AN OUTBREAK OF BOTULISM AMONG SHEEP AND GOATS IN NORTHERN LOCALITIES OF NORTH KORDOFAN STATE, SUDAN

A.A.Ibrahim[1*] and M.T.A. Shigidi[2]

المستخلص

ورد بلاغ الى ادارة الثروة الحيوانية بشمال كردفان بظهور134 حالة مرضية من مجموع 3480 رأسا من الضأن و الماعز خلال شهر بالمحليات الشمالية لولاية شمال كردفان. قدرت نسبة النفوق بخمسين فى المائة. و نسبة لاشتباه مرض التسمم الوشيقى، فان الدراسة هدفت لتشخيص و التعرف على البكتيريا المسببة له و عترتها.

اجرى تقصى حقلى لجمع المعلومات من الرعاة، حيث اخذت 10 عينات مصل من الحيوانات المريضة، 9 عينات من محتويات الكرش ، و 6 عينات من انسجة الحيوانات النافقة. تم تشريح 9 جثث لحيوانات نفقت حديثا.

باختبار التعادل فى الفيران تم كشف ذيفان بكتيريا التسمم الوشيقى فى عينة واحدة من الامصال ، وعينة واحدة من محتويات الكرش، و 3 عينات من انسجة الحيوانات لنافقة. اكد الاختبار ان مسبب الوباء هو المطثية الوشيقية العترة C.

Abstract

The study reports the results of an investigation of an outbreak of a disease in sheep and goats in the northern localities of North Kordofan State which was diagnosed on clinical signs to be botulism. One hundred and thirty four out of 3480 sheep and goats under natural grazing, were affected within a month time. Field investigation was made to collect information about the disease by interviewing the owners. Necropsy was made on 9 fresh carcasses of dead animals.

Toxin neutralization test in mice detected Type C botulinum toxin in 1 of 10 serum samples, in 1 of 9 samples of rumenal contents from dead animals, and in 3 of 6 bones and muscle tissues from carcasses. The test confirmed *Clostridium botulinum* Type C toxin to be the possible cause of the outbreak.

[*1] Department of Microbiology, Faculty of Veterinary Science, University of West Kordofan, El-Nuhud. E-mail- abduadam19@yahoo.com

[2] Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, Khartoum North, P.O. box, 32.

Introduction

Botulism is a neuroparalytic intoxication that is often fatal and is characterized by a generalized muscle weakness, and in severe cases, by a flaccid paralysis. It is caused by a potent neurotoxin produced by any of seven (A-G) strains of *Clostridium botulinum* (Sesardic *et al.*, 2000 and Chaddock *e tal*.,2002).

The disease usually causes major economic losses in farm animals due to high mortality which may be as high as 50% (Jensen and Swift, 1982; Trueman *et al*., 1992).

Sheep botulism was first reported in Southern and Western Australia (Bennetts and Hall, 1938).Thereafter, the disease was reported in Southern parts of Africa and in Gulf coasts of the United States of America (Merchant and Packer, 1967).

Botulism is closely associated with drought, as in such instances, animal pastures become scarce and animals suffer from nutritional deficiencies. In trying to satisfy the craving, animals usually chew decomposed carcasses, which are the major sources of botulinum toxin.

According to Lisboa *et al.* (1996), animals grazing on such extensive ranges with inadequate supply of nutrients, are liable to develop pica which when seen in ruminants, is a strong evidence of botulism (Radastits *et al.*, 2000).

An outbreak of suspected botulism was noticed for the first time among sheep and goats in North Kordofan State, with a death rate of more than 50% of the affected animals. Clinical signs, disease epidemiology and animal behavior, lead to the suspicion of botulism .The purpose of this study was to identify the causative agent of the disease.

Materials and Methods

Samples:

Blood for sera was collected from 79 sick animals; 20 sera from goats and 59 from sheep. About 5 ml of blood were collected in sterile (5ml) plain vaccutainer tubes, which were kept overnight in the refrigerator, then the clear sera were siphoned with sterile capillary pipettes into sterile bijou bottles.

After necropsy of fresh carcasses, about 250g of rumenal contents were collected from 9 carcasses in sterile plastic bags for toxin determination. From 106 carcasses, 250g of bones and tags of flesh were collected from each carcass in sterile plastic bags for toxin detection.

