BJVP
Brazilian Journal of Veterinary Pathology
ISSN 1983-0246

Original Full Article

Granulomatous enteritis associated with porcine proliferative enteropathy

Mariana A. Machuca*[1], Javier A. Cappuccio[1], Maria A. Quiroga[1], Lucas Insarralde[1], Jose L. Cancer[2], Silvio Arrospide[2], Carlos J. Perfumo[1]

[1]Cátedra de Patología Especial, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata (1900), Buenos Aires, Argentina. [2] Private practitioner, Roque Perez, Buenos Aires, Argentina.

*** Corresponding Author:** Cátedra de Patología Especial, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, 60 y 118, La Plata (1900), Buenos Aires, Argentina.Tel.: 54 221 423 6663 int 416 E-mail address: mmachuca@fcv.unlp.edu.ar

Submitted November 16th 2011, Accepted February 2nd 2012

Abstract

An outbreak of proliferative hemorrhagic enteropathy in a commercial pig farm, characterized by granulomatous enteritis, was studied by slaughter checks and by histopathological, histochemical and immunohistochemical studies. Six of the postmortem examined pigs (117-122-day-old) with diagnosis of porcine proliferative enteropathy (PPE) showed granulomatous enteritis and 4.3% of the 465 slaughtered pigs showed gross lesions of PPE. A total of 33 of the 66 ileum samples had histopathological changes, whereas 17 of them had granulomatous enteritis and PPE lesions. *Lawsonia intracellularis* was immunolabeled in 52 of the ileum samples in epithelial cells and within granulomatous inflammation in Peyer´s patches and in 28 samples of mesenteric lymph nodes. Porcine circovirus type 2 was only detected by immunohistochemistry in 4 ileum samples and in 5 mesenteric lymph nodes. Since there was a strong statistically significant association between granulomatous enteritis and *L. intracellularis* infection, granulomatous enteritis could be considered as a part of the PPE complex.

Key Words: Swine, enteritis, granulomatous, immunohistochemistry, *Lawsonia intracellularis*.

Introduction

Porcine proliferative enteropathy (PPE), caused by *Lawsonia intracellularis* (*L. intracellularis*), is a worldwide enteric infectious disease that affects weaned and growing-finishing pigs (19, 20). The PPE complex includes a group of acute and chronic conditions characterized by anorexia, diarrhea, retarded growth and increased mortality (6, 20). The acute form, proliferative hemorrhagic enteropathy (PHE), is characterized by the thickening of the intestinal wall and blood clots or bloody fluid into the gut lumen. Young adult pigs, replacement gilts, and finishing pigs close to market age are frequently affected by PHE. The chronic form include porcine intestinal adenomatosis (PIA), often seen in post weaned pigs between 6 and 14 weeks of age, necrotic enteritis (NE) and, the relatively rare form, regional ileitis (RI). While PIA is considered the result of an uncomplicated infection with *L. intracellularis*, NE and RI lesions are associated with secondary bacterial infections or prolonged inflammation (6, 20). Regardless of the PPE form, the thickening of the mucosa of the small intestine and colon is characteristic. Histologically, the affected intestine shows a marked proliferation of immature crypts epithelial cells (20).

Postmortem diagnosis is performed by histopathology, histochemistry and immunohistochemistry (IHC). Slaughter checks for PPE seem to be of low sensitivity and therefore unreliable for farm monitoring (13). However, they are considered more effective in detecting cases of PPE than the clinical inspection of a herd (23).

Porcine circovirus type 2 (PCV-2) has been associated with a number of syndromes and diseases in pigs (2, 4, 11). Porcine circovirus associated diseases (PCV-AD) include systemic infection (previously known as postweaning multisystemic wasting syndrome, PCV-2 associated pneumonia, and PCV-2 associated enteritis (22). PCV-2 associated enteritis affects pigs between 40 and 112 days of age (15, 22). Clinical signs and gross lesions resemble those of a subacute or chronic ileitis associated with *L. intracellularis* (14). The most consistent histopathological feature is granulomatous enteritis with infiltration of epithelioid and multinucleated giant cells and lymphoid depletion in Peyer´s patches (4, 14, 22). Both immunohistochemistry and in situ hybridization techniques have demonstrated the presence of PCV-2 antigens or viral DNA in the cytoplasm of histiocytes and multinucleated giant cells in Peyer´s patches (4, 28). In addition, PCV-2 positive cells have been observed in the lamina propria, submucosa and crypt epithelium of affected intestines (14).

