

THE MORPHOPATHOLOGIC STUDY OF PROLIFERATIVE ENTEROPATHIES, INTESTINAL ADENOMATOSIS COMPLEX, IN SWINES

STUDIUL MORFOPATOLOGIC AL ENTEROPATIILOR PROLIFERATIVE, COMPLEXUL ADENOMATOZEI INTESTINALE, LA SUINE

A. OLARIU-JURCA^{1),*}, Iasmina LUCA¹⁾,
M. MAREK¹⁾, K. IMRE¹⁾,
Mihaela Marigena MOLDOVAN²⁾,
Liliana Elena TESLICI¹⁾

ABSTRACT | REZUMAT

The study was conducted between January 2016 and April 2019, at the Discipline of Forensic Medicine and Necropsy Diagnosis of F.V.M. Timisoara. A total of 188 pigs, with the age between 6 and 20 weeks, were necropsied. The carcasses came from intensive growth systems. The necropsy was performed in order to determine the cause of death. Only 62 pigs showed lesions of proliferative ileo-typhlocolitis.

After evisceration, a macroscopic examination of the organs was performed. The histopathological examination was made after taking samples from the stomach, ileum, ileocecal valve, the first part of the colon, the mesenteric (ileocolic) lymph nodes, spleen, caecum and colon. The samples were fixed in a 10% formaldehyde solution and processed using the paraffin method, stained by the Masson trichrome method (H.E.A.). The microscopic examination was performed using an Olympus Cx41 microscope, with increasing objectives (6x, 10x, 20x, 40x). The most significant lesions were microphotographed.

For bacteriological examination, in order to exclude swine salmonellosis, samples from the stomach, caecum, colon, liver, heart and long bone were taken and sent to the Discipline of Infectious Diseases. The bacteriological exams were negative for infection with *Salmonella spp.* To differentiate from *Brachyspira hyodysenteriae* infection, it was taken into account that morphopathological changes in swine dysentery are mainly confined to the check and colon, covering the appearance of haemorrhagic inflammation in acute evolution, or haemorrhagic-diphtheroid in subacute-chronic evolution. Cecocolic lesions are reflected throughout the body, leaving fingerprints in most organs, but especially in the liver, kidneys and heart. These aspects determined the widening of the sphere of morphopathological investigations in order to record the structural alterations in the dynamics, both at

Studiul a fost efectuat în perioada ianuarie 2016 - aprilie 2019, la Disciplina de Medicină Legală și Diagnostic Necropsic a Facultății de Medicină Veterinară din Timișoara. Au fost necropsiate 188 de cadavre (suine), cu vârste cuprinse între 6 - 20 săptămâni. Acestea au provenit din sisteme intensive de creștere. Examinarea cadavrelor a fost efectuată în vederea elucidării cauzei morții. Doar 62 de suine au prezentat leziuni de ileo-tiflocolită proliferativă. După eviscerare s-a efectuat examinarea macroscopică amănunțită a organelor luate în studiu. Din stomac, ileon, valvula ileocecală, prima porțiune a colonului, limfonodurile mezenterice (ileocolice) și splină, cec, colon, am prelevat probe pentru examen histopatologic.

Probele au fost fixate în soluție de formaldehidă 10%, prelucrate prin metoda la parafină, colorate prin metoda tricromică Masson (H.E.A.). Preparatele histopatologice obținute au fost examinate la un microscop Olympus Cx41, cu obiective crescânde (x6, x10, x20, x40), leziunile cele mai semnificative au fost microfotografiate. În vederea excluderii salmonelozei porcine, au fost recoltate probe, pentru examenul bacteriologic din stomac, cec, colon, ficat, cord și os lung. Examenul bacteriologic a fost efectuat la Disciplina de boli infecțioase, examenul a fost negativ pentru infecția cu *Salmonella spp.* Pentru diferențierea de infecția cu *Brachyspira hyodysenteriae* s-a avut în vedere faptul că modificările morfopatologice în dizenterie la suine sunt cantonate cu precădere în cec și colon, îmbrăcând aspectul inflamației hemoragice în evoluția acută, sau hemoragico-difteroidă în evoluția subacută- cronică. Leziunile cecocolice din dizenterie au fost reflectate în tot organismul lăsând, amprente în majoritatea organelor, dar cu precădere în ficat, rinichi și cord. Aceste aspecte au determinat lărgirea sferei investigațiilor morfopatologice pentru a consemna alterările structurale în dinamică, atât la locul de colonizare și internalizare a brachispirelor, cât și a unor organe cu importanță vitală, în vederea stabilirii diagnosticului morfopatologic de dizenterie suină.

