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CASES OF PORCINE PROLIFERATIVE ENTEROPATHY IN BULGARIA AND TESTING OF SOME ALTERNATIVE METHODS OF DIAGNOSTICS

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Summary

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Clinico-epidemiological investigations were performed in 11 affected pig farms in different regions of the country. The morbidity rate (12.2% to 26.5%) was evaluated on the basis of clinical signs, gross and slaughterhouse findings. Gross pathology, histological (specific stainings) and cytological studies were performed with a view to their application for detecting the disease, All known clinico-morphological forms of manifestation of porcine proliferative enteropathies (PPE) were observed throughout our inevstigations. The cases of porcine intestinal adenomatosis and regional ileitis predominated (over 80%). Taking into consideration the totality of gastrointestinal problems faced by pig breeding in Bulgaria, it could be stated that the share of PPE was continuously increasing.

Key words: diagnostics, *Lawsonia intracellularis,* porcine proliferative enteritis, swine

INTRODUCTION

Porcine proliferative enteropathy (PPE) includes several acute and chronic states manifested by softened faeces, bloody diarrhroea and stunted growth (McOrist & Gebhard, 1999). The disease affects weaned pigs at a different age, primarily growing and fattening (Jones *et al.,* 1993a). The syndrome consists of porcine intestinal adenomatosis (PIA), porcine hemorrhagic enteropathy (PHE), necrotic enteritis (NE) and regional ileitis (RI) (Love *et al.*, 1977). In each of these states, a characteristic lesion is present − thickened mucosa of the small intestine (mainly the ileal one), the caecum and/or the proximal colon.

The principal histological lesion of PIA is the adenomatous hyperplasia of intestinal crypt cells due to proliferation of immature epithelial cells and almost complete lack of cup cells (Barker, 1993). Immature enterocytes contain a large amount of the intracellular bacterium *Lawsonia intracellularis* in the apical cytoplasm (McOrist & Gebhart, 1999). The first cases of PPE were reported by Schwartz and Beister in 1931 in the USA (Schwartz & Beister, 1931). The authors described some cases in pigs with the characteristic proliferative changes in ileal and colonic mucosa. The disease results in a consideraby reduction in productive traits in pigs in many herds throughout the world (McOrist, 1996).

PPE is caused by the Gram-negative bacterium *Lawsonia intracellularis*, that is very similar to rickettsiae in many aspects, although not taxonomically related (McOrist *et al.*, 1995). This is an obligate intracellular agent that can not be cultivated on nutrient media. Its identification as well its etiological role were confirmed in 1993 following succesfull cultivation in cell culture and reproduction of a disease using cell suspension (Lawson *et al.*,1993; McOrist *et al.*,1995).

Various organizational activities in farms such as transportation of pigs, changes in the feeding regimen or the diet, sudden temperature variations, overpopulation, impaired hygienic or immune status as well as a genetic predisposition could influence the appearance, the development and severity of PPE (Hagen & Bilkei, 2003; Bona & Bilkei, 2003).

PPE is a widely distributed disease, reported in many countries in Europe, North and South Aemrica, New Zealand and Japan (Rowland & Lawson, 1992). According to some reports, the incidence of lesions in affected animals was low – 0.7–2.0% (Kubo *et al.*, 1984; Christencen & Cullinane, 1990). Other communications show that the prevalence of lesions amounted to 40%, esepcially in some herds (Poiton, 1989).

Because of the difficult cultivation of *L. intracellularis*, the quest for alternative methods and techniques for its detection and proving began shortly after its determination (Jones *et al.*,1993b). The process is going on by now with regard to the optimization and rapid performance of diagnostics.

The aim of the present report was to present the results from the test of some alternative methods of PPE diagnostics with the intention of their use in the detection of the disease as well as to determine the epidemiological status of several affected farms in Bulgaria.

