut fermenting frugivore/concentrate selector [3,5].

Previous studies of the stomach concentrated on the gross anatomical and light microscopic structure and largely made use of museum material or specimens not collected immediately after death [3,6–9]. 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. The quality of fixation permitted scanning electron microscopic investigations and allowed the imaging of the bacterial flora in the stomach. On one occasion, scrapings of the mucosal layer could also be taken and processed for routine non-pathogenic investigative microbiology. The investigations revealed a previously unknown, bacteria covered microscopic structure at the luminal surface of the tunica mucosa of the cardiac gland region of the stomach.

Table 1

Details of the individual babirusa from which stomach samples were collected and the method of fixation employed. The animals are identified by their international studbook number according to [10]

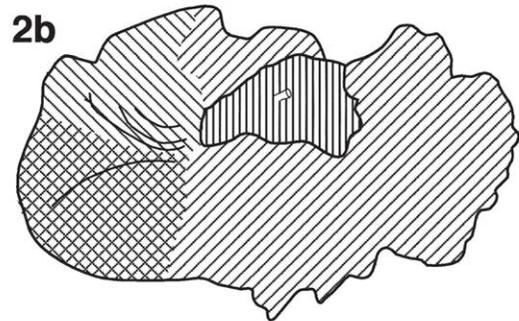
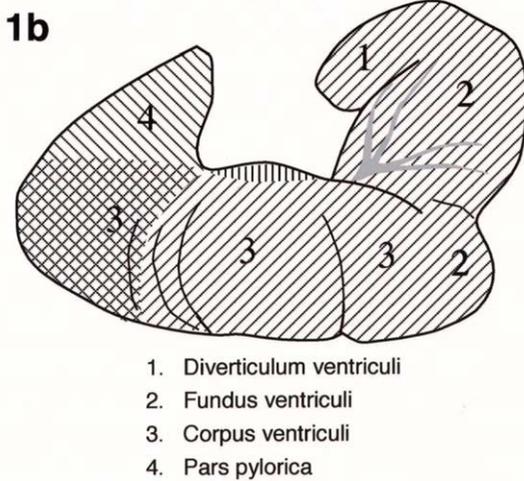
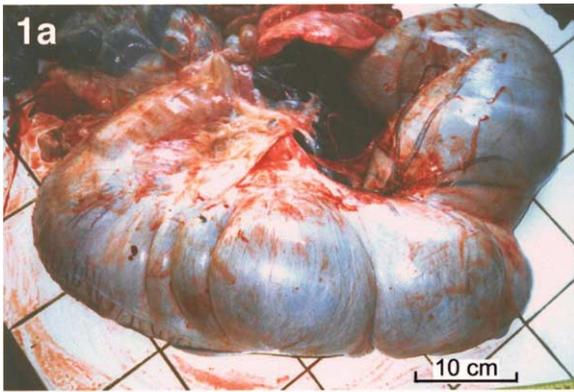
Studbook number	Sex	Place of death	Fixative used
8	F	Stuttgart Zoo	10% Formalin
12	M	Frankfurt Zoo	Klötz
47	M	Antwerp Zoo	10% Formalin
48	F	Antwerp Zoo	Bouin's
49	M	Antwerp Zoo	10% Formalin
52	F	Rotterdam Zoo	10% Formalin
80	M	Rotterdam Zoo	10% Formalin
355	F	Surabaya Zoo	Bouin's
347	M	Surabaya Zoo	Bouin's

## 2. Material and methods

### 2.1. Specimens

The stomachs of nine babirusa from five zoological collections were used for this study. Details of the animals and the fixation materials employed are contained in Table 1. The animals are identified by their international studbook number [10]. All of these individuals were euthanised for veterinary reasons not related to the gastrointestinal system.

Tissue samples from all stomach regions and gland types (see Figs. 1 and 2) were collected during autopsies carried out shortly after death. Samples were fixed in either 10% formalin, Klotz or Bouin's fluid. Those fixed in the latter were kept in Bouin's fluid for 24 h only, after which they were stored in 70% alcohol. This paper will concentrate on the cardiac gland region of the stomach, which was coloured dark grey-brown to greenish black in freshly dead specimens (Fig. 2).



-  Stratified squamous epithelium
-  Cardiac glands
-  Pyloric glands
-  Gastric glands

Fig. 1. Intact stomach of an adult male babirusa (Studbook # 347). (a) Photograph. (b) Line drawing based on photograph.

