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NEONATAL DIARRHEA IN PIGS CAUSED BY *Clostridium perfringens*

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Abstract

The outbreaks of enteritic infections in piglets caused by *Clostridium perfringens* belongs to the disease group with marked age incidence i.e. it normally occurs in suckling piglets aged to 7 days, usually on 2nd or 3rd day. At necropsy, the predominant pathomorphological lesions are most frequently observed in small intestine, especially in jejunum. However, in some cases the pathomorphological lesions may macroscopically be absent. For that reason, diagnostic criteria should consider: the disease history data (mortality pattern), clinical signs of the disease (bloody diarrhea in suckling piglets), pathomorphological lesions and bacteriologic findings. The material for research included samples from 5 swine farms, where health problems (diarrhea, increased mortality) in suckling piglets of different age were detected. In total 69 piglet carcasses were submitted to necropsy. In typical cases the presence of bloody content in small intestine, snake appearance of affected intestinal loops, the presence of emphysema in the intestinal wall were observed. Applying bacteriology testing (anaerobic cultivation) in the most examined cases *Clostridium perfringens* was detected in tissue samples.

Key words: suckling piglets, necrotic enteritis, *Clostridium perfringens*

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NEONATALNA DIJAREJA PRASADI UZROKOVANA SA *CLOSTRIDIUM PERFRINGENS*

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Kratak sadržaj

Crevne infekcije prasadi izazvane *Clostridium perfringens* pripadaju grupi oboljenja kod kojih je izražena zakonitost incidence u pogledu uzrasta obolelih jedinki tj. infekcija se ustanovljava u uzrastu do 7 dana, najčešće oko 2-3 dana života prasadi na sisi. Patomorfološkim pregledom lezije se najčešće ustanovljavaju u tankom crevu, naročito u jejunumu. Međutim, patomorfološki nalaz se ponekad može karakterisati i odsustvom makropatoloških lezija specifičnih za klostridijalnu infekciju. Zato se kao dijagnostički kriterijumi uzimaju u obzir i anamnestički podaci (uginuće u prvim danima života prasadi), klinička slika oboljenja (hemoragična dijareja prasadi na sisi), patomorfološke promene i bakteriološki nalaz. Materijal za ispitivanje je obuhvatao pet farmi svinja, na kojima su utvrđeni zdravstveni problemi (pojava proliva i uginuća) kod prasadi na sisi različitog uzrasta. Ukupno je patomorfološki pregledano 69 leševa prasadi na sisi. U tipičnim slučajevima, patomorfološkim pregledom ustanovljeno je prisustvo krvavog sadržaja u lumenu tankih creva, zmijoliki izgled crevnih uvojaka kao i prisustvo mehurića vazduha u zidu tankih creva. Bakteriološkim ispitivanjem (anaerobna kultivacija) iz tkiva uginule prasadi u najvećem broju slučajeva izolovan je *Clostridium perfringens*.

Ključne reči: prasad na sisi, nekrotični enteritis, *Clostridium perfringens*

INTRODUCTION

Clostridium perfringens is a Gram-positive, spore forming bacterium that can cause a variety of toxic-specific lesions and gastrointestinal diseases in domestic and wild animals as well as in humans (*van Asten et al., 2010*). Owing to its ability to produce spores under adverse environmental conditions, it is one of the most widespread potential bacterial pathogens in nature as well as in the gastrointestinal tract of most animal species (*Backer et al., 2010*). Based on the production of 4 major toxins, alpha (CPA), beta (CPB), epsilon (ETX) and iota

(ITX), *C. perfringens* isolates are classified into 5 toxinotypes (A-E) (Miclard et al., 2009; Songer and Taylor, 2006). Two other toxins, enterotoxin (CPE) and beta2 (CPB2) can be produced by all types of *C. perfringens*, although they are not used in typing (Songer and Uzal, 2005).

C. perfringens type C infection occurs in all swine-producing areas of the world (Backer et al., 2010; Jäggi et al., 2006). Type C causes frequently hemorrhagic, often fatal, necrotic enteritis in young piglets (Songer and Taylor, 2006). It is mostly seen in the first 7 days of life (Jackson and Cockcroft, 2007; Prodanov-Radulović et al., 2013). Enteric disease, necrotic enteritis, caused by these organisms impacts producers, veterinary practitioners, and diagnosticians, despite long-term availability of immunoprophylactic products (toxoid vaccines) for swine protection (Songer and Uzal, 2005). In view of high morbidity and mortality rates, necrotic enteritis is a cause of serious financial losses in pig rearing (Springer and Selbitz, 1999).

MATERIAL AND METHODS

The material for this research originated from five swine farms, where certain disorders and health problems in suckling piglets were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: anamnestic evaluation and clinical investigation, pathomorphological examination, standard bacteriological examination for detection of the presence of aerobic and anaerobic bacteria in the organs and tissue samples derived from diseased and/or died suckling piglets.

