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PATOMORPHOLOGICAL DIAGNOSIS OF HEMORRHAGIC PROLIFERATIVE ENTEROPATHY IN SWINE*

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SUMMARY: In this paper seven samples of the intestines originated from the naturally infected pigs, macroscopic, histochemically and immunohistochemically were examined. The caudal part of jejunum and ileum were affected. The affected intestine was thickened and dilated. The lumen of the ileum and colon contained one or more blood clots without feed contents. The rectum contained black tarry feces of mixed blood and digesta. The mucosal surface of the affected portion of intestine was markedly thickened without macroscopic erosions. Histological examination revealed: extensive degeneration, congestion, and hemorrhage within the proliferative epitelium, as well as a marked accumulation of the bloody cellular debris above affected mucosa. Clusters of argyrophilic, slightly curved rod-shaped microorganisms in the apical cytoplasm of enterocytes by Warthin-Starry silver stain were demonstrated. Immunohistochemical staining confirmed the presence of L. intracellularis in the apical cytoplasm of hyperplastic enterocytes and in lamina propria. In conclusion, diagnosis of hemorrhagic proliferative enteropathy is based on detection of the histologic lesions and detection of L. intracellularis by Warthin Starry silver stain, as well as by immunohistochemistry, where immunohistochemistry and Warthin-Starry silver method can be a complementary methods to confirm the diagnosis of L. intracellularis infection in pigs.

Key words: *hemorrhagic proliferative enteropathy, swine, Lawsonia intracellularis.*

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INTRODUCTION

Porcine proliferative enteropathy (PPE) is the infectious, intestinal hyperplastic disease, characterized by thickening of the intestinal mucosa due to enterocyte proliferation. The disease affects weaned pigs, primarily growing and fattening. Porcine proliferative enteropathy is worldwide in distribution and occurs commonly in all pigs-rising regions and in all pig farm managment (McOrist et al., 2003). The incidence of lesions in pigs at normal slaughter age is generally low, at 0.7-2.0%, and therefore unreliable for farm monitoring (Jensen et al., 1999).

The etiological agent is the obligatory intracellular bacterium *Lawsonia intracellularis*, which preferentially grows within the cytoplasm of intestinal epithelial cells. *L. intracellularis* forms curved to straight vibrioid-shaped rods and measure 1.25-1.75 μm in length by 0.25-0.43 μm in width (Dale et al., 1998). From epizootiological point of view it is important that *L. intracellularis* can remain viable in feces at 5 to 15°C for 2 weeks (Collins et al., 2000). It is known from literature that infectious dose is relatively low and fecal excretion may be high in some infected "spreader" pigs (McOrist et al., 1993; Guedes et al., 2003). There are two forms of the PPE: clinical and subclinical. Clinical forms can be acute and chronic. Acute clinical form is hemorrhagic proliferative enteropathy. Chronic forms are: porcine intestinal adenomatosis, necrotic enteritis and regional ileitis. In each of these states, the characteristic lesion is present: thickened mucosa of the small intestine (mainly the ileal one), the caecum and/or the proximal colon. The main histological lesion of the PPE is the adenomatous hyperplasia of the intestinal crypt cells due to proliferation of the immature epithelial cells and almost lack of goblet cells. In most cases, no significant inflammatory reaction occurs and the organisms remain in the epithelium at this stage (Yates et al., 1979; Ivetić et al., 2006; McOrist and Gebhart, 2006; Ivetić et al., 2009). In severe cases of PPE, *L. intracellularis* can also be observed in the mesenteric lymph node and tonsils (McOrist and Gebhart, 2006). Because of the difficulty of culturing *L. intracellularis*, it has been necessary to develop alternative methods for its detection. Confirmation of clinical diagnosis may be obtained by demonstration of *L. intracellularis* in feces and intestinal tissue by PCR or by indirect serologic assays for detection of antibodies (Nathues, 2007). At necropsy, the use of modified Ziehl-Neelsen stain or the Giminez stain on mucosal smears to demostrate the intracellular organisms is a simple presumptive technique, requiring minimal time and equipment (Love et al., 1977). Histopahological examination of affected tissues will reveal the distinctive morphology of the proliferative lesions. Specific identification of *L. intracellularis* in these lesions can be achieved by immunohistochemical staining of fixed embedded tissues (Ladinig et al., 2009). In the absence of specific immunological reagents, modifications of the Warthin-Starry silver impregnation technique are satisfactory for routine use (Young, 1969). The affected crypts need to be examined carefully at high magnifications due to the small size of *L. intracellularis.*

In this work patomorpholological diagnosis included macroscopic examination and using histochemical and immunohistochemical methods for diagnose of this infection.

