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Gastric ulcers in pigs affected with postweaning multisystemic wasting syndrome[1]

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ABSTRACT.- Corrêa A.M.R., Zlotowski P., Barcellos D.E.S.N., Cruz C.E.F. & Driemeier D. 2008. **Gastric ulcers in pigs affected with postweaning multisystemic wasting syndrome.** *Pesquisa Veterinária Brasileira 28(12):601-605.* Departamento de Patologia Clínica Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. E-mail: davetpat@ufrgs.br

Samples of gastric lymph nodes and the stomachs from 24 pigs selected from herds affected by postweaning multisystemic wasting syndrome (PMWS) and sudden death associated with gastric ulcers were studied. Pigs were selected on the basis of unthriftiness, decreased feed intake, and wasting. The stomachs were opened, inverted, and classified into 0-3 score according the severity of the gross lesions present in *pars oesophagica* (non-glandulargastric mucosa). Selected samples were processed for paraffin embedding and stained with hematoxylin and eosin. Immunohistochemistry using anti-PCV2 (porcine circovírus type 2) antibody, anti-*Helicobacter pylori* antibody and a wide-spectrum anti-cytokeratin antibody was performed. Gross changes in *pars oesophagea* were classified according to the severity of lesions as score 3, 2, and 1 in 8, 6, 5 stomachs respectivelly. Microscopically, hyperplastic lymphoid follicles, lymphohistiocytic inflammatory infiltrates and focci of necrosis in the gastric mucosa were common findings. Large amounts of PCV2 antigen were observed in the cytoplasm and nuclei from intralesional cells and debris from the gastric glandular mucosal zone; however, in the fundus, anti-PCV2 immunostaining was restricted to the surface mucosal cells and foveolar compartment. All gastric lymph nodes were positive for PCV2 antigen. Anti-*H. pylori* immunostaining was seen in eleven cases, mainly in the antrum, on the mucosal surface and foveolar compartment. The association of the anti-PCV2 immunostaining with the glandular mucus-producing cells suggests a role for PCV2 as an additional factor for the swine ulcer development.

INDEX TERMS: Diseases of swine, gastric ulcer, PMWS, PCV2, *Helicobacter pylori*.

RESUMO.- [**Úlceras gástricas em suínos afetados pela síndrome multissistêmica do definhamento.**] Amostras de linfonodos gástricos e os estômagos de 24 leitões selecionados de rebanhos afetados pela síndrome multissistêmica do definhamento suíno e mortes súbitas por úlceras gástricas foram estudados. Os animais foram selecionados por baixa performance, baixo consumo de alimento e desnutrição. Os estômagos foram abertos, invertidos e classificados, conforme a severidade das lesões presentes na *pars oesophagica* (porção não-glandular da mucosa gástrica). Amostras selecionadas foram processadas por método histológico convencional para coloração de hematoxilina-eosina. Testes imuno-histoquímicos utilizando anticorpos anti-PCV2 (circovírus suíno tipo 2), anti-*Helicobacter pylori* e anticitoqueratina de largo espectro foram feitos. As alterações macroscópicas na *pars oesophagica* foram classificadas como de escore 3, 2 e 1 respectivamente em 8, 6 e 5 estômagos. Microscopicamente, foram notados folículos linfóides hiperplásicos, infiltrados linfohistiocitários e focos de necrose na mucosa gástrica. Grandes quanti-

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dades de antígeno do PCV2 foram observadas no citoplasma, núcleo e restos necróticos de células intralesionais das glândulas gástricas nas regiões do antro e cárdia; entretanto, na região do fundo, a marcação anti-PCV2 foi restrita às células da superfície mucosa e fossetas gástricas. Todos os linfonodos gástricos foram positivos para PCV2. Coloração anti-*H. pylori* foi identificada em 11 casos, principalmente, na superfície mucosa e fossetas gástricas no antro. A associação de antígenos PCV2 com células produtoras de muco lesadas na zona glandular gástrica sugere o envolvimento de PCV2 como um fator adicional para o desenvolvimento da úlcera gástrica suína.

TERMOS DE INDEXAÇÃO: Úlcera gástrica, SMDS, PCV2, *Helicobacter pylori*, suínos.

