Anatomy and histology of the babirusa (*Babyrusa babyrussa*) stomach

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Abstract – Stomachs from six adult and one 12-month-old babirusa (*Babyrusa babyrussa*) were collected from zoological gardens. The babirusa stomach was larger than that of the domestic pig (*Sus scrofa*), and possessed a large *diverticulum ventriculi*. Its gastric glands were confined to a small, easily identifiable unit at the end of the *corpus ventriculi*, and the connections between the different stomach parts were wide and unrestricted, with the exception of the well-defined connection between the *diverticulum ventriculi* and the *fundus ventriculi*. Microscopically, the structure of the cardiac, gastric and pyloric glands was similar to that of *Sus scrofa*. However, the mucus-producing cardiac glands of babirusa occupied a larger area within the stomach (>70 % versus 33 %). The pH in the lumen of the cardiac gland area lay between 5.3 and 6.4 and micro-organisms were found here. It was hypothesised that the babirusa is a non-ruminant foregut-fermenting frugivore/concentrate selector. © 1999 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

anatomy / babirusa / digestion / foregut fermentation / histology / pig / stomach / stomach glands / Suidae / Mammalia

1. Introduction

The babirusa (*Babyrusa babyrussa*) is a remarkable pig native to the tropical rain forests of Sulawesi, Buru and the Sula and Togian Islands of Indonesia [1]. The species is threatened by illegal hunting and habitat degradation [2] and forms the subject of an international conservation breeding programme [3]. Despite a long history of captive management, the scarcity of data on the natural diet and digestive strategies of the babirusa [4] have caused the food offered to these animals in captivity to be largely the result of trial and error. In addition, popular opinion often still considers all pig species to be similar to *Sus scrofa* with respect to their gastro-intestinal anatomy and nutrition. Taking into account the wide differences in habitat preferences among the Suidae, together with the paucity of studies seriously investigating their digestive anatomy and feeding strategies, this conclusion is considered to be

premature [5]. This may be particularly so for the babirusa with its unusual stomach anatomy.

The anatomy of the babirusa stomach has been studied by four investigators in the last 150 years [6–10]. All other accounts in the literature have been based on these pieces of original research. The results indicated that the anatomy of the babirusa stomach was substantially different from that of the other pigs. Vrolik [6] recognised two clearly defined areas in the stomach; a cardiac part with a well-delineated *appendice en capuchon* and a pyloric part. These were later redefined by Langer [10] as, respectively, the *corpus ventriculi* with the *fundus ventriculi* (and its *diverticulum ventriculi*), and the *pars pylorica*. The apparent complexity of the babirusa stomach suggested to the early workers that it represented a transitional form between the simple stomach of *Sus scrofa* and the composite stomach of the ruminants [6, 8]; Davis [8] even implied that he had recognised all the chambers of the ruminant stomach, except the *omasum*.

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Table 1. Details of the animals from which the stomach was collected, method of fixation employed and the use made of individual stomach specimens.

No.	Sex	Born	Died	Fixative	T.a.d.	Musc	Area	Histol
0004	F	Antwerp 05/04/1975	Antwerp 11/11/1991	10 % formalin	90 min	–	–	+
0012	M	Stuttgart 29/07/1976	Frankfurt 17/09/1992	Klötz	90 min	–	–	+
0015	M	Surabaya ca. 1975	Rotterdam 29/10/1992	10 % formalin	?	+	+	–
0020	M	Stuttgart 04/01/1979	Leipzig 09/01/1993	10 % formalin	12 h	–	+	–
0030	M	Frankfurt 26/02/1981	Leipzig 17/03/1993	10 % formalin	> 12 h	–	–	–
0048	F	Jakarta ca. 1982	Antwerp 02/05/1993	Bouin's	60–90 min	–	–	+
0156	M	Antwerp 18/07/1991	München 21/08/1992	10 % formalin	12 h	+	+	–

The animals are identified by their studbook number according to Plasa [3]. No. = studbook number, F = female, M = male, T.a.d. = time after death when the stomach was collected (min = minutes, h = hours), + = used for that aspect of the study, – = not used for that aspect of the study, Musc = organisation of the muscle layers of the stomach, Area = measurement of the surface area of the stomach, Histol = histology and histochemistry.

The most recent examination of the gross anatomy of the babirusa stomach has been carried out by Langer [9, 10]. Although he too stressed the relatively large size of the *fundus* and *diverticulum ventriculi*, and agreed that the stomach of the babirusa was more complex in shape than that of *Sus scrofa*, the results of his studies led to the opposite conclusion, that a direct correspondence with the stomach of a ruminant was not justified. However, the possible function of the stomach's shape remained largely unexplored and unexplained. In addition, the glandular composition of the mucosal lining of the stomach remained undescribed in detail, possibly because of the age of the museum specimens studied, or the methods used for their conservation.