Clinical PCV-AD appears to be triggered by several infectious and non-infectious factors. Coinfection with viruses, bacteria or Mycoplasma spp. can enhance PCV-2 replication and exacerbate clinical signs (7, 11, 22). The association between *L. intracellularis* and PCV-2 has been previously reported. However, few reports have described this coinfection with diarrhea or granulomatous enteritis in swine herds or wild boars (14, 25, 28).

Granulomatous inflammation is a distinct form of chronic inflammation in which cells of the monocyte-macrophage system are predominant, and which occurs as a secondary response to endogenous or exogenous antigens. Development or regulation of the granulomatous inflammation requires multiple factors, such as: 1) the presence of indigestible or poorly degradable and persistent antigen in the tissues, 2) the development of the host immune response, usually an intense T-lymphocyte-mediated response, or 3) the interplay of various cytokines produced by cells within a chronic inflammatory lesion (1).

The aim of the present study was to report clinical, histopathological, histochemical and immunohistochemical findings of granulomatous enteritis associated with PPE in growing and slaughter-weight pigs.

Material and methods

Herd and clinical presentation

A 6000 sow-farm using a multi-site production system reported a PHE outbreak that began in December 2008 and ended in March 2009. At the beginning of the outbreak, clinical signs including acute diarrhea with brownish-to-red discoloration of the feces, pallor, weakness and sudden death affected 17-week-old pigs. In addition, an increased mortality from 1.5 to 2.5% and the reduction of average daily gain in 17- to 23.3-week-old pigs.

Pathological studies

At the beginning of the outbreak, seven 17-week-old clinically affected pigs were submitted to Cátedra de Patología Especial, Facultad de Ciencias Veterinarias, UNLP, for postmortem examination. Tissue samples were obtained from lungs, lymph nodes, liver, kidney and ileum. Samples were fixed in 10% neutral buffered formalin and processed for histopathologic and immunohistochemical examination.

A total of 465 pigs (23.3-week-old) with an average of 113 kg/body weight were examined in three different slaughter inspections. A total of 66 randomly selected ileum samples were taken 10 cm from the ileocecal junction. In addition, 46 mesenteric lymph nodes were randomly collected. Samples were fixed in 10% buffered formalin for histopathologic and IHC studies.

Tissue samples were embedded in paraffin and stained with haematoxylin and eosin. In order to detect fungi and Mycobacterium spp, ileum samples were also analyzed by histochemistry using special stains such as PAS and Ziehl Neelsen. Lesions of PPE were categorized in PHE or PIA. PIA lesions were graded as follows: 1= a few crypts with isolated proliferation of immature enterocytes with or without cellular debris in the lumen; 2= multifocal proliferative changes of cryptal immature enterocytes, lack of goblet cells and cellular debris in the lumen; and, 3= diffuse crypts hyperplasia.

Immunohistochemical studies

Lawsonia intracellularis IHC was performed on ileum and mesenteric lymph nodes samples. Tissue sections were deparaffinized and rehydrated by sequential immersions of the slides in xylene followed by graded concentrations of ethanol. Endogenous peroxidase activity was blocked by incubation in 3% H2O2 in phosphate buffered saline (PBS) for 30 min at room temperature. Antigen retrieval was performed by proteinase K (Sigma Chemical Co. St. Louis, USA P2308) in 1/200 PBS dilution (4 mg/ml) for 15 min at room temperature. A polyclonal rabbit antibody anti-*L.intracellularis* (kindly provided by Dr. RMC Guedes) (10), diluted at 1/6000 with PBS was applied for 1 hour at 37ºC in a humid chamber. Then, tissue sections were washed in PBS-tween (0.05%) for 5 min, and incubated for 20 min at room temperature with biotinylated secondary antibody (LSAB2® System HRP K0673, DAKO Laboratories Co., California, USA). The slides were washed three times with PBS-tween before flooding and incubating with labeled streptavidin biotin for 20 min at room temperature. The final reaction was produced by immersing the sections in a solution of diaminobenzidine (LSAB2® System HRP K0673, DAKO Laboratories Co., California, USA) for 10

Table 1. Histopathological and immunohistochemical results in seven clinically affected pigs with gross lesions of PPE.