MATERIALS AND METHODS

Epidemiological and clinico-morphological investigations were performed in 11 affected pig farms (5 in North and 6 in South Bulgaria). All farms were of an industrial type with a capacity for more than 500 sows and 5 out of them – with over 1000 sows. The total number of studied categories of pigs (growing and fattening) was over 14 000. The pigs from all studied farms were housed in pens under different technologies: pens with raised slatted floor, 8–15 pigs in a pen, pens with perforated plastic floor for 15–30 animals or pens with brick floors. In all cases, the feeding and water supply were mechanized and in some farms – automatic. These differences in rearing technologies determined a different hygienic level.

The epidemiological studies included analysis of the susceptible population of animals and follow-up of the parameters: morbidity rate, death rate and lethality. For this purpose, survey cards were filled in.

The clinical observations and physical examination monitored the appearance, the course and the character of symptoms, the development and the outcome of the disease.

Necrospy specimens were obtained from dead pigs (parts of the ileum), collecting 5–6 samples from each affected farm for cytological and histological study.

For cytological study, smear preparations of ileal mucosa were prepared, air-dried and stained with hemacolor (Diff Quik).

For the histological study, the specimens were fixed in 10% neutral formalin and embedded in paraffin. The cross-sections of 5 µm, were impregnated with silver nitrate (Wartin-Starry silver staining) and stained with hematoxylin & eosin (H/E).

RESULTS

The results of the *epidemiological survey* showed that in the different farms, the morbidity rate ranged between 12.2% and 26.5%. The lethality in untreated animals was over 40%, and cumulative death rate – between 2 and 6 %.

In all studied farms, the majority of affected piglets were at the age of 50–80 days (3–7 weeks after weaning), and in two farms, a higher morbidity rate was observed in the period immediately after their transfer into the growing sector. The survey data showed a higher incidence of the disease during the winter-spring season.

In clinical aspect, over 80% of fattening pigs exhibited a retarded growth compared to their group mates and thus, an ununiformity of the population was observed (Fig. 1). The affected animals showed inappetance and/or fastidious appetite (apathy to forage). In a part of pens with such pigs, an intermittent diarrhoea was noticed.

In 60–70% of affected animals, a remission occurred within 6–7 weeks from the onset of clinical signs. It was characterized by a gradual return of the appetite and a slow attainment of normal fattening parameters. In the other 30–40%, a permanent and irreversible delay in growth was observed, and the final average body weight was by about 20% lower than that of unaffected pigs. Some animals were cachectic.

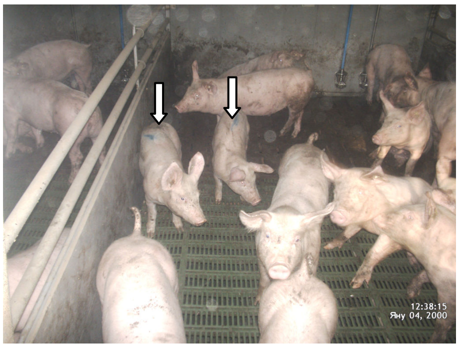

Fig. 1. Retarded growth (ununiformity) in pigs from a fattening herd.

Fig. 2. Melena in a porcine haemorrhagic enteropathy case.

In about 10% of clinically studied pigs, the death occurred rapidly or suddenly, in most of them, after a marked anaemia. Generally, these animals were not losing weight. Sometimes, melena (Fig. 2) was found out in their pens.

The main *gross* finding at slaughtering in pigs with clinically manifested retarded growth (ununiformity) was the considerable thickening of ileal wall. It was due to the significant growth of mucosa resulting in a noticeably narrowed intestinal lumen (Fig. 3). The mucosal surface was moist, but not covered with mucus. Sometimes, it was scattered with haemorrhages (Fig. 4). In some pigs from this group, coagulation

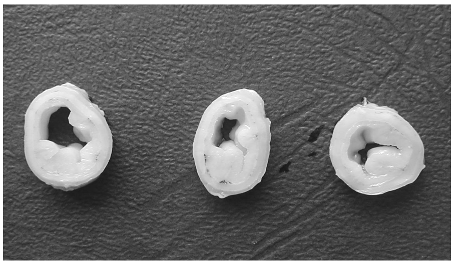

Fig. 3. An almost complete obstruction of the ileal lumen in a case of porcine intestinal adenomatosis (transverse cross-section of an intestine; slaughterhouse finding).

