ニワトリ・ボツリヌス症発病への食糞の関与

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Implication of Coprophagy in Pathogenesis of Chicken Botulism

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ABSTRACT. Oral administration of 1×10^7 viable spores of *Clostridium botulinum* type C killed the chickens kept on a board floor to allow them coprophagy, whereas the same dose of the spores failed to develop symptoms in those kept on a wire-net floor not to allow them coprophagy. Type C toxin was detected in the cecal droppings of the chickens of both the groups after feeding the spores and also in serum of symptomatic as well as asymptomatic chickens kept on a board floor. Thus, coprophagy, by which chickens ingest type C toxin (C_1 L toxin) and the bacterial cells, seems to be a prerequisite for development of chicken botulism.—KEY WORDS: broiler botulism, cell-bound toxin, *Clostridium botulinum* type C, coprophagy, C_1 L toxin.

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Botulism is caused usually by ingestion of food or feed containing *C. botulinum* toxin produced in it. Since 1969, outbreaks of type C broiler botulism have been reported in many countries [3]. In many of the outbreaks, however, no source of preformed toxin has been detected. Roberts *et al.* [10] proposed, therefore, that type C spores orally ingested by broiler chickens would proliferate and produce the toxin intraintestinally ("toxico-infection"). In the chicken, the cecum was suggested to be the site of *in vivo* toxin production [9]. Using the cecoligated and untreated pheasants, Kurazono *et al.* [8] demonstrated intracecal toxin production by *C. botulinum* type C. We reported more recently that the cell-bound type C toxin given perorally to the chicken was much more potent than the cell-free toxin, and purified type C progenitor toxin (C_1 L toxin with a molecular weight of 500,000) specifically bound to type C cells, resulting in markedly enhanced oral toxicity to the chicken as well as the mouse [6]. It appeared that the cell-bound C_1 L toxin plays a crucial role in pathogenesis of chicken botulism.

Since the toxin absorbed from the cecum may not be enough to intoxicate the host chicken, which is relatively insusceptible to type C (C_1) toxin [4], we presumed that coprophagy of cecal droppings containing type C toxin (C_1 L toxin) and type C bacterial cells is a prerequisite for the development of chicken botulism. If this presumption is the case, feeding type C spores to the chickens being allowed coprophagy would develop botulism, whereas that to those being not allowed coprophagy would not.

In the present experiments, chickens were grouped into two; the first group were kept on a board floor and the second one on a wire-net floor. *C. botulinum* type C spores of the same doses were fed to both the groups. The first group developed botulism and died, whereas the second one did not.

MATERIALS AND METHODS

Bacterial strains and preparation of spore suspensions: *C. botulinum* type C strains 003–9 and 006–9 were used. The origins of these strains were described elsewhere [6]. For obtaining spores, trypticase-peptone-glucose medium (TPG) was used. The

medium consisted of 5.0% trypticase (BBL, Becton Dickinson, Cockeysville, Md.), 0.5% Bacto peptone (Difco Lab., Detroit, Mich.), 0.5% yeast extract (Oriental Yeast, Tokyo), 1.0% glucose, and 0.1% L-cysteine-HCl (pH 7.6). The culture was incubated for 3 days at 37°C in anaerobic jars (Oxoid, Basingstoke, Hampshire, England) filled with hydrogen gas and room temperature catalyst. The cultures were centrifuged at 3,000×g and the precipitate was resuspended in distilled water to a 0.1 volume of the original culture. The suspension was heat-treated for 15 min at 80°C to destroy the toxin, determined for the viable population, and kept frozen at −20°C until used.

Viable spore count: For viable spore count, serial 10-fold dilutions in distilled water of the spore suspension were each inoculated in 0.5-m*l* quantities into two plastic pouches [1], to which 0.4% egg-yolk agar [5] consisting of 0.1% L-cysteine-HCl, 0.8% of a 50% egg yolk suspension, and 7.4% GAM agar (Nissui Seiyaku, Tokyo), pH 7.3, was added in 15-m*l* quantities. The pouches were incubated for 48 hr at 37°C to count the developed colonies.

Administration of type C spores to chickens: One-day-old chicks (Male, white leghorn, Hy-Line) were purchased from a near-by brooder and maintained in a battery of brooders by feeding on commercial chicken feed. Type C spores were administered orally to them at 2 or 5 weeks old. A 1.0-m*l* portion of a spore suspension containing 1.0×10^7 viable spores/m*l* was administered into the crop with a metal catheter. The chickens were grouped into two; group 1 were housed in wire-net cages (62×90×30 cm) with metal-board floors to allow them access to the droppings and group 2 in cages of the same quality with wire-net (with 2.7-cm meshes) floors not to allow them access to the droppings. All cages were placed in an air-conditioned room. The chickens were fed on commercial feed for growing chickens in troughs, supplied with drinking water in drip-type waterers, and observed for a week for the characteristic symptoms and deaths.