Nº pigs	Age (days)	PPE Lesion	Granulomatous Inflammation	IHC *L. intracelullaris*	IHC PCV-2
1	120	PIA 2	(+)	(+)	(-)
2	120	PIA 3	(-)	(+)	(-)
3	120	PIA 2	(+)	(+)	(-)
4	122	PHE	(+)	(+)	(+)
5	122	PHE	(+)	(+)	(-)
6	117	PHE	(+)	(+)	(-)
7	117	PHE	(+)	(+)	(+)

min at room temperature. Sections were washed and counterstained with Harris´s haematoxylin. Positive and negative control sections were included.

PCV-2 immunohistochemistry was performed with a polyclonal anti-PCV2 antibody (VMRD, Inc Pullman, WA, USA, 210-70 PCRV) on ileum samples and mesenteric lymph nodes. Briefly, tissue sections were deparaffinized with xylene and rehydrated through graded alcohols. Slides were flooded for 15 min with 3% H2O2 to remove endogenous peroxidase activity. Tissues were rinsed for 5 min in 0.1M PBS (pH 7.5) and then incubated with preheated 0.05% protease XIV for 40 minutes. Tissue sections were rinsed in PBS and flooded with 0.5% skim milk in PBS for 20 min at room temperature. PCV-2 antibody was used at a 1/200 dilution in 0.1M PBS, and incubated for 1 hour at 37ºC. Biotinylated G Protein (1/500) was used as a secondary antibody and was incubated for 40 min at room temperature. Streptavidin-peroxidase (LSAB2® System HRP K0673, DAKO Laboratories Co., California, USA) was applied for 15 min at room temperature. Sections were finally incubated in diaminobenzidine–hydrogen peroxide solution for 8 min and counterstained with Harris's haematoxylin. Positive and negative controls were used. Immunohistochemistry findings were graded based on the intensity of immunolabeling as follows: += slight, ++= moderate, +++= abundant.

Statistical evaluation

Histopathologic findings and IHC results were evaluated for statistical significance by Chi square test. Values of $p \leq 0.05$ were considered significant.

Results

All seven pigs postmortem examined at the UNLP had gross lesions compatible with acute or chronic forms of PPE. Microscopic lesions of PIA, grades 2 and 3, were observed in three cases. The remaining 4 cases had marked congestion and hemorrhages within the proliferative epithelium, associated with PHE form. In addition, the lamina propria and Peyer´s patches of six cases were infiltrated with histiocytic cells and a few giant cells, characteristic of granulomatous inflammation. Necrosis and cell depletion were occasionally observed in Peyer´s patches. Only two mesenteric lymph nodes had slight lymphoid depletion. The remaining tissues did not have other microscopic changes. *L. intracellularis* antigen was detected in the apical cytoplasm of ileal enterocytes of hyperplastic crypts, within the cytoplasm of macrophages in the lamina propria, and in the cytoplasm of histiocytes and giant cells in Peyer´s patches. Moreover, in only two cases PCV-2 antigen was observed, with slight intensity in the cytoplasm of histiocytic cells. The immunolabeling was detected in the lamina propria of one case and in esenteric lymph nodes of another. The results of the istopathology and IHC studies against *L. intracellularis* in the ileum and PCV-2 are shown in Table 1.

