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Clinical and Epidemiological Aspects of an Outbreak Suggesting Botulism in Free-Range Chicken (*Gallusgallus domesticus*) in the State of Maranhão, Brazil

Aspectos Clínico-Epidemiológicos de um Surto Sugestivo de Botulismo em Galinha Caipira (*Gallus gallus domesticus*) no Estado do Maranhão, Brasil

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Abstract

Birds may experience outbreaks of disease and exhibit symptoms of progressive flaccid paralysis, which may be related to poisoning through botulinum toxins arising from anaerobic sources in the rearing regimen. The botulism outbreaks in birds are associated more closely with type C toxins, and they are among the most vulnerable species, showing a high degree of susceptibility to this poison. This study was conducted with the objective of characterizing the outbreak of botulism in free-range chickens, considering of the clinical and epidemiological features of this case. Care was taken to provide reliable data in order to enable wise decisions and implementation of a course of prophylactic actions in these farming systems. A rustic breeding system suddenly experienced an outbreak in which birds of all ages were affected, around 50 % of the birds (15/30), where the 15 birds affected with peripheral nervous system and muscular dysfunctions, typical of botulism were evaluated, exhibiting easily detached feathers and flaccid paralysis ascending up the legs, wings and neck, resulting in the demise of all the affected chickens. Unfortunately, the antibiotic-based treatment did nothing to slow down or stop the progression of clinical signs, and no improvement was observed in the affected birds. Thus, the rural farming systems must necessarily ensure preventive measures as a priority. These include vaccination and eradication of the sources that promote high levels of contamination by botulinum toxins, like stagnant water puddles, decaying food waste, rotting carcasses, and soiled chicken litter, besides other anaerobic sources.

Keywords: Birds. *Clostridium botulinum.* Flaccid Paralysis. Feather Drop. Toxins.

Resumo

Surtos com paralisia flácida progressiva podem ocorrer em aves e estão associadas à intoxicação por toxinas botulínica provenientes de fontes anaeróbicas existentes no sistema de criação. Os surtos de botulismo em aves estão mais associados às toxinas do tipo C, estando entre as espécies mais vulneráveis, apresentando alto grau de suscetibilidade. O objetivo do trabalho foi caracterizar o surto sugestivo de botulismo em galinhas caipiras, considerando os aspectos clínicos e epidemiológicos do caso, assegurando o conjunto de dados confiáveis para tomada de decisões e adoção de medidas profiláticas em sistemas de criação de galinhas caipiras. O surto ocorreu em um sistema de criação caipira, acometendo aves de todas as idades, cerca de 50% das aves do plantel (15/30), onde foram avaliadas as 15 aves acometidas com disfunções do sistema nervoso periférico e muscular, típicos de botulismo, com penas facilmente desprendidas e paralisia flácida ascendentes das pernas, asas e pescoço, culminando na morte de todas as aves acometidas. O tratamento realizado a base de antibiótico não influenciou na progressão dos sinais clínicos, portanto, sem melhora clínica das aves acometidas. Desta forma, medidas preventivas devem ser priorizadas no sistema de criação caipira, como vacinação e eliminação de fontes que apresentem alto risco de contaminação por toxinas botulínicas, como poças d'águas estagnadas, restos alimentares em putrefação, carcaças, cama de frango contaminada, dentre outras fontes anaeróbicas.

Palavras-chave: *Aves. Clostridium botulinum. Paralisia Flácida. Queda de Penas. Toxinas.*

1 Introduction

The ingestion of *Clostridium botulinum* induces the release of botulinum neurotoxins which cause Botulism, a neuromuscular intoxication producing progressive flaccid paralysis in birds (DE OLIVEIRA JUNIOR *et al.*, 2016). In birds, the botulism outbreaks reported are associated more closely with type C toxins, and they are among the most vulnerable species, showing a high degree of susceptibility to this poison (OLINDA *et al.*, 2016; WLODARCZYK *et al.*, 2014). The botulinum toxins are highly prevalent in anaerobic habitats, where *C. botulinum*, can spread swiftly. This anaerobic spore-forming bacterium can induce severe poisoning in birds, with the typical and distinctive symptoms such as flaccid paralysis, resulting in high mortality (SOUILLARD *et al.*, 2016; SILVA *et al.*, 2017).