The procedure for preparing suspensions from rumenal contents and carcass tissues was as described by Haim and Timothy (1998). About 2g of rumenal contents or carcass tissues were weighed in a sterile piece of aluminum foil and transferred to a sterile mortar. Then 2 ml of cold gelatin diluents (o.2% gelatin, o.4% Na2HPO4 in 100 ml normal saline)) per gram of tissue, were added and mixed until a uniform suspension was obtained. The prepared suspension was held overnight in refrigerator and then centrifuged at 12000 × g in a refrigerated centrifuge at 4C for 20 minutes.

The supernatant was centrifuged for the second time and sterilized by filtration. The clarified supernatant was adjusted to pH 6.2 with N NaOH and used for toxin-testing.

To 1.8ml of each supernatant, 0.2ml of 0.5% trypsin were added.
Trypsin-treated preparations were incubated at 35 -37C for 1 hour with occasional gentle agitation. Parallel tests with untreated duplicates and trypsin-treated preparations were conducted.

For detection of toxin, the neutralization test in mice described by Haim and Timothy (1998) was used. A pair of adult mice (2g wt. each) was injected intraperitoneally with 0.5ml of each untreated supernatant fluid and another pair with 0.5ml of the trypsin-treated supernatant fluid, using 1 or 3 ml-syringes with $^{5}/_{8}$ inch, 25 gauge needles. One separate tube containing 1.5 ml of supernatant fluids (2 for carcass tissues, 2 for rumenal contents) were heated at 100C for 10 minutes, to inactivate the toxin and then cooled to room temperature.

Pairs of mice were injected intraperitoneally with 0.5ml of each inactivated supernatant. To detect botulinum toxin in the serum of sick animals, 0.4ml of each serum were injected intraperitoneally in pairs of adult mice (30g wt each).
Typing of suspected toxin was done simultaneously with the above tests using the mouse neutralization test as follows:
0.25ml of *Cl. botulinum* Type C or Type D antitoxin supplied by Onderstepport Veterinary Institute of South Africa, were added to each 1ml of serum, rumenal contents, or carcass extract, mixed well by swirling the tubes and incubated at room temperature for 30 to 60 minutes.

For toxin detection in rumenal contents and carcass extracts, mice were divided into 6 groups of 2 mice each: group1was injected with 1ml of test material only, group 2 was injected with 1ml of heated test material, group 3 was injected with 1ml of trypsinized test material, group 4 was injected with 1ml of test material + 0.25 ml of Type C antitoxin, group 5 was injected with 1ml of test material + 0.25 ml of Type D antitoxin, group 6 was kept as an uninoculated control.

For detection of toxin in serum samples, mice were grouped into 4 groups of 2 mice each: group 1 was injected intramuscularly with 1ml of a test serum, group 2 was injected with 1 ml of a test serum + o.25 ml of Type C antitoxin, group 3 was injected with 1ml of the test serum + o.25 ml of Type D antitoxin, and group 4 was kept as an uninoculated control. All mice were periodically observed for 48 to 96 hours for symptoms of botulism.
Because of the limited amount of the antitoxin, only few samples were tested,10 samples of suspected sera, 9 samples of rumenal contents, and 6 samples of carcass tissues.

Results

Field investigation showed that 50% of the affected animals had died since the onset of the disease. Some animal died suddenly, whereas others showed stiff gait, lowered head, bent neck, drooling of saliva, protruding of the tongue, and swinging of the tail sideways. Paralysis of descending pattern was frequently seen in the terminal stages of the disease. Pathological lesions were not found at necropsy, but there were fragments of chewed bones in the rumen.

The plant carrying capacity of the disease area was nil and the land was quite bare (Fig.1). Animals were noticed chewing carcasses that were scattered in the bare area.

The response of mice to differently treated botulinum toxin in differently treated, serum samples, rumenal contents, and carcass extracts are shown in Tables: 1, 2, 3. Deaths were evident in the groups that had received untreated preparations, the groups that had trypsinized preparations, and the groups that had been injected with preparations to which type D antitoxin was added.

Death occurred within 24 hours and was preceded by signs such as ruffled fur, posterior paresis, labored breathing, and pinched appearance at the waist (Fig.2). *Clostridium botulinum* type C was identified. Animals that survived were included in the groups that had received heated preparations, preparations to which type C antitoxin was added, and the uninoculated controls.

Table (1). Response of mice inoculated intraperitoneally with suspected serum samples.

Type of inoculum	No. of mice group	Response
1ml of serum	2	All died
1 ml of serum + 0.25 ml of type C antitoxin	2	All survived
1ml of serum + 0.25 ml of type D antitoxin	2	All died
Uninoculated control	2	All survived

Table (2). Response of mice inoculated intraperitoneally with suspected rumenal contents.