Fig. 2. Internal stomach lining of the stomach of an adult female babirusa (Studbook # 355). Stomach opened along the greater curvature. (a) Photograph (note the dark colouring of the cardiac gland area). (b) Line drawing based on photograph.

## 2.2. Bacteriology

Scrapings from the epithelial surface of the different stomach regions of the male babirusa studbook # 47 were immediately smeared on glass slides and stained with Gram's stain. Additional scrapings taken from these epithelial surfaces were also immediately inoculated into screw-capped tubes containing Cooked Meat Medium (Oxoid) for transportation to the bacteriology lab. The supernatant layer was cultured onto both Horse Blood Agar (Columbia Blood Agar Base to which had been added 1% defibrinated horse blood) and MacConkey's Agar (Oxoid) and incubated overnight at 37 °C. Material from the meat fraction was inoculated onto Horse Blood Agar and incubated overnight at 37 °C under anaerobic conditions produced by Anaerogen sachets (Oxoid) in a

sealed culture box. Following culture, colonies from the aerobic culture were described and then stained by Gram's method. The lactose fermentation activity on MacConkey's Agar was noted. Colonies from the anaerobic culture were described and the appearance of the Gram stain noted.

Subcultures were made of all colonies and the subsequent colony types reassessed and additional Gram stains made. Preliminary identification of the isolated bacteria from aerobic cultures was determined by means of the Microbact24E system (Microgen Products) and the API 20E system (Bio-Mérieux) following the manufacturers' protocols. Comparable preliminary screening of the anaerobic bacteria was undertaken using the API 20A system (Bio-Mérieux).

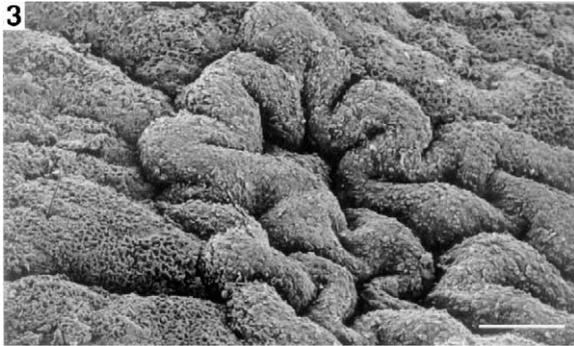


Fig. 3. A scanning electron micrograph of the boundary between the squamous (centre and lower right) and cardiac gland region (left and top right) in an adult male babirusa (studbook # 12). Note the sharp convoluted edge between the two types of epithelia. Note also the 'honeycomb' structure of the luminal surface in the glandular part. Scale bar = 1.0 mm.

### 2.3. Histology

Paraffin sections of 6  $\mu\text{m}$  in thickness were stained with haematoxylin and eosin (H&E), phosphotungstic acid haematoxylin (PTAH) and Gram stain.

### 2.4. Scanning electron microscopy

For scanning electron microscopy, all specimens were placed in a solution of 3% glutaraldehyde 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 [11]. They were subsequently post-fixed in 2% osmium tetroxide in distilled water for 8 h. Dehydration in graded acetones was followed by critical point drying using carbon dioxide [12]. After they were mounted on aluminium stubs, the specimens were sputter coated with 20- $\mu\text{m}$  gold/palladium [13] and viewed in a Phillips 505 scanning electron microscope.

## 3. Results

### 3.1. Scanning electron microscopy

The appearance of the border between the stratified squamous and cardiac gland region of the stomach is illustrated in Fig. 3. The transition from the non-glandular epithelium (centre and right lower) to



Fig. 4. A scanning electron micrograph of the surface of the squamous epithelium in an adult male babirusa (studbook # 47). A mixed population of bacteria including rods of different sizes (occurring singly or in colonies), and chains of cocci can be seen. The surface of the squamous epithelial cells shows a patterned series of convoluted ridges about 0.3  $\mu\text{m}$  in width and of variable length. Scale bar = 5.0  $\mu\text{m}$ .

the glandular epithelium of the mucosa of the cardiac gland area (left and top right) was very abrupt. The stratified squamous epithelium was considerably folded into ridges and troughs and as a consequence there was a convoluted boundary with the cardiac gland epithelium. Between the folds, and also on the surface of the squamous epithelium, a mixed population of bacteria was observed, which included rods of different sizes, occurring singly or in colonies, and chains of cocci (Fig. 4). The surface of the squamous epithelial cells showed a patterned series of convoluted ridges about 0.3  $\mu\text{m}$  in width and of variable length.