RESULTS AND DISCUSSION

On the first two examined farms, the evaluation of anamnestic data indicated apparent health problems and increased mortality in suckling piglets. Clinical manifestations occurring in suckling piglets (first 5 and 10 days after farrowing) included severe diarrhea and signs of body dehydration. Despite the fact that the piglets were therapeutically treated, there was no evident response to applied medication. Decrease in growth rate was evident in some survived nursed piglets, which did not die but they remain stunted. On the second evaluated farm, the sows are vaccinated during pregnancy but recently the vaccine has been changed (i.e. vaccine from another producer was introduced). During data control, the difference in vaccine composition was noticed: the old one had 3 types of toxoid *C. perfringens* (type B and purified toxoid type C and D) while the newly applied contains only one type of C beta-toxoid.

The control of farm anamnestic data, it was discovered that severe diarrhea in piglets was most frequently in the litters deriving from first litter and older sows. By clinical examination of piglets aged 11-15 days yellow-brownish colored diarrhea accompanied by staining of peritoneum was detected. In the 4-days old litters, traces of the reddish-brown diarrhea on the piggery floor were discovered. In the piglets from the first litter sow, bloody diarrhea in the first day of life was evident. The pathomorphological examination of the dead suckling piglets revealed lesions dominantly on the mucosal surface of the digestive tract: hemorrhagic and necrotic enteritis, catarrhal and hemorrhagic gastritis, diphteroid-necrotic gastritis and enteritis (*Enteritis necroticans porcellorum*). The stomach was often full of milk and mucosa of the small intestine, dark red and necrotic. Bacteriological examination (anaerobic cultivation) of tissue samples (spleen, liver, kidneys and mesenterial lymph nodes) from dead suckling piglets, revealed presence of *Clostridium perfringens*.

The feces of sows contained small numbers of type-C organisms and these multiply rapidly in the small intestine of piglets, out-competing other bacteria and becoming the dominant organisms in the population (Songer and Uzal, 2005; Songer and Taylor, 2006). Oral infection of piglets, acquired in most cases through teats contaminated with feces, leads to replication of *C. perfringens* type C in the intestines resulting in the production of protein toxins (exotoxins), including β -toxin. The β -toxin is not degraded because of poor synthesis of digestive enzymes by piglets and high anti-trypsin content in sows' milk. It consequently has a decisive influence on the pathogenesis of necrotic enteritis (Springer and Selbitz, 1999). Lethal and necrotizing effects of CPB play key roles in tissue damage. Death is likely due to the effects of intestinal damage and toxemia (Miclarad et al., 2009). Hypoglycemia and secondary bacteremia due to *C. perfringens* or *Escherichia coli* may raise the fatality rate (Songer and Uzal, 2005).

Clinical signs vary according to immune status and age of affected piglets (Songer, Uzal 2005). Clinical disease can take the per acute, acute or chronic course with signs of intense abdominal pain, depression, and bloody diarrhea, which begins 8 to 22 hours after exposure to *C. perfringens* type C. The course of the disease is usually 24 or fewer hours in 1- to 2-day old piglets (Jackson and Cockcroft, 2007), but chronic disease (usually in older animals) can persist for 1 or 2 weeks, and is characterized by persistent diarrhea without blood and dehydration (Songer, Uzal 2005). Disease is most common in 3-dayold piglets, but may occur as early as 12 hours after birth (Jackson and Cockcroft, 2007; Prodanov-Radulović et al., 2013). Piglets become weak, move with reluctance and rapidly become moribund, risking crushing by the sow. Many of them are found dead within 12-36 hours after birth. However, death also occurs in some

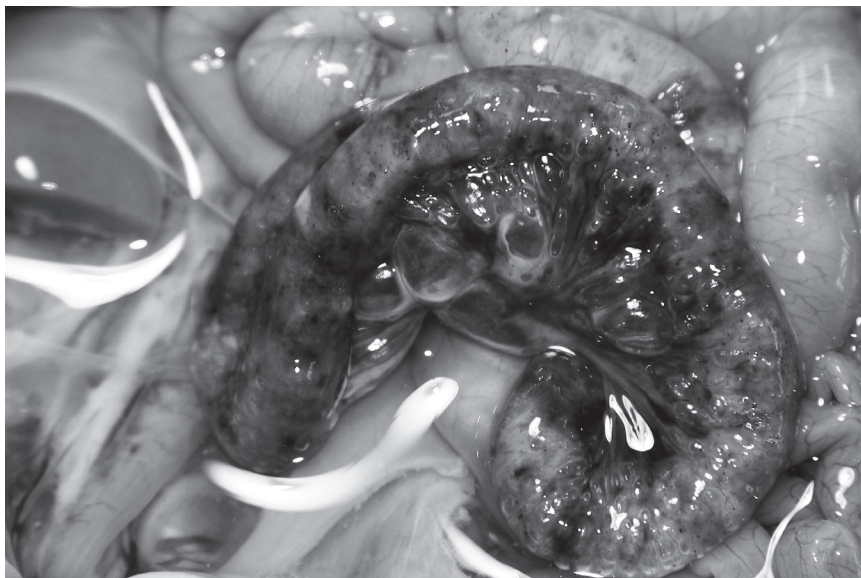
animals without diarrhea being seen (Songer and Taylor, 2006).