At slaughter, 10 out of 465 intestinal samples (2.15%) had thickening of the ileal wall. Subserosal and mesenteric edema was observed in other 10 cases (2.15%). Histopathological changes in 33 of the 66 intestinal samples collected were characterized by a marked proliferation of ileal crypt enterocytes, cellular debris and inflammatory cells in the crypt lumen and the lack of goblet cells in the overlayer monolayer. In addition, histiocytes and giant cells infiltration were observed in the lamina propria and Peyer´s patches (Figure 1 A and B). Furthermore, occasional lymphoid depletion, necrosis and neutrophil infiltration were observed in Peyer´s patches. Two cases (6.1%) were classified as PHE, seven cases (21.2%) as PIA grade 1, ten cases (30.3%) as PIA grade 2, and finally, fourteen cases (42.4%) as PIA grade 3. In addition, 16/66 (24.2%) cases of PPE showed only crypt epithelial hyperplasia and absence of goblet cells. Crypt epithelial proliferation and granulomatous inflammation were observed in 17 cases (25.7%). Granulomatous inflammation without PPE lesions was observed only in 3 cases (0.5%), and they were only positive to *L.intracellularis* by IHC. No histopathological changes were observed in the remaining 30 samples (45.5%). A statistically significant association (p=0.00049) was detected between PPE and granulomatous inflammation (Table 2).

Histopathological examination of the 46 mesenteric lymph nodes collected at slaughter revealed granulomatous inflammation with histiocytic and giant cells in six cases (13%) and mild lymphoid depletion was

detected in five (10.8%) samples. Hyperplastic changes were observed in 10 (21.7%) cases. No histopathological changes were observed in the remaining samples. No statistical association was detected between PPE ileum lesions and granulomatous lymphadenitis in mesenteric lymph nodes (p= 0.74). PCV-2 detection (p=0.57). *L. intracellularis* antigens were detected in the epithelial cells of the ileum and in the cytoplasm of mononuclear cells, including macrophages/histiocytes in the lamina propria as well as in histiocytic and multinucleated giant cells in the Peyer´s patches (Figure 1 C). PCV-2 antigens were occasionally observed in the cytoplasm of macrophages/histiocytic cells in the lamina propria, but none of them were observed associated with granulomatous inflammation in the lamina propria (Figure 1 D). A statistically significant relationship was detected (p=<0.006) between granulomatous inflammation and IHC detection of *L.intracellularis* (Table 3).

Table 2. Relationship between PPE and granulomatous inflammation, and IHC detection of *L. intracellularis* and granulomatous inflammation in pig ileum samples obtained at slaughter.

	Granulomatous +	Granulomatous -	Total
PPE +	17	16	33
PPE -	3	30	33
Total	20	46	66

p=0.00049

Table 3. Relationship between IHC detection of *L. intracellularis* and granulomatous inflammation in ileum samples obtained at slaughter.

	Granulomatous		Total
L.intracellularis	+	-	
+	20	32	52
-	0	14	14
Total	20	46	66

p<0.006

In mesenteric lymph nodes, *L. intracellularis* antigens alone were demonstrated in 22 samples (47.8%), whereas *L.intracellularis* PCV-2 antigens were detected in only four cases (8.6%). Fourteen (30.4%) samples were negative for both pathogens. There was no statistical association between immunohistochemical results and granulomatous inflammation in mesenteric lymph nodes (p=0.624).

All ileum samples (n=66) were negative for Mycobacterium spp or fungi by PAS and Ziehl Neelsen.

Discussion

Clinical cases of PPE are most commonly observed in pigs older than 6 weeks of age, whereas PHE is frequently reported in young adults from 4 to 12-month-old (20). In the present study, clinically affected pigs were 17.4 - week-old. The outbreak began as a PHE form followed by PIA cases that lasted 4 months. The occurrence of PIA in growing pigs after an outbreak of PHE in swine herds has been previously reported (8, 17, 24). Outbreaks of PHE are now very frequent in high health, multi-site farms. Clinical cases of PHE in market-aged pigs are not frequently reported (20) even though they happen very often. The influences of a multi-site production system, management situations and use of antibiotics have been suggested by serological status and infection patterns (5, 8). However, how these variables influence the clinical course or the pathological form of PPE is not completely known.

