Brief Note

Isolation of *Clostridium septicum* from Diseased Chickens in Broiler Farms

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Though *Clostridium septicum* has been known as a causative agent of malignant edema in various animal species, there appeared only few papers [2, 4] dealing with the isolation of this organism from chickens in Japan and the serological characterization of the isolates has not been carried out by those authors.

In 1978, we met outbreaks of malignant edema in chickens on three different broiler farms (A, B and C) in Ibaraki prefecture. This paper describes the isolation of *C. septicum* from diseased and dead chickens in these outbreaks and serological characteristics and the pathogenisity against chickens of the isolates.

These three farms adopted the concrete-floor-feeding system with the pens made of wood and wire-netting. On Farm A, 350 of about 5,000 chicken of 30 to 45 days of age died at in a few hours to several days after the onset of such symptoms as loss of activities and reddening and/or swelling of the skins. On Farm B, 250 of about 8,000 chickens of 45 to 55 days of age died with the same symptoms as on Farm A. On Farm C, the mortality rate was about 5% in a group of about 7,000 chickens. The main autopsy findings in diseased and dead chickens were bloody-serous infiltration of subcutaneous tissues and cloudy degeneration of their pectoral muscles.

Bacteriological examinations were made with eight chickens, of which four were 33 to 35-day-old and other three were 55-day-old diseased chickens from Farm A and B, respectively, and the remaining one was a 48-day-old dead chicken with reddening and swelling of the skin from Farm C. Tripticase-soy agar (BBL) supplemented with 1% glucose and 1% sheep blood and Cooked-meat medium (Difco) were used and incubations were made at 37°C for 48 hours under aerobic and anaerobic conditions. Isolates were identified according to Anaerobe Laboratory Manual [1].

Thirty-three strains of *C. septicum* were isolated mainly from the subcutaneous lesions, liver, blood and bone marrow and less frequently from the spleen and the kidney of the eight chickens. In addition to *C. septicum*, *Staphylococcus* sp. and *Escherichia coli* were also isolated from five of the eight chickens.

Then, serological characterization of the isolates was made by the tube agglutination test. The antigens were prepared by centrifuging the broth cultures which had
been incubated for 18 hours and washing the pellets three times with PBS. The pellets were resuspended in PBS with or without added 0.2% formalin. The former was heated at 100°C for one hour. Antisera against five NCTC strains (Nos. 281, 284, 286, 504 and 551) as well as three isolates (IB-86, IB-146 and IB-214) were obtained by immunizing rabbits intravenously with the formalinized antigens. Agglutination tests were carried out with heat-killed antigens and read with an agglutinoscope after 3-hour incubation at 37°C and then overnight standing at 2°C. The results of the agglutination tests are shown in Table 1. As can be seen, the present isolates were grouped into two distinct serovars. According to the criteria of Moussa [3], Nos. 284, 504 and 551 of NCTC strains belonged to O-antigen type 1, while Nos. 281 and 286 to type 2. The antigen of strains IB-86, IB-214, IB-71, IB-82 and IB-111 were agglutinated by antisera to strain NCTC 284, NCTC 551, IB-86 and IB-214 and therefore these strains were identified as O-type 1 of Moussa’s criteria. On the other hand, strains IB-146, IB-1 and IB-144 were identified as O-type 2. In the present tests, however, strain NCTC 504, that was regarded as O-type 1 by Moussa [3], did not cross-react with any other strain of O-type 1. Further studied will be necessary to elucidate this discrepancy. The five strains identified as O-type 1 were originated from Farm A & C, and the three strains of O-type 2 were from Farm B. The fact that different serological types were detected on different farms seems to be significant in relation to epizootiology of the disease caused by _C. septicum_ in chickens.

To examine the isolates for pathogenicity 10-day-old chickens were inoculated into the pectoral muscles with 0.2 to 0.8 ml of 24-hour culture fluids in cooked meat medium with or without added 2.5% CaCl₂. Most chickens died in 24 to 48 hours after inoculation of 0.4 ml of the culture fluid (corresponding to about 5.0 ml per kg of body weight) of each IB strain, while chickens receiving 0.8 ml of the culture fluid diluted 1:10 with physi-
ological saline (equivalent to 1 ml of the culture fluid per kg of body weight) survived. When 2.5% CaCl₂ was added to the culture fluid, the susceptibility of the chickens increased remarkably. Those inoculated with even a 10⁻⁴ dilution died within 2 days. Differences in the virulence among serovars and strains of *C. septicum* were not observed in the present investigations.

REFERENCES


要約

ブロイラー種異常鶏からの *C. septicum* の分離（短報）：白坂昭治・弁野義己（筑城大学農学部家畜衛生学教室，理化学研究所動物薬理研究室）——筑城県下3ブロイラー農場の皮下血漿液浸潤を主徴とする異常鶏8羽の主として皮下病変部、肝、骨髄、血液などから *C. septicum* 33菌株を分離した。血清学的に、2農場由来の菌株は Moussa の凝集反応型別 (1959) の O-type 1 に、他の1農場由来の菌株は O-type 2 に相当したが、10日齢ひな筋肉内接種による病原性試験では両型菌間に差異は認められなかった。