CONTRIBUTION OF CLOSTRIDIUM PERFRINGENS TYPE A WITH β2 TOXIN GENE IN AETIOLOGY OF PORCINE ENTERIC DISEASES. A CASE REPORT

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Abstract

The aim of the report was to describe three cases of fatal enteritis in piglets, from which Clostridium perfringens type A strains carrying β2 toxin gene were isolated as the only specific pathogen. Six animals submitted for the study originated from three farms. The age of the piglets ranged from two days to three weeks. Animals from each farm showed at necropsy different intensity of pathological changes. The typing of the isolated strains was performed by the use of PCR method. All the strains presented the expression of a toxin in ELISA.

Key words: piglets, Clostridium perfringens, enteritis, toxins.

Clostridium perfringens is an important pathogen for humans and animals. The virulence of the bacterium is associated with exotoxins production. Based on the production of four major lethal toxins, designated α, β, ε, and ι, C. perfringens strains are classified into five types: A, B, C, D, and E (11). Type A produces only α-toxin, type B - α, β- and ε-toxins, type C - α- and β-toxins, type D - α- and ε-toxins, type E - α- and ι-toxins. Apart of the major toxins, over ten other toxins / hydrolytic enzymes produced by different C. perfringens strains are known (11, 18). One of them is β2 toxin (CPB2) (5). The cpb2 gene encoding CPB2 was found in the genome of some isolates belonging to all of the mentioned above toxino-types. Prevalence of cpb2 in C. perfringens strains isolated from cases of porcine enteritis is higher than in other animal species. In swine, toxino-types C and A are recognised as the principal among causative agents of clostridial enteritis (16). Several authors presented recently data indicating the association between carrying cpb2, non-enterotoxigenic C. perfringens strains, and enteric diseases in piglets. The aim of presented report was to describe three cases of fatal enteritis in piglets, from which Clostridium perfringens type A strains carrying β2 toxin gene were isolated as the only specific pathogen.

Material and Methods

Dead piglets from three farms designated as P-1, P-2, and P-3 were submitted for diagnostic examination. One piglet at the age of three days from the farm P-1, one piglet three-week-old from the farm P-2, and four piglets at the age of two days from the farm P-3 were submitted.

The P-1 and P-2 farms were small farms managing farrow-to-finish herds with less than 15 sows. In the P-3 farm, the farrow-to-finish herd with 750 sows were managed.

Enteric problems in piglets at the P-1 farm were observed in one litter. Two of the piglets died suddenly and about half of the litter demonstrated creamy diarrhoea. The examined piglet was not treated with antibiotics. No diarrhoea was observed in the sow who delivered the mentioned litter.

In the farm, P-2 sporadic creamy and mucoid diarrhoea appeared in 30% of piglets from a litter at 3 weeks of age. One of the piglets died suddenly and about half of the litter demonstrated creamy diarrhoea. The examined piglet was not treated with antibiotics. No diarrhoea was observed in the sow who delivered the mentioned litter.

In the farm, P-2 sporadic creamy and mucoid diarrhoea appeared in 30% of piglets from a litter at 3 weeks of age. One of the piglets died suddenly and was delivered to the laboratory. The animals from the litter were not treated with antibiotics until the time of the examination. As the distance between the farms P-1 and P-2 and the laboratory was not long, the piglets were submitted for the examination shortly after death.

In the farm, P-3, enteric disorders in piglets appeared about one month before submission of the animals for the examination to the laboratory. Clinical symptoms were observed in 17 out of 35 litters born in this period on the farm. Sudden death was the main symptom in some litters at the age of 2-3 d. The mortality in particular litters reached up to 25%. The diarrhoea was observed sporadically. Watery, yellowish, and green diarrhoea was observed much more often in
piglets at the age of 4 d and older ones. The morbidity in the litters achieved 60 – 90 %. No diarrhoea was observed in sows. The diarrhoeic piglets were weak and often not able to suck. Dehydration was observed in some of them. The treatment of the affected litters with amoxicillin (administered i.m. for 3-5 d) proved to be effective and allowed for the recovery of the piglets. The dead piglets that were submitted for laboratory examination, were not treated with antibiotics before their death.