The increased co-operation and communication between zoological collections in the last 10 years has now allowed the gathering of relatively fresh tissues. This has enabled us to re-examine the gross anatomy of the babirusa stomach and to study in some detail the histology of its mucosal lining.

2. Methods

2.1. Specimens

Stomachs from seven babirusa which had died in zoological gardens in Europe were used for this study. Details of the animals, fixation materials used, and which specimens were used for which studies are contained in table 1. Babirusa are numerically identified according to the international babirusa studbook [3]. With exception of the 1-year-old male (0156), all animals were mature adults. In three cases autopsies were carried out 60–90 min after death. The stomachs collected at that time were opened along the greater curvature, emptied and bathed in fixative in order to obtain the best possible histological samples. The other stomachs were fixed with the contents intact in order to better preserve the natural shape of its structure for gross anatomical observations.

The cause of death in all but one animal was due to causes which were unrelated to the gastro-intestinal system. The exception (0020) had suffered from chronic diarrhoea as a result of gastric mycosis (Eulenberger, pers. comm.) and was used solely for gross anatomy.

2.2. Gross anatomy

The identification of the anatomical parts of the stomach was carried out according to the organisation of the muscle layers within the stomach wall [11, 12]; each part of the stomach is demarcated from its neighbour by a different combination of two of the three muscle layers. The *fundus ventriculi* is built up of the external *stratum longitudinale* (longitudinal muscle layer) and the internal *fibrae obliquae internae* (internal oblique fibres). The *corpus ventriculi* is mainly lined by the *stratum circulare* (circular muscle layer) externally and the internal oblique fibres internally, with some external longitudinal fibres on its lesser and greater curvatures. The *pars pylorica* is made up of the external longitudinal and internal circular muscle layers. The orientation of the internal muscle layers across the stomach was studied after removal of the *tunica mucosa* by dissection. The orientation of the outer layers was often clear following inspection; the serosa was only dissected away in regions where orientation was in doubt.

For measurements of the luminal surface area of the stomach, pieces of paper were fitted against the mucosal surface of the stomach, care being taken to follow the major sacculations and muscular folds. The method did not allow incorporation of the small mucosal folds. The pieces of paper were dried to constant weight in an oven at 45 °C, together with six papers with known surface areas. The surface areas of the mucosal regions were then calculated from the weight of the papers compared to the weights of the papers with known surface areas. Estimates of the surface area of the unopened diverticulum were derived from measurements of the extraluminal surface.

2.3. Histology

Most samples from stomach 0004 were taken from the fresh stomach and fixed in Bouin's fluid, some additional samples were later taken from the formalin (10 %)-fixed stomach. Samples of stomach 0012 were taken after the stomach had been stored in Klötz fluid for 1 day and were then placed in Bouin's fluid. All samples fixed in Bouin's fluid were kept in the fluid for 24 h only, after which they were stored in 70 % alcohol. Paraffin sections of 6 µm in thickness were stained with Ehrlich's haematoxylin and

eosin (H&E) and a range of histochemical methods were used to differentiate the glycoproteins present. The periodic acid-Schiff (PAS) technique stained neutral glycoproteins alone (deep red colour) [13, 14]. Alcian blue at pH 2.5 (AB) indicated with a blue colour the presence of sulphated and non-sulphated acid glycoproteins [13, 14]. The combination of the AB and PAS techniques (AB/PAS) stained neutral glycoproteins red, acid glycoproteins blue and a mixture of the two types was stained purple to dark blue in colour [13, 14]. The high iron diamine/Alcian blue at pH 2.5 method (HID/AB) allowed differentiation of sulphated and non-sulphated acid glycoproteins. The former stained grey–purple–black and the latter coloured blue [14, 15]. Mast cells were also coloured blue with the AB stain and stained grey–purple–black with the HID stain because of the presence in the cells of heparin, a sulphated and carboxylated mucosubstance [14].

The presence of micro-organisms within the mucus layer covering the stomach surface was investigated by the application of Gram's stain [13] to microscopic sections from each of the different regions of the stomach. On one occasion (babirusa 0004) the pH in the stomach lumen was measured with a digital pH meter 90 min after death (and 3 h after feeding).

3. Results

3.1. Gross anatomy

The stomach of the adult babirusa had an elongated shape and a bulky appearance (figure 1). To the left of the oesophagus a blindsac was found which itself possessed a diverticulum at its extremity. The central part of the stomach was long in shape, and on the right of the abdomen formed an approximately 90° bend cranially. The terminal part of the stomach was relatively short, funnel-shaped, and gave rise to the duodenum.