The paralysis occurs due to the toxins acting upon the presynaptic nerve ends of the neuromuscular junction, lowering the acetylcholine produced, thus inhibiting muscle contraction. The signs of paralysis become evident progressing cranially, commencing with the legs, moving upwards to the wings, neck and eyelids, and ultimately leading to the birds' death . The cause of death is due to heart and respiratory failure induced by the paralysis of the muscles responsible for the cardiorespiratory functions (DOLLY, 2003).

As botulism outbreaks always culminate in the high birds mortality, the farmers suffer significant economic

losses; hence, in certain countries, botulism is a mandatory notification disease (SOUILLARD *et al.*, 2016). Although it has a crucial sanitary aspect, in birds, botulism is rarely reported, and necessarily requires greater investigation from both clinical and epidemiological perspectives. This is because in farming systems for free-range birds preventive measures need to be established and implemented, and the clinical and epidemiological data of such poisoning must be used as a guide to introduce direct measures to reduce and eliminate the risks that this poisoning can cause (SOUILLARD *et al.*, 2016, ANIMAL & PLANT HEALTH AGENCY, 2016).

Thus, this work aims at characterizing the outbreak indicative of botulism in farms raising free-range chickens, due to the clinical and epidemiological features of the case. The study ensures the provision of a reliable dataset to enable wise decisions and the implementation of prophylactic procedures in free-range chicken farming regimens.

2 Material and Methods

The rural area of Montes Altos in the State of Maranhão, northeastern Brazil, experienced an outbreak, suggesting botulism, which affected 15 free-range chickens of all ages, raised extensively on feed which included corn grain, cooked rice and domestic food waste. The water sources included pond and stagnant water available in the breeding environment, or sources either from rainwater or domestic use.

The region where the outbreak occurred revealed features typical of the Cerrado biome namely, rainfall over a clearly defined period, frequently commencing with mild rain-showers in November, and extending until April-May of the next year, as well as a sharply distinctive and characteristic dry period from approximately mid-May to June and lasting till October end. This outbreak was observed towards the close of the dry period, during which pools of stagnant water low in oxygenation are noted.

Breeding involves the free-range raising of chickens of all ages, of no specific breed, where the complete breeding cycle is performed, beginning with reproduction until the final stage, when the birds are slaughtered. Out of the the 30 birds (in total) present at the beginning, 15 (50 %) of them started to show symptoms and became gradually affected. They stopped feeding and began to show clinical symptoms characteristic of botulism, linked to dysfunction in the muscles and peripheral nervous system, such as easily detached feathers and flaccid paralysis ascending from the legs and wings to the neck (Figure 1A, 1B, 1C).

Figure 1 - Clinical signs suggesting botulism in free-range chicken. A) Conscious bird with flaccid pelvic limb paralysis. B) Conscious bird with flaccid wing paralysis. C) Comatose bird with flaccid paralysis of the neck

Source: The authors'.

The birds thus affected were conscious, but lacked symptoms that implied no dysfunction in the central nervous system (1A, 1B). The symptoms were observed to occur generally in an ascending pattern, commencing first with paralysis of the pelvic limbs, making any movement impossible for the birds. This paralysis then spread to the wings, neck and eyelids, within 3 to 5 days, until the birds succumbed due to respiratory failure. All the affected birds struggled until the final stages displaying symptoms of agony and general muscle flaccidity, with a flexible neck and open wings, ending in the death of all of them.

The affected birds were treated with an antibiotic based on sulfamethoxazole (10 %) + trimethoprim (2 %) (Trissulfin Pó, Ouro Fino, São Paulo/Brazil) diluted in water and administered orally in a dosage of 40 mg /kg/day for three days. Further, an antibiotic based on oxytetracycline (10%) + lidocaine (2%) (Tormicin 100 injectable, Fabiani Animal Health, São Paulo/Brazil) was given in a dose of 1 ml/kg as a single administration. Unfortunately, this treatment was not effective, and the birds failed to show clinical improvement; all the affected birds displayed similar clinical evolution, suggesting that the antibiotic-based drug treatment exerted no effect on the clinical signs of these t birds.