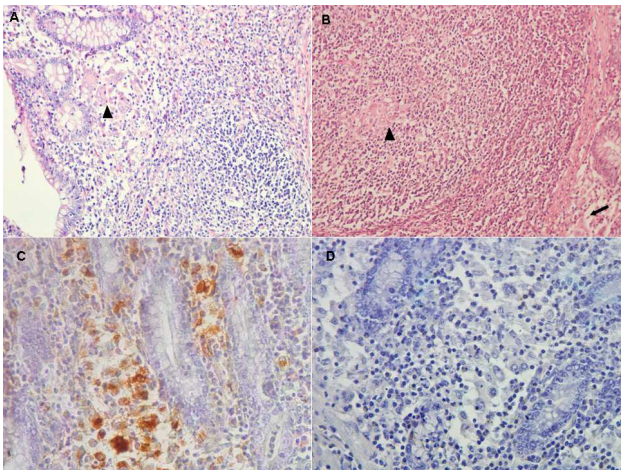

Figure 1: Granulomatous enteritis in pigs with proliferative enteropathy. A: Macrophages and multinucleated giant cells infiltration in the lamina propria (arrowhead). H&E stain, 20 x obj.; B: Focal granulomatous inflammatory infiltrate in Peyer´s patches (arrowhead). Moderate crypt epithelial hyperplasia and histiocytic cells infiltration in the lamina propria (arrow). H&E stain, 20 x obj.; C: Marked immunolabeling of L. intracellularis in macrophages and histiocytic cells in the lamina propria. LSAB, 40x obj.; D: Lack of PCV-2 detection in granulomatous infiltrates in the lamina propria. LSAB, 40x obj.

The percentage of PPE lesions reported at slaughter is low (0.7-2.0%) (13). In the present study, 4.3% of the ileum samples examined had PPE gross lesions. Previous studies reported that PPE lesions found at slaughter are more closely associated with PHE outbreaks (12, 23), corroborating the findings in the present study. Furthermore, in this study, 50% of the ileum samples had histopathological changes of PPE, 90% of them associated with chronic forms (PIA grade 1, 2 or 3). The high percentage of lesions found at slaughter suggests that either the *L. intracellularis* infection could be delayed to close-to-market aged pigs (23) or that the immunological response against the bacterium was not able to control efficiently the infection (1). In addition to this, 17/33 (51.5%) of the samples with PPE lesions had a granulomatous inflammation in the lamina propria and Peyer´s patches.

PCV-2 associated enteritis is an increasing diagnosis of PCV-AD (21). The presence of diarrhea and granulomatous enteritis with abundant PCV-2 DNA in Peyer´s patches are considered hallmarks of PCV-2

associated enteritis (15, 21). In the present study, PCV-2 antigens were not found in Peyer´s patches with granulomatous inflammation and only a few PCV-2 positive cells were observed in the lamina propria. Therefore, a diagnosis of PCV-2 associated enteritis might be ruled out.

Coinfection of *L. intracellularis* and PCV-2 as a cause of enteritis has been previously reported (7, 15, 22, 25, 27, 28). In a retrospective study, Jensen and others in 2006 (14), found 7.5% of samples positive for both agents and a statistical association between PCV-2 and *L. intracellularis* as a cause of enteritis. In the present study, there was no significant association between PCV-2 and *L. intracellularis* coinfection causing granulomatous enteritis, based on immunohistochemical results. These findings suggest that *L. intracellularis* may produce a granulomatous inflammation of Peyer´s patches in the absence of a PCV-2 infection (11, 14).

In the present study, granulomatous inflammation was observed in 30% (20/66) of the ileum samples taken at slaughter. Ziehl-Neelsen and PAS negative results significantly reduced the chances of mycobacterial or fungal involvement. Moreover, 100% of the intestines were IHC positive for *L. intracellularis* and a relationship ($p< 0.006$) was detected between *L. intracellularis* infection and granulomatous inflammation.

Although rare, granulomatous inflammation in Peyer´s patches and lamina propria with PPE lesions has been previously reported (14, 16, 25). However, in those studies only few cases were evaluated. In this study, multinucleated giant cells and histiocytic cells were IHC positive for *L. intracellularis* only.

In this study, about 50% of the lymph nodes and ileum samples were IHC positive for *L.intracellularis*. Detection of this bacterium in both the intestinal epithelial cells and mesenteric lymph nodes indicate an infection not older than 3-4 weeks (3). These results could indicate the persistence of *L. intracellularis* for a long period of time in some tissues and the appropriate chronic inflammatory response.