3.1.1. Muscular anatomy

The outer smooth muscle coat of the stomach was composed of either the *stratum longitudinale* or the *stratum circulare* (figure 1a, b). The former completely covered the blindsac of the stomach. However, this muscular layer was then reduced to a narrow band along the lesser and greater curvatures of the central portion of the stomach. Thereafter, towards the outflow from the stomach, these narrow bands of muscle fanned out again over this funnel-shaped stomach region. On the ventral surface, a plaque of connective tissue fibres was found at the lesser curvature between the central area of the stomach and the funnel-shaped part. An additional band of longitudinal muscle fibres was found anchored to this plaque. Thus, the *stratum longitudinale* completely covered the funnel-shaped part of the stomach.

Most of the outer muscle coat of the central stomach region was composed of circularly orientated smooth muscle fibres (figure 1a, b). To the right of the oesophagus, these could be identified as the *stratum circulare*. On the

Figure 1. Internal and external muscle layers of the tunica muscularis of the babirusa stomach. O = oesophagus, P = pylorus, — stratum longitudinale, - - - stratum circulare, fibrae obliquae internae, - - - singular muscle layer with circular orientation, → incisura angularis, ⇨ incisura cardiaca.

lesser curvature, at the border between the central stomach part and the terminal funnel-shaped part, an external notch could be found, the *incisura angularis*. To the left of the oesophagus only a single muscle layer seemed to comprise the stomach wall. The identity of this muscle layer could not be determined with certainty; the alignment of the fibres suggested that they could belong to either the *stratum circulare*, or to the *fibrae obliquae internae* if it were running in a circular fashion.

The inner muscle coat of the stomach (figure 1c, d) was composed of either the *fibrae obliquae internae* or the *stratum circulare*. The *fibrae obliquae internae* comprised the inner muscle coat of the blindsac, its diverticulum and the central stomach region. This muscle layer was involved in the formation of a fold of tissue which started at the entrance of the blindsac and spiralled into it. The same layer of muscle also formed a spiral fold at the entrance to the diverticulum.

Most of the internal smooth muscle fibres covering the central stomach region originated from a small area just to the left of the oesophagus, thereby forming a notch on the lesser curvature, the *incisura cardiaca* (figure 1c, d). The muscle tissue of the *fibrae obliquae internae* lying closest to the lesser curvature was strongly developed and formed a hoop around the oesophageal opening, the arc of which lay in the *incisura cardiaca*. The 'legs' of this hoop of smooth muscle were thickened. They comprised the foundations of two mucosal folds which formed a groove running along the lesser curvature of the stomach towards the *pars pylorica*, the gastric groove. The muscular tissue underlying the mucosal floor of this groove was formed by the *stratum circulare* of the central stomach region.

The rest of the *fibrae obliquae internae* ran almost parallel to the lesser curvature, before fanning out over the stomach and taking on an almost circular orientation in the central stomach region (figure 1c, d). Thickened fibres of the *fibrae obliquae internae* formed three taenia-like

Table II. Surface area measurements of the gross anatomical parts of the babirusa stomach.

Animal	<i>Fundus ventriculi</i>		<i>Diverticulum ventriculi</i>		<i>Corpus ventriculi</i>		<i>Pars pylorica</i>		Total	
	(cm ²)	(%)	(cm ²)	(%)	(cm ²)	(%)	(cm ²)	(%)	(cm ²)	(%)
0015	940	32	208	7	1 642	55	192	6	2 982	100
0020	1 139	36	231	7	1 576	49	256	8	3 202	100
0156	529	36	205	14	608	42	113	8	1 455	100

The animals are identified by their studbook number according to Plasa [3].

folds separating relatively shallow haustra-like sacculations in this region. Towards the right side of the stomach the *fibrae oblique internae* split into two main bands of fibres running to opposite ends of that part of the stomach forming the sharp bend towards the funnel-shaped end. Only a few thin fibres covered the rest of the wall of this bend. The inner muscle coat of the funnel-shaped region of the stomach was formed by the *stratum circulare*.