Examinations of the chicken specimens for type C toxin: Blood samples were taken at the moribund stage from diseased chickens and on day 5 from asymptomatic ones by heart puncture and the serum was tested for type C (C_1) toxin. The cecum droppings were collected on the indicated days from the tray underneath the wire-net floor and from the surface of the board floor. Each specimen was macerated in five volumes of gelatin diluent, pH 6.2 [2], centrifuged for 20 min at 8,600×g, and a 0.5-m*l* portion of the supernatant was injected intraperitoneally into a mouse. The mice injected with serum or fecal extract were observed for 4 days for the typical symptoms and deaths. The toxic specimens were subjected to neutralization tests with anti-C_1 serum (goat serum, Chiba Serum Institute, Ichikawa-shi, Chiba).

RESULTS

Viable spores (1×10^7/chicken) of strains 006–9 and 003–9 were each administered orally to a group of four or eight 2-week-old chickens (Table 1). All eight chickens of group 1 given the spores of strain 006–9 developed symptoms and died on day 3. Five of eight chickens given spores of strain 003–9 died on day 4; one of the other three developed mild symptoms but survived as did the other two without any symptoms. No chicken of group 2 given spores of either strain developed symptoms for a week. Type C toxin was detected in the cecum droppings of group 1 sampled on days 3 and 6 (the survivors fed spores of strain 003–9 only) and in those of group 2 sampled on day 2 but not on day 6. Serum toxin was not looked for with these groups.

Spores of the same strains in the same

Table 1. Oral administration of spores[a] of *C. botulinum* type C to 2-week-old chickens kept on a wire-net or board floor

Floor	Strain	Chickens died /number fed	Toxin in the cecal droppings on day		
			2	3	6
Board	006–9	8/8	ND[b]	+	NA[c]
	003–9	5/8	ND	+	+
Wire net	006–9	0/4	+	ND	−
	003–9	0/4	+	ND	−

a) 1×10^7 viable spores per chicken. b) Not determined. c) Not applicable.

Table 2. Oral administration of spores[a] of *C. botulinum* type C to 5-week-old chickens kept on a wire-net or board floor

Floor	Strain	Chickens died /number fed	Toxin in fecal droppings on day			Toxin in serum/number tested[b]
			1	3	5	
Board	006–9	4/4	+	∓	NA[c]	2/2
	003–9	2/4	+	+	+	4/4
Wire net	006–9	0/4	+	+	−	0/2
	003–9	0/4	+	+	−	0/2

a) 1×10^7 viable spores per chicken.
b) Blood samples were taken from asymptomatic chickens on day 5 after feeding spores and at the moribund stage from ill chickens.
c) NA: not applicable.

doses were each fed to both the groups of four 5-week-old chickens (Table 2). All chickens of group 1 given spores of strain 006–9 developed symptoms and died on day 3. Two of four chickens given spores of strain 003–9 died on day 4. One of the other two developed mild symptoms but survived as did the other asymptomatic one. The serum toxin was detected in all the diseased chickens examined at the moribund stage on day 3 or 4 and in mildly ill and asymptomatic chickens tested on days 5. No chicken of group 2 developed symptoms for a week. Regardless of the symptoms, type C toxin was detected in all the cecum droppings sampled on day 1 but not on day 5 (the survivors only). The amount of cecal droppings on the board floor was always smaller than that on the tray underneath the wire-net floor in both the experiments with 2- and 5-week-old chickens.

The characteristic symptoms observed were progressive paralysis of the wings, legs and eyelid, diarrhea, and limberneck.

DISCUSSION

Broiler chickens are relatively insusceptible to *C. botulinum* type C toxin [4] but occasionally affected by type C botulism without detection of the source of preformed toxin. Toxico-infection was suggested by Roberts *et al.* [10] to explain broiler botulism. Intracecal toxin produc-

tion by *C. botulinum* type C was postulated for the chicken [9] and proved in the pheasant [8]. We found that C_1 L toxin binds to type C bacterial cells, by which the toxin becomes orally more toxic to the chicken (chicken oral MLD of purified C_1 L toxin was larger than 5,000,000 mouse ip LD_{50}, whereas that in the presence of 1.5×10^{10} cells was only 500,000 mouse ip LD_{50}), and stated that this binding would play a crucial role in the pathogenesis of chicken botulism [6].