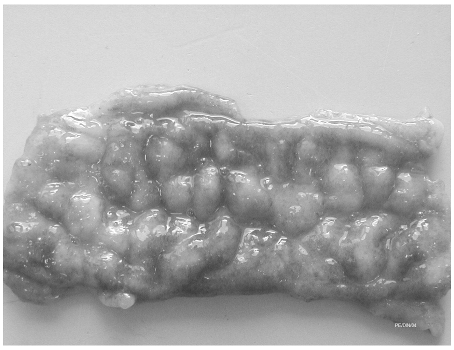

Fig. 4. Porcine intestinal adenomatosis – haemorrhages among the proliferated mucosa (slaughterhouse finding).

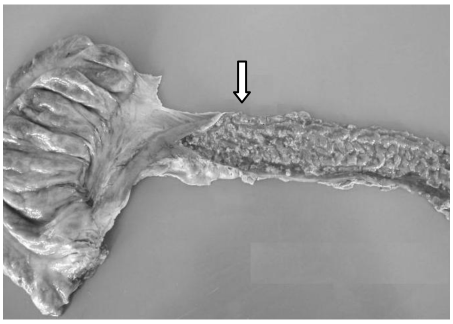

Fig. 5. Necrotic enteritis – necrosis of ileal mucosa (slaughterhouse finding).

necroses of ileal mucosa were observed. The mucosa appeared as covered with grey-yellowish cheese-like substance (Fig. 5).

In pigs with rapid or sudden lethal issue, with or without signs of anaemia, profuse haemorrhages were detected in the end part of the ileum or the caecum. The blood was generally clotted (Fig. 6).

Histologically, enlargement of glandular crypt cells due to growth of glandular epithelial cells was noticed. They were usually arranged in several layers, 5–6 or more. There were microorganisms in their cytoplasm. In lamina propria mucosae, focal or diffuse mononuclear cell proliferation resulting in extensive thickening of this layer was observed (Fig. 7). In longer-continuing cases, growth of granulation tissue, hypertrophied intestinal muscular layer and subserosal oedema were detected among proliferates.

Silver-impregnated tissue sections revealed several or multiple intracellular microorganisms in superficial epithelial cells of mucosa (Fig. 8).

Cytological smear preparations revealed intracellular microorganisms. They appeared as straight or slightly curved rods in the cytoplasm of intestinal epithelial cells (Fig. 9).

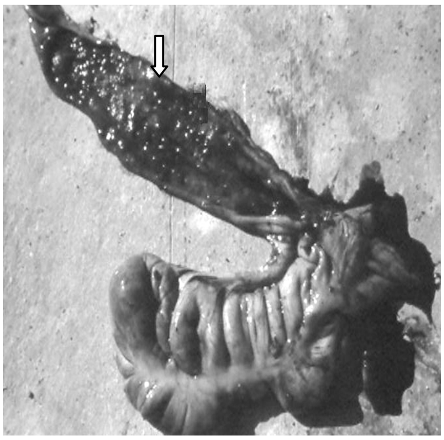

Fig. 6. Porcine haemorrhagic enteropathy – haemorrhages in ileal lumen in a sudden death.