The cardiac gland region was typically characterised by a 'honeycomb' pattern (Figs. 3 and 5). This 'honeycomb' pattern covered the whole of the cardiac gland area. The entrance to each 'honeycomb cell' had a diameter of approximately 0.07 mm. The walls of the 'honeycomb' appeared to extend in height by about 0.20–0.25 mm above the level of the glandular epithelium (Fig. 5). The thickness of these walls varied. The surface of the 'honeycomb cells' had a very granular appearance at low magnification (Fig. 5). Higher magnification revealed that the walls appeared to be almost entirely composed of a bacterial microflora (Figs. 6 and 7). Rods of various sizes occurred in colonies with individual and chains of cocci. A network of extracellular material seemed to provide some measure of support to these colonies and was most obvious at the rim of each 'honeycomb cell' (Fig. 7).

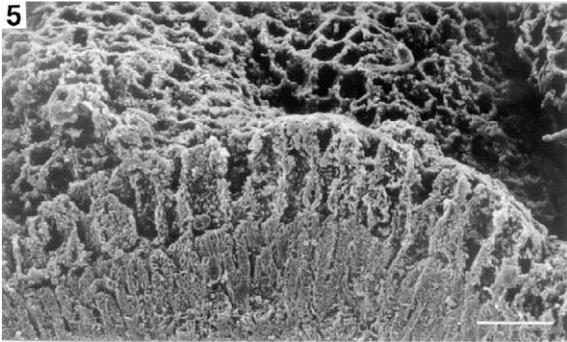


Fig. 5. A scanning electron micrograph of a vertical section through the wall of the cardiac gland area of an adult female babirusa (studbook # 8). The glandular epithelium underlies the ‘honeycomb’ layer. Note the tubular appearance of the ‘honeycomb cells’ that extend about 0.20–0.25 mm above the level of the glandular epithelium. Note also that the walls taper towards the lumen. The surface of the ‘honeycomb cells’ has a granular appearance. Scale bar = 0.2 mm.

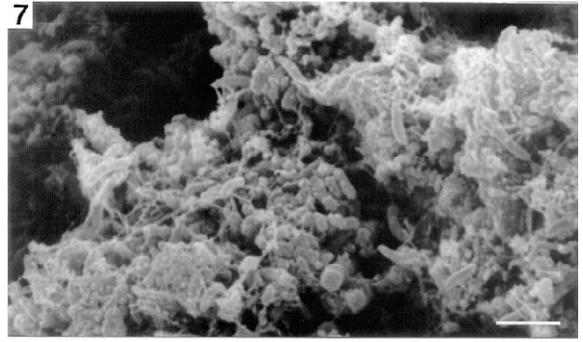


Fig. 7. A scanning electron micrograph of the surface rim of three adjacent ‘honeycomb cells’ of the cardiac gland area of an adult male babirusa (studbook # 49). Note the mixed population of rods and cocci that causes the granular appearance of the walls. Note also the network of extracellular material that seems to provide support to these colonies of bacteria. Scale bar = 5.0  $\mu\text{m}$ .

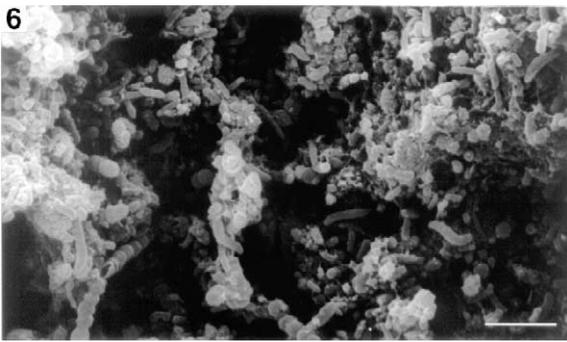


Fig. 6. A scanning electron micrograph looking down towards the bases of several ‘honeycomb cells’ of the cardiac gland area of an adult male babirusa (studbook # 47). Note the mixed population of rods and cocci that causes the granular appearance of the walls. Scale bar = 5.0  $\mu\text{m}$ .