The presence of multinucleated giant cells, as a part of chronic inflammation, could be explained as a failure of the organism to degrade exogenous or endogenous antigens (1). Many species of intracellular bacteria avoid the damaging effects of phagolysosomal fusion, an activity which is associated with cytolysins or hemolysins (26). The hemolytic activity in *L. intracellularis* has been reported at least in vitro (26). In addition, a release of the macrophage chemoattractants that affect the host's ability to mount a cellular response by hyperplastic crypt cells has been suggested (18). These two different factors associated with granulomatous inflammation have been described in *L. intracellularis* and could be important in the development of granulomatous inflammation. The persistence of intracellular organisms could be caused by an inadequate Th-1 response or an exacerbated Th-2 response (1, 9, 26). However, the cytokines involved in the inflammatory response mounted against *L. intracellularis* infection require further investigation.

In conclusion, the epidemiological and pathological features observed in this study were distinctive from those previously reported for PPE and PCV-2 associated enteritis. In this study, a strong statistical association was detected between granulomatous enteritis and *L. intracellularis* infection. Thus, granulomatous enteritis could be considered as a part of the PPE complex. Further studies are needed to evaluate possible predisposing factors of the herd, differences in bacterial pathogenicity of *L. intracellularis*, and individual factors associated with this particular form of PPE.

Acknowledgements

We would like to thank Carolina Aralda and Lorena Diaz for their excellent technical help. This work was supported by grants from Fondo para la Investigación Científica y Tecnológica 05-33987, Secretaría de Ciencia y Técnica, Universidad Nacional de La Plata, and Cabaña Argentina SA, Argentina.

References

1. ACKERMANN MR. Chronic inflammation and wound healing. In: MD McGavin, JF Zachary, Eds. **Pathologic Basis of Veterinary Disease**, 4th ed., St. Louis, USA: Mosby Inc., 2007, 153-192.
2. ALLAN GM, ELLIS JA. Porcine circovirus: a review. **J. Vet. Diag. Invest.**, 2000, 12, 3-14.
3. BRANDT D, KAIM U, BAUMGÄRTNER W, WENDT M. Evaluation of Lawsonia intracellularis infection in a group of pigs in a subclinically affected herd from weaning to slaughter. **Vet. Microbiol.**, 2010, 146, 361-365.
4. CHAE C. A review of porcine circovirus 2-associated syndromes and diseases. **Vet. Journal**, 2005, 169, 326-336.
5. CHOUET S, PRIETO C, MIELI L, VEENHUIZEN MF, MCORIST S. Patterns of exposure to Lawsonia intracellularis . **Vet. Rec.**, 2003, 152, 14-17.
6. COOPER DM, GEBHART CJ. Comparative aspects of proliferative enteritis. **J. Am. Vet. Med. Assoc.**, 1998, 212, 1446-1451.
7. ELLIS J, CLARK E, HAINES D, WEST K, KRAKOWKA S, KENNEDY S, ALLAN GM. Porcine circovirus-2 and concurrent infections in the field. **Vet. Microbiol.**, 2004, 98, 159-163.
8. GUEDES RMC, GEBHART CJ, ARMBRUSTER GA, ROGGOW BD. Serologic follow-up of a repopulated swine herd after an outbreak of proliferative hemorrhagic enteropathy. **Can. J. Vet. Res.**, 2002, 66, 258-263.
9. GUEDES RMC, GEBHART CJ. Evidence of cell-mediated immune response and specific local mucosal immunogobulin (Ig) A production against Lawsonia intracellularis in experimental infected swine. **Can. J. Vet. Res.**, 2010, 74, 97-101.
10. GUEDES RMC, GEBHART CJ. Preparation and characterization of polyclonal and monoclonal antibodies

against Lawsonia intracellularis. **J. Vet. Diagn. Invest.**, 2003, 15, 438–446.