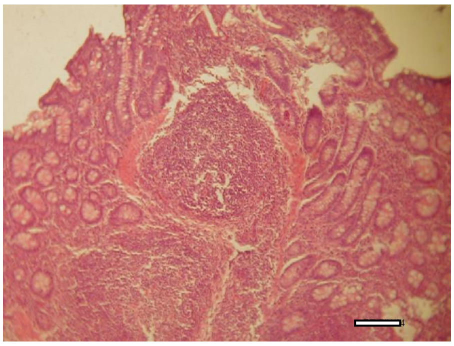

Fig. 7. Mononuclear cell proliferation in lamina propria mucosae resulting in severe thickening of this layer in regional ileitis, H/E, bar ≈ 5 µm.

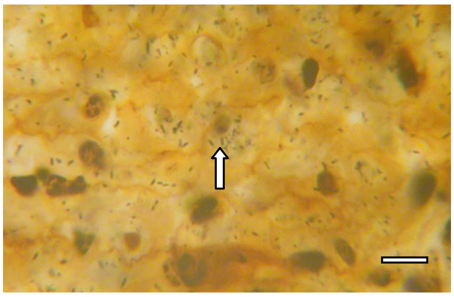

Fig. 8. Intracellular microorganisms in superficial epithelial mucosal cells in sliver-impregnated tissue cross-sections, Wartin-Starry silver staining, bar ≈ 3 µm.

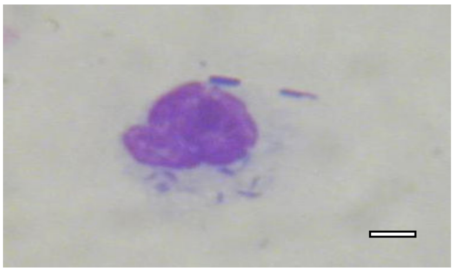

Fig. 9. Chronic PPE, ileum (smear preparation) – intracellular bacteria in the cytoplasm of epithelial cells. Diff Quik, bar ≈ 3 µm.

DISCUSSION

Our results showed a morbidity rate of PPE in Bulgaria (12.2–26.5%), similar to that reported in other countries where the disease is common (Pejsak *et al.*,1997; Lawson & Gebhart, 2000; Pejsak *et al.*, 2001). It should be said that the cause for the later detection of the problem in Bulgaria is its absence during the last 10–15 years. In the early 90-ties, PPE cases were observed in our country (unpublished data from diagnostic studies of Dinev *et al.*, 1995) but at that time, they were associated with *Campylobacter spp*. as an etiological agent (Poiton, 1989). In fact, *Campylobacter spp.* organisms were not isolated and as reported in other countries too, the disease has somewhat subsided in that the period (McOrist & Gebhart, 1999). The prevalence of PPE during winter and spring could be associated with extreme climatic changes, especially with significant differences between day and night temperatures or with the hot and humid weather as also stated by other authors (McOrist & Gebhart,1999).

In our studies, all known clinicomorphological forms of PPE were manifested (PIA, RI, NE and PHE). The cases of PIA and RI were dominating (over 80%). These results correspond to most data reported (McOrist & Gebhart,1999). The prevaling chronic form of the disease, characterized by a moderate diarrhoea and retarded growth should be differentiated from non-specific enterites (Dufresne, 1998). Taking into consideration the other gastrointestinal problems of pig breeding in Bulgaria, it could be said that the share of PPE is becoming more and more important.

Necrospy and macroscopic evaluation of lesions are an extremely important steps in PPE detection. In some instances, when gross changes are perceptible, the pathoanatomical study could be reliable (Guedes *et al.*, 2002). The imprint cytological study is a rapid, easy and not expensive method that could largely support the diagnosis. The modified method of Warthin-Srarry with silver impregnation of tissue cross-sections is a satisfactory method for routine use. The histological examination revealed the chanacteristic morphology of proliferative lesions. The obtained results by diagnostic techniques used by us correspond to the opinion of some investigators that the combination of cytological and some modified histological studies (silver impregnation of tissue cross-sections) could be an alternative to detect the etiological agent *L. intracellularis* that is difficult for cultivation (Jones *et al.*, 1993b; McOrist & Gebhart, 1999; Guedes *et al.*, 2002).

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