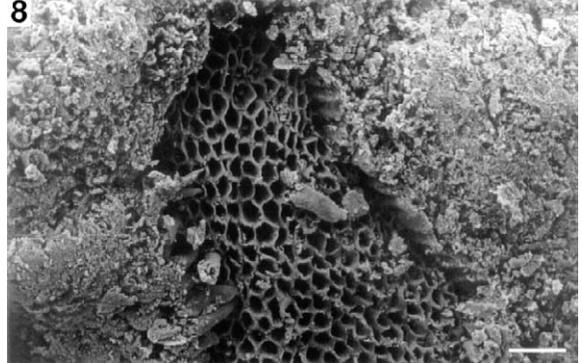


Fig. 8. A scanning electron micrograph of a small area of the cardiac glands of an adult female babirusa (studbook # 48) where the ‘honeycomb’ layer and the underlying epithelium have been removed. A smooth-walled ‘honeycomb’ pattern can be seen. This comprises the connective tissue support for the glandular epithelium. Scale bar = 0.2 mm.

In small areas where the ‘honeycomb’ layer and the underlying epithelium of the cardiac glands had been removed, a different, smooth-walled, ‘honeycomb’ pattern was evident (Fig. 8). This comprised the connective tissue support for the glandular epithelium.

### 3.2. Light microscopy

At the luminal border of the cardiac gland epithelium, on top of the ridge between each glandular pit, non-glandular cellular tissue was seen to extend, ribbon-like, into the lumen (Fig. 9). Sheets of squa-

mous epithelial-like cells formed thin, tube-like structures that effectively extended the lumen of the glandular pit into the lumen of the stomach. As many as ten to twenty cells, stacked one on top of another, comprised the sheet arising from the ridges of the glandular pits. Although bacteria were detected along the length of these tube-like structures, a dense layer of mixed Gram-negative and Gram-positive bacteria covered the surfaces of the luminal third.

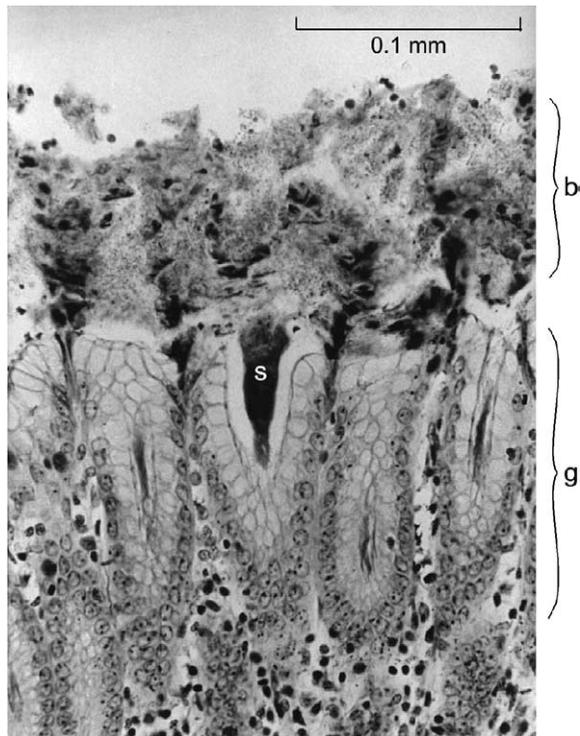


Fig. 9. Histological section of the cardiac glands of an adult male babirusa (studbook # 47) stained with phosphotungstic acid haematoxylin (PTAH). Note the darkly stained, thin, cellular walls of the 'honeycomb' structure, positioned on top of the luminal border of the cardiac gland epithelium of the stomach. Also note the dense population of bacteria associated with the 'honeycomb' layer. **b** = honeycomb layer with bacteria; **g** = tunica mucosa of the cardiac gland region; **s** = glandular secretion. Scale bar = 0.1 mm.

### 3.3. Microbiology

Aerobic culture on blood agar of tissue scrapings from the stratified squamous region yielded domed colonies of large Gram-negative bacilli. Some small colonies of small Gram-positive cocci were also present. Aerobic culture of similar scrapings on MacConkey's agar yielded a few very small, pale, colonies of Gram-positive cocci, and much larger numbers of lactose fermenting Gram-negative bacilli.

Samples from the cardiac gland region that were cultured aerobically with blood agar formed dome-shaped colonies of Gram positive bacilli as well as grey dome-shaped colonies of Gram negative bacilli. Numerous small colonies of Gram-positive cocci were also formed. Culture of similar scrapings on Mac-

Conkey's agar yielded full and partial fermentation associated mainly with Gram-negative bacilli, although some Gram-positive bacilli were also found.