MATERIAL AND METHODS

Intestine samples of seven growing pigs from two herds, on which necropsies were performed at Department of Pathology of the Institute of Veterinary Medicine of Serbia, were examined. Samples of the distal ileum originated from the infected pigs, macroscopic, histochemically and immunohistochemically were examined.

Samples of distal ileum were fixed in 10% buffered formalin, and after standard processing cast in paraffin blocks. Paraffin sections 3-5 μm thick were stained with hematoxylin and eosin (HE) and with Warthin-Starry silver stain for light microscopic examination. Three-step indirect immunohistochemical technique was performed at Veterinary Diagnostic Laboratory at Iowa State University, Ames, USA. After antigen retrieval and inactivation of endogenous peroxidase, the sections were incubated with primary non commercially monoclonal antibody against *Lawsonia intracellularis* diluted in PBS. All rinsing procedures and serum dilutions were done in PBS (pH 7.2). The detection kit was LSAB2 System-HRP, Rabbit/mouse (DAKO, K0675). Reactions were visualized by using DAB+ (Dako, K3468) and counterstaining with hematoxylin. Intestine sections of infected pigs were used as positive controls. Intestine section not treated with the primary antibody were used as negative controls.

RESULTS AND DISCUSSION

Macroscopic examination revealed that the small intestine contained bile-stained mucus to the level of mid-jejunum where hemorrhage began and became gradually more copious with large blood clots. Caudal part of jejunum and ileum were affected. The affected intestine was thickened and dilated. The lumen of the ileum and colon contained one or more blood clots without feed contents (Figure 1). The rectum contained black tarry feces of mixed blood and digesta. The mucosal surface of the affected portion of intestine was markedly thickened without macroscopic erosions. Literature data shown the same macroscopic finding (McOrist and Gebhart, 2006). In this case there were no lesions in large intestine.

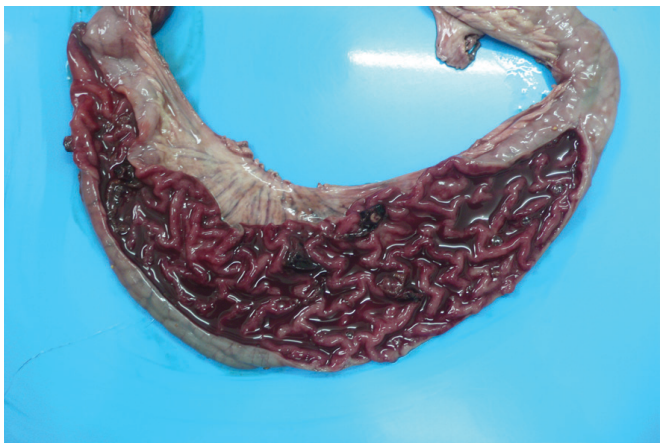

Figure 1. Swine ileum, thickened mucosa, hemorrhages and blood clots in the lumen
Slika 1. Ileum svinje, sluznica je zadebljala, krvavljenje i krvni ugrušci u lumenu

Histological examination revealed: extensive degeneration, congestion, and hemorrhage within the proliferative epitelium, as well as, a marked accumulation of the

bloody cellular debris above affected mucosa. In the cranial part of jejunum and in ileum there was flattening of villous architecture. The mucosa was markedly thickened by irregular hyperplastic crypts in which there was piling up of young epithelial cells, a high mitotic index, and a lack of goblet cells (Figure 2). Many crypts contained copious cellular debris and neutrophils and there was infiltration of the lamina propria by a mixed population of cells, including macrophages, lymphocytes, eosinophils, plasma cells and neutrophils. As well known, with severe disease, bacteria are present in macrophages in lamina propria. This may cause release of tumor necrosis factor-α, resulting in vascular permeability and hemorrhage (McGavin and Zachary, 2007). It is also known from literature that described lesions are characteristic for *Lawsonia intracellularis* infection in swine (Ivetić et al., 2006; McOrist and Gebhart, 2006; Ivetić et al., 2009).

