Immunohistochemical Study on the Distribution of Endocrine Cells in the Gastrointestinal Tract of the Babirusa, *Babirusa babirusa* (Suidae)

S. AGUNGPRIONO¹, A. A. MACDONALD², K. Y. G. LEUS³, N. KITAMURA^{4*}, I. K. M. ADNYANE¹, G. P. GOODALL², E. HONDO⁴ and J. YAMADA⁴

Addresses of authors: ¹Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor 16151, Indonesia; ²Department of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Summerhall, Edinburgh EH9 1QH, Scotland, UK; ³Royal Zoological Society of Antwerp, Koningin Astridplein 26, B-2018 Antwerpen, Belgium; ⁴Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan; *Corresponding author. fax: +81 155495354; e-mail: kitamura@obihiro.ac.jp

Summary

The distribution and relative frequency of endocrine cells in the gastrointestinal tract of the babirusa were studied immunohistochemically using the avidin-biotin-peroxidase complex method. Thirteen types of gut endocrine cells were detected; they were immunoreactive for chromogranin, serotonin, somatostatin, gastrin, bovine pancreatic polypeptide (BPP), glucagon, secretin, cholecystokinin (CCK), methionine-enkephalin-Arg⁶-Gly⁷-Leu⁸ (MENK8), motilin, gastric inhibitory polypeptide (GIP) and peptide tyrosine tyrosine (PYY). Cells that were immunoreactive for chromogranin, serotonin, somatostatin and glucagon were found in all portions of the gastrointestinal tract. MENK8-immunoreactive cells were observed in the stomach and small intestine. Gastrin-immunoreactive cells were detected in the pyloric region and duodenum. PYY-immunoreactive cells were found in the small and large intestine. Cells immunoreactive for motilin, CCK, GIP, and secretin were observed in the proximal small intestine and those immunoreactive for neurotensin were found only in the ileum. Although the distribution pattern of endocrine cells in the gastrointestinal tract of babirusa was similar to those reported for pig, restricted distribution of several endocrine cells, gastrin, BPP, MENK8, motilin, CCK, GIP, secretin and neurotensin and wider distribution of glucagon and PYY were observed in the babirusa. The unexpected presence of MENK8 in all glandular regions of the stomach and PYY in the small intestine was also noted. The distribution of gut endocrine cells might be related to the regulatory characteristics of the babirusa digestive tract.

Introduction

Gastrointestinal hormones are secreted by endocrine cells which are distributed throughout the mucosa of the gastrointestinal tract. They play important functions in the overall regulation of digestive processes such as nutrient absorption, the secretion of intestinal and associated glands, gut motility and intestinal blood flow (Deveney and Way, 1983). Morphological studies at light and electron microscopic level have demonstrated the presence and distribution of hormone-producing endocrine cells in the gastrointestinal tract of man and domestic mammals (Facer et al., 1985; Rindi et al., 1986; Kawakita et al., 1990).

Data on the immunohistochemical distribution of endocrine cells are available for both domesticated pigs (*Sus domestica*) (Ito et al., 1987; Ito et al., 1988) and the Eurasian wild boar (*Sus scrofa*) (Dall'Aglio et al., 1998). However, until the present, no comparable information has been reported for other genera of wild pigs.

The babirusa (*Babirusa babirusa*) is considered to be unique among the family of Suidae (pigs) partly because of its long isolation. The animal is found only on the Indonesian islands of Sulawesi, Buru and the Sula and Togian Island groups. There are three living subspecies: *Babirusa babirusa babirusa* (Sula islands and Buru), *Babirusa babirusa togeanensis* (Togian islands) and *Babirusa babirusa celebensis* (Sulawesi). The Sulawesi subspecies is the only one currently held in captivity and the animals used in the present study were from this subspecies. The animal is most easily recognized by the almost naked grey skin and the very large maxillary canines of the male that grow vertically upwards, pierce through the skin of the snout and curve in front of the forehead. The male mandibular canines are equally enlarged but grow alongside the snout (Macdonald et al., 1993).