Type of inoculums	No. of mice group	Response
1ml of rumenal contents.	2	All died
1 ml of heated rumenal contents.	2	All survived
1ml of trypsinized rumenal contents.	2	All died
1ml of rumenal contents+0.25ml of type C antitoxin	2	All survived
1ml of rumenal contents+0.25ml of type D antitoxin.	2	All died
Uninoculated control	2	All survived

Table (3). Response of mice inoculated intraperitoneally with suspected carcass extracts.

Type of inoculum	No. of mice group	Response
1 ml of carcass extract	2	All died
1 ml of heated carcass extract	2	All survived
1 ml of trypsinized carcass extract	2	All died
1 ml of carcass extract + 0.25 ml of Type C antitoxin	2	All survived
1 ml of carcass extract + 0.25 ml of Type D antitoxin	2	All died
Uninoculated control	2	All survived

Fig.1. Bare Rangeland.

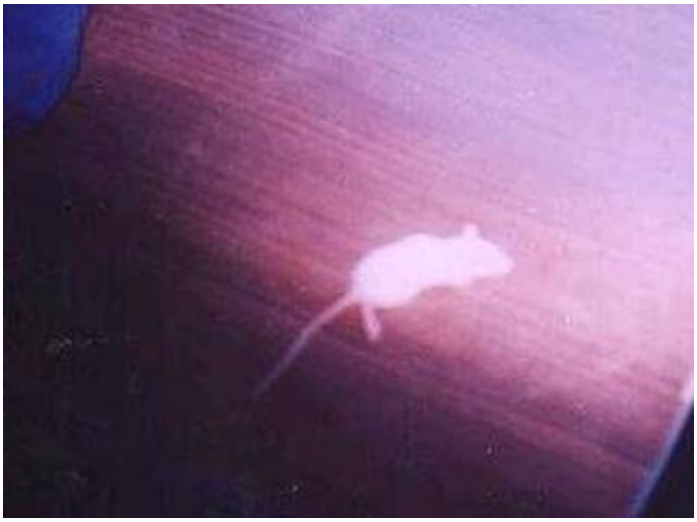

Fig.2. Posterior paralysis of a mouse (experimental observations).

Discussion

The mortality rate during the outbreak (50%) and signs of the disease, resembled those reported by Sesardic et al., 2000, and Chaddock *et al*., 2002.
Bareness of rangeland was due to the drought waves that struck North Kordofan during the years 1967, 1973, 1984, and 1992 (Musa and Musa, 2003). This

resulted in severe deterioration of vegetation, disappearance of palatable plants ,and emergence of new plant species with poor nutritive value.

Lisboia *et al.(*1996) reported that pica was exhibited by some of the animals grazing on ranges with inadequate supply of nutrients. When pica is seen in ruminants, it is a strong evidence of carrion poisoning (Blood *et al.*,1989). Lack of pathological lesions in necropsied animal agrees with the findings of Jensen and Swift (1982).

Botulinum Type C toxin was detected in sera, rumenal contents, and tissue extracts by the mouse neutralization test, whereas Type D toxin was not detected.

Radostits *et al*. (2000) reported that types C and D were common in warm climates and that most outbreaks of carrion eating botulism were associated with these types, because they produce higher concentrations of toxins which could survive in carrion for a year.

Botulism in sheep is usually caused by type C. Our results are consistent with those of Lught *et al*. (1996) who diagnosed botulism in sheep in South Africa as being caused by type C. Types C and D of *Clostridium botulinum* affect sheep and of these, Type C is by far the most common (Lewis *et al.,* 2000).

Bioassay tests yielded few positive results; 1out of 10 serum sample, 1out of 9 rumenal contents, and 3 out of 6 samples of carcass tissues. Mouse bioassay is not always a perfect test as many times animals which die of botulism had such low levels of toxin in their blood or tissue that could not be detected (kirk and Adaska,2001).

Furthermore, harsh environmental conditions may affect the stability and activity of the toxin. It is common knowledge, that it may be difficult to prove the presence of botulinum neurotoxin and in outbreak of botulism, it is not uncommon to have only a portion of clinically affected animals test positive or none (Smith and Sugyama, 1988)). However, despite its drawbacks and ethical and practical disadvantages, mouse bioassay is still the recommended as method for Botulium neurotoxin detection and culture identification in most countries (Szabo *et al*, 1994).

Serum samples rarely have detectable levels of neurotoxin (Hatheway,1979) which explains why out of 10 serum samples, only one sample gave a positive result. The toxin of *Cl. botulinum* binds almost irreversibly to individual nerve terminals, thus serum and cerebrospinal fluid specimens have low levels of toxin which may be difficult to detect (Jacobs *et al*.,994).