All the piglets were necropsied, and from each of them the samples of the small intestine, stomach, liver, spleen, and kidney for bacteriological examinations were collected. For the isolation of C. perfringens, the samples were cultured under anaerobic conditions on the tryptose-sulphite-cycloserine (TSC) agar (Oxoid) supplemented with egg yolk emulsion (Oxoid) (Fig. 1) and on the Columbia agar with 5% of sheep blood. The inoculated media were incubated overnight at 37°C. To demonstrate the possible presence of pathogenic strains of Escherichia coli, all the samples were also cultured aerobically at 37°C on plates with horse blood agar and on the MacConkey agar.

The microorganisms whose colonies resembled morphologically C. perfringens were initially identified microscopically (Gram-stained smears). Colonies of Gram-positive rods were subcultured and identified biochemically with use of Api ID32 A test (Biomerieux).

The isolated C. perfringens strains were typed by polymerase chain reaction (PCR). Multiplex PCR was used for the typing of the indicated fragments of α-, β-, ε-, τ-, β2- toxin genes and enterotoxin gene.

The preparation of the samples for PCR was carried out on the basis of a procedure described before (12). Briefly: two or three colonies of pure culture were picked up and boiled for 10 min in 200 µl of PBS and then centrifuged at 10 000 rpm for 20 s. The supernatant was diluted 1:10 in deionised water.

The set of specific primers (Table 1) proposed by Songer and Bueschel (15) was used for the amplification.

The amplification was carried out in a 50 µl volume. The mixture contained 10 mM Tris HCL, pH 8.8; 50 mM KCl; 3 mM MgCl2 (Fermentas); 0.6 mM of each dNTP (Fermentas); 0.25 µM each of primers (Oligo IBB PAN); 1.5 U of Taq polymerase (Fermentas), and 3 µl of boiled and diluted specimen. The reaction was performed by initial denaturation for 3 min at 94°C followed by 34 cycles consisting of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. After cycling, the final extension was continued for 10 min at 72°C.

The PCR products were visualised by electrophoresis in 2% agarose gel containing 1 µg/mL of ethidium bromide.

The expression of plc gene in the isolated C. perfringens strains was checked by the use of α-toxin Bio-X Alpha Toxin Elisa Kit (Bio-X Diagnostics).

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Gene</th>
<th>Sequence 5’ – 3’</th>
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<td>α</td>
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<td></td>
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Results

Post mortem examination. During necropsy of the piglet from P-1 farm, intensive haemorrhagic inflammatory changes with partial necrosis of mucosa in the jejunum and ileum were found. Most intensive, dark red or purple coloured necrohaemorrhagic lesions were localised in sharply demarcated portion of the jejunum about 7-8 cm in length. Posterior to this portion of the intestine, milder hemorrhagic inflammatory changes (without necrosis) extending to the end of the jejunum and including the proximal part of the ileum were observed. The surface of mucosal membrane was covered by increased amount of mucus with small addition of blood.

The piglet from farm P-2 showed haemorrhagic mucosal inflammation in the jejunum. Most intensive lesions were observed in the portion about 10 cm in length. Anterior and posterior to this part of the jejunum, milder, reddish coloured haemorrhagic inflammatory lesions were visible. No necrotic lesions were found. In the lumen of the intestine, increased amount of mucus with addition of blood was observed.

Piglets from farm P-3 showed another type of lesions in the abdominal cavity. The stomach and small intestines were gas-filled. In the case of two piglets, the stomachs were ruptured and their content (clotted milk) was in the abdominal cavity. Intestinal walls in all piglets were thin and flaccid. Various amounts of clotted milk were found in the jejunum. No necrotic and haemorrhagic inflammatory lesions were observed. On the surface of the liver of two piglets, the areas of greyish colour were observed. The kidneys in the two animals were slightly enlarged.