Tissue scrapings from the stratified squamous region yielded domed colonies of Gram-negative yeasts following anaerobic culture with blood agar. These were associated with Gram-negative bacilli and a few Gram-positive bacilli. The latter were also found associated with small colonies as well as colonies showing a feathered outline (stratovolcano). Filamentous Gram-positive bacilli were found in the stratovolcanic shaped colonies. Some haemolytic colonies were observed and these were associated with numerous Gram-negative bacilli as well as chains of Gram-positive cocci and Gram-positive bacilli.

Samples from the cardiac gland region cultured anaerobically with blood agar formed moist domed colonies of Gram positive, granular stained bacilli. Haemolysis was noted with some moist domed colonies and these comprised Gram-negative bacilli of various sizes, some with spores. A sporing bacillus that stained Gram negatively was isolated (and was likely to be *Clostridium piliformi*, a gut flora). Other domed colonies comprised short, broad shaped Gram-positive bacilli, Gram-positive bacilli with a swollen central segment, and Gram-negative bacilli. Florets of small colonies containing Gram-negative bacilli were also present, as were numerous small colonies containing short thin Gram-positive bacilli. Also present in these colonies were other microorganisms that, although staining Gram negatively, exhibited yeast-like morphology. Some small colonies contained Gram-positive bacilli, which appeared to be segmented and others seemed to be formed from individual and short chains of cocci. Still others demonstrated bipolar staining.

Identification of the bacteria is incomplete and of a preliminary nature. The aerobic test results indicated the presence of *Escherichia coli* and *Enterobacter cloacae* in both the squamous and cardiac gland regions. There was also evidence of *Citrobacter freundii* on the squamous epithelium. The anaerobic test results demonstrated the presence of *Lactobacillus acidophilus* together with *Streptococcus* and *Clostridium* spp. on the squamous region. The cardiac gland region had evidence of *Bifidobacterium* spp. together with *Lactobacillus fermentum* and *Streptococcus* spp.

Other bacteria were present but could not be identified with the methods available to us.

#### 4. Discussion

The cardiac glands of the domestic pig were first recognised and described by Greenwood [14] who also reported the presence of mucous substances in the cells of these glands. Further details of the histological characteristics of these mucous glands were subsequently published by Ellenberger and Hofmeister [15], Edelmann [16], Bensley [17–19], Haane [20], Barthol [21], Trautmann [22], and Schulz [23]. In the first histological study of the babirusa stomach, the cardiac glands were found to be short, tubular, branched and coiled at their bases, like those of the domestic pig [3]. However, no histological study to date has drawn attention to anything like the ‘honeycomb’ structure so evident on the scanning electron micrographs of the present report. No evidence of a similar structure could be found in the text or illustrations of any of the detailed histological studies of the domestic pig mentioned above. Freshly collected samples of the cardiac gland area of the domestic pig, processed in the same way as the babirusa samples failed to reveal the presence of a ‘honeycomb’ layer [24]. There is as yet no histological evidence of a comparable structure in the stomach of any wild pig species [5,25].

A significant area of cardiac or other mucogenic glands can also be found in those regions of the stomach where fermentation takes place in families such as the peccaries (Tayassuidae), sloths (Bradypodidae and Megalonychidae) and the camels and their relatives (Camelidae) [9,26]. 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 the macropod marsupials (kangaroos and wallabies) [9,26–29]. A honeycomb layer overlying the surface epithelium of the cardiac gland region of the stomach was not found in the White-lipped peccary, *Tayassu pecari*, or the Bennett’s Wallaby, *Macropus rufrogriseus* [24]. Further research is needed to investigate its absence or presence in the stomachs of additional examples of the macropod marsupials and the colobine monkeys.

The cells that comprise the structure of the ‘honeycomb’ have the general appearance of squamous epithelia. The quality of the fixation was sufficient for histology and scanning electron microscopy, but did not permit a careful examination of the ultrastructure of these cells and the underlying structures. The important relationships with the adjacent cells of the cardiac mucosa remain to be studied following the harvesting of material specifically fixed for transmission electron microscopy.

Cardiac glands are mucogenic glands. Modern histochemical techniques have provided information on the different types of mucosubstances produced by the cardiac glands of the domestic pig [23], and several other mammals [30–33], including the babirusa [3]. In the babirusa, the cardiac gland cells proper produced neutral glycoproteins whereas the epithelial cells lining the gastric pits of the cardiac glands produced neutral glycoproteins together with sulphated and non-sulphated acid glycoproteins [3]. One would therefore expect the walls of the ‘honeycomb’, which extend from the tops of the ridges between the glandular pits, to be coated in a mixture of the different glycoproteins, whereas the neutral glycoproteins would extrude through the centres of the ‘honeycomb cells’ into the lumen of the stomach.