3.1.2. Main stomach parts

The main parts of the stomach could be identified from the paths taken by the different muscle layers (see Materials and methods, figure 2a, b). The blindsac represented the *fundus ventriculi* with its *diverticulum ventriculi*; it bordered the central part of the stomach, the *corpus ventriculi*. The presence of only a single circularly orientated muscle layer in the border region between the *corpus* and *fundus ventriculi* might have complicated the positioning of this border. However, two further observations helped to locate it; the outer *stratum longitudinale*, which in the *corpus ventriculi* was only present as a narrow strip along the greater curvature, widened and passed onto the walls of the *fundus* and *diverticulum ventriculi* to form the outer muscle layer; and secondly, the internal muscular spiral fold at the entrance of the *fundus ventriculi* started at the same position. The central part of the stomach, the *corpus ventriculi*, bordered on the terminal part of the stomach, the *pars pylorica*, at the place where the *fibrae obliquae internae* of the *corpus ventriculi* stopped. This corresponded to the place where the narrow bands of the *stratum longitudinale* widened to form the external muscle coat of the *pars pylorica*. On the lesser curvature, to the right of the oesophagus, an external constriction was found, the *incisura angularis*, which coincided with the border between the *corpus ventriculi* and the *pars pylorica* on the lesser curvature (figure 1a, b).

The establishment of objective borders between the different parts of the stomach enabled measurements to be made of the mucosal surface areas represented by each of these parts (table II). The adult babirusa stomach had a total luminal surface area of around 3 000 cm². The total mucosal surface area of the 1-year old animal (0156) was about half the size of those of the adult animals. The relative proportions of the stomach surface area represented by the different stomach parts did not seem to be markedly different in the younger animal, with the exception of the proportionately larger size of the *diverticulum ventriculi*. In all animals the *corpus ventriculi* formed the

largest part of the stomach closely followed by the *fundus ventriculi*. The mucosal surface area of the *pars pylorica* represented less than 10 % of the stomach's total area.

On both the parietal and the visceral sides, the *fibrae oblique internae* extended a long way towards the *pars pylorica* and thereby included the bend of the *corpus ventriculi* (figure 1c, d). This latter region was observed to form a clearly identifiable entity within the *corpus ventriculi*. Its structure was 'wedge shaped'. The narrow edge of the wedge pointed towards the *incisura angularis* on the lesser curvature and the wide edge of the wedge was found on the greater curvature where the stomach bent towards the *pars pylorica* (figure 3a, b). The region was characterised by a smoothly stretched outer surface, a non-sacculated convex border with the greater curvature and a plentiful supply of blood vessels running from the greater curvature towards the lesser curvature in the direction of the point of the wedge. The connective tissue insertion of the extra band of longitudinal muscle fibres was clearly visible, on its ventral surface, towards the lesser curvature (figure 3a). According to the degree of filling of the stomach the impression was sometimes created of two external constrictions on the greater curvature separating this region from the remaining *corpus ventriculi*

Figure 2. Gross anatomical stomach parts and areas with different mucosal lining within the babirusa stomach. O = oesophagus, P = pylorus,
 ■ *corpus ventriculi*, ■ stratified squamous epithelium,
 ■ *fundus ventriculi*, ■ cardiac glands,
 ■ *diverticulum ventriculi*, ■ gastric glands,
 ■ *pars pylorica*, ■ pyloric glands.

Figure 3. Detail of the terminal part of the babirusa stomach (drawn from the stomach of the 1-year-old animal – studbook number 0156). **a.** External view of the ventral wall. **b.** The ventral wall has been removed from the *pars pylorica* and the gastric unit to demonstrate the internal surface differentiations of the dorsal wall. A: *pars pylorica*, B: gastric unit = the wedge-shaped entity representing the distal part of the *corpus ventriculi*, C: remainder of the *corpus ventriculi*. 1: blood vessels, 2: connective tissue anchor point of a band of external longitudinal muscle fibres covering the *pars pylorica*, 3: taenia-like folds in the *corpus ventriculi*, 4: haustra-like succulations of the *corpus ventriculi*, 5: mucosal fold separating the gastric unit from the remainder of the *corpus ventriculi*, 6: *incisura angularis*, 7: shallow mucosal folds at the transition of the gastric unit into the *pars pylorica*, 8: *torus pyloricus*.

Tunica mucosa,
Tunica muscularis.

on the left side and the *pars pylorica* on the right side. However, there were no internal muscular folds corresponding to these apparent external constrictions. The rest of the *corpus ventriculi* had a very thin and much folded wall with haustra-like succulations. The *tunica muscularis* of the *pars pylorica* was much thicker than that of the wedge-shaped structure (figure 3b).

3.1.3. Internal stomach lining

A small area of non-glandular epithelium was found at the lesser curvature (figure 2c, d). This surrounded the oesophagus and stretched out mainly in the direction of the *pars pylorica*. It covered the two folds and the floor of the gastric groove and ended before the *incisura angularis*. It also extended for a short distance into the *fundus ventriculi*.