In the present investigation, we demonstrated that the chickens kept on a wire-net floor, being not allowed coprophagy, and fed with 1×10^7 viable spores of *C. botulinum* type C did not develop botulism, whereas those kept on a board floor, being allowed coprophagy, developed symptoms fairly rapidly. The toxin was demonstrated in the cecal droppings of both the groups for at least 3 days after the spore feeding. No serum toxin was detected in those kept on the wire-net floor but detected in those kept on the board floor regardless of the symptoms. The results were interpreted as that type C spores fed orally to the chickens germinated, proliferated and produced the toxin in the cecum, but the amount of the toxin absorbed from the cecum was too small to intoxicate the host. The chickens kept on the board floor were able to ingest the cecal droppings containing the toxin (C_1 L toxin) and the bacterial cells, whereas those kept on the wire-net floor were unable to do so. It is conceivable that C_1 L toxin in the cecal droppings bound to the bacterial cells via the cell-wall peptidoglycan [7] in the proventriculus, where pH is low, having made highly stabilized toxin. The cell-bound toxin, being resistant to the gastric juice and pepsin, came to the duodenum, where the toxin (it is not known whether it is free or still in a form of a complex with the peptidoglycan) was absorbed to cause botulism in relatively insusceptible chichens because of the possible accumulation of the toxin in the circulation due to the slow detoxification of the absorbed toxin [11]. It was noted that spores of strain 006–9 caused illness more rapidly giving a higher fatality rate than did those of strain 003–9. The difference can be explained by our previous finding [7] that the peptidoglycan fraction of strain 006–9 is capable of binding a larger amount of C_1 L toxin than that of strain 003–9.

REFERENCES

1. de Waart, J. and Smit, F. 1967. The enumeration of obligatory anaerobic bacteria using pouches made from plastics with a low oxygen permeability. *Lab. Prac,* 16: 1098–1099, 1105.
2. Dowell, V. R., Jr. and Hawkins, T. M. 1973. Laboratory methods in anaerobic bacteriology. CDC laboratory manual. Centers for Disease Control, Atlanta, Ga. 92p.
3. Eklund, M. W. and Dowell, V. R., Jr. 1987. Avian Botulism. Charles C. Thomas Publisher, Springfield, Ill. 405 p.
4. Gross, W. B. and Smith, L. DS. 1971. Experimental botulism in gallinaceous birds. *Avian Dis* 15: 716–722.
5. Hauschild, A. H. W. and Hilsheimer, R. 1977. Enumeration of *Clostridium botulinum* spores in meats by a pour-plate procedure. *Can. J. Microbiol.* 23: 829–832.
6. Hyun, S. and Sakaguchi, G. 1988. Cell-bound toxin of *Clostridium botulinum* type C and its pathogenic significance. *Jpn. J. Vet. Sci.* 50: 495–501.
7. Hyun, S. and Sakaguchi, G. 1989. Association between the cell-wall peptidoglycan and the progenitor toxin of *Clostridium botulinum* type C. *Jpn. J. Vet. Sci.* 51: 169–176.
8. Kurazono, H., Shimozawa, K., and Sakaguchi, G. 1987. Experimental botulism in pheasants. pp. 267–281. *In:* Avian botulism (Eklund, M. W. and Dowell, V. R., Jr., eds.). Charles C. Thomas Publisher, Springfield, Ill.
9. Miyazaki, S. and Sakaguchi, G. 1978. Experimental botulism in chickens: the cecum as the site of production and absorption of botulinum toxin. *Jpn. J. Med. Sci. Biol.* 31: 1–15.
10. Roberts, T. A., Thomas, A. I., and Gilbert, R. J. 1973. A third outbreak of type C botulism in broiler chickens. *Vet. Rec.* 92: 107–109.
11. Sakaguchi, G., Sakaguchi, S., Kurazono, H., Kamata, Y., and Kozaki, S. 1987. Persistence of

specific antigenic protein in the serum of chickens given intravenously botulinum toxin type B, C, D, E or F. *FEMS Microbiol. Lett.* 43, 355–359.

要　　約

ニワトリ・ボツリヌス症発病への食糞の関与：玄 順 浩・阪口玄二（大阪府立大学農学部獣医公衆衛生学教室）——C型ボツリヌス菌芽胞（1羽当たり1×10^7個）を経口投与すると，食糞を可能にしたニワトリは発症，死亡したが，食糞を不可能にしたニワトリは発症しなかった．食糞可能群，不可能群とも，芽胞投与後数日間，盲腸糞にC_1毒素の排泄がみられた．また，食糞可能群の発症鶏，無発症鶏とも，血中毒素が検出されたが，食糞不可能群からは検出されなかった．以上の結果から，ニワトリに経口投与されたC型ボツリヌス菌芽胞は，消化管（盲腸）内で発芽，増殖すると共に毒素を産生し，盲腸糞に一旦排泄されたのち，菌体と共に再摂取され，毒素（C_1L毒素）は腺胃内で菌体と結合して安定化され，上部小腸から吸収され，ニワトリを発症させたと考えられる．このように，食糞は，ニワトリ・ボツリヌス症発病に重要な役割を果たすと考えられる．