Cuvinte cheie: enteropatii proliferative,
complexul adenomatozei intestinale,
Lawsonia intracellularis

1) Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania”
Faculty of Veterinary Medicine of Timișoara, Romania

2) Laborator Anatomopatologic Dr. Moldovan Mihaela-Marigena,
Timișoara, Romania

*) Corresponding author: olariujurca_adrian@yahoo.com

the place of colonization and internalization of the *B. hyodysenteriae*, and of some organs of vital importance, in order to establish the morphopathological diagnosis of swine dysentery.

Keywords: proliferative enteropathies, intestinal adenomatosis complex, *Lawsonia intracellularis*

The proliferative enteropathies (intestinal adenomatosis complex) caused by *Lawsonia intracellularis* include a series of morphoclinical manifestations reported in pigs, characterized by more or less severe inflammation of the ileum, caecum and first portion of the colon. The common morphopathological change in these segments is hyperplasia of the mucosal and glandular epithelium. These enteropathies are found in all categories of pigs, most commonly in piglets in their first weeks after weaning, up until the age of 24 weeks, or even up to the age of 38 weeks, according to other authors (3, 10, 12, 13, 14).

Porcine proliferative enteritis (PPE) was first observed in 1931 in American slaughterhouses. The disease was later reported and studied in several countries on five continents. In Romania, proliferative enteritis was identified by Caraivan et al. (1988) (13).

Currently, the etiological agent of proliferative enteritis is considered to be an ileum symbiont, a strictly intracellular bacteria, morphologically similar to *Campylobacter* spp. isolated by Lawson et al. (1975). *Lawsonia intracellularis* was approved as a new species of the new genus *Lawsonia*, proposed by McOrist et al., in 1995 (7). Studies conducted by McOrist et al. (1993) on some enteroviruses and rotaviruses (viruses normally present in swine intestines), isolated from pig lesions, presumed to be involved in the proliferative enteropathy, have shown that these agents have no etiological significance (11, 12, 13, 14, 15).

The proliferative enteritis evolves as a gastrointestinal syndrome with different stages of severity, depending on the lesions' type and extension (1, 2, 10). Changes in the lining of the ileum (regional ileitis) are often accompanied by numerous perforations of the hypertrophic ileal wall, which causes the development of generalized peritonitis, with a fatal outcome (1, 5, 6, 7, 10). Advanced intestinal adenomatosis, in the form of proliferative haemorrhagic enteropathy (PHE), is found in young pigs in fatteners and it is clinically manifested as acute haemorrhagic anaemia. Sometimes the animals die without digestive disorders and only a paleness of the skin and mucous membranes can be observed. Abortion can be present in pregnant sows with clinical signs of this disease. In general, animals with clinical manifestations of uncomplicated proliferative enteritis heal in about 6 weeks (4, 11, 12, 13, 14).

MATERIALS AND METHODS

The necropsy was performed using specific swine techniques (1,8,9). After evisceration, a macroscopic examination of the organs was performed. The histopathological examination was made after taking samples from the stomach, ileum, ileocecal valve, the first part of the colon, the mesenteric (ileocolic) lymph nodes, spleen, caecum and colon. This examination was meant to show the modification in structural features (shape, size, colour, appearance, lobulation, consistency and examination per section). The samples were fixed in 10% formaldehyde solution for 24 hours, they were modelled and reintroduced into a new formaldehyde bath for final fixation for two days. The fixed samples were passed into the dehydration battery, consisting of alcohol in increasing concentrations, from 70 degrees to absolute alcohol. In each of the 5 baths, the samples were kept for 2 hours, after which the alcohol was removed by introduction into benzene to clarify the section, then the paraffin bath was placed in a thermostat at 56°C. By paraffining, blocks were obtained containing samples (fragments) of injured organs, which were sectioned at the microtome at 6 µm. The obtained sections were fixed on well-degreased slides with Mayer albumin. The sections glued to the slides were stained by the Masson trichomic method (H.E.A.) modified by V. Ciurea with methyl blue for overall examination and the Giemsa method for cellular details (8).