It may be concluded that, as the land of the study area was bare with zero carrying capacity, animals developed the habit of chewing carcasses (pica), and hence contracted botulinum toxin which was detected in sera, rumenal contents, and carcass tissues of the affected animals. Clostridium botulinum type C was responsible for the disease outbreak. Prevention of pica by supplementation of essential nutrients and Injection of animals with type C antitoxin, are possible measures to control such outbreak.

References

Bennetts, H. W. and Hall, H. T. (1938). Botulism of sheep and cattle in Western Australia; its cause and its prevention by immunization. Aust. Vet. J., 14: 105-119. Cited by Jensen and Swift (1982). Diseases of Sheep 2nd edition. Philadelphia. p. 287-290.

Blood, D. C., Radostits, C. M. and Henderson, J. A. (1989). Veterinary Medicine. 9th ed. Bailliere Tindall. London, U. K. pp 1283.

Chaddock, J. A., Melling, J. and Sussman, M. (2002). Clostridium botulinum and associated neurotoxins. Molecular Medical Microbiology, Volumes: 1-3, 1141-1152. Academic Press; Londo.Davies, E. T. (ed) (1984). Manual of Veterinary Investigation. Vol. 1. Ministry of Agriculture, Fisheries and Food (MAFF). U.K. Pp. 210.

Haim, M. S. and Timothy, J. R. (1998). Bacteriological Analytical Manual Online. 8th ed. U.S. Food and Drug Administration. Center for Food Safety and Applied Nutrition. P. 400.

Hatheway, C. I. (1979). Laboratory procedures for cases of suspected infant botulism. Review of Infectious Diseases, 1, 647-651.

Jacobs, D. S. (ed) (1994). Laboratory Test Handbook, 3rd ed. Cleveland. Pp. 1513.

Jensen, R. and Swift, B. L. (1982). Diseases of Sheep 2nd Ed. Philadelphia, USA., P. 287-290.

Kirk, J. and Adeska, J. (2001). Botulism in Cattle. A Report, University of California, Davis Veterinary Medicine Extension, USA.

Lewis,C.J., Martin, W. B. and Aitken, I. D. (2000). Diseases of Sheep. 3rd ed., Pp. 131-143. Blackwell Science, Oxford, UK.

Lisboa, J., Kuchembuck, M. R., Dutra, I. S., Goncalves, R. C., Almedia, C. T. and Barros, F. I. (1996). Epidemiology and clinical picture of an outbreak of botulism on Sao Paulo State, Brazil, (in Portuquese) Pesquisa-Veterinaria-Brazileira., 16(2-3), 67-74.

Lught, L. L., Van der Henton, M. M. and Steyn, B. G. (1996). Type C botulism in sheep associated with feeding of Poultry Litter. J. of the South Afric. Vet. Association, 67 (1), 3-4.

Merchant, L. A. and Packer, R. A. (1967). Veterinary Bacteriology and Virology (7th Ed.). Iowa State University Press. Lowa, USA. P. 276.

Musa, M. M. and Musa, A. S. (2003). The impact of drought and desertification on the Western Region of the Sudan. Sud. J. Vet. Sci. Anim. Husb., 42 (1, 2), 9-15.

Radostits, C. M., Gay, C. C., Blood, D. C. and Hinchcliff., K. W. (2000). Veterinary Medicine. A text book of the diseases of cattle, sheep, pigs, goats and horses 9th ed. W. B. Saunders Co. Ltd., London, New York, Philadelphia, San Francisco, St. Lois, Sydney. Pp. 1197.

Smith, L. D. and Sugyiama, H. (1988). Botulism: The Origin, Its Toxin, The Disease. Springfield, IL Thomas Books.

Sesardic, D., Martin, H., Tierney, R., Bigalke, H., Ball, M., Zeller, A. M. and Halder, M. E. (2000). An in vitro assay for testing of neutralization antibodies to botulinum toxins. Proceedings of the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, 1001-1008.

Szabo, E. A., Pemberton, J. M., Gibson, A. M., Eyles, M. J. and Desmarcheller, P. M. (1994). Polymerase chain reaction for detection of Cl. botulinum type A, B and E in food, soil and infant faeces. J. Appl. Bacteriol., 76, 539-545.

Trueman, K.F., Bock, R. E., Thomas, R. J., Taylor, J. D. and Green, P. A. (1992). Suspected Botulism in Three Intensively Managed Australian Cattle Herds. Vet. Rec., 130, 398-400.