The digestive function of the babirusa is not yet fully understood, although previous studies have revealed certain basic aspects such as foraging behaviour and food selection, diet digestion and gastrointestinal anatomy (Langer, 1973; 1988; Leus, 1994, 1996; Leus and Morgan, 1996; Leus and Vercammen, 1996; Leus and Macdonald, 1997; Leus et al., 1999). These studies revealed that the babirusa is likely to be a non-ruminant forestomach-fermenting frugivore/concentrate selector. The animal has a unilocular stomach with a large fundus and diverticulum ventriculi. Mucus-producing cardiac glands occupy more than 70% of the internal stomach surface area (compared to about 30% in *Sus scrofa*), whereas the oxyntic glands are confined to a comparatively small oxyntic gland unit at the distal end of the corpus ventriculi. Numerous micro-organisms are present in the cardiac gland area which shows a near-neutral pH. The intestinal tract does not appear to be substantially different in gross anatomical structure from that of *Sus scrofa*. Very little is known about the food habits in the wild. Their diet appears to mainly consist of fruits supplemented with leaves, herbs, roots, invertebrates and small mammals and birds.

Table 1. List of animals used in the present study

No*	Sex	Zoo	Fixative
8	F	Stuttgart	10% Formalin
47	M	Antwerp	10% Formalin
49	M	Antwerp	10% Formalin
52	F	Rotterdam	10% Formalin
347	M	Surabaya	Bouin
355	F	Surabaya	Bouin

* International studbook number.

The present study was conducted to identify the type, distribution and relative frequency of gut endocrine cells in the gastrointestinal tract of the babirusa using specific immunohistochemical methods. The data obtained will be compared mainly with those of domestic and Eurasian wild pigs, and thereby contributes to an understanding of the digestive function of babirusa and of Suidae in general.

Materials and Methods

Tissue materials were obtained from six adult babirusa (*Babirusa babyrussa celebensis*), as soon as possible after euthanasia for veterinary reasons (Table 1). None of the animals had macroscopic or microscopic (haematoxylin and eosin staining) lesions in the gastrointestinal tract. Stomachs were either opened along the greater curvature, emptied and completely bathed in fixative, or tissue samples were taken immediately after opening. Samples were taken from the following parts of the gastrointestinal tract: from the stomach, the squamous, cardiac, oxyntic and pyloric regions; from the small intestine, the duodenum, jejunum and ileum; and from the large intestine, the caecum, colon and rectum. Tissues fixed in Bouin's fixative (Table 1) were submerged in Bouin's fluid for 24 h and then kept in 70% ethanol until further processing. The samples were dehydrated in graded series of ethanol-xylol and embedded in paraffin.

Sections were cut at 5 µm thickness, mounted on gelatin-coated glass slides and stained immunohistochemically using the avidin-biotin-peroxidase complex (ABC) method (Hsu et al., 1981) with the Vectastain Elite ABC Kit (Vector Laboratories Inc., Burlingame, CA). The primary antisera to gut hormones used in the present study are listed in the Table 2. The reaction sites were revealed by Tris buffer (pH 7.4) solution containing 3-3' diaminobenzidine hydrochloride and H₂O₂. Negative and positive controls were included in the staining procedures. Negative control was carried out by incubating sections with phosphate-buffered saline and non-immune serum instead of the primary antiserum, and positive control was conducted with sections of pig and cattle known to contain the hormones (Kitamura et al., 1985; Ito et al., 1987, 1988). Sections were lightly counterstained with Mayer's haematoxylin, dehydrated, cleared and placed under a cover-slip. Inspection of the slide was conducted by light microscopy. Only immunoreactive endocrine cells with discernible nuclei were considered. The relative frequencies of endocrine cells were graded subjectively into five groups: (-), absent; (+/-), rare and not found in every section; (+), few; (++), moderate in number; and (+++), numerous.

Results

The present study revealed 13 kinds of endocrine cells immunoreactive for chromogranin, serotonin, somatostatin, gastrin, bovine pancreatic polypeptide (BPP), glucagon, secretin, cholecystokinin (CCK), methionine-enkephalin-Arg⁶-Gly⁷-Leu⁸ (MENK8), motilin, gastric inhibitory polypeptide (GIP) and peptide tyrosine tyrosine (PYY) in the gastrointestinal tract of the babirusa (Table 3). The antibodies to MENK8 demonstrated not only endocrine cells but also nervous elements. Immunoreaction against substance P was detected only in the nervous elements. No positive reactions were detected in the negative control sections. The endocrine cells showed the characteristic form of both open and closed types. The shape of the cells included round, oval, triangular, spindle or flask-like appearances. Open-type cells which had contact with the lumen through their apical cytoplasm were found in the pyloric region of the stomach, the epithelial villi of the small intestine and in the intestinal glands. They were not found in the oxyntic gland region of the stomach. Endocrine cells in the oxyntic gland region were of the closed type. No endocrine cells were found in the mucosa of the squamous region of the stomach.