Microbiological examination. *C. perfringens* was isolated from all examined piglets. In the case of piglets from farms P-1 and P-2, the bacterium was isolated from scrapings collected from pathologically changed mucosal membrane in the jejunum and from intestinal mucus. In the case of piglets from the farm P-3, *C. perfringens* strains were isolated from samples of intestinal mucus, from clotted milk from the stomach, from the jejunum, and from sections of pathologically changed liver and kidneys. No pathogenic *E. coli* strains were isolated from the examined piglets.

Multiplex PCR evidenced the presence of plc and cpb2 genes in all isolated *C. perfringens* strains (Fig. 2). Apart of plc no genes encoding other main lethal toxins were found, implying that only type A strains were isolated. No strains carrying cpe gene (encoding enterotoxin) were found.

All the isolated *C. perfringens* strains demonstrated in ELISA the production of α-toxin (CPA). The mentioned results confirm the phenomenon of opalescence around colonies caused by lecithinolytic activity of CPA produced by the strains, observed at cultivation of the isolates on TSC agar with egg yolk emulsion (Fig. 1). The ability to produce β2 toxin in the isolated *C. perfringens* strains was identified only genetically.

Discussion

The examined piglets, originating from three different farms, demonstrated various intensity and type of pathological changes. The intestinal inflammatory lesions observed in the piglet from farm P-1 were more advanced than in the piglet from farm P-2 and their portion of the jejunum affected by necrosis could
resemble the changes found in cases caused by *C. perfringens* type C (16). On the other hand, necrohaemorrhagic lesions in demarcated portions of the jejunum or ileum have been described also in cases of enteritis caused by type A (16). Necrotic changes in the piglet from farm P-1 could be also a consequence of a longer pathological process than in the case of the piglet from farm P-2. However, it is noteworthy that the owners of all examined piglets informed about sudden death of the animals.

The intestinal lesions similar to those found in piglets from farm P-3 have been described in reports from cases of enteric infections caused by *C. perfringens* type A (16). The changes, however, are not always connected with the mentioned pathogen. The isolation of *C. perfringens* from the liver and kidneys of piglets from farm P-3 could be a consequence of post-mortem migration of the bacteria from the intestines to other organs (3).

Despite various pathological changes found in necropsied piglets originating from particular farms, nonenterotoxigenic *C. perfringens* type A strains carrying *cpb2* were isolated from the animals. The role of CPA and CPB2 in the pathogenesis of porcine neonatal enteritis is not enough elucidated. However, association of *C. perfringens* type A subtype β2 with enteric diseases in piglets was recently described in reports from different countries (1, 2, 6, 7, 10, 14, 18). The strains of this subtype were isolated from suddenly died piglets (6), from cases of acute and subacute enteritis (1, 10, 14, 18), and from piglets without diarrhoeal symptoms (7). The detection of CPA and CPB2 in clinical specimens or the demonstration of the toxins in cultures of the isolated strains provides additional indications concerning potential pathogenicity of the isolates. No commercial kits for the detection of CPB2 are available until now. However, the data indicate the expression of *cpb2* in over 90% of *cpb2* positive *C. perfringens* strains isolated from cases of piglet enteritis (2).

The data from the mentioned reports and observations of some veterinarians seem to suggest increasing role of *C. perfringens* type A in the aetiology of piglet enteritis in recent years. Laboratory diagnosis of these infections can be hampered because the microorganism is considered to be a part of the natural flora of the intestine in pigs. However, frequent isolation of *cpb2* subtype strains from cases of enteric disorders in pigs and low occurrence of these strains in healthy animals seem to confirm contribution of the subtype to the pathological process.

The piglets described in the present report displayed various types of lesions. Many of them have been listed in descriptions of pathological changes found in pig enteritis caused by *C. perfringens* type A (16). It is not known which of the lesions are caused by CPA and/or CPB2. Further studies concerning the mode of the action of CPA and CPB2 and the role of *C. perfringens* type A *cpb2* positive strains in aetiology of piglet enteric diseases are necessary.

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References

15. Songer G.J., Bueschel D.: Multiplex PCR procedure for genotyping of *Clostridium perfringens* 1999,