The *diverticulum ventriculi*, *fundus ventriculi* and the largest part of the *corpus ventriculi*, up to the 'wedge-shaped' structure, were covered by the *glandulae cardia-*

cae or cardiac glands. The luminal surface of this region was dark grey-brown in colour in freshly dead material. The *glandulae gastricae* or gastric glands were confined to the 'wedge-shaped' structure at the end of the *corpus ventriculi*. This structure was defined as the 'gastric unit'. The terms gastric pouch or gastric chamber were avoided because of the wide internal connections between the gastric unit and the remainder of the *corpus ventriculi*. The surface of the gastric gland region was greyish-pink in colour in freshly dead specimens and was characterised by a very thick *tunica mucosa* with well-developed gastric fields and grooves. The internal border between the gastric unit and the remainder of the *corpus ventriculi* was formed by a fold composed of *tunica mucosa* and *tela submucosa* (figure 3b). The fold was very prominent on the dorsal wall of the stomach but became less clear as it ran from the greater to the lesser curvature on the ventral wall. The side of the fold bordering the remainder of the *corpus ventriculi* had a thin *tunica mucosa* which thickened as it reached the top of the fold and ran over onto the side of the fold bordering the gastric unit (figure 3b).

The thickness of the *tunica mucosa* of the gastric unit decreased as it reached the border with the *pars pylorica*, whereas the thickness of the *tunica muscularis* increased (figure 3b). Some shallow mucosal folds could be seen at the transition of the gastric unit into the *pars pylorica*. The *pars pylorica* was lined with *glandulae pyloricae* or pyloric glands and its luminal surface was smooth and beige in colour in freshly dead specimens. The border between the non-glandular area (white-yellow in colour in freshly dead specimens) and the different glandular areas was abrupt to the naked eye. However, no similarly clear demarcation lines could be seen between the various types of glandular areas which seemed to gradually run into one another.

The surface areas represented by each of the different gland types in three babirusa stomachs are shown in table III. The cardiac gland area represented the largest proportion of the mucosal surface area (> 70 %), leaving only relatively small regions occupied by gastric and pyloric glands, and stratified squamous epithelium. The proportions of the total stomach surface represented by the different gland types and the non-glandular epithelium in the 1-year-old animal (0156) were very similar to those in the two adult animals.

Table III. Surface area measurements of the different types of mucosal lining within the babirusa stomach.

Animal	Squamous epithelium		Cardiac glands		Gastric glands		Pyloric glands		Total	
	(cm ²)	(%)	(cm ²)	(%)	(cm ²)	(%)	(cm ²)	(%)	(cm ²)	(%)
0015	129	4	2 438	82	223	8	192	6	2 982	100
0020	112	4	2 286	71	548	17	256	8	3 202	100
0156	86	6	1 077	74	179	12	113	8	1 455	100

The animals are identified by their studbook number according to Plasa [3].

Figure 4. Histological section of the *tunica mucosa* of the cardiac gland area of the babirusa stomach. a. Alcian blue/periodic acid-Schiff (AB/PAS) staining: the apical regions of the gastric pit epithelial cells change in colour from predominantly PAS-red (indicating the presence of neutral glycoproteins) to PAS-violet, PAS-purple and eventually PAS-dark blue (indicating the presence of both neutral and acid glycoproteins) when progressing from the base to the surface of the gastric pits. The cardiac gland cells proper show only a PAS positive reaction in their apex. Scale bar = 25 μ m. b. High iron diamine/Alcian blue (HID/AB) staining: the layer of material covering the luminal surface of the stomach and the apical region of the epithelial cells of the lumen and gastric pits are stained grey-purple (indicating the presence of both sulphated and non-sulphated acid glycoproteins). In the bottom of the gastric pit blue staining (non-sulphated acid glycoproteins) predominated. The cardiac gland cells remain completely unstained by HID/AB. Scale bar = 25 μ m.

3.2. Histology

The *tunica serosa* was composed of a mesothelium lining and a thin layer of connective tissue. The *tunica muscularis* was mostly composed of two layers, usually a thinner outer layer and a thicker inner layer. A clear outer longitudinal and inner circular layer could not always be observed in the *fundus* and *corpus ventriculi* of the babirusa stomach. However, clearly arranged outer longitudinal and inner circular muscle layers were present in the very thick *tunica muscularis* of the *pars pylorica*. The *tunica submucosa* contained predominantly connective tissue, blood vessels, adipose cells and small ganglia of the *plexus submucosus*. The *tunica mucosa* of the three glandular regions is discussed in more detail below.

3.2.1. Cardiac glands

The *tunica mucosa* of the cardiac gland region was thin. The gastric pits regularly occupied 1/3 to 1/2 of its depth

(figure 4a, b). The density and degree of branching and coiling of the glands seemed to be somewhat variable, but in general the glands were tubular, branched and coiled at their bases. At regular intervals, several gastric pits were found emptying into a single depression or gastric groove on the surface of the mucosa. When stained with H&E, a dark purple layer of material was sometimes seen to cover the luminal surface of the cardiac gland region.

