An Episode of Proliferative Hemorrhagic Enteropathy
Associated with Lawsonia Intracellularis in a Pig Farm from Romania

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Abstract. In swine, Lawsonia intracellularis is known to be responsible for porcine proliferative enteropathy. The syndrome can be divided in an acute intestinal hemorrhage (proliferative hemorrhagic enteropathy) affecting naïve adult pigs and a wasting disease (porcine intestinal adenomatosis) in growing pigs. As new diagnostic techniques develop, there is increased number of pig farms where L. intracellularis is being identified worldwide. There are few reports of L. intracellularis outbreaks in Romania. We aimed to describe the clinical signs, treatment, outcome, gross necropsy and histopathological lesions from an episode of proliferative hemorrhagic enteropathy associated with L. intracellularis in a farm from Transylvania. A farm of 4000 pigs (TOPIGS line) from Bistrita-Nasaud County. The microscopical examination was composed of Hematoxilin and Eosin exam, Warthin-Starry silver stain and immunohistochemistry for Multi-Cytokeratin. Over a period of 6 days, a total number of 10 pigs died (from 2000 animals in the age group 90-120 days), and 1 pig died (from 1000 animals in the age group 60-90 days) with pallor, anorexia or with no clinical signs. Gross lesions were represented by pallor of the carcass and were restricted to the ileum. The intestinal wall was thicker (cerebriform aspect) and turgid. A mixture of blood and fibrin was present in the ileum, impregnating the faeces in the large intestine. Histologically the mucosa was thicker due to epithelial proliferation (Multi-Cytokeratin positive), with few Goblet cells, with severe erosion, necrosis and haemorrhage. Curved rod-shaped bacteria with morphology consistent with L. intracellularis were observed in the apical cytoplasm of epithelial cells using the Warthin-Starry silver stain. The food was medicated with chlortetracycline (20 - 40 mg a.s./kg b.w./day) and the pigs remained healthy, with no further mortality. We report here an outbreak of proliferative hemorrhagic enteropathy associated with L. intracellularis in a pig farm from Romania, emphasizing the importance of early diagnostic and control measures for this disease.

Keywords. Transylvania, pig production, ileitis, Warthin-Starry silver stain

INTRODUCTION

Proliferative hemorrhagic enteropathy (PHE) was classified as a distinct syndrome of porcine intestinal adenomatosis complex in swine. Currently, porcine proliferative enteropathy (PPE), a condition which is caused by Lawsonia intracellularis groups together the different syndromes (porcine intestinal adenomatosis, necrotic enteritis, regional ileitis, and proliferative hemorrhagic enteropathy) that were previously referred to as intestinal adenomatosis complex (Brown et al., 2007). PHE affects mainly naïve adult pigs while porcine intestinal adenomatosis is seen in growing pigs (Yeh et al., 2011).

Lawsonia is prevalent in pig farms all-over the world, and, although it may infect other species as well, it is in swine that the infection is most important. Following an initial infection, the animals can shed the organism for weeks ((Brown et al., 2007).). However, there is only one report about the incidence of Lawsonia intracellularis in swine farms from Romania, where the authors report that in 10 out of 12 samples they have found positive samples, with almost 50% of the analyzed samples being serologically positive (Costinar et al., 2008).
As new diagnostic techniques develop, there is increased number of pig farms where *L. intracellularis* is being identified worldwide. As there are few reports of *L. intracellularis* outbreaks in Romani, we aimed to describe the clinical signs, treatment, outcome, gross necropsy and histopathological lesions from an episode of proliferative hemorrhagic enteropathy associated with *L. intracellularis* in a pig farm from Transylvania.

**MATERIALS AND METHODS**

The biological material was selected from a mixed farm (breeding and meat production) composed of approximately 4000 pigs (TOPIGS line) from Bistrita-Nasaud County. The diseased animals were observed and clinical data was recorded. The necropsy exam of the dead pigs was performed in the farm by the farm veterinarian, and a selection of organs with lesions were quickly transported to the Pathology Department of the Faculty of Veterinary Medicine, Cluj-Napoca., there, following description of lesions, the organs were immediately immersed in 10% buffered formalin (Chempur, Poland) for fixation, for at least 24 hours and labelled accordingly. Microscopical examination from the ileum was performed on sections prepared by embedding the tissues in paraffin according to standard histological techniques. Briefly, as previously described in our department, we used a standardized lab protocol. Following fixation, intestinal biopsies were dehydrated in ascending concentration of ethanol (70%, 95%, and 100%), cleared in xylene and embedded in paraffin (Histowax, Histo-Lab. Ltd, Gothenburg, Sweden). Serial sections of 5 μm were cut and processed for standard hematoxylin and eosin staining (H&E).