INTRODUCTION

Porcine circovirus type 2 (PCV2) is widespread in the commercial swine population and has been associated with several disease complexes (Allan & Ellis 2000, Segalés & Domingo 2002, Ellis et al. 2004, Segalés et al. 2004). PCV2 is the essential infectious component of postweaning multisystemic wasting syndrome (PMWS) (Allan et al. 2000, Meehan et al. 2001, Krakowka et al. 2005), which is an economically important disease of piglets characterized by wasting and multisystemic lesions, including granulomatous lymphadenitis with lymphoid depletion, hepatitis, pneumonia, and myocarditis (Allan & Ellis 2000, Chae 2004). The demonstration of high or moderate amounts of PCV2 antigen or nucleic acids in close association with characteristic microscopic lesions confirms the diagnosis of PMWS (Sorden 2000). PCV2 antigens have been detected by immunohistochemistry in a wide range of cells from pigs naturally or experimentally infected with PCV2 (Rosell et al. 1999, Kennedy et al. 2000, Opriessnig et al. 2007, Pérez-Martín et al. 2007).

Gastric ulceration is an important disease entity in pigs (Embaye et al. 1990, Friendship 2006). Ulceration of the *pars oesophagica* (non-glandulargastric mucosa) can affect pigs of any age but the highest rate of ulceration occurs in pigs 3-6 months of age. The exact cause of ulceration is not completely understood, but many of the risk factors are well known and include management, nutrition, infectious agents and others (Friendship 2006). The stratified squamous epithelium of the *pars oesophagica* of the stomach is devoid of mucous-producing glands and lacks the sodium bicarbonate buffering system characteristic of the gastric glandular mucosa. As a consequence, the *pars oesophagica* is subjected to damage by the acidic contents of the stomach. Others have described this pathogenesis in gastric ulcers lesions (Embaye et al. 1990, Krakowka et al. 1995, Argenzio & Eisemann 1996, Friendship 2006). In addition, Krakowka et al. (2005) suggested that the porcine gastric ulcer disease complex may involve gastric colonization with *Helicobacter pylori*-like bacteria, but additional studies would help to clarify the association of the bacteria with the gastric ulcers development.

Experimental infections of gnotobiotic pigs with various viral pathogens, including porcine reproductive and respiratory syndrome virus, has not resulted in gastric ulceration; however, experimental infection with porcine circovirus type 2 (PCV2) has caused multisystemic lesions, including gastric lesions (Harms et al. 2001). Although gastric ulceration of the *pars oesophagica* has been reported in PMWS-affected pigs (Segalés et al. 2000), no direct relationship between these lesions and PCV2 has been established (Segalés et al. 2005). The aim of the present study was to establish an association between PCV2 infection and the development of gastric ulcers in PMWS affected pigs.

MATERIALS AND METHODS

The study included 24 3-5-month-old pigs from three different herds, which had been experiencing cases of PMWS and sudden death associated with gastric ulcers. Animals were selected on the basis of unthriftiness, decreased feed intake, and wasting. The stomachs and samples of gastric lymph nodes were analyzed. The stomachs were opened along the greater curvature, inverted, and scored into 0-3 according to the severity of the gross lesions (Embaye et al. 1990) present in *pars oesophagica*. Classification of lesions was performed using a scoring system. In it, score 1 lesions were limited in extent, occurring mainly near the oesophageal opening or at the margin adjacent to the cardiac portion. Lesions designated as score 3 severely affected the entire non-glandular area and score 2 lesions had intermediate severity. Samples of the formalin-fixed gastric lymph nodes, transitional *pars oesophagica*-glandular cardiac mucosa, glandular antral mucosa, and fundic mucosa were processed for routine HE and immunohistochemical (IHC) staining. A polyclonal antibody against PCV2[4] at 1:1.000 dilution in the streptavidin–biotin–immunoperoxidase[5] technique, and with diaminobenzidine[5] as chromogen was applied (Sorden et al. 1999). Anti-*Helicobacter pylori* IHC staining was performed in the cardiac and antral sections similarly as in anti-PCV2 IHC, but at 1:200 dilution and with a rabbit anti-*H. pylori* antibody[5]. Anti-cytokeratin IHC studies were performed using the avidin-biotin-peroxidase complex detection system (Mills 1992) and a wide-spectrum polyclonal bovine anti-cytokeratin[5] antibody at 1:100 dilution. Alcian blue counterstaining was applied in selected sections.

RESULTS

Gross findings

In eight stomachs, the *pars oesophagica* damage was scored 3 with extensive and severe erosive lesions affecting its entire area, leaving a deep ridge at the margin with the cardiac mucosal portion of the stomach. Extensive granulation tissue was observed on the affected areas and in four cases, erosions reached the muscular layer. Two of the eight stomachs were filled with clotted or partially digested blood and gastric mucus. All of these eight stomachs showed focal edema of the gastric serosa in the lesser curvature. Six stomachs were classified score

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Fig.1. Score 3 gastric ulcer. Extensive and severe erosive lesion affecting the entire area of the *pars oesophagica* (Swine stomach nº 4).