The epithelial cells lining the gastric pits reacted positively to the different stains for glycoproteins. When stained with PAS, the epithelial cells towards the bottom of the gastric pits showed a small PAS positive area in their cell apex. This stained area became larger towards the top of the gastric pits, and filled the entire apical region of the cells on the luminal border. A similar staining pattern in blue colour occurred when using the AB method. With AB/PAS the coloured region in the apex of the cells appeared larger. It also changed in colour from predomi-

nantly PAS-red to violet, purple and eventually dark blue when progressing from the base to the surface of the gastric pits (figure 4a). In those gastric pits with a wide lumen, an intensely PAS-red coloured substance could sometimes be observed in the centre of the lumen, contrasting sharply with the dark purple colour of the apex of the epithelial cells lining both the higher regions of the gastric pit and the stomach lumen. HID/AB caused the layer of material covering the luminal surface of the stomach and the apical region of the epithelial cells to be stained grey-purple with a somewhat AB-blue haze (figure 4b). Travelling from the bottom to the surface of the gastric pit, the stained area became larger, less blue and more grey.

The cardiac gland cells proper showed a PAS positive reaction in their apex, although the staining was much weaker than that in the epithelial cells of the gastric pit and luminal surface. With AB/PAS, only a PAS positive reaction was present in these cells (figure 4a). The cardiac gland cells remained completely unstained by AB or HID/AB (figure 4b). The *lamina propria mucosae*, surrounding the cardiac glands, contained many lymphocytes. Mast cells were numerous in the inner region of the *lamina propria mucosae* surrounding the bases of the cardiac glands.

3.2.2. Gastric glands

The *lamina propria mucosa* in this region was markedly thickened. The gastric pits were short, extending about 1/5 of the depth of the *tunica mucosae*. The glands themselves were densely packed, long straight tubular glands. They were rarely branched and only showed coiling at their bases (figure 5). Gastric grooves were formed in the same way as in the cardiac gland region.

The surface and gastric pit epithelial cells reacted strongly PAS positive in their supranuclear region. This PAS positive staining remained prominent throughout the gastric pit. When stained with AB only a few epithelial cells of the gastric pit and luminal surface reacted positively. With AB/PAS, the majority of the surface and gastric pit epithelium cells stained PAS positive only, and just a few of the cells stained dark purple-blue in colour. HID/AB caused a few cells of the surface and gastric pit epithelium to be stained blue and an even smaller number of cells to be stained purple-grey.

The parietal cells were most numerous in the neck region of the glands and their numbers gradually decreased towards the base of the gland. Conversely, the number of chief cells increased towards the base of the glands. When stained with PAS, cells with a PAS positive supranuclear cytoplasm were present at several places along the length of the gland, surrounded by unstained chief and parietal cells. In some places these PAS positive cells occupied almost the entire base of the gland. None of the cell types within the glands, except for the mast cells in the connective tissue, reacted AB positive. With AB/PAS, the reaction was as with PAS, except for the blue staining

Figure 5. Histological section of the thick *tunica mucosa* of the gastric gland area of the babirusa stomach. H&E staining. The gastric pits are short. The long straight tubular glands are densely packed. The parietal cells are most numerous in the neck region of the glands and their numbers gradually decrease towards the base of the gland. Conversely, the number of chief cells increase towards the base of the glands. Scale bar = 100 μ m.

of the mast cells in the connective tissue. HID/AB caused only the mast cells to be stained dark purple-grey.

Because of the thick stacking of the gastric glands only small amounts of *lamina propria mucosae* connective tissue were found running up between the individual glands. Somewhat thicker strands of connective tissue could be observed clumping several individual glands together. Lymphocytes were few in number. Mast cells were again most numerous in the inner region of the *lamina propria mucosae* surrounding the bases of the glands.

3.2.3. Pyloric glands

The *tunica mucosa* of the pyloric region was thinner than that of the gastric gland region but thicker than that of the cardiac gland region. The gastric pits were deep. The pyloric glands themselves were short, tubular, branched

gastric unit in that region. The *tunica mucosa* became thicker and more and more parietal and chief cells appeared as the fold was traversed from the cardiac gland to the gastric gland side. The gastric glands did not reach as far as the lesser curvature but were separated from it by cardiac glands. The transition from gastric to pyloric glands was marked by an increase in the thickness of the *tunica muscularis*, a thinning of the *tunica mucosa*, an increase in the depth of the gastric pits, and a reduction in the number of parietal and chief cells until the glands were composed of pyloric gland cells only. A transition from glands with predominantly cardiac gland characteristics to pyloric glands was found at the point where the non-glandular epithelium extends furthest towards the *incisura angularis* on the lesser curvature.