Clusters of argyrophilic, slightly curved rod-shaped microorganisms in the apical cytoplasm of enterocytes by Warthin-Starry silver stain were demonstrated (Figure 3). Modifications of the Warthin-Starry silver impregnation technique are satisfactory for routine use (Young, 1969). However, this method is not specific for *L. intracellularis* and cannot always detect the organism in necrotic debris or in autolyzed tissue. More specific identification of *L. intracellularis* can be achieved by immunohistochemistry staining of fixed tissues. Immunohistochemical staining confirmed the presence of *L. intracellularis* in the apical cytoplasm of hyperplastic enterocytes and in lamina propria (Figure 4). This observation suggests that bacteria were mainly phagocytized and lysed within the cytoplasm of macrophages. It is known from literature that this technique is more sensitive than the silver stain because it reveals organisms within macrophages of the lamina propria during recovery from PPE (Ladinig et al., 2009). In addition, extracellular *L. intracellularis* can be identified either in exudate or necrotic debris in superficial mucosa. In a study comparing diagnostic methods, immunohistochemistry staining detected nearly twice as many pigs PPE lesions as did silver staining of formalinized tissues. The most sensitive tests for diagnosing PPE are mucosal PCR and tissue immunohistochemistry, but both require necropsy to perform. The antemortem methods are less accurate, with serology being most sensitive (Ladinig et al., 2009).

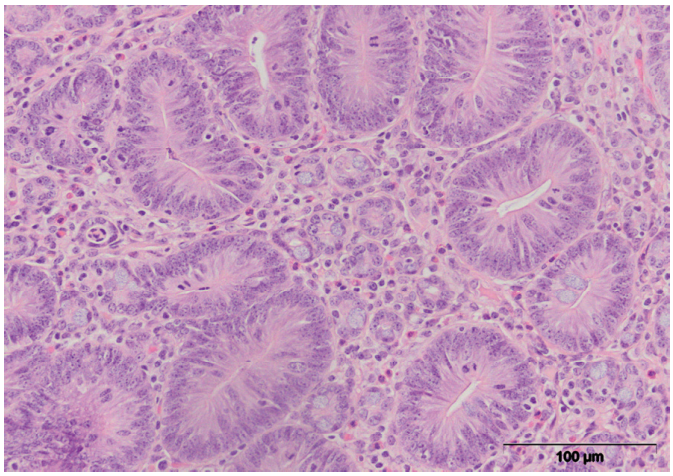

Figure 2. Swine ileum, hyperplastic crypts in which there is piling up of young epithelial cells, a high mitotic index, and a lack of goblet cells, HE stain

Slika 2. Ileum svinje, hiperplastične kripte sa umnoženim mladim epitelnim ćelijama, visokim mitotskim indeksom i nedostatkom peharastih ćelija, HE bojenje

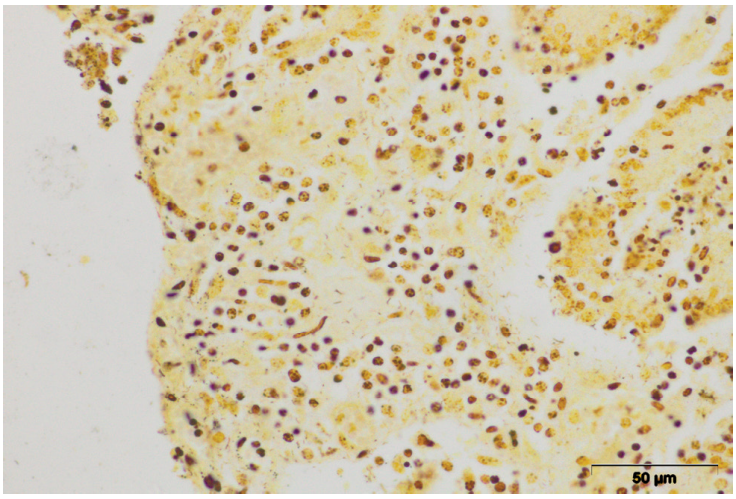

Figure 3. Swine ileum, argyrophilic, slightly curved rod-shaped microorganisms within epithelial cells, Warthin-Starry silver stain

Slika 3. Ileum svinje, argirofilni, blago savijeni štapićasti mikroorganizmi u epitelnim ćelijama, Warthin-Starry bojenje