For bacteriological examination, in order to exclude salmonellosis, samples from the stomach, caecum, colon, liver, heart and long bone were taken and sent to the Discipline of Infectious Diseases.

RESULTS AND DISCUSSIONS

External examination. Most of the swine had a poor or mediocre state of maintenance, being weak, cachectic and anaemic (Fig. 1). The skin was dehydrated, covered with large hair (hypertrichosis) and without luster. The eyes were clogged in their orbits. The conjunctiva and sclera were grey-whitish, dull or anaemic. In some cases, grey-brown faces were observed in the posterior part of the body.

Internal examination. In the thoracic and abdominal cavities, a citrine or reddish fluid, in varying a-

mounts (10-60 ml), was observed.



Fig. 1. Pig carcass: poor state of maintenance; cachectic; dehydrated skin covered with large hair (hypertrichosis) and without luster; eyes clogged in their orbits

After stomach and intestine dissection, the gastrointestinal content was removed. The mucosa and the intestinal segment had variable colours, from light pink to red and different shades, uniform or in outbreaks. Small ulcers were present, in some cases.

The lung had a normal appearance or congestion, with areas of oedema and bronchopneumonia present. These nonspecific lesions are a result of the combined action of various saprophytic germs that enhance their virulence due to decreased resistance of the body as a consequence of inadequate microclimate conditions.

After inspection and dissection, large organs, such as the heart, the liver and the kidneys, had a lighter shade, with brownish-yellow or grey, yellowish colour and low consistency. Either matte or glossy appearance was observed.

All these physical-structural features suggest the presence of protide-lipid dystrophies.

The most relevant lesions for establishing the presumptive diagnosis of proliferative enteropathies were reported mainly in the ileum and to a small extent in the ileocecal valve and the first part of the colon.

The terminal portion of the small intestine, the ileocecal valve and the first portion of the colon on inspection and after dissection, had thickened walls, increased consistency and narrowed lumen. A pale pink or red mucosa with various shades was identified. A cerebriform appearance was present.

The macroscopic ileum had a thickened, swollen wall, more or less oedematous and a narrow lumen.

The mucosa, in some cases, presented red colour and different shades, uniform or in outbreaks. In other cases, a pink-grey colour with irregular surface and cerebriform appearance (hyperplastic ileitis) were observed (Fig. 2).



Fig. 2. Hyperplastic ileitis: irregular surface, cerebriform appearance and pink-grey colour

In 21 cases, oedema and thickening of the mucosa and intestinal wall were observed in the terminal part of the ileum, at the level of the ileocecal valve and in the first part of the colon, having a cerebriform appearance - hyperplastic ileo-typhlocolitis.

Microscopically (100x, 200x, 400x objectives), the ileum showed an accentuated proliferation of the granulation tissue in the lamina propria of the mucosa, covering the appearance of cell piles (Fig. 3).

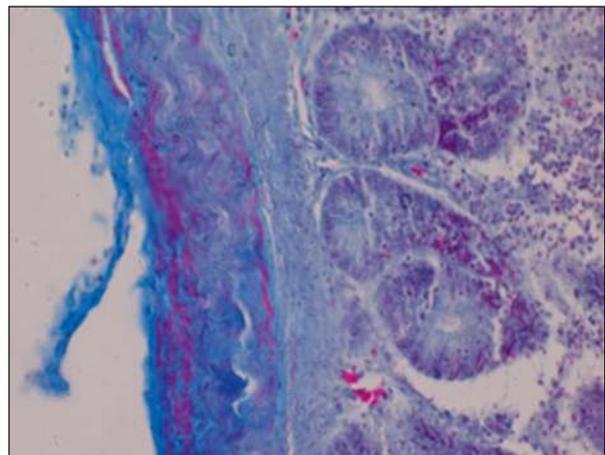


Fig. 3. Hyperplastic ileitis: granulation tissue and haemorrhagic infiltrate in the lamina propria and muscularis mucosae; massive submucosal and muscular fibrosis (muscular hypertrophy). Col. HEA 400x

