A Report on an Outbreak of Botulism in Broilers in Pakistan

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ABSTRACT

An outbreak of type C botulism in four week-old broilers in an open farm house is described. Botulism is an intoxication caused by exotoxins of Clostridium botulinum. At 4 weeks of age, an increase in mortality was observed in the broilers. Clinically, the birds presented with paralysis of the legs, wings, and neck. Affected birds were sitting and reluctant to move. Necropsy failed to find any specific lesions. Except water, investigations of environmental samples to detect the source of the toxin were not successful despite repeated testing. DNA of C. botulinum type C was detected by PCR in liver, heart, muscles and crop. The result was confirmed by a mouse lethality neutralization test. During the 2 weeks after the onset of the clinical signs the mortality was about 47.8%. After 2 weeks, clinical signs and mortality abated.

Botulism is a severe flaccid paralytic disease caused by ingestion of feed or water contaminated with the botulinum neurotoxins. Clostridium botulinum is a Gram-positive, obligatory anaerobic, spore forming bacterium. While growing, this organism can produce exotoxins that are potent neurotoxins. The toxins can be released during autolysis under the right environmental conditions (Bonventre and Kempe, 1960). Seven botulinum neurotoxins (A, B, Cα, D, E, F, G) are known, which are produced by different toxovars of C. botulinum (Collins and East, 1998; Souillard et al., 2014). Human disease has been mainly associated with botulinum neurotoxins types A, B, E and (more rarely) F, whereas animal botulism is mainly associated with botulinum neurotoxins types C and D (Dohms, 2008). Most avian cases are caused by C. botulinum toxin type C. Rarely, toxin types A, D, and E have caused disease in birds (Dohms, 2008). Botulism was first reported in chickens in 1917 (Dickson, 1917) and has been reported many times in the literature since 1970s. Avian botulism is a serious problem in developing countries, leading to significant economic losses (Lindberg et al., 2010; Abudabos, 2013). A fairly large number of case reports have been published about botulism in broiler chickens in Australia (Harrigan, 1980), USA (Dhom et al., 1982; Pecelunas et al., 1999; Schockenitturino et al., 1985), and Europe (Haagsma, 1974; Roberts et al., 1973). Because et al., 2014; Sharpe et al., 2011). No data are available chickens seem to become increasingly resistant against botulism with age (Dohms, 2008; Hardy and Kaldhusdal, 2013; Abudabos, 2013), the disease has rarely been described in laying hens (Fossum et al., 2009; Souillard et al., 2014). 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with partially pale areas, while skeletal muscles of the breast and legs were pale. Histopathology investigations by routine methods showed acute fatty degeneration of the myocardium, as well as acute, multifocal degeneration of skeletal muscles without cellular infiltration, which seemed to indicate bacterial intoxication. Further, liver, spleen, and kidney were severely hyperemic due to congestion. Diagnostic services were provided by Veterinary Research Institute (VRI) Lahore and Quality operation Laboratories University of Veterinary and Animal sciences Lahore Pakistan. Feed was analyzed by high-performance liquid chromatography (HPLC) for mycotoxins (Popp et al., 2012). Only aflatoxin was detected at a level of 104 mg/kg, which was in the normal range. Examination of livers by HPLC did not reveal the presence of other mycotoxins except aflatoxin at a concentration of 80–120 mg/kg. Miscellaneous gram-positive cocci were isolated from the heart blood, lungs and liver on blood agar (Oxoid, Wesel, Germany). In addition, Clostridium perfringens was isolated from the liver and identified as C. perfringens type A by multiplex PCR as described previously (Souillard et al., 2013; Gad et al., 2011; Vidal et al., 2013). By mouse lethality neutralization test (Smith, 1980), botulinum neurotoxin type C was detected in pooled crop, liver and gizzard contents. Using PCR primers for the botulinum toxin gene, the bacteria were detected in the samples from the liver, heart, muscles, crop, and gizzard, as well as from intestinal contents (Williamson et al., 1999; Sharp et al., 2011; Vidal et al., 2011). In the first attempt, samples of mud, feed, stored straw, fresh water, slaughterhouse waste, old litter deposited outside the farm were taken and analyzed using PCR for the C. botulinum toxin C gene DNA. All the samples revealed negative results. In the second attempt, water samples from poultry farm pond were taken revealed positive results for C. botulinum. Due to the reason of unreliable electric supply in Pakistan, water is usually stored in artificial ponds and used at the time of need. Several measures were taken to prevent the spread of disease within the affected house. Immediately after the onset of clinical signs the birds were treated with penicillin (Penivet®, Star laboratories (Pvt) Ltd, Pakistan) at a dose of 1 g/liter in the drinking water for 5 days. Furthermore, all the water utensils were washed with disinfectant and fresh water was provided and new litter was added daily. Dead birds were frequently collected and removed. After 1 week, 478 birds (47.8%) had died. After 2 weeks, clinical signs and mortality abated in remaining birds. In order to decide how to proceed with the flock, the possibility of slaughtering the flock without compromising food safety had to be clarified. Poultry meat originating from flocks infected with C. botulinum, even if there is confirmation of botulism, can be used for human consumption. This recommendation explicitly also applied to cases like the one described here, where the disease of the flock was caused by C. botulinum type C, which is not considered to cause disease in humans (Popp et al., 2012; Skarin et al., 2013). Finally, in agreement with the responsible authorities and the local veterinary office, remaining birds were raised to 6 weeks and then sold in local market for human consumption. After cleaning, disinfection, as well as after pest control, poultry house was restocked with broiler chicks, which remained free from infection during the entire rearing period.