Two important roles have been ascribed to mucus produced in the mammalian stomach: protection against physical and chemical abrasion and the provision of a suitable microenvironment or biofilm for an autochthonous bacterial population living on the luminal surface of the stomach, and/or on the surface of ingested food particles [34,35]. The present, and earlier studies have confirmed the presence of very large numbers of bacteria in the cardiac gland area of the babirusa stomach. Results of digestibility studies on the babirusa have also strongly suggested that microbial fermentation in the stomach is an important step in the digestive process in this species [5,36]. In order for the fermenting microorganisms to survive and multiply, careful control of the pH in that part of the stomach where fermentation takes place is essential. The cardiac gland mucus, perhaps together with saliva, may perform an important buffering role with respect to volatile fatty acids produced by bacterial fermentation. In one case where it could be measured the pH in the cardiac gland area of the babirusa stomach ranged from 6.4 in the diverticulum ventriculi to 5.3

in the corpus ventriculi [3]. This falls within the range of 5 to 7 found in the forestomachs of colobine monkeys [37], and the range of 5 to 8 deemed suitable for the growth of most fermenting bacteria [38]. Different bacteria do have different pH optimums, which affects their ability to colonise different surfaces within the stomach [38]. Further studies are needed to investigate the range and control of pH values within the honeycomb layer, along the surface of the layer, at the surface of food particles and within the lumen of the cardiac gland area of the stomach of the babirusa.

A number of other considerations remain as questions that cannot be answered by the present study. The mucous produced by the cardiac glands may provide additional or specific nutrients to the microflora of the 'honeycomb' structure. The 'honeycomb' structure itself may be a unique adaptation for surface enlargement in order to increase attachment space for, and retention time of, bacteria in a stomach of large volume without strong compartmentalisation [3]. The possibility that the honeycomb structure may provide an increase in the surface area for absorption of fermentation products does not seem to be supported by the apparent absence of blood vessels in the walls of the 'honeycomb', but alternative mechanisms may be present.

The identification of the bacteria in the cardiac gland region of the babirusa remains incomplete and of a very preliminary nature. The number and range of types of bacteria seen in the cardiac gland area following scanning electron microscopy (Figs. 6 and 7) was unexpected and quite different to what has been reported for domestic pigs (e.g., [39–42]). It is clear that specialised microbiological studies are necessary to reveal the full spectrum of microorganisms present, the functions they perform and the role that they play in the digestive process of the babirusa. It is also clear that further studies are required to explore the details of the growth, form and functions of the 'honeycomb' structure we have found in the cardiac gland region of the babirusa stomach.

### Acknowledgements

The authors gratefully acknowledge the assistance of the veterinarians and the veterinary staff of the zoos which housed the babirusa used in this study, particularly Dr Francis Vercammen and Dr Walter

De Meurichy of the Royal Zoological Society of Antwerp, Dr Willem Schaftenaar of Rotterdam Zoo, Dr Bernd Matern of Frankfurt Zoo, Dr Wolfram Rietschel of Stuttgart Zoo, and the whole veterinary team of Surabaya Zoo. Mr Colin Warwick provided professional assistance with photography and computer graphics. The study was financially supported by the Development Trust of the University of Edinburgh. The Centre for Research and Conservation of the Royal Zoological Society of Antwerp gratefully acknowledges the structural support of the Flemish Government.