In 18 cases, the microscopical findings were as follows: cells hyperplasia from villi epithelium which became bistratified or tristratified after cylindrical epithelium transformation into pseudostratified columnar; muscular hypertrophy of the ileum wall - hyperplastic ileitis; the intestinal glands became elongated, branched, having a papillomatous or pseudoadenomatous appearance - adenomatous ileitis (Fig. 4).

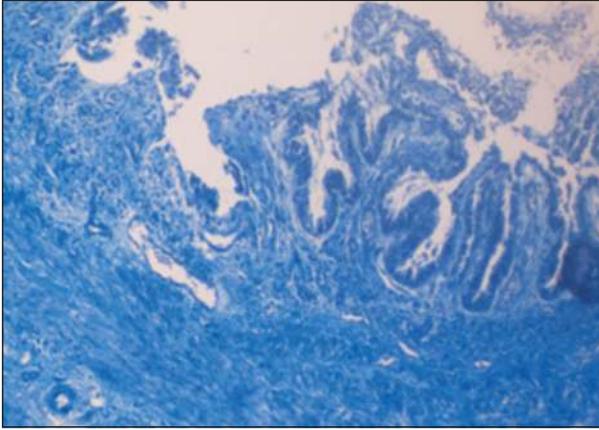


Fig. 4. Hyperplastic ileitis: cells hyperplasia villi epithelium and from the intestinal glands' epithelium; elongated and branched intestinal glands with papillomatous appearance. Col. HEA 400x

Based on this aspect, the disease has been called intestinal adenomatosis, which is an inflammatory process and not a tumour. According to Mc Orist et al. (1992), cited by Paul (2005), the presence of massive lymphocytic infiltrates in the lamina propria of the mucosa, certifies the inflammatory nature of the pathological process (10). Macroscopically, in 12 cases, the ileum wall was thickened, turgid red and with a weak cerebriform appearance, visible through the transverse serosa. The lumen contents had a red-blackish colour, with a repulsive smell (melena - haemorrhagic proliferative ileitis/proliferative haemorrhagic enteropathy (PHE) (Fig. 5).



Fig. 5. Haemorrhagic proliferative ileitis - the ileum wall was thickened, red turgid

Microscopically, degenerative processes (in different stages) covering the epithelium were found; glandular pseudoadenomatous hyperplasia and areas of mucosa with necrosis coagulation; lymphocytic infiltrations and haemorrhagic exudates in the mucosa and submucosa (Fig. 6).

These morphological changes certify the presence of proliferative haemorrhagic enteropathy (PHE),

which, according to Barker and Van Drumel (1985), quoted by Paul (2005), would be the consequence of the hypersensitivity reaction to intracellular bacterial antigens, released after cell degeneration or phagocytosis (10).

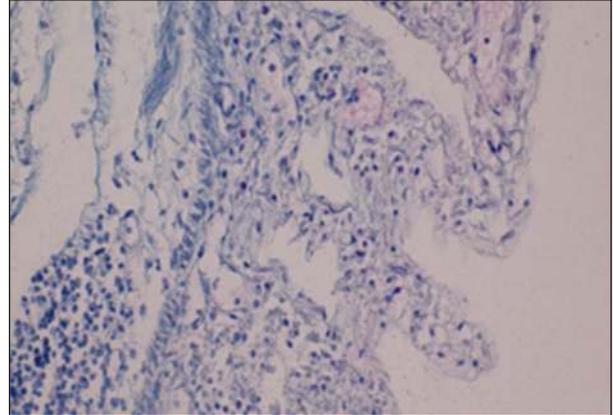


Fig. 6. Proliferative haemorrhagic ileitis: gaps with haemorrhagic exudate in the lamina propria; degenerative processes of different intensities in the covering epithelium; lymphocytic infiltrations in the mucosa and submucosa. Col. HEA 400x

In 11 cases, the ileum mucosa, the ileocecal valve and the first part of the colon were covered with necrotic detritus or caseous grey-yellow pseudomembranes, strongly anchored on the substrate, which caused the intestinal wall to thicken. These modified physical-structural features certify the morphopathological diagnosis of fibrinonecrotic ileitis (Fig. 7).