3.2.5. Gram-positive micro-organisms

Stomach samples from animal 0048 which had been rapidly preserved in Bouin's fixative retained fragments of the surface mucus gel. These contained a dense population of Gram-positive bacteria in the cardiac and gastric gland areas. Much smaller numbers seemed to be present in the area of the pyloric glands. Only a few micro-organisms could be seen on the surface of the stratified squamous epithelium. Gastric fluids collected from the stomach of babirusa 0012 confirmed the presence of numerous Gram-positive bacteria in the lumen of the *fundus ventriculi* and proximal *corpus ventriculi*. A smaller number of micro-organisms was found in the distal part of the *corpus ventriculi* and in the *pars pylorica*. No protozoa were observed. The results from the pH measurements in animal 0004 were as follows: *diverticulum ventriculi* pH = 6.4, *fundus ventriculi* pH = 5.6, *corpus ventriculi* pH = 5.3, gastric unit pH = 3.7, *pars pylorica* pH = 4.4, duodenum pH = 7.2.

4. Discussion

On a gross anatomical level, the main feature that distinguishes the babirusa stomach from that of other Suidae is the large surface area of the stomach covered with cardiac glands (> 70 %). In *Sus scrofa* the cardiac glands occupy approximately 33 % of the total glandular stomach area, the gastric glands 33 % or somewhat more and the pyloric glands 33 % or somewhat less [10, 16–19]. The area covered by stratified squamous epithelium in the babirusa was small, and was similar to that in the domestic pig (6 %) [10]. Recent reviews of the available information on the stomach anatomy of wild pigs other than *Sus scrofa* indicated that they were very similar in their anatomy to that of the domestic pig [5, 20]. No comparable surface area measurements for *fundus ventriculi*, *diverticulum ventriculi* and total cardiac gland area could be found, but descriptions and drawings of the stomachs of the other wild pigs suggested that the babirusa is the only member of the Suidae showing enlargements of these structures. The spatial distribution of the different

gland types in the babirusa stomach, as described in the current study, corresponded largely to the drawings published by Langer [9]. However, a combination of the diagrams illustrating the gross anatomical stomach parts (figure 70 in Langer [10]) and those showing the distribution of the different gland types over the inner surface of the stomach (figure 8 in Langer [9]) suggested that the gastric glands are completely incorporated within the *pars pylorica*. The results of the present study indicated that this was not the case; the gastric glands were confined to the gastric unit and were therefore part of the *corpus ventriculi* (figure 2).

The histological evidence indicated that the unique aspect of the babirusa stomach lies more with the different proportions occupied by the different gland types than with the structure of the individual glands or the substances they produce. A variety of studies have shown that the main sites of mucus production in the mammalian stomach are: the surface and gastric pit epithelial cells, the cardiac glands, pyloric glands and the mucous neck cells of the gastric glands [15, 21, 22]. The different staining methods for mucosubstances employed in the present study indicated, for the first time, the pattern of occurrence of mucosubstances in the babirusa stomach. Both the microscopic structure of the stomach glands and the cellular distribution of the different types of mucosubstances within these glands appeared to be similar to those found in the domestic pig [16, 23–28]. Greenwood [16], Bensley [23, 24] and Ito [28] mentioned the presence of mucous neck cells throughout the entire length of the gastric glands of *Sus scrofa* to the extent that some glands were only composed of mucous neck cells and parietal cells to the exclusion of chief cells. We observed PAS positive cells throughout the entire length of the babirusa gastric glands. These may also represent mucous neck cells.

The presence of a large area of mucus-producing cardiac glands in the stomach of the babirusa (table III) raises questions about the function(s) of this large amount of mucus with respect to the digestive mechanisms of this species. Two important roles, often ascribed to the mucus present in the mammalian stomach, are those of protection of the mucosal lining, and the creation and maintenance of an environment suitable for the survival of micro-organisms [29, 30]. Protection against the endogenous secretions of acid and pepsin by a mucus gel adhering to the surface of the mucosal lining is likely to be of special importance in those regions of the babirusa stomach which are in direct contact with acid and pepsin activity, i.e. the gastric unit, the *pars pylorica* and that area of the *corpus ventriculi* which borders on the gastric unit. However, an adherent mucus gel may also form the equivalent of a biofilm with an autochthonous bacterial population on the luminal surface of the digestive organ or on the surface of ingested food particles [29–31]. The present study confirmed the presence of Gram-positive bacteria in both the mucus gel adhering to the stomach surface and the luminal mucus of the babirusa stomach.