### References

- [1] A.A. Macdonald, The Babirusa, in: W.L.R. Oliver (Ed.), Status survey and conservation action plan for Pigs, Peccaries and Hippos, IUCN, Gland, 1993, pp. 161–170.
- [2] J. Manansang, A.A. Macdonald, D. Siswomartono, P. Miller, U. Seal, Population and Habitat Viability Assessment for the babirusa (*Babyrousa babyrussa*), IUCN/SSC Conservation Breeding Specialist Group, Apple Valley MN, 1996.
- [3] K. Leus, G.P. Goodall, A.A. Macdonald, Anatomy and histology of the babirusa (*Babyrousa babyrussa*) stomach, C. R. Acad. Sci. Paris, Ser. III 322 (1999) 1–12.
- [4] S. Agungpriyono, A.A. Macdonald, K.Y.G. Leus, N. Kitamura, I.K.M. Adnyane, G.P. Goodall, E. Hondo, J. Yamada, Immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the babirusa, *Babyrousa babyrussa* (Suidae), Anat. Histol. Embryol. 29 (2000) 173–178.
- [5] K. Leus, A.A. Macdonald, From babirusa (*Babyrousa babyrussa*) to domestic pig: the nutrition of swine, Proc. Nutr. Soc. 56 (1997) 1001–1012.
- [6] W. Vrolik, Recherches d'anatomie comparée sur le Babyrussa, Nieuwe Verh. K. Ned. Inst. Wet. 1. Klasse, Amsterdam 10 (1843) 207–248.
- [7] D.D. Davis, Notes on the anatomy of the babirusa, Field Mus. Nat. Hist. Chicago (Zool. Ser.) 22 (1940) 363–911.
- [8] P. Langer, Vergleichend-anatomische Untersuchungen am Magen der Artiodactyla (Owen, 1848). I. Teil: Untersuchungen am Magen der Nonruminantia (Suiformes), Gegenbaurs Morphol. Jahrb. 119 (1973) 514–561.
- [9] P. Langer, The mammalian herbivore stomach, comparative anatomy, function and evolution, Gustav Fischer, Stuttgart, New York, 1988.
- [10] L. Plasa, Internationales Zuchtbuch für den Hirscheber 1999 (International studbook for the babirusa 1999) *Babyrousa babyrussa*, Wilhelma Zoologisch-botanischer Garten, Stuttgart, 2001.
- [11] T. Murakami, K. Yamamoto, T. Itoshisha, S. Irino, Modified tannin-osmium conductive staining method for non-conductive S.E.M. specimens, Arch. Histol. Cytol. 40 (1977) 35–40.
- [12] A.L. Cohen, Critical point drying principles and procedures, Scanning Electron Microsc. (2) 1979 (1979) 303–323.