3 Results and Discussion

The clinical symptoms and progression of the disease in the birds affected with flaccid paralysis and unaltered psychic state, concurred closely with the clinical signs of botulism in birds. The cranial progression of the paralysis, leads to wing drop and flaccid neck, dyspnea and ultimately death, due to severe cardiorespiratory dysfunction (DOLLY, 2003). The clinical signs and absence of specific pathological alterations, together with the epidemiological data, directed the diagnosis to the possibility of botulism because of the lack of evidence to suggest differential diagnoses like transient cerebral palsy, Newcastle disease, encephalomyelitis, avian cholera, musculoskeletal disorders, toxicity and other bacterial and fungal diseases which could result in a similar clinical sign (DOLLY, 2003). According to Souillard *et al.* (2016)

the susceptibility of birds to botulinum toxin-poisoning declines with age; however, in this outbreak, chickens of all ages showed symptoms, with no particular age group being predominant. Upon considering all the environmental and management features involved in the breeding, as well as the high percentage of birds affected, it became clear that the likely sources of contamination could be linked to the intake of these preformed toxins, from a common and abundant place of origin. The original source of the botulinum toxins was hard to confirm in bird poisoning, but there was a strong suspicion that the stagnant puddles of water could have been the possible cause. This assumption was made because the outbreak took place at the end of the drought period, when the toxins were found in higher concentrations in these water bodies. However, the possibility of the birds ingesting these toxins from other food sources, whenever available, cannot be ruled out. This is due to the extensive rearing system, where little or no control can be exerted in terms of the food consumption of these birds (ANZA *et al.*, 2014; DE OLIVEIRA JUNIOR *et al.*, 2016). When the rains commenced, the cases were nonexistent, probably because of the increase in the availability of water for the birds to drink, and which possibly diluted the toxins that were likely present in the stagnant water pools. This state of events, involving the heightened rainfall and nonexistence of cases, strengthens the inclination to accept this hypothesis; however, further research work is mandatory before this assumption can be confirmed.

The birds affected were not of any specific breed, and showed no signs of resistance to clinical suspicion because all of them displayed identical symptoms and clinical evolution of the disease. According to de Oliveira Junior *et al.* (2016) based on the intensity of the poisoning, different clinical conditions are visible in birds with botulism. This can range from mild cases, with instability during movement and drowsiness, but with recovery within 4 days, to severe cases, revealing extreme prostration and paralysis of the leg muscles, ascending to the wing and neck, and culminating in death. All the cases observed during this outbreak revealed very similar disease progression, within a period of up to five days, and considered as severe because all the birds suffered agonizing deaths, with flaccid paralysis, typical of botulism.

The high mortality observed during this outbreak is a significant characteristic of clinical suspicion and is a highlight of this case because one-half of the poultry failed to recover and exhibited identical clinical evolution until they succumbed to death. As the high mortality in the botulism cases results in significant economic losses to the breeding system, preventive measures must definitely be implemented (SOUILLARD *et al.*, 2016; SATO *et al.*, 2016). In birds, the presumptive diagnosis of botulism needs to be based on recording a successful patient history plus as well as the characteristic clinical symptoms, as in some cases instances the toxin may not be detected, as in the sero-neutralization in mice (ANNIBALLI *et al.*, 2013; DE OLIVEIRA JUNIOR *et al.*, 2016). The birds affected by this outbreak showed the typical clinical symptoms of botulism hich, on analysis, due to the patient's history and epidemiological aspects, bolstered the evidence to confirm botulism in these birds.

In order to reach a confirmatory diagnosis of botulism, serum neutralization in mice is accepted as the 'gold standard' method but is not always successful in detecting botulinum toxins in intoxicated birds. A negative result in this test, however, does not exclude the presence of botulism. In such cases, as encountered in the present study, a complete clinical epidemiological assessment is necessary to make the diagnosis, and therefore confirmatory tests were not done for confirmation (SILVA *et al.*, 2017). All the clinical signs displayed by the infected birds during the outbreak concurred with the case studies of botulism in birds, and significantly support the presumptive diagnosis of botulism (LOBATO *et al.*, 2009; SATO *et al.*, 2016).

The treatment given to the infected birds, based on antibiotic administration, enabled the possibility of bacterial diseases sensitive to the antibiotics given, to be ruled out because the evolution and clinical states of the affected birds remained unchanged by the treatment. The treatment indicated for botulism is based on the administration of serum, antitoxin C/D; however, it was not possible to administer it to the sick birds (SILVA *et al.*, 2017).

4 Conclusion

Negligence and underreporting of botulism cases in free-range chickens can raise the degree of damage in this type of breeding system. Hence, in rustic farms, it is mandatory to implement preventive measures to keep these birds from gaining access to sources that are potentially high-risk sites of contamination by botulinum toxins. Such sites include stagnant pools of water, decaying food waste, rotting carcasses and soiled chicken litter. Vaccination, as a preventive measure, is very effective in these creations in regions where avian botulism is endemic. More research applied to botulism in free-range chickens is needed, and should be encouraged, in order to prevent this type of disease in these birds.

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