As a special stain to demonstrate the presence of *Lawsonia spp.* in the lesions, we used the Warthin-Starry silver method. Moreover, to demonstrate the proliferation of intestinal glandular epithelium we used the immunohistochemical (IHC) method, with a Multi-Cytokeratin ready-to-use antibody (AE1, AE3, Leica Biosystems). Automated IHC was performed using the Bond-max system (Leica Microsystems), an automated machine which is able to process up to 30 slides at a time. Slides carrying tissue sections cut from formalin-fixed, paraffin-embedded tissue blocks following previously H&E evaluation were labelled and dried for 1 hour at 60°C. They were then covered by Bond Universal Covertiles (Leica Microsystems) and placed into the Bond-max instrument. All subsequent steps were performed by the automated instrument according to the manufacturer’s instructions (Leica Microsystems).

**RESULTS AND DISCUSSIONS**

In a pig farm composed of approximately 4000 pigs of different ages, over a period of 6 days, a total number of 10 pigs dyed (from 2000 animals in the age group 90-120 days), and 1 pig dyed (from 1000 animals in the age group 60-90 days) with pallor, anorexia or with no clinical signs. Necropsy of the dead pigs was performed at the farm and gross lesions were represented by pallor of the carcass and intestinal lesions, restricted to the ileum. In all animals, the intestinal wall was thicker (cerebriform aspect) and turgid (figure 1A). A mixture of blood and fibrin was present in the ileum, impregnating the faeces in the large intestine. The thicker aspect of the intestine was observed also post formalin fixation (figure 1B).

Histological exam of the ileum sections revealed that there were severe, diffuse areas of erosion and necrosis of the intestinal mucosa. In the lumen, we noticed the presence of a fibrinous-hemorrhagic exudate. A striking feature was represented by massive hemorrhage, as a consequence of increased vascular permeability due to tumor necrosis factor-α release by the macrophages in the lamina propria (Gelberg, 2007). The hemorrhage involved the mucosa and lamina propria, with edema in the submucosa and reduction in number of the mucus
secreting cells (Goblet cells). There was mildly hyperplasia of intestinal crypts with cellular debris and neutrophils. In the lamina propria there was a mixed inflammatory exudate composed of lymphocytes, plasma cells and neutrophils (figure 1C and 1D).

Fig. 1 Proliferative hemorrhagic enteropathy. A: Gross lesions, unfixed specimen at the level of distal ileum with turgid aspect; B: Formalin fixed specimen, distal ileum, thickened (cerebriform) aspect of the intestine; C and D: Terminal ileum: superficial mucosal necrosis, hemorrhagic and fibrinous effusions, mildly hyperplastic crypts with cellular debris and neutrophils; lamina propria: mixed inflammatory exudate with lymphocytes, plasma cells and neutrophils. E and F: Warthin-Starry stain of curved Lawsonia spp. bacteria located in the apical cytoplasm of proliferating enterocytes.
The histochemical stain revealed curved rod-shaped bacteria with morphology consistent with *L. Intracellularis* in the apical cytoplasm of intestinal gland epithelial cells (figure 1E and 1F). There was multifocal proliferation of epithelial cells, confirmed also by immunostaining for Multi-Cytokeratin. The areas with proliferating epithelial cells had an increased mitotic index. The Peyer’s patches were hypertrophied with lymphocyte migration.

Proliferative hemorrhagic enteropathy is a sporadic disease, with low morbidity but mortality that can go up to 50% of affected animals (Brown et al., 2007). In our case, for the rest of the affected age groups from the same shelter, the food was medicated with chlortetracycline (20 - 40 mg a.s./kg b.w./day) and the pigs remained healthy, with no further mortality.

CONCLUSIONS

We report here an outbreak of proliferative hemorrhagic enteropathy associated with *L. intracellularis* in a pig farm from Romania, emphasizing the importance of early diagnostic and control measures for this disease.

REFERENCES


