Case Report of Necrotic Enteritis in Neonatal Pigs Caused by Clostridium perfringens Type C

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Necrotizing or necrotic enteritis caused by C. perfringens type C, which is called also C. perfringens type C enteritis, of pigs has been reported in England [5], the United States [1], Denmark [6–8] and East Germany [9]. In Japan, though C. perfringens type A enteritis of pigs has been written [10, 14], there has been no report on type C enteritis. Recently the disease occurred in two prefectures, situated with a long distance from the northern to the southern parts of Japan, in 1979. This paper deals with some findings obtained in the field and the laboratory on these cases.

In the first outbreak, during March to April, 1979, 110 of 259 neonatal pigs, two to three days old, died on a farm (mortality 42.5%), which was raising 60 sows and others in Fukushima prefecture (outbreak F). The piglets succumbed, after having shown bloody, watery diarrhea.

In the second outbreak, during October to November, 1979, 48 piglets, one to three days old (mortality 25.1%) and 21, four to 14 days old (mortality 10.9%), of 191 died on a farm in Oita prefecture (outbreak O). The former of an acute form did not show any recognizable clinical sign, but the latter, less acute one [3], hemorrhagic or non-hemorrhagic diarrhea, listless, and/or undernourishment. The disease appeared more frequently in piglets farrowed by gilts than those by sows.

Main autopsy findings in both outbreaks were as follows: Dark red or reddish orange lesion of hemorrhage and necrosis was confined in the jejunum, differentiated sharply from the normal lower portion. The affected intestinal wall became abnormally thick and its mucosal surface gave a corrugated appearance. A subtle fibrin attached to the serosal surface of the lesion and it also induced adhesion of the latter with the peritoneum. There was copious serosanguineous fluid in the peritoneal cavity. Beside them, in outbreak O, acute case had larger areas of lesion than did less acute cases and emphysema in the mesenterium and also the intestinal serosal surface.

The intestinal contents taken from three piglets in outbreak F were ex-
amined for \textit{C. perfringens} toxin. The specimen was diluted 5-fold with saline and centrifugated at 5,000 rpm for 15 min. The supernatant fluid was filtered through 0.45 \(\mu\)m Milipore filter. After twofold dilution, 0.4-ml portions of the dilution with and without the same volume of \textit{C. perfringens} type A antiserum (Chiba Serum Institute, Ichikawa-shi, Chiba) of 100 U/ml were injected intravenously to a pair of mice. Two of the three filtrates with and without the antiserum killed the mice. From this fact, it became clear that the toxin in the intestinal contents was not type A.

Bacteriological examinations were performed on the intestinal contents, lungs, livers, spleens and kidneys of three piglets from each outbreak. \textit{C. perfringens}-like colonies were obtained on Zeissler’s blood agar plates and Kanamycin-added CW agar plates (Nissui), which were used for viable counting, from the above materials by the Gas-pak method (BBL). The numbers of such colonies in the intestinal contents were \(10^5\)–7/g in outbreak F and \(10^4\)–6/g in outbreak O. Aerobically, coliforms together with or without \textit{Staphylococcus} sp. were recognized on blood agar and/or DHL agar plates from some of the materials in outbreak F but not in significant numbers. Six \textit{C. perfringens}-like organisms selected received further detailed bacteriological studies. From the results obtained, all six strains were identified as \textit{C. perfringens} by referring to Bergey’s manual.

The neutralization tests with \textit{C. perfringens} antiserum were performed on the strains. The supernatants of 18-hr cultures in Nishida’s chopped meat medium [11] were obtained by centrifugation at 5,000 rpm for 15 min. At first the test was carried out to confirm that they were not type A. A mixture of the supernatant, type A antiserum and saline was kept at 37°C for 45 min. and injected intravenously to a pair of mice (II in Table 1). As control, saline-added supernatant was injected to mice in the same way as above (I in Table 1). In both tests, all mice died within an hour. Therefore, it was verified that the strains were not type A. Then, neutralization tests with type C and type D diagnostic antiserum (Wellcome, Kent, U.K.) were attempted. The mixtures were prepared as shown in Table 1 (III, IV, V and VI). The procedure of mouse inoculation was the same as above. to mixtures III and IV, a tryp-

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Supernatant</th>
<th>Saline</th>
<th>Type of antiserum</th>
<th>1% trypsin sol.</th>
<th>Neutralization</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A  C  D</td>
<td></td>
<td>Outbreak F</td>
</tr>
<tr>
<td>I</td>
<td>1*</td>
<td>1</td>
<td>-    -    -</td>
<td>-</td>
<td>1J**</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>0.8</td>
<td>0.2  -    -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>0.3</td>
<td>0.2  -    0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>0.1</td>
<td>0.2  0.2  -</td>
<td>0.5</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>0.6</td>
<td>0.2  -    0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VI</td>
<td>1</td>
<td>0.4</td>
<td>0.2  0.2  0.2</td>
<td>-</td>
<td>++</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Identification</td>
</tr>
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<td>(\alpha)-toxicogenicity</td>
</tr>
</tbody>
</table>

*: ml, **: strain, ***: -; death of a mouse, +; survival of a mouse.
sin solution was added to inactivate β toxin [2] produced by type C and B organisms and to activate ε toxin [12] produced by type D and type B organisms. From the result with mixture III, it was presumed that the strains were type D. However, this presumption was denied by the results with mixture IV. Because in this test, the supernatant was neutralized completely with type C antiserum, in spite of the presence of trypsin. All mice survived for 48 hr in the tests with mixtures IV and VI, both containing type C antiserum. Thus, the strains were identified as *C. perfringens* type C. α-Toxigenicity was examined by the method of Evans [4], in which an egg yolk solution was prepared by the procedure of van Heyningen [13]. The titers of the six strains were in a range of 2.0 to 3.0 as shown in Table 1. This fact is worthwhile remarking, because such high titers are comparable to those of highly toxigenic *C. perfringens* type A in our experiences.

### References


### 要 約

*Clostridium perfringens* C型菌による新生豚の壊症性腸炎例について（短報）：東 量三・浜岡隆文1)・塗井一三2)・丹治敏夫3)・山口弘之3)・志賀一穂3)・近藤房生4)（農林水産省家畜衛生試験場北海道支場, 1)農林水産省家畜衛生試験場, 2)福岡県家畜保健衛生所, 3)大分県家畜保健衛生所, 4)宮崎大学農学部家畜公衆衛生学教室）1979年福島・大分両県下の100農家で新生豚の壊症性腸炎が発生した。福島県では1日に2日間で48/191頭（25.1％）、4～14日間で21/91頭（10.9％）の死亡率を示した。両例とも臨床的には出血性下痢が、剖検では空腸の出血性変状が主であった。各例3株，計6株についてC. *perfringens*の同定がなされ、毒素・抗毒素中和試験の結果，C型菌と同定され，α－毒素原性は2.0～3.0の値を示した。