Fig. 7. Fibrinonecrotic ileitis: fibrinonecrotic deposits on the mucosal surface

Microscopically, the pseudomembranous hyperplasia was camouflaged, covered by necrotic detritus infiltrated with fibrin, present on the mucosal surface, in the lamina propria. Leukocyte infiltrate was observed on the base of the mucosa (Fig. 8).

Macroscopically, on inspection, the kidneys were enlarged. A greyish-whitish colour and a dull appearance without luster similar to „boiled or scalded meat“

(protido-lipid dystrophy) were identified (Fig. 9).

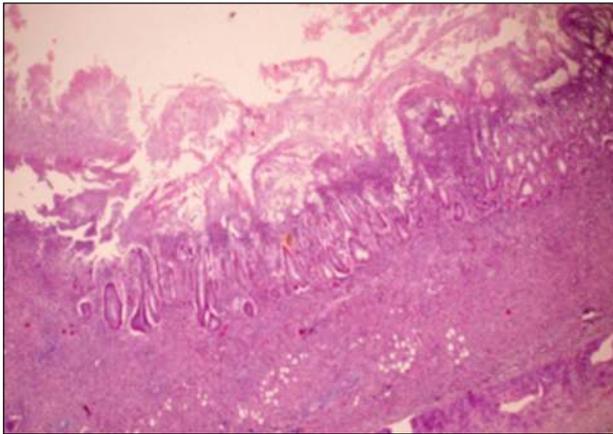


Fig. 8. Fibrinonecrotic ileitis: necrotic detritus infiltrated with fibrin, present on the mucosal surface and lamina propria. Col. HEA 100x



Fig. 9. Enlarged kidney with grey-whitish colour

Microscopically, numerous optically empty vacuoles and remnants of renocyte membranes were observed in the lumen of the urinary tract with congestion and interstitial haemorrhage. The renal glomeruli presented vacuolar tubular necrosis and vascular changes (Fig. 10). All the samples sent for bacteriological examination were negative.

Summarizing the morphological aspects of the proliferative inflammations/intestinal adenomatosis complex found in all the investigated swine, we would like to mention some elements for differential diagnosis compared to branchispiric dysentery and subacute-chronic salmonellosis.

Intestinal adenomatosis is mainly confined to the terminal part of the ileum, the ileocecal valve and the first third of the colon. Salmonellosis is most commonly found in the spiral colon in the form of diffuse colitis diphtheria. Branchispiric dysentery is commonly found in the caecum and spiral colon, being morphopathologically exteriorized with haemorrhagic or haemorrhagic necrotic typhlitis.

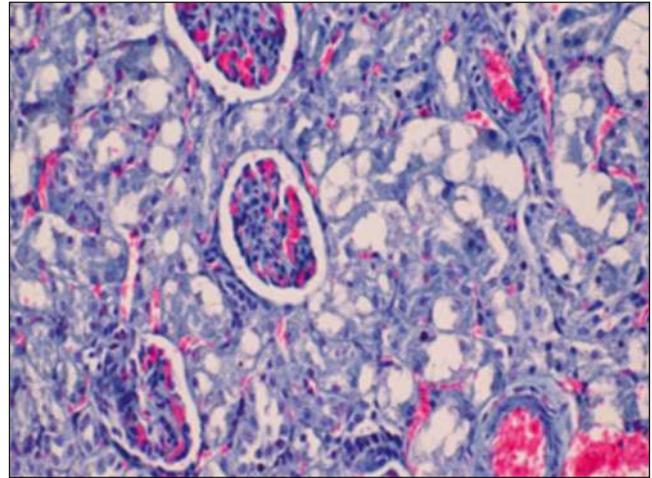


Fig. 10. Proto-lipid nephrosis - vacuolar tubular necrosis and vascular changes (congestion and intraparenchymal haemorrhage). HEA 400x

CONCLUSIONS

From a total of 188 necropsied cases, proliferative enteritis and/or intestinal adenomatosis complex were histopathologically diagnosed in 62 cases (32.95%).