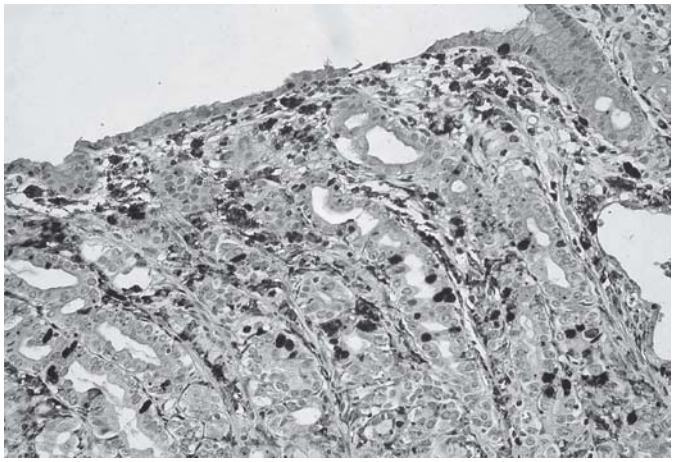

Fig.2. Anti-PCV2 immunostaining in the gastric mucosa, which also shows necrosis and decreased number of mucus-producing cells (Swine stomach nº 10). Anti-PCV2 immunostaining counterstained with Alcian blue and hematoxylin, obj. 20x.

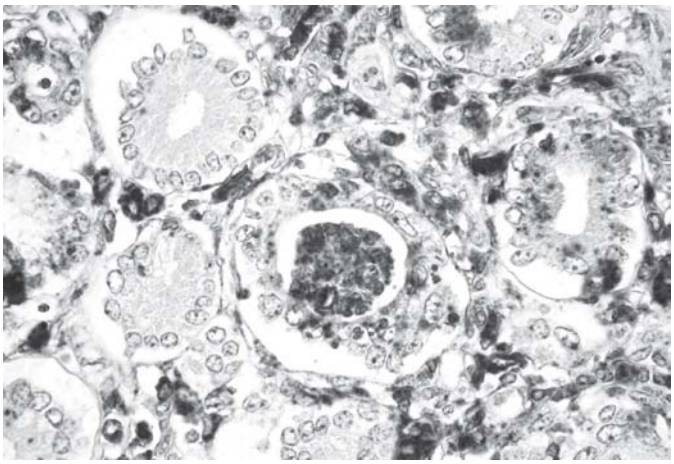

Fig.3. Intense anti-PCV2 immunostaining in the cytoplasm, cellular debris, and lymphohistiocytic infiltrates in the lamina propria of the gastric glands at the cardia zone (Swine stomach nº 5). Anti-PCV2 immunostaining counterstained with hematoxylin, obj. 40x.

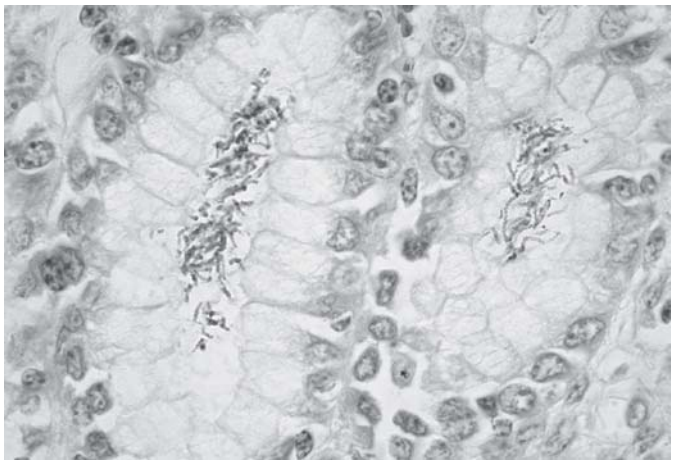

Fig.4. Anti-*Helicobacter pylori* immunostaining in the foveolar compartment (Swine stomach nº 14). Anti *H. pylori* immunostaining counterstained with hematoxylin, obj. 100x.

2, and the ulcerative lesions reached more than half of the *pars oesophagica*. In five cases, the *pars oesophagica* was graduated as score 1, when the epithelial lesions did not exceed a half part of the region of stratified squamous epithelial tissue. Five stomachs did not present gross lesions (score 0) in the *pars oesophagica*, whose squamous epithelium was smooth, white and glistening, and the junction with the cardiac glandular zone was abrupt and distinct. No ulceration lesions were observed in the glandular mucosa.

Histological findings

The presence of hyperplastic lymphoid follicles embedded in the gastric mucosa was a common finding in the stomachs with any degree of gross lesions. In score 3 gastric lesions on the *pars oesophagica*, there was extensive granulation tissue. Lymphohistiocitic inflammatory infiltrates were common in the lamina propria and, in one case scored 3, giant cells were also detected. Areas of necrosis in the gastric glands were often seen associated with cellular debris within the glands, frequently more conspicuous in the glandular mucosa (cardia and antrum) of the gastric mucosa.