**Discussion**

The present report describes an outbreak of botulism in commercial broilers. Although a number of case reports about botulism in chickens have been published, reports about the disease in Pakistan are rare. Generally, clinical signs of botulism in chickens, turkeys, and other birds are similar. As in this case, flaccid paralysis of legs, wings, neck, and eyelids are the most predominant features of the disease, and death is caused by cardiac and respiratory failure. As in other described cases with type C botulism in birds, necropsy findings were un-remarkable (Harrigan, 1980; Haagsma, 1974; Schockeniturrino et al., 1985; Smart et al., 1983). Field cases of type C botulism in broiler chickens has generally been described as single-farm–related problem and mostly limited to certain farms. Birds in one farm experienced a high mortality rate, whereas birds in adjacent farm showed no illness or deaths (Roberts and Collings, 1973; Dohms et al., 1982). In the present investigation, a similar observation was made, as only one broiler farm was affected, due to provision of contaminated water, perhaps because of its proximity to the slaughter house. The slaughter house site is a favored place for pests such as rats, flies and maggots. One possible explanation for contamination of water sources is leakage of dead debris of slaughter house in rainy weather seems to be the major cause of water source contamination. In the present case, DNA of C. botulinum was detected in stored ponds water, indicating this possibility. However, examination of several other environmental samples by PCR for detection of C. botulinum toxin C gene DNA revealed negative results.

In the past, source of toxin could not be determined as in the present study, when feed, water and litter were tested after confirmed outbreak of botulism (Dohms et al., 1982; Trampel et al., 2005). In the case described here, after cleaning and disinfection as well as pest control, house was restocked with broilers, which remained free from infection during the next rearing period.
periods. This also is in accordance with the experience previously reported (Dohms, 2008; Popp et al., 2012). Human disease is mainly associated with type A, B, E, and F (Collins and East, 1998). Thus the public health significance of avian type C botulism is considered minimal (Dohms et al., 1982; Raymundo et al., 2012; Popp et al., 2012). The only case relating human botulism to poultry was the intoxication of humans with type A botulism by consumption of a precooked chicken meat vegetable mix, which had been stored for a prolonged period of time at room temperature (King, 2008; Skarin et al., 2010). So, the flock in the case described here sold to the consumer level and complete cleanliness and disinfection of farm was performed. Moreover, source of stored water was destroyed completely to prevent spread of infection to other flocks. In conclusion, it should be noted that despite the relatively small number of outbreaks in poultry, botulism still is a problem. This is mainly because of lack of biosecurity measures and poor management conditions in developing countries like Pakistan.

References