Cells immunoreactive for chromogranin, serotonin, somatostatin and glucagon were found in all parts of the gastrointestinal tract (Table 3). Cells immunoreactive for the first two hormones were numerous in almost all regions of the gastrointestinal tract (Figs 1b, 2h). Somatostatin-immunoreactive cells varied in number from moderate to numerous in the stomach (Fig. 1d). They then decreased gradually from moderate numbers in the duodenum to only a few in the rectum. Glucagon-immunoreactive cells were present in moderate numbers in the oxyntic gland region and were seldom seen in other regions of the stomach, small intestine (Fig. 2e) and caecum. These cells were absent in the distal parts of the large intestine.

Cells that were immunoreactive for MENK8 were observed in the stomach and small intestine. In the stomach, these cells varied from a few to moderate numbers in the cardiac gland region. There were few in the oxyntic gland region but moderate numbers in the pyloric region (Fig. 1e). In the small intestine, MENK8-immunoreactive cells were moderate in number in the duodenal and jejunal regions, but then became few in the ileum.

Gastrin-immunoreactive cells were found numerous in the pyloric region (Fig. 1c). The cells were moderate in number in the duodenum. Their number declined aborally and disappeared in the ileum. Cells immunoreactive for BPP were distributed in the stomach. These cells were moderate in number in the cardiac (Fig. 1a) and oxyntic gland regions and then decreased to a few in the pyloric region.

Cells that were immunoreactive for PYY were found in both small and large intestine, whereas those immunoreactive for motilin, CCK, GIP, secretin and neurotensin were found only in the small intestine. The number of PYY-immunoreactive cells was few in the duodenum and jejunum, but their number increased from the ileum (Fig. 2g) to the large intestine. The largest number was observed in the rectum. The number of cells that were immunoreactive for motilin, CCK and GIP were moderate in the duodenum but few were found in the jejunum (Fig. 2b-d). Secretin-immunoreactive cells were found only in the duodenum in moderate number (Fig. 2a). Small numbers of cells that were immunoreactive for neurotensin were found only in the epithelial villi of the ileum (Fig. 2f). Cells immunoreactive for somatostatin, BPP, MENK8, CCK and PYY were also observed in the duodenal (Brunner's) glands. BPP endocrine

Table 2. List of primary antisera used in the present study

Antiserum	Code	Dilution	Source
Chromogranin	8808014	1: 5000	Incstar, Stillwater, MN, USA
Serotonin	43H2T	1: 50 000	Immuno Nuclear Corp., Stillwater, MN, USA
BPP	Lot 615-R110-146-17	1: 100 000	R.E. Chance, Lilly Lab., Indianapolis, IN, USA
Somatostatin	—	1: 10 000	S. Ito, Niigata University, Niigata, Japan
Gastrin	GP-1304	1: 5000	N. Yanaihara, Yanaihara Institute, Shizuoka, Japan
CCK	RPN1742	1: 4000	Amersham International plc., Amersham, UK
MENK8	R-0171	1: 8000	N. Yanaihara
Motilin	R-1104	1: 8000	N. Yanaihara
Neurotensin	R-3501	1: 10 000	N. Yanaihara
Secretin	R-801	1: 10 000	N. Yanaihara
GIP	G/R/34-IIID	1: 80 000	Guildhay, Guildford, UK
Glucagon	RPN1602	1: 5000	Amersham International plc.
PYY	IHC7173	1: 5000	Peninsula Lab. Inc., Belmont, CA, USA
Substance P	IHC7451	1: 8000	Peninsula Lab. Inc.