11. HARDING JCS. The clinical expression and emergence of porcine circovirus 2. **Vet. Microbiol.**, 2004; 98: 131-135.
12. HOLYOAKE PK, CUTLER RS, CAPLE IW. A diagnostic dilemma: detecting proliferative enteritis in pigs at slaughter. **Aust. Vet. J.**, 1994, 71, 308-309.
13. JENSEN TK, MOLLER K, CHRISTENSEN G, LESER TD, JORSAL SE. Monitoring ileitis and Lawsonia intracellularis in abattoir pigs. **Vet. Rec.**, 1999, 145, 613-615.
14. JENSEN TK, VIGRE H, SVENSMARK B, BILLE-HANSEN V. Distinction between porcine circovirus type 2 enteritis and porcine proliferative enteropathy caused by Lawsonia intracellularis. **J. Comp. Pathol.**, 2006, 135, 176-183.
15. KIM J, HA Y, JUNG K, CHOI C, CHAE C. Enteritis associated with porcine circovirus 2 in pigs. **Can. J. Vet. Res.**, 2004, 68, 218-221.
16. LOMAX LG, GLOCK RD. Naturally occurring porcine proliferative enteritis: pathologic and bacteriologic findings. **Am. J. Vet. Res.**, 1982, 43, 1608-1614.
17. LOVE RJ, LOVE DN, EDWARDS MJ. Proliferative haemorrhagic enteropathy in pigs. **Vet. Rec.**, 1977, 100, 65-68.
18. MacINTYRE N, SMITH DGE, SHAW DJ, THOMSON JR, RHIND SM. Immunopathogenesis of experimentally induced proliferative enteropathy in pigs. **Vet. Pathol.**, 2003; 40, 421-431.
19. McORIST S, GEBHART CJ. Porcine proliferative enteropathies. In: **Diseases of Swine**, 8th ed., BE Straw, S D´Allaire, W Mengeling, DJ Taylor, Eds., Iowa State University Press, Ames, 1999, 521-534.
20. McORIST S, GEBHART CJ. Proliferative enteropathies. In: **Diseases of Swine**, 9th ed., BE Straw, JJ Zimmerman, S D´Allaire, DJ Taylor, Eds., Blackwell Publishing, Oxford, 2006, 727-738.
21. OPRIESSING T, MENG XJ, HALBUR P. Porcine circovirus type 2-associated disease. Update on current terminology, clinical manifestations, pathogenesis, diagnosis and intervention strategies. **J. Vet. Diag. Invest.**, 2007, 19, 591-615.
22. OPRIESSNIG T, MADSON DM, ROOF M, LAYTON SM, RAMAMOORTHY S, MENG XJ, HALBUR PG. Experimental reproduction of porcine circovirus type 2 (PCV2)-associated enteritis in pigs infected with PCV2 alone or concurrently with Lawsonia intracellularis or Salmonella typhimurium. **J. Comp. Path.**, 2011, 145, 261-270.
23. POINTON AM, DAVIES PR, BAHNSON PB. Disease surveillance at slaughter. In: **Diseases of Swine**, 8th ed., BE Straw, S D´Allaire, W Mengeling, DJ Taylor, Eds., Iowa State University Press, Ames, 1999, 1111-1132.
24. ROWLAND AC, ROWNTREE PGM. A haemorrhagic bowel syndrome associated with intestinal adenomatosis in the pig. **Vet. Rec.**, 1972, 91, 235-241.
25. SEGALÉS J, FERNÁNDEZ-SALGUERO JM, FRUCTUOSO G, QUINTANA J, ROSELL C, POZO J, DE ARRIBA ML, RUBIO P, DOMINGO M. Granulomatous enteritis and lymphadenitis in Iberian pigs naturally infected with Lawsonia intracellularis . **Vet. Pathol.**, 2001, 38: 343-346.
26. SMITH DGE & LAWSON GHK Lawsonia intracellularis: getting inside the pathogenesis of proliferative enteropathy. **Vet. Microbiol.**, 2001, 82, 331-345.
27. WELLENBERG GJ, STOCKHOFE-ZURWIEDEN N, BOERSMA WJA, DE JONG, MF, ELBERS AR. The presence of co-infections in pigs with clinical signs of PMWS in The Netherlands: a case-control study. **Res. Vet. Sci.**, 2004, 77, 177-184.
28. ZLOTOWSKI P, CORREA AM, BARCELLOS DE, CRUZ CE, ASANOME W, FERNANDES BARRY A, AMAURI AA, DRIEMEIER D. Intestinal lesions in pigs with postweaning multisystemic wasting syndrome. **Pesq. Vet. Bras.**, 2008, 28, 313-318.