Proliferative enteritis was morphopathologically externalized by: hyperplastic ileo-typhlocolitis (10.83%), adenomatous ileitis (9.32%) and proliferative-haemorrhagic ileitis (6.19%); necrotic and hyperplastic colitis (5.76%), necrotic detritus on the mucosal surface, young connective tissue (granulation) and/or mature fibrous tissue in the mucosa and submucosa; muscular hypertrophy with hyperplasia of the internal and external muscular layer of the intestine.

Intestinal adenomatosis may develop as primary enteritis following *Lawsonia intracellularis* infection, but in most cases, it is secondary, a consequence of colibacillary, salmonella, branchispiric and parasitic, fungal and mechano-irritative enteritis chronicity.

The use of technological norms for breeding, feeding, hygiene, prophylaxis and preventing diseases in pigs are recommended to be correctly applied.

REFERENCES

1. Cătoi C., (2003), Veterinary necropsy diagnosis, (Ed.) Academic Press, Cluj-Napoca, Romania [in Romanian]
2. Coman M., (2006), Special pathological anatomy, (Ed.) Art Press & Cosmopolitan Art, Timișoara, Romania [in Romanian]
3. Herman V., Moga Mânzat R., Râmneanțu M., (2008), Diagnosis in infectious diseases of animals, (Ed.) Mirton, Timișoara, Romania [in Romanian]
4. Jacobson M., Fellström C., Jensen-Waern M., (2010), Porcine proliferative enteropathy: an important disease with questions remaining to be solved. *Vet J*, 184(3):264-268.

5. Jubb K.V.F., Kennedy P.C., Palmer N., (1993), Pathology of Domestic Animals, (Ed.) Academic Press, New York, USA
6. McGavin M.D., Carlton W.W., Zachary J.F., (2001), Special veterinary pathology, (Ed.) Mosby, USA
7. McOrist S., Gebhart C.J., Boid R., Barns S.M., (1995), Characterization of *Lawsonia intracellularis* gen. nov., sp. nov., the obligately intracellular bacterium of porcine proliferative enteropathy. Int J Syst Bacteriol, 45(4):820-825
8. Olariu-Jurca A., Olariu-Jurca I., Coman M., Stancu A., (2015), Compendium of practical veterinary pathological anatomy, (Ed.) Eurobit, Timișoara, Romania
9. Olariu-Jurca I., Olariu-Jurca A., (2018), Necropsy diagnosis by species and syndromes, (Ed.) Eurobit, Timișoara, Romania [in Romanian]
10. Paul I., (2005), Etiomorphopathology of bacteriosis in animals, (Ed.) Pim, Iași, Romania [in Romanian]
11. Pedersen K.S., Ståhl M., Guedes R.M., Angen Ø., Nielsen J.P., Jensen T.K., (2012), Association between faecal load of *Lawsonia intracellularis* and pathological findings of proliferative enteropathy in pigs with diarrhoea. BMC Vet Res, 8:198
12. Pascu C., Costinar L., Mernea I., Tătar D., Herman V., (2015), Prevalence of *Lawsonia intracellularis* infections in pig herds from the Western Romania, Agriculture and Agricultural Science Procedia, 6: 378-381
13. Perianu T., (2003), Infectious diseases of animals. Bacteriosis, vol. I, (Ed.) Venus, Iași, Romania [in Romanian]
14. Răpunțean Gh., Răpunțean S., (2005), Special Veterinary Bacteriology. (Ed.) Academic Pres, Cluj-Napoca, Romania, 282-287 [in Romanian]
15. Vasiiu C., (2017), Treaty of bacterial diseases in animals, (Ed.) Napoca Star, Cluj-Napoca, Romania [in Romanian].