- [13] P. Echlin, Sputter coating techniques for scanning electron microscopy, *Scanning Electron Microsc.* (2) 1975 (1975) 217–224.
- [14] M. Greenwood, Observations of the gastric glands of the pig, *J. Physiol.* 5 (1882) 195–208, vii–ix.
- [15] W. Ellenberger, V. Hofmeister, Der Magensaft und die Histologie der Magenschleimhaut der Schweine, *Arch. Wiss. Prakt. Thierheilk.* 11 (4 Heft) (1885) 249–268.
- [16] R. Edelmann, Vergleichend anatomische und physiologische Untersuchungen über eine besondere Region der Magenschleimhaut (Cardiadrüsenregion) bei den Säugetieren, *Deutsche Ztschr. Thiermed.* 1889–1890 (15) (1889) 165–214.
- [17] R.R. Bensley, The cardiac glands of mammals, *Am. J. Anat.* 2 (1902) 115–156.
- [18] R.R. Bensley, The cardiac glands of the mammalian stomach, *Anat. Rec.* 4 (1910) 375–390.
- [19] R.R. Bensley, The Gastric Glands, in: E.V. Cowdry (Ed.), *Special Cytology*, vol. 1, Section VI, Paul B Hoeber Inc., New York, 1928, pp. 139–167.
- [20] G. Haane, Ueber die Cardiadrüsen und die Cardiadrüsenzonen des Magens der Haussäugetiere, *Arch. f. Anat. u. Physiol.*, Anat. Abt. 1 (1905) 1–32, 463–464.
- [21] A. Barthol, Beiträge zur Anatomie und Histologie der Magenschleimhaut von *Sus scrofa domestica*, Inaug. Diss. Tierärztlichen Hochschule zu Dresden, Buchdruckerei Otto Franke, Dresden, 1914.
- [22] A. Trautmann, Die Funktionszustände der Cardiadrüsenzonen und der Cardiadrüsen im Magen von *Sus scrofa*. Die Beschaffenheit der Cardiadrüsen Schleimhaut im langer bestehenden sog. 'kleinen Magen', *Pflug. Arch. Ges. Physiol.* 211 (1926) 440–453.
- [23] H. Schulz, Zur Kenntnis der Cardiadrüsen im Magen der Säugetiere, *Z. Wiss. Zool.* 166 (1962) 99–133.
- [24] K. Leus, A.A. Macdonald, G. Goodall, S. Mitchell, L. Bauwens, Cardiac glands with a difference – scanning electron microscopy of the cardiac gland region in the stomach of the *Babirusa Babirusa babyrussa*, domestic pig *Sus scrofa domestica*, White-lipped peccary *Tayassu pecari* and Bennett's wallaby *Macropus rufrostratus*, in: A. Fidgett (Ed.), *Zoo Nutrition 2*, Filander Verlag, Furth, 2003, pp. 209–217.
- [25] A.A. Macdonald, Comparative study of the functional soft tissue anatomy in pigs and peccaries, *Bongo* 18 (1991) 273–282.
- [26] C.E. Stevens, I.D. Hume, *Comparative physiology of the vertebrate digestive system*, 2nd ed., Cambridge University Press, Cambridge, 1995.
- [27] H.J. Kuhn, Zur Kenntnis von Bau und Funktion des Magens der Schlankaffen (Colobidae), *Folia Primatol.* 2 (1968) 193–221.
- [28] R.T. Gemmel, W.V. Engelhardt, The structure of the cells lining the stomach of the tamar wallaby (*Macropus eugenii*), *J. Anat.* 123 (1977) 723–733.
- [29] D.J. Chivers, Functional anatomy of the gastrointestinal tract, in: A.G. Davies, J.F. Oates (Eds.), *Colobine Monkeys: their ecology, behaviour and evolution*, Cambridge University Press, Cambridge, 1994, pp. 205–227.
- [30] S.S. Spicer, Diamine methods for differentiating mucosubstances histochemically, *J. Histochem. Cytol.* 13 (1965) 211–234.
- [31] D.G. Sheahan, H.R. Jervis, Comparative histochemistry of gastrointestinal mucosubstances, *Am. J. Anat.* 146 (1976) 103–132.
- [32] T. Katsuyama, S.S. Spicer, Histochemical differentiation of complex carbohydrates with variants of the concanavalin A-horseradish peroxidase method, *J. Histochem. Cytochem.* 26 (1978) 233–250.
- [33] A.S.A.M. Dougbag, R. Berg, Morphological observations on the normal cardiac glands of the camel (*Camelus dromedarius*), *Anat. Anz.* 148 (1980) 258–264.
- [34] A. Allen, Gastrointestinal mucus, in: B.B. Rauner (Ed.), *Salivary, gastric, pancreatic and hepatobiliary secretion, Handbook of physiology. The gastrointestinal system*, vol. III, American Physiological Society, Oxford University Press, New York, 1989, pp. 359–382.
- [35] J.W. Costerton, K.-J. Cheng, G.G. Geesey, T.I. Ladd, J.C. Nickel, M. Dasgupta, T.J. Marrie, Bacterial biofilms in nature and disease, *Annu. Rev. Microbiol.* 41 (1987) 435–464.
- [36] K. Leus, Feeding babirusa (*Babirusa babyrussa*) in captivity, in: J. Nijboer, J.-M. Hatt, W. Kaumanns, A. Beijnen, U. Gansloßer (Eds.), *Zoo Animal Nutrition*, Filander Verlag, Fürth, 2000, pp. 237–250.
- [37] R.N.B. Kay, A.G. Davies, Digestive physiology, in: A.G. Davies, J.F. Oates (Eds.), *Colobine Monkeys: their ecology, behaviour and evolution*, Cambridge University Press, Cambridge, 1994, pp. 229–249.
- [38] W.N. Ewing, D.J.A. Cole, *The Living Gut – an introduction to micro-organisms in nutrition*, Context, Dungannon, N. Ireland, 1994.
- [39] G.W. Tannock, J.M.B. Smith, The microflora of the pig stomach and its possible relationship to ulceration of the pars oesophagea, *J. Comp. Pathol.* 80 (1970) 359–367.
- [40] R. Fuller, P.A. Barrow, B.E. Brooker, Bacteria associated with the gastric epithelium of neonatal pigs, *Appl. Environ. Microbiol.* 35 (1978) 582–591.
- [41] D.J. McGillivray, P.D. Cranwell, Anaerobic microflora associated with the pars oesophagea of the pig, *Res. Vet. Sci.* 53 (1992) 110–115.
- [42] B.B. Jensen, Possible ways of modifying type and amount of products from microbial fermentation in the gut, in: A. Piva, K.E. Bach Knudsen, J.E. Lindberg (Eds.), *Gut environment of Pigs*, Nottingham University Press, Nottingham, UK, 2001, pp. 181–200.