All antisera were raised in rabbit except that against gastrin, which was raised in guinea pig. BPP, bovine pancreatic polypeptide; CCK, cholecystokinin; MENK8, methionine-enkephalin-Arg⁶-Gly⁷-Leu⁸; GIP, gastric inhibitory polypeptide; PYY, peptide tyrosine tyrosine.

Table 3. Distribution of immunoreactive endocrine cells in the gastrointestinal tract of the babirusa, *Babirusa babirusa*

Endocrine cell type	Stomach			Small intestine			Large intestine		
	Cardiac	Oxyntic	Pyloric	Duodenum	Jejunum	Ileum	Caecum	Colon	Rectum
Chromogranin	+++~++++	+++	+++~++++	+++~++++	+++~++++	+++~++++	+++	+++	+++
Serotonin	+++~++++	+++~++++	+++~++++	+++~++++	+++~++++	+++~++++	+++	+++	+++
Somatostatin	+++~++++	+++	+++~++++	+++*	+++~++++	+++~++++	+	+	+
Gastrin	—	—	+++	++	+/++	—	—	—	—
BPP	++	++	+	—*	—	—	—	—	—
Glucagon	+	++	+	+	+	+	—	—	—
MENK8	+++~++++	+	++	+++*	++	+	—	—	—
Motilin	—	—	—	++	+/—	—	—	—	—
CCK	—	—	—	+++*	+	—	—	—	—
GIP	—	—	—	++	+++~++++	—	—	—	—
Secretin	—	—	—	++	—	—	—	—	—
Neurotensin	—	—	—	—	—	+	—	—	—
PYY	—	—	—	++*	+	+++~++++	++	++	+++

—, absent; +/—, rare and not detected in every section; +, few; ++, moderate; +++, numerous.

Cells were also detected in Brunner's glands. See footnote of Table 2 for the abbreviation of cell type.

Fig. 1. Photomicrographs of immunoreactive endocrine cells in the stomach of the babirusa. (a) Bovine pancreatic polypeptide-immunoreactive cells in the cardiac gland region; (b) serotonin-immunoreactive cells in the oxyntic gland region; (c) gastrin- (d) somatostatin- and (e) methionine-enkephalin-Arg⁶-Gly⁷-Leu⁸-immunoreactive cells in the pyloric gland region. (a)–(e) magnification, ×400.

Fig. 2. Photomicrographs of immunoreactive endocrine cells in the intestine of the babirusa. (a) Secretin- (b) cholecystokinin- and (c) gastric inhibitory polypeptide-immunoreactive cells in the duodenum. (d) Motilin- and (e) glucagon-immunoreactive cells in the jejunum. (f) Neurotensin- and (g) peptide tyrosine tyrosine-immunoreactive cells in the ileum. (h) Chromogranin-immunoreactive cells in the colon. (a), (b), (d), (g), (h) magnification, $\times 400$; (c), (e), (f) magnification, $\times 600$.

cells were observed in Brunner's glands, although they were not found in the intestinal glands.

Discussion

Gut endocrine cells in pig were studied by Capella and Solcia (1972) using histochemical methods. Recent advances in immunohistochemistry have made it possible to demonstrate the presence of a number of endocrine cells in the gastrointestinal tract of mammals. Specifically with regard to the family Suidae, immunohistochemical studies on the type and distribution of gut endocrine cells have been performed on the gastrointestinal tract of domestic pig (Ito et al., 1987) and wild boar (Dall'Aglia et al., 1998). This is the first study to report the distribution of immunohistochemically identifiable endocrine cells in the gastrointestinal tract of the babirusa.

The present study revealed 13 types of endocrine cells in the gastrointestinal tract of the babirusa. The endocrine cells were immunoreactive for chromogranin, serotonin, somatostatin, gastrin, BPP, glucagon, MENK8, motilin, CCK, GIP, secretin, neurotensin and PYY.

The general pattern of distribution of endocrine cells in the gastrointestinal tract of the babirusa was similar to those reported so far in other mammalian species. However, when compared with that of pig (Ito et al., 1987), the specific distribution of cells immunoreactive for gastrin, BPP, MENK8, motilin, CCK, GIP, secretin and neurotensin was more restricted in the babirusa, whereas the distribution of glucagon- and

PYY-immunoreactive cells was wider. Additional interesting findings were noted which included the presence of MENK8 in all glandular regions of the stomach and PYY in the small intestine of the babirusa.