Immunohistochemical findings

The most remarkable findings were observed in the gastric glandular mucosa (cardia and antrum), where large amounts of PCV2 antigen were observed within the cytoplasm and nuclei of cells and in celluar debris associated with the lesions. In the fundus, IHC staining was observed exclusively on the surface mucosal cells and foveolar compartment. No IHC staining was seen in the oxyntic glands. While no anti-PCV2 immunostaining could be detected in the gastric tissues from six stomachs, all gastric lymph nodes were positive for PCV2 antigen. Anti-*H. pylori* immunostaining was positive in eleven ca-

ses, mainly in the antrum, on the mucosal surface and foveolar compartment. The *pars oesophagica* lesions and the IHC results are summarized in Table 1. Double immunostaining for simultaneous demonstration of PCV2 and cytokeratin confirmed the presence of PCV2 within epithelial cells of gastric glandular mucosa.

DISCUSSION

A number of risk factors have been described in association with gastric ulcers in swine, including PCV2 infection, but the role of PCV2 infection in the ulcer development is not yet clarified (Friendship 2006). Among the methods used to detect PCV2 in tissues and to correlate it with lesions, the *in situ* hybridization (ISH) and immunohistochemistry are the most widely used (McNeilly et al. 1999, Rosell et al. 1999, Segalés et al. 2005).

Table 1. Gastric ulcers scores and immunohistochemical results[b] observed in stomachs from 24 PMWS confirmed pigs

Stomach	Lesion score[a]	IHC PCV2 Lymphonode	IHC PCV2 Antrum mucosa	IHC PCV2 Cardia mucosa	IHC PCV2 Fundus mucosa	IHC *H. pylori*
01	2	+++	++	++	+	Æ
02	0	++	+	Æ	Æ	+
03	3	+	Æ	Æ	Æ	++
04	3	++	+	+	+	Æ
05	2	+++	++	+	+	+
06	3	+	Æ	Æ	Æ	Æ
07	0	++	Æ	Æ	Æ	Æ
08	1	+++	+++	+++	+	Æ
09	2	+++	++	+	+	+
10	2	++	+	+	+	Æ
11	3	+	+	+	Æ	+++
12	3	+	Æ	Æ	Æ	Æ
13	0	+++	Æ	Æ	Æ	++
14	1	++	+	++	Æ	++
15	3	+	+	Æ	Æ	Æ
16	0	+	++	+	+	+
17	1	+++	++	++	+	Æ
18	1	++	++	++	+	Æ
19	3	+	Æ	Æ	Æ	++
20	2	+++	++	++	+	+
21	0	+	+	+	Æ	Æ
22	1	++	++	+	+	+
23	3	+	+	+	+	Æ
24	2	++	+	+	+	Æ

[a] Lesion score: 0 = no lesions; 1 = mild; 2 = moderate; 3 = accentuated.
[b] IHC staining: Æ = negative; + = slight; ++ = moderate; +++ = marked.

Considering the hypothesis in which the mucus-producing cells of the gastric glandular zones are targets for the PCV2 infection in the stomach of pigs, the necrosis induced by PCV2 in these cells may be associated with the decreased stomachal pH by the imbalance of the sodium bicarbonate buffering system and subsequently, with the ulceration of the *pars oesophagica*. The main target cell for PCV2 replication in the swine stomach mucosa may have not been established in this study; however, the association of the anti-PCV2 immunostaining with the glandular mucus-producing cells indicates that PCV2 should be considered as an additional factor for the swine ulcer development.

The lack of anti-PCV2 staining in stomachs from six pigs, mostly of them with score 3 gastric ulcers samples, could be explained by a decreased PCV2 viremia in chronic cases. Initial or terminal stages of diseases may be associated with mild or even absence of PCV2 antigen (Segalés et al. 2004). In some PCV2 infected pigs, it is possible that the viremia has to reach high systemic levels to infect others cell lines, and then to develop its full expression of ulcerogenic potential. This hypothesis or individual resistance may explain why 5 pigs had no gastric ulcers.

We can not rule out the participation of other risk factors in the development of the ulcers seen in these cases, but a strong link between histopathological lesions and anti-PCV2 immunostaining at the glandular mucus-producing cells may help us to understand the pathogenic role of PCV2 infection and gastric ulcer development in pigs.

Although *Helicobacter pylori* may occasionally cause gastric ulcers (Krakowka et al. 1995), we could neither find a significant relationship between *H. pylori* and gastric ulcers scores nor between *H. pylori* and PCV2, but this lack of relationship does not exclude *H. pylori* as a potential agent in the development of swine gastric ulcers.

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