

Avian Botulism Type C in a Commercial Poultry Farm: First Report in Central America

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Abstract

Avian botulism outbreaks are frequently produced by type C neurotoxin secreted by *Clostridium botulinum* proliferating in decomposing bird carcasses and contaminated soils or water sediments. In this study, a botulism outbreak was diagnosed in broilers from a Costa Rican commercial farm through clinical signs, absence of postmortem histopathological lesions, and the confirmation of toxin in the serum of the birds. *C. botulinum* was furthered isolated from the intestine of these animals. This is the first report of avian botulism due to *C. botulinum* type C in Central America.

Keywords: Avian botulism; Central america; Poultry

Case Report

Botulinum neurotoxins (BoNTs) are potent neurotoxins which cause botulism disease. These are produced by various species of clostridia and eight types of BoNTs have been identified (designated with a letter A-H) [1]. *C. botulinum* produces BoNT types A-F and H. *C. baratii* produces type F, *C. butyricum* BoNT type E and *C. argentinense* BoNT type G [1,2]. BoNT types A, B, E, F and H cause disease in humans, types B and D cause botulism in herbivorous mammals (equine and cattle) and type C mainly affects birds [3]. Also, recombinant toxins (C/D) have been described in some cases of avian botulism [2].

C. botulinum spores can survive in soils and water sediments and produce toxins in anaerobic microenvironments including stagnant waters, carcasses of dead animals, and decaying organic matter [4]. Floods, pesticides and other agricultural pollutants can lead to changes in the ecosystem that promote BoNT production [5]. Insect larvae and other flying invertebrates may concentrate BoNTs in their body [2,4,5], then vertebrate animals may ingest these invertebrates along with the toxin. In vertebrates, BoNT alters the neuromuscular junction and prevents the release of acetylcholine in nerve synapses, hindering muscle contraction [2,6]. Finally, progressive flaccid paralysis occurs in intoxicated animals resulting in respiratory failure and death [6].

One human case of botulism has been reported in Costa Rica [7]. Also, *C. botulinum* has been isolated from soils in this country [8]. However, there are no reports of botulism in other Central American countries. Thus, the aim of the present study is to report the first botulism outbreak in poultry in Central America.

An initial group of broiler chickens (15 weeks old) showed tremors, flaccid paralysis in the wings, closed eyes, and twisted neck. Four weeks after the onset of symptoms, 2000 animals died. Dead animals showed no macroscopic or histopathological alterations in their liver,

lungs, kidney, gizzard, spleen, intestine and heart. Parasites (protozoa and helminthes), and bacterial pathogens (mainly *Salmonella* and *Escherichia coli*) were not detected in the organs and blood cultures.

Intestinal and serum samples from sick animals as well as wood shavings, water and food samples given to the chickens, were sent to the Laboratorio de Investigación en Bacteriología Anaerobia (LIBA), University of Costa Rica under suspicion of botulism.

In order to demonstrate the presence of BoNT, lethality and neutralization assays were performed in Swiss mice (20-25 g) (approved by the Animal Care and Use Committee of the Universidad de Costa Rica, protocol CICUA 07-13). Serum samples were inoculated intraperitoneally and *C. botulinum* monovalent anti-toxin types A, B, C, D and polyvalent (ABCDEF) anti-toxins (provided by the Center for Disease Control and Prevention (CDC, Atlanta, USA) were used for neutralization tests. Serum samples heated for 10 min at 80°C were used in the control group [6].

For bacterial isolation 25 ml of water and 25 g of intestine, wood shavings and food samples were homogenized in 50 ml of 0.85% saline solution. Each suspension was inoculated in two tubes of pre-reduced Chopped Meat (CM) (Oxoid, Cheshire, England). One of the inoculated broths for each sample was heated at 80°C for 10 min in order to eliminate other bacteria and induce sporulation, and then these were incubated at 30°C for 5 days. The rest of the samples were incubated under the same conditions [9,10]. Then, all tubes were centrifuged at 12,000 rpm for 10 min at 4°C and supernatants were used for lethality assays in mice, as indicated above. Pellets of each tube were inoculated onto Egg Yolk Agar (Oxoid, Cheshire, England) plates, which were then incubated at 30°C for 48 h, under anaerobic conditions. Lipase positive isolates were furthered identified using ID 32A system (BioMerieux, Marcy l'Etoile, France) [2]. *C. botulinum* isolates were inoculated in CM broth and incubated at 30°C for 72 hr. Supernatants from these cultures were used for toxigenic and neutralization assays in male Swiss mice (20-25 g), as noted above.

Toxicity assays in mice indicated that a heat-labile toxin was present in the serum of sick poultry, as animals treated with serum samples died, while animals treated with heated serum survived. The activity of BoNT in the sera was neutralized with polyvalent antiserum and with a specific serum anti-toxin C. Inoculated animals treated with anti- A, B, or D did not survive.

The supernatants of the cultures from food and water samples were non-toxicogenic in the animal model assay. On the other hand, supernatants of intestinal and wood shavings samples that had been previously heated for 10 min at 80°C and incubated for 5 days were toxicogenic. The effect induced by the supernatants was neutralized with anti-toxin C. *C. botulinum* was then isolated from wood shavings from the poultry houses and its toxicogenic potential was demonstrated in the animal model. This effect was neutralized with anti-toxin C.

Discussion

The *C. botulinum* type C outbreak reported occurred on a poultry commercial farm. Possible causes for this outbreak could be that the animals may have fed from fly larvae and dead birds, which contain a high concentration of toxins [2,4]. Furthermore, in the surroundings of the henhouses a small swamp was located. This body of water eventually became dry and may have encouraged an increase in temperature providing the necessary conditions for the rapid replication of *C. botulinum* [9]. In fact, the presence of *C. botulinum* was demonstrated in the sediment around poultry houses (data not shown).

The occurrence of type C botulism is quite rare in carnivores. There are few reports in dogs, lions and cats [3] and although there is risk for human disease, no cases have been reported in humans [10]. To our knowledge, this is the first report of a documented case of avian botulism in Central America. This situation should be of concern for veterinarian health and scientific authorities.

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