Chromogranin is an acidic protein found in almost all mammalian gut endocrine cells (Facer et al., 1985; Rindi et al., 1986; Kawakita et al., 1990) and was therefore used as a general marker of endocrine cells in the present study. Numerous chromogranin-immunoreactive cells were found distributed in all the gut regions examined. This finding is consistent with previous reports of studies in pig (Ito et al., 1988), lesser mouse deer *Tragulus javanicus* (Agungpriyono et al., 1994), Java treeshrew *Tupaia javanica* (Agungpriyono et al., 1998) and Philippine carabao *Bubalus bubalis* (Baltazar et al., 1998). The distribution of chromogranin-immunoreactive cells consistently reflects that of major populations of endocrine cells in the gastrointestinal tract of babirusa.

Some kinds of endocrine cells are present in the Brunner's glands of pig (Ito et al., 1987), cat *Felis catus* (Kitamura et al., 1982), horse *Equus caballus* (Kitamura et al., 1984), cattle *Bos taurus* (Kitamura et al., 1985), sheep *Ovis aries* (Calingasan et al., 1984), lesser mouse deer (Agungpriyono et al., 1994), mink *Mustela vison* (Kawano et al., 1983) and several species of bats *Desmodus rotundus*, *Pipistrellus abramus* and *Plecotus auritus* (Yamada et al., 1984, 1988), but not in the Java treeshrew (Agungpriyono et al., 1998) and rat *Rattus rattus* (Walter, 1986). In the present study, cells that were immuno-

reactive for somatostatin, BPP, MENK8, CCK and PYY were detected in the Brunner's glands of the babirusa. Endocrine cells in the glands may regulate the secretion by the glands. It is interesting to note the presence of BPP-immunoreactive cells in the Brunner's glands, as these cells were not found in the intestinal glands.

Gastrin-immunoreactive cells were found only in the pyloric gland region and the intestinal glands of duodenum and jejunum. This distribution was smaller compared to that of the pig (Ito et al., 1987). In the pig, a few gastrin-immunoreactive cells can also be found in the large intestine. Their distribution in the babirusa was similar to that found in ruminant animals such as the lesser mouse deer (Agungpriyono et al., 1994), sheep (Calingasan et al., 1984), fallow deer *Dama dama* (Ceccarelli et al., 1995), impala *Aepyceros melampus* (Schoeman et al., 1998) and domestic cattle (Kitamura et al., 1985).

Several different types of endocrine cells are found in the cardiac gland region of the stomach of the calf (Kitamura et al., 1985), pig (Ito et al., 1987), bat (Yamada et al., 1988), honey possum *Tarsipes rostratus* (Yamada et al., 1989), musk shrew *Suncus murinus* (Kitamura et al., 1990) and Java treeshrew (Agungpriyono et al., 1998). In the babirusa, cells immunoreactive for chromogranin, serotonin, somatostatin, BPP, glucagon and MENK8 were detected in this region of the stomach. The numbers of BPP- and MENK8-immunoreactive cells in the cardiac gland region were large in contrast to the numbers found in the domestic pig (Ito et al., 1987).

The distribution pattern of cells that are immunoreactive for glucagon in the babirusa is, in general, similar to that of the domestic pig (Ito et al., 1987). However, their distribution in the babirusa is slightly wider, in that glucagon-immunoreactive cells are not detected in the pyloric gland region and jejunum of the domestic pig.

MENK8-immunoreactive cells are distributed from the stomach (except the oxyntic gland region), to the large intestine in the domestic pig (Ito et al., 1987). The cells are confined to the duodenum in the lesser mouse deer (Agungpriyono et al., 1994) and Philippine carabao (Baltazar et al., 1998) and are absent from the gastrointestinal tract of the Java treeshrew (Agungpriyono et al., 1998). In the babirusa, MENK8-immunoreactive cells occurred in all glandular regions of the stomach and small intestine but were not present in the large intestine. In addition, the presence of MENK8-immunoreactive cells in the oxyntic gland region of the babirusa differed from that reported in the pig and from other mammals for which they have not been reported.