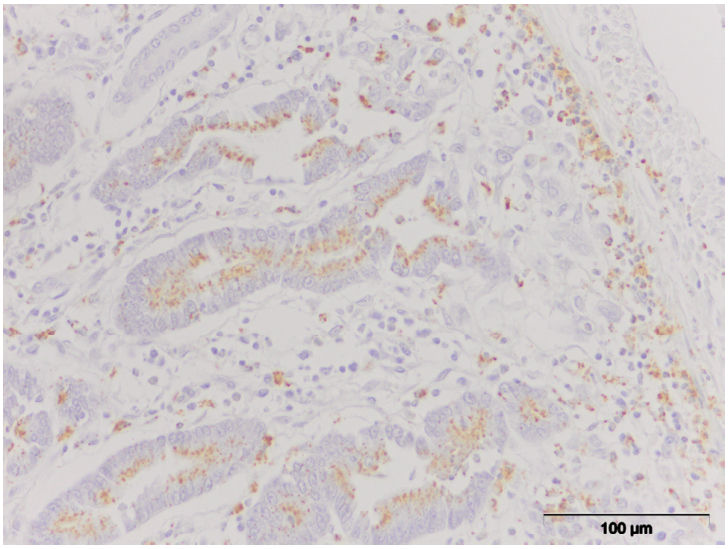

Figure 4. Swine ileum, clusters of immunopositive intracellular microorganisms (brown) are seen in the apical cytoplasm of hyperplastic epithelial cells and in lamina propria, Immunohistochemistry, LSAB2

Slika 4. Ileum svinje, grupe imunopozitivnih intracelularnih mikroorganizama (smeđe) se uočavaju u apikalnoj citoplazmi hiperplastičnih epitelnih ćelija i u lamini propriji, Imunohistohemija, LSAB2

CONCLUSION

In conclusion, diagnosis of hemorrhagic proliferative enteropathy is based on detection of the histologic lesions and detection of *L. intracellularis* by Warthin Starry silver stain, as well as by immunohistochemistry, where immunohistochemistry and Warthin-Starry silver method can be a complementary methods to confirm the diagno-

sis of *L. intracellularis* infection in pigs. The "gold standard" for the histopathological diagnosis of *L. Intracellularis* infection is immunohistochemical examination, however, due to restricted availability of polyclonal antiserum and monoclonal antibodies, not all diagnostic laboratories can perform this test.

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PATOMORFOLOŠKA DIJAGNOSTIKA HEMORAGIČNE PROLIFERATIVNE ENTEROPATIJE SVINJA

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Izvod

U ovom radu je makroskopski, histohemijski i imunohistohemijski ispitano 7 uzoraka creva prirodno inficiranih svinja. Patomorfološke promene u vidu zadebljanja crevnog zida i proširenog lumena su ustanovljene na kaudalnom delu jejunuma i ileumu. U lumenu ileuma i kolona su uočeni ugrušci krvi bez prisustva hrane. U rektumu je ustanovljen katranasti feces koji potiče od svarene krvi i crevnog sadržaja. Površina sluznice zahvaćenog dela creva je pokazivala značajno hiperplastično zadebljanje. Erozije sluznice nisu ustanovljene. Histološkim ispitivanjem u proliferisanom epitelu uočena je degeneracija epitelnih ćelija, kao i kongestija i obimna krvavljenja. Pored toga, zapaženo je i nakupljanje hemoragičnog ćelijskog debrisa iznad površine sluznice. Warthin-Starry bojenjem ustanovljene su grupe argirofilnih, blago savijenih štapićastih mikroorganizama u apikalnoj citoplazmi enterocita. Imunohistohemijskim ispitivanjem upotrebom monoklonskog antitela protiv *L. intracellularis* potvrđeno je prisustvo mikroorganizama u apikalnoj citoplazmi hiperplastičnih enterocita i u lamini propriji. Dijagnoza hemoragične proliferativne enteropatije je postavljena na osnovu karakterističnih histoloških lezija i korišćenjem Warthin-Starry bojenja, kao i upotrebom imunohistohemijske metode, korišćenjem monoklonskog antitela protiv *L. intracellularis*. Imunohistohemijska metoda i Warthin-Starry metod bojenja mogu biti komplementarne metode za potvrdu infekcije izazvane sa *L. intracellularis* kod svinja.

Ključne reči: hemoragična proliferativna enteropatija, svinja, *Lawsonia intracellularis*.

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