Lesions are typically noticed in the jejunum and ileum; they may extend anterior to within a few centimeters of the pylorus and posterior to the proximal colon. Gross mucosal lesions are reddish or black in color, with intense hemorrhage and gas bubbles in the intestinal wall. Contents of the affected area contain blood, and may be found as far distal as the rectum. Jejunum portion of intestine may be loosely adherent to adjacent segments by acute fibrinous peritonitis. Intestinal wall is usually thickened and yellow or grayish, and its contents may be bloodstained and contain necrotic debris. Hallmark lesions include profound mucosal necrosis and emphysema in small intestine, sometimes extending into cecum and proximal colon. Type C is a primary pathogen but can apparently colonize lesions associated with other diseases such as transmissible gastroenteritis (TGE) (Songer and Taylor, 2006). Characteristically, dead piglets are in good condition (Jackson and Cockcroft, 2007). At necropsy, the predominant lesions are most frequently observed in small intestine, especially jejunum; occasionally lesions may be confined to large intestine. Lesions are similar in all segments of the intestines and in acute cases consisting of diffuse or segmental extensive fibrinonecrotic (pseudomembranous) enteritis with emphysema and bloody gut contents. There may be fibrin strands on the intestinal serosa, and adhesions may develop between intestinal loops (Songer, Uzal 2005). One may see localized peritonitis, but the piglet usually dies before this can develop (Jackson and Cockcroft, 2007).

Figure 1. Necrohemorrhagic enteritis in 3-day old piglet



Figure 2. Acute *C. perfringens* type C enteritis in 3-day-old piglet: an emphysematous segment of the intestine (jejunum)



On the third examined swine farm, the health problems in suckling piglets were connected with the purchasing of breeding animals (gilts). Despite the fact that all gilts originated from one farm, after the farrowing, all litters died in the first 2 days of life. Clinically, severe dehydration, depression, piglets' cohorting, the yellow or light brown colored diarrhea was observed. In some animals the purple-red-colored watery feces was evident already on the first days of life. Eventually, all farrowed litters died. The pathomorphological examination of the dead suckling piglets aged 2-3 days revealed catarrhal gastritis, angry purplish-red color of jejunum (i.e. color like rot-cherry). In some cases, the small intestines manifesting snaky appearance of affected intestinal loops, the presence of emphysema in the intestinal wall or with extensive whitish sediment (gypsum-like content) were observed. Applying anaerobic cultivation, *C. perfringens* was isolated from the organs and tissue samples (liver, spleen, kidney, mesenterial lymph nodes) of died suckling piglets.

Outbreaks of *C. perfringens* infection often follow the introduction of infected breeding stock, and the disease persists in herds for up to 2 months; however, in herds where new stock is constantly introduced, the outbreaks may continue for up to 15 months (Prodanov-Radulović et al., 2013; Songer and Taylor, 2006). Farrowing houses or areas may become heavily contamina-

ted. Typically, three or four litters or part of a litter in a herd may be affected by severe disease but up to 50 litters have been reported to be affected in some outbreaks. Herds may be infected with the organism but typical disease may be absent (Songer and Uzal, 2005). In some cases, this is attributed to early administration of antimicrobials as either treatment or prevention, but most commonly, it is the results from increasing practice of including *C. perfringens* type C toxoid in vaccines given to sows to prevent piglet diarrhea (Springer and Selbitz, 1999). Where protective antibody is present in the colostrum at adequate levels, no disease will be seen. Where levels are inadequate or the intake is insufficient, clinical signs may develop somewhat later and be mild and difficult to recognize (Songer and Taylor, 2006). However, Prodanov-Radulović et al. (2011) suggested that enteric disease of suckling piglets could be provoked with the feed quality, i. e. the presence of mycotoxins in the feed for lactating sows and in the piglets' first feed. The authors reported the occurrence of hemorrhagic enterotoxaemia in piglets despite the fact that dams were vaccinated twice during gestation.

Clostridium perfringens is characterized by a very short generation time, and type C organisms can multiply to numbers approaching 10^8 - 10^9 per gram of contents in only a few hours (Songer and Taylor, 2006).