The evidence to date, from most of the mammalian species studied, indicates that cells that are immunoreactive for CCK, secretin, motilin and GIP are mainly located in the proximal part of the small intestine, whereas neurotensin-immunoreactive cells are mainly found in the distal part of small intestine (Polak, 1989). A similar situation was observed in the babirusa. However, when compared with the situation in the domestic pig (Ito et al., 1987), the distribution of these cells in the babirusa was more restricted.

The immunolocalization of PYY in the gastrointestinal tract has not been reported in the family of Suidae. In other mammals, PYY-immunoreactive cells are usually present in the distal portion of small intestine and in the large intestine (El-Salhy et al., 1983a,b; Ali-Rachedi et al., 1984; Agungpriyono et al., 1994, 1998; Baltazar et al., 1998). In the babirusa, PYY-immunoreactive cells were observed in all portions of small and large intestine, although they were few in number in the

duodenum and jejunum. This finding resembles the reports of studies on the rat (El-Salhy et al., 1983a).

In conclusion, the distribution and relative frequency of gut endocrine cells in the babirusa has been described in the present study. The pattern of distribution of endocrine cells in the gastrointestinal tract was generally similar to that reported for the pig. However, we noted restricted distribution of endocrine cells that were immunoreactive for gastrin, BPP, MENK8, motilin, CCK, GIP, secretin and neurotensin in the babirusa, although the distribution of glucagon- and PYY-immunoreactive cells was wider in this species. The unexpected presence of MENK8 in all glandular regions of the stomach and PYY in the small intestine was noted and discussed. Further physiological studies are required to elucidate the ways in which the distribution of gut endocrine cells may be related to the regulatory characteristics of the babirusa digestive tract.

Acknowledgements

We are greatly indebted to the directors, veterinary, laboratory, curatorial and keeper staff of the various zoological institutions for their support and for making it possible to collect the tissues, specifically: Pak Stany Subakir, Dr Endah Wahyuni, Dr Liang Kaspe (Surabaya Zoo), Dr Wolfram Rietschel and Dr Lutz Plasa (Wilhelma Zoological and Botanical Garden, Stuttgart), Dr Bernd Matern (Frankfurt Zoo), Dr Walter De Meurichy and Dr Francis Vercammen (Royal Zoological Society of Antwerp), Dr Willem Schaftenaar (Rotterdam Zoo). The financial support of the following organizations is gratefully acknowledged: the Commission of the European Communities, the Development Trust of the University of Edinburgh and the Balloch Trust. We are grateful to Drs R. E. Chance, S. Ito and N. Yanaihara for the donations of antisera that are listed in Table 2. The experiment was conducted in Obihiro during the stay of S.A. as a visiting research fellow supported by a grant (No. S98250) from Japan Society for the Promotion of Science.

References

- Agungpriyono, S., J. Yamada, N. Kitamura, Y. Yamamoto, N. Said, K. Sigit, and T. Yamashita, 1994: Immunohistochemical study of the distribution of endocrine cells in the gastrointestinal tract of the lesser mouse deer (*Tragulus javanicus*). *Acta Anat.* **151**, 232–238.
- Agungpriyono, S., D. L. Kusindarto, M. Akmal, E. Hondo, M. Kurohmaru, and J. Yamada, 1998: Immunohistochemical study on the distribution of gut endocrine cells in the gastrointestinal tract of Java treeshrew (*Tupaia javanica*). *J. Primatol. Indonesia* **2**, 21–29.
- Ali-Rachedi, A., I. M. Varndell, T. E. Adrian, D. A. Gapp, S. van Noorden, S. R. Bloom, and J. M. Polak, 1984: Peptide YY (PYY) immunoreactivity in endocrine cells of the gut and pancreas. *Histochemistry* **80**, 487–491.
- Baltazar, E. T., N. Kitamura, E. Hondo, J. Yamada, C. P. Maala, and L. T. Simborio, 1998: Immunohistochemical study of endocrine cells in the gastrointestinal tract of the Philippine carabao (*Bubalus bubalis*). *Anat. Histol. Embryol.* **27**, 407–411.
- Calingasan, N. Y., N. Kitamura, J. Yamada, Y. Oomori, and T. Yamashita, 1984: Immunocytochemical study of the gastroenteropancreatic endocrine cells of the sheep. *Acta Anat.* **118**, 171–180.
- Capella, C., and E. Solcia, 1972: The endocrine cells of the pig gastrointestinal mucosa and pancreas. *Arch. Histol. Jap.* **35**, 1–29.
- Ceccarelli, P., V. Pedini, and A. M. Gargiulo, 1995: The endocrine cells in the gastro-enteric tract of the adult fallow deer (*Dama dama* L.). *Anat. Histol. Embryol.* **24**, 171–174.
- Dall'Aglio, C., P. Scocco, P. Ceccarelli, and V. Pedini, 1998: Neuroendocrine cells in the gastrointestinal tract of wild boar. *Anat. Histol. Embryol.* **27**, 381–385.

