Pakistan J. Zool., vol. 48(1), pp. 298-300, 2016.

Short Communication

A Report on an Outbreak of Botulism in Broilers in Pakistan

Sajid Umar,[1,3]* Muhammad Irfan Khan,[2] Muhammad Younus,[2] Muhammad Yaqoob[1] and Qamar-un-Nisa[2]

[1]*Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan*
[2]*University of Veterinary and Animal Sciences, Lahore, Pakistan*
[3]*National Veterinary School, Toulouse, France*

Article Information
Received 9 May 2015
Revised 13 July 2015
Accepted 31 July 2015
Available online 1 January 2016

Authors' Contributions
SU designed the study and performed PCR analysis. MIK performed feed and water analysis. MY performed necropsy studies, MY performed clinical analysis of live birds. QUN isolated and identified bacteria.

Key words
Botulism, poultry, broilers, *Clostridium botulinum*.

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, C_α, 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; Schockeniturrino *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 regarding contamination by *C. botulinum* spores of poultry houses and surroundings after a botulism poultry outbreak in Pakistan. Equally rare are descriptions of outbreaks in chicken in Pakistan. Until now, no case has been published in scientific journals. In other cases botulism was suspected but could not be reported. The lack of well-documented cases is surprising, since according to practitioners broilers are susceptible to *Clostridium* toxin due to lack of biosecurity measures and poor management of most of the poultry farms in Pakistan. The present paper describes a case of botulism in a commercial broiler flock.

The outbreak occurred in a broiler farm, just opposite to a slaughter house. The slaughter house waste products and other organic wastes are often deposited at the site, which leads to severe rat, flies and maggot infestation. In poultry house, 1000 broilers were reared. At 2 weeks of age, broilers suffered from *Escherichia coli* infection, characterized by polyserositis. The infection was treated with colistin sulfate.

At 4 weeks of age, mortality of the broilers suddenly increased within a few hours. This was accompanied by paralysis of legs, wings, and neck. Affected birds were sitting and reluctant to move. Gasping was observed in some birds shortly before death. At necropsy no specific lesions or findings were detected. Livers and spleens were swollen, and spleens and kidneys were marbled. Heart muscles revealed a shining red color

* Corresponding author: s.umar@envt.fr
0030-9923/2016/0001-0298 $ 8.00/0

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. perferingens* 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_{α} 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 the 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

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

Abudabos, A.M., 2013. *Pakistan J. Zool*., **45**: 371-376.

Bonventre, P.F. and Kempe, L.L., 1960. *J. Bact*., **79**:18–23.

Collins, M. D. and East, A.K., 1998. *J. appl. Microbiol*., **84**:5–17.

Dickson, E.C., 1917. *J. Am. Vet. Med. Assoc*., **50:** 612–613.

Dohms, J.E., 2008. In: *Diseases of poultry* (eds. Y.M. Saif, A.M. Fadly, J.R. Glisson, L.R. McDougald, L.K. Nolan and D.E. Swayne) 12th edn. Blackwell Publishing Ames, pp. 879–885.

Dohms, J.E., Allen, P.H. and Rosenberger, J.K., 1982. *Avian Dis.*, **26:**204–210.

Fossum, O., Jansson, D.S., Etterlin, P.E. and Vagsholm, I., 2009. *Acta Vet. Scand*., **51**:3.

Gad, W., Hauck, R., Kruger, M. and Hafez, H.M., 2011. *Arch. Geflkd.*, **75**:74–79.

Haagsma, J., 1974. *Tijdschr. Diergeneesk.*, **99**:1069–1070.

Hardy, S.P. and Kaldhusdal, M., 2013. *Vet. Microbiol*., **165**: 466–468.

Harrigan, K. E., 1980. *Aust. Vet. J*., **56**:603–605.

King, L.A., 2008. *Eurosurveillance*, **13**:18978.

Lindberg, A., Skarin, H., Knutsson, R., Blomqvist, G. and Båverud, V., 2010. *Vet. Microbiol*., **146**:118–123.

Pecelunas, K. S., Wages, D.P. and Helm, J.D., 1999. *Avian Dis.*, **43**:783–787.

Popp, C., Hauck, R., Gad, W. and Hafez, H.M., 2012. *Avian Dis.*, **56**:760–763.

Raymundo, D.L., Von Hohendorf, R., Boabaid, F.M., Both, M.C., Sonne, L., Assis, R.A., Caldas, R.P. and Driemeier, D., 2012. *J. Zoo Wildl. Med.,* **43**:388–390.

Roberts, T. A. and Collings, D.F., 1973. *Avian Dis.*, **17**:650–658.

Roberts, T. A., Thomas, A.I. and Gilbert, R.J., 1973. *Vet. Rec.* **92**:107–109.

Schockeniturrino, R. P., Deavila, F.A., Pinese, J.E. and Yokoya, F., 1985. *Brazil. Rev. Microbiol.*, 16:31–35.

Sharpe, A. E., Sharpe, E.J., Ryan, E.D., Clarke, H.J. and McGettrick, S.A., 2011. *Vet. Rec.*, **168**:669.

Skarin, H., Lindberg, A., Blomqvist, G., Aspán, A. and Båverud, V., 2010. *Avian Path.*, **39**:511–518.

Skarin, H., Tevell Åberg, A., Woudstra, C., Hansen, T., Löfström, C., Koene, M., Bano, L., Hedeland, M., Anniballi, F., De Medici, D. and Olsson Engvall, E., 2013. *Biosec. Bioterr.,* **11**: S183–S190.

Smart, J.L., Laing, P.W. and Winkler, C.E., 1983. *Vet. Rec.*, **113**:198–200.

Smith, L.D.S., 1980. Clostridial infections. In: *Isolation and identification of avian pathogens*, 2nd ed. (eds. S.B. Hitchner, C.H. Domermuth, H.G. Purchase and J. E. Williams), American Association of Avian Pathologists, Endwell, NY, pp. 33–35.

Souillard, R., Woudstra, C., Dia, M., Léon, D., Toux, J.Y., Guimaraes, V., Bayon-Auboyer, M.H., Michel, V., Le Bouquin, S. and Fach, P., 2013. *Uses of real-time PCR assay for detection of* Clostridium botulinum *neurotoxinogen in diseased and healthy poultry farms*. In: *10ème Journées de la Recherche Avicole et Palmipèdes à Foie Gras*, pp. 341–345, La Rochelle.

Souillard, R., Woudstra, C., Le Maréchal, C., Dia, M., Bayon-Auboyer, M.H., Chemaly, M., Fach, P. and Lebouquin, S., 2014. *Avian Path.*, **43**:458-464.

Trampel, D.W., Smith, S.R. and Rocke, T.E., 2005. *Avian Dis.*, **49**:301–303.

Vidal, D., Anza, I., Taggart, M.A., Perez-Ramirez, E., Crespo, E., Hofle, U. and Mateo, R., 2013. *Appl. environ. Microbiol.*, **79**: 4264–4271.

Vidal, D., Taggart, M.A., Badiola, I. and Mateo, R., 2011. *J. Vet. Diagn. Invest.*, **23**: 942–946.

Williamson, J., Rocke, T. and Aiken, J., 1999. *Appl. environ. Microbiol*., **7**:3240–3243.