Attachment to jejunal epithelial cells at villous apices is followed by desquamation of these cells and proliferation of the organism along the basement membrane. Necrosis of the villous lamina propria is extensive, and hemorrhage accompanies necrosis (Gurtner et al., 2010). The necrotic zone advances to involve crypts, muscular mucosa and sub mucosa, and occasionally the muscular layers. Perforation of intestinal wall leads to emphysema in muscle layers, beneath the peritoneum, and in mesenteric lymph nodes (Songer and Taylor, 2006).

On the last two examined swine farms, by controlling the epizootical and anamnestic data, certain irregularities in the implementation of immunoprophylactic measures were found. The sows were vaccinated, but not according to manufacturer's recommendation (i.e. only once before farrowing). In suckling piglets aged 5 days, the signs of diarrhea are observed but not in all litters. After controlling the piggeries, it was established that there was a full-floor facility with straw bedding, while the boxes were separated by the wooden wall. The health problem has been apparent for about one year, but diarrhea was not recorded in each litter. A large number of piglets got sick and died in 12 to 24 hours after farrowing. After farrowing, the piglets had good birth weight, but pinky-colored diarrhea occurred as soon as on the second day of life. Treatment with antimicrobials was of little use in diseased piglets

and all diseased animals eventually die. Also, the problem was a high mortality rate in nursing piglets. Clinical examination revealed that diseased piglets were dehydrated, with loss of body condition. Nursing was minimal, and these piglets rapidly lost condition and became gaunt and weak. They had reddish-brown to yellow-brown colored diarrhea accompanied by staining of perineum as well as redness and swelling of the anus.

By gross pathological examination in dead suckling piglets, distinct lesions on small intestine were evident: snaky appearance of affected intestinal loops and the presence of emphysema (i.e. gas bubbles) in the intestinal wall. The jejunum of affected piglets was also swollen with an angry purplish-red color, with bloodstained fluid content. In some cases, the mucous surface of small intestine was covered by a grayish-yellow deposits and the intestinal wall was thickened and friable. Also, hemorrhagic gastritis, diffuse hemorrhage of the kidneys, enlarged and reddened mesenteric lymph nodes could be found. In a laboratory testing (anaerobic cultivation), *C. perfringens* was isolated from the examined tissue samples.

The disease occurs epizootically in non-vaccinated populations, and prevalence of affected litters can reach 100%. The case fatality rate varies, but 100% mortality in litters from non-immune sows is not unusual, and herd mortality may be > 50% (Jäggi et al., 2009). The study of Springer and Selbitz (1999) clearly demonstrate that vaccination of sows with the toxoid vaccine and concomitant administration of a penicillin preparation in the piglets leads to a drastic reduction in piglet losses. With increased herd immunity, the disease may become enzootic, with mild cases developing over a period of months. Continued appearance of acute disease suggests herd immune deficiency (such as by frequent introduction of immune-naïve gilts) or failure of piglets to receive adequate amounts of colostrum (Songer and Uzal 2005).

Diagnostic criteria - mortality pattern, clinical signs of the disease, and pathological gross lesions are sufficient basis for a presumptive diagnosis of *C. perfringens* type-C enteritis in piglets. More detailed herd infection history, exclusion of other causes of necrotic enteritis and bacteriological culture may be needed to establish a presumptive diagnosis in chronic cases (Songer and Taylor, 2006). Final diagnosis, however, should be based on bacteriological cultivation of intestinal contents (isolation of large numbers of *C. perfringens* followed by genotyping of isolates and/or CPB detection from intestinal contents) (Songer and Uzal, 2005; van Asten et al., 2010). Although *C. perfringens* type C can be found in the intestines of clinically healthy pigs, this is not frequently the case, which usually advocates the diagnostic relevance of isolation of this microorganism from the intestines of diseased pigs. Also, small amounts

of *C. perfringens* type C are usually isolated from the intestines of clinically normal pigs, whereas large numbers of the organism are usually isolated from pigs with necrotic enteritis (Songer and Uzal, 2005). The clostridial enteritis infections have a complex pathogenesis, thus the diagnosis cannot be based on mere isolation of the clostridia involved, and other factors should be taken into consideration to establish the final diagnosis (Songer and Taylor, 2006; Prodanov-Radulović et al., 2011; Prodanov-Radulović et al., 2013).

CONCLUSION

The early age at which this disease occurs, the rapid course, compatible clinical signs, mortality pattern and typical necropsy findings suggest the diagnosis, which can be readily confirmed by bacteriological examination. However, in some cases, diagnosis should be based upon findings in the herd as a whole rather than on the examination of individual diseased animals. The treatment is of little use in animals with clinical signs, and prophylaxis is the preferred approach. The disease can be effectively prevented by vaccination of pregnant sows, by re-evaluation and correction of the environmental conditions and management system since these factors may have considerable influence on the occurrence of the disease.

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