The most frequently discussed function of micro-organisms inhabiting the surface mucus gel and/or lumen mucus of the stomach is bacterial fermentation of plant structural polysaccharides by means of enzymes which the host is unable to produce itself [30]. In order for the fermenting micro-organisms to survive and multiply, a careful control of the luminal stomach pH is essential. Stomach mucus is considered to play an important role in this control by performing a buffering function, not only against acid produced by the gastric glands, but also against the acidity resulting from the presence of volatile fatty acids (VFAs), the main products of fermentation [32–35]. In the present study, the highest acidity occurred in the gastric unit and the *pars pylorica*, whereas the pH in the cardiac gland region ranged from 5.3 to 6.4. These values fall within the range of 5 to 7 found in other animals with significant forestomach fermentation [34, 36–38]. All of the above observations are in line with the hypothesis that bacterial fermentation takes place in the cardiac gland area of the babirusa stomach.

The presence of cardiac glands in forestomach fermentation chambers is not a feature unique to the babirusa. It also occurs in Bradypodidae and Megalonychidae (tree sloths) [10, 39], Tayassuidae (peccaries) [9, 10] and Camelidae (camels and llamas) [35, 40]. However, only two groups of animals appear to share with the babirusa the possession of an enlarged and elongated stomach part lined almost exclusively with cardiac glands: the colobine monkeys and macropodid marsupials (kangaroos and wallabies) [10, 33, 39, 41]. Furthermore, it has been shown in the Colobinae [37, 39] and Macropodidae [32, 33] that VFAs cannot only be efficiently transported across non-glandular stratified epithelium but can also cross cardiac gland epithelium. There, therefore, appears to be no structural reason why VFAs, if produced in the stomach of the babirusa, would not be absorbed through the mucosa of the cardiac gland area into the blood stream.

Many animals employing forestomach fermentation show a system of folds, blindsacs or narrow constrictions in the stomach which help to increase the time available for fermentation (by slowing down the passage of digesta) and which separate the fermentation chamber from the low pH of the gastric gland area. The spiral fold found at the entrance of the *fundus ventriculi* and the shallow haustra-like sacculations in the *corpus ventriculi* did not fully compartmentalise the babirusa stomach. A preliminary study of passage time using 2-mm-sized plastic markers did not reveal any clear difference between the retention time for babirusa and that found for domestic pigs [42]. Similarly, Conklin and Dierenfeld [43], found a single marker (chromium mordanted fibre) excretion peak and low metabolic faecal N loss in babirusa fed on a zoo diet. This suggested that there was no selective retention of the digesta in any part of the gastro-intestinal tract, and that caeco-colic fermentation may be less important for the babirusa [43]. The babirusa in the present study appeared to have a caecum and colon which was structurally similar to that of the domestic pig (Leus and Mac-

donald, pers. obs.). Apart from a shallow mucosal fold, no internal structures were found which separated the gastric unit of the babirusa from the *corpus ventriculi*. However, the sheer size of the cardiac gland area (table III) in the babirusa stomach may be large enough to permit sufficient separation of the food ingested from the acid and pepsin produced in the gastric unit, thereby allowing some microbial fermentation to take place. All of the above might enable the babirusa to better digest soluble fibres and hemicellulose than *Sus scrofa*, but be less efficient in the digestion of the tougher cell wall components such as cellulose. Recent digestibility studies on babirusa have found indications that this may indeed be the case [5, 43, 44]. We would therefore expect the babirusa to choose plants and plant parts which contain relatively large amounts of cell solubles and small amounts of insoluble cell wall components [45, 46]. This would be consistent with a frugivore/concentrate selector feeding strategy. A behavioural study on two babirusa in a semi-natural enclosure in France found indications for this type of food selection [47]. In the wild, fermentation may also be of special importance to the babirusa for the breakdown of mechanical fruit and seed protection mechanisms such as peels, and for the detoxification of chemical plant protection mechanisms [45, 48].

Information on the diet of babirusa in the wild is at this moment scarce and anecdotal in nature. They are said to eat fruits and leaves, as well as some roots and animal material [4, 49]. A detailed and quantitative study of the foodstuffs consumed in the wild, is needed to confirm or reject the hypotheses suggested above.

5. Conclusions

We conclude from this study of the anatomy and histology of the babirusa stomach that the basic structure closely resembles that of the other Suidae; it is a unilocular stomach (with a diverticulum), the luminal surface of which is largely glandular in nature. The histological composition of the glands is similar to that of *Sus scrofa*. The main feature that distinguishes the babirusa stomach from those of other Suidae is its bigger volumetric size and the proportionately large surface area of the lumen covered by mucus producing cardiac glands (> 70 %). This mucus secreting area appears to provide an environment which is able to support a bacterial flora. These results lead to the hypothesis that the babirusa is a non-ruminant forestomach fermenter.

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