- Deveney, C. W., and L. W. Way, 1983: Regulatory peptides of the gut. In: Basic and Clinical Endocrinology (F. S. Greenspan and P. H. Forsham, eds), Asian edn. Singapore: Maruzen, pp. 479–499.
- El-Salhy, M., E. Wilander, L. Juntti-Berggren, and L. Grimelius, 1983a: The distribution and ontogeny of polypeptide YY (PYY)- and pancreatic polypeptide (PP)-immunoreactive cells in the gastrointestinal tract of rat. *Histochemistry* **78**, 53–60.
- El-Salhy, M., L. Grimelius, E. Wilander, B. Ryberg, L. Terenius, J. M. Lundberg, and K. Tatemoto, 1983b: Immunocytochemical identification of polypeptide YY (PYY) cells in the human gastrointestinal tract. *Histochemistry* **77**, 15–23.
- Facer, P., A. E. Bishop, R. V. Lloyd, B. S. Wilson, R. J. Hennessy, and J. M. Polak, 1985: Chromogranin: a newly recognized marker for endocrine cells of the human intestinal tract. *Gastroenterology* **89**, 1366–1373.
- Hsu, S., L. Raine, and H. Fanger, 1981: Use of Avidin-Biotin-Peroxidase Complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *J. Histochem. Cytochem.* **29**, 577–580.
- Ito, H., J. Yamada, T. Yamashita, Y. Hashimoto, and N. Kudo, 1987: An immunohistochemical study on the distribution of endocrine cells in gastrointestinal tract of the pig. *Jpn. J. Vet. Sci.* **49**, 105–114.
- Ito, H., Y. Hashimoto, H. Kitagawa, Y. Kon, and N. Kudo, 1988: Distribution of chromogranin-containing cells in the porcine gastroentero-pancreatic endocrine system. *Jpn. J. Vet. Sci.* **50**, 395–404.
- Kawakita, S., K. Takatsuki, M. Tsukada, M. Yoneda, T. Takano, A. Kawakubo, and H. Nagura, 1990: Localization of chromogranin A-immunoreactivity in bovine gastrointestinal endocrine cells with special reference to Grimelius Silver Stain. *Endocrinol. Jpn.* **37**, 299–308.
- Kawano, H., T. Yamashita, J. Yamada, and N. Kitamura, 1983: A light microscopic study of the gastro-entero-pancreatic endocrine cells of mink (*Mustela vison*). *Arch. Histol. Jap.* **46**, 559–573.
- Kitamura, N., J. Yamada, T. Yamashita, and N. Yanaiharu, 1982: Endocrine cells in the gastrointestinal tract of the cat. *Biomed. Res.* **3**, 612–622.
- Kitamura, N., J. Yamada, N. Y. Calingasan, and T. Yamashita, 1984: Immunocytochemical distribution of endocrine cells in the gastrointestinal tract of the horse. *Equine Vet. J.* **16**, 103–107.
- Kitamura, N., J. Yamada, N. Y. Calingasan, and T. Yamashita, 1985: Histologic and immunocytochemical study of the endocrine cells in the gastrointestinal tract of the cow and calf. *Am. J. Vet. Res.* **46**, 1381–1386.
- Kitamura, N., J. Yamada, T. Watanabe, and T. Yamashita, 1990: An immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the musk shrew, *Suncus murinus*. *Histol. Histopathol.* **5**, 83–88.
- Langer, P., 1973: Vergleichend-anatomische Untersuchungen am Magen der Artiodactyla (Owen, 1848). I. Teil: Untersuchungen am Magen der Nonruminantia (Suiformes). *Gegenbaurs morph. Jahrb.* **119**, 514–561.
- Langer, P., 1988: The Mammalian Herbivore Stomach, Comparative Anatomy, Function and Evolution. Stuttgart, New York: Gustav Fischer.
- Leus, K., 1994: Foraging behaviour, food selection and diet digestion of *Babirusa babirusa* (Suidae, Mammalia). PhD Thesis. Edinburgh: The University of Edinburgh.
- Leus, K., 1996: The Habitat and Diet of the Sulawesi Babirusa (*Babirusa babirusa celebensis*): Population and Habitat Viability Assessment Workshop for the Babirusa (*Babirusa babirusa*). Report (J. Manansang, A. A. Macdonald, P. Miller and U. Seal, eds). Apple Valley, MN: Conservation Breeding Specialist Group (SSC/IUCN), pp. 121–134.
- Leus, K., and C. A. Morgan, 1996: Analyses of diets fed to babirusa (*Babirusa babirusa*) in captivity with respect to their nutritional requirements. In: Proceedings of the 2nd International Symposium on wild boar (*Sus scrofa*) and on order Suiformes. Turin, Italy. IBEX J. Mount. Ecol. **3**, 41–44.
- Leus, K., and P. Vercammen, 1996: Behaviour of a male and female babirusa (*Babirusa babirusa* Suidae, Mammalia) during the first five days after their move to a semi-natural enclosure. *Zool. Gart. N.F.* **66**, 133–155.
- Leus, K., and A. A. Macdonald, 1997: From babirusa (*Babirusa babirusa*) to domestic pig: the nutrition of swine. *Proc. Nut. Soc.* **56**, 1001–1012.
- Leus, K., Goodall, G. P., and A. A. Macdonald, 1999: Anatomy and histology of the babirusa (*Babirusa babirusa*) stomach. *Comptes Rendus l'Academie Des Sci., Serie III – Sci. la Vie* **322**, 1–12.
- Macdonald, A. A., Bowles, D., Bell, J., and K. Leus, 1993: Agonistic behaviour in captive babirusa (*Babirusa babirusa*). *Z. Säugetierk.* **58**, 18–30.
- Polak, J. M., 1989: Endocrine cells of the gut. In: Handbook of Physiology. A Critical, Comprehensive Presentation of Physiological Knowledge and Concept, Vol. II. Section VI: The Gastrointestinal System (S. G. Schultz section ed.). Bethesda, USA: American Physiological Society, pp. 79–96.
- Rindi, G., R. Buffa, F. Sessa, O. Tortora, and E. Solcia, 1986: Chromogranin A, B and C immunoreactivities of mammalian endocrine cells. Distribution, distinction from costored hormones/prohormones and relationship with the argyrophil component of secretory granules. *Histochemistry* **85**, 19–28.
- Schoeman, J. H., V. De Vos, and G. Van Aswegen, 1998: Distribution of endocrine cells in the gut of the impala (*Aepyceros melampus*). *Onderstepoort J. Vet. Res.* **65**, 31–35.
- Walter, H. J., 1986: Endocrine cells do not exist in rat Brunner's glands. *Brain Res.* **367**, 141–150.
- Yamada, J., V. J. M. Campos, N. Kitamura, A. C. Pacheco, T. Yamashita, and U. Caramaschi, 1984: Immunocytochemical study of gastro-entero-pancreatic endocrine cells in the vampire bat (*Desmodus rotundus*). *Gegenbaurs Morph. Jahrb.* **130**, 845–856.
- Yamada, J., N. Kitamura, T. Yamashita, and C. J. Phillips, 1988: An immunohistochemical study of gut endocrine cells in two species of insectivorous vespertinid bats (Chiroptera: *Pipistrellus abramus* and *Plecotus auritus sacrimontis*). *Gegenbaurs Morph. Jahrb.* **134**, 1–8.
- Yamada, J., K. C. Richardson, and R. D. Wooller, 1989: An immunohistochemical study of gastrointestinal endocrine cells of nectarivorous marsupial, the honey possum (*Tarsipes rostratus*). *J. Anat.* **162**, 157–168.