BOTULISM IN FOUR CAPTIVE LION CUBS; CLINICAL MANIFESTATIONS AND AN ENVIRONMENTAL SURVEY.

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ABSTRACT

Acute quadriparesis is described in four lion cubs (Panthera leo) with lower motor neuron paralysis and diminished spinal reflexes that progressed over the first 72 hrs. All lions gradually recovered and were back to normal by the end of four weeks. All lion cubs had eaten the same dead pony that had died 24 hrs earlier. Fecal samples of all four lions were positive for Clostridium botulinum type C. C. botulinum type C was also isolated from soil samples around the carcass but not from other sites in the lions' feeding area. Serology for C. botulinum was negative. Based on the typical clinical presentation, the laboratory results and the environmental samples, a diagnosis of botulism intoxication was made .This is to the best of our knowledge, only reported once in the veterinary literature.

INTRODUCTION

Naturally occurring botulism is a rare neurological disease in felids. Five circus lions (Panthera leo) were previously reported to suffer from clinical botulism (2). The lions presented with ataxia and weakness of the hind limbs and inability to swallow. Four of the five lions made a slow recovery over a period of few months. Another report of botulism in felids is of a group of eight cats, four of which died after being fed pelican carrion (1). Clostridium botulinum type C was isolated from one cat and the microorganism and its toxin were found in the pelican.

We report a cluster of four lion cubs (Panthera leo) raised at the Safari-Zoological Center (SZC), Tel-Aviv, Israel that presented with acute onset of ataxia and paresis after eating a carcass of a pony that died 24 hrs earlier. Although the sera tested for C. botulinum toxin were negative, the typical clinical presentation and the isolation of C. botulinum type C from feces and soil samples from the lions' feeding area, strongly suggest the lions suffered from botulism.

CASE REPORT

Four lion cubs, two littermates at the age of 6 months and another two littermates aged 12 months were affected. The two younger cubs presented acute onset of non-ambulatory tetraparesis with diminished spinal reflexes of all four limbs, weak jaw tone, weak tail tone and difficulty in apprehending food. Both had slightly dilated pupils and weak palpebral reflexes, and were easily fatigued. All other cranial nerves and mental status seemed normal. The older cubs were only mildly affected, one had a subtle weakness of the hind limbs but was otherwise normal, and the other presented four limbs' weakness but was still ambulatory. Spinal reflexes could not be evaluated in the older less affected lions. The four lion cubs were part of a larger group of 11 lions kept at the SCZ at the time of occurrence of the clinical signs. All 11 lions were housed during the night and left to roam free in an area of about 11 hectares during the day of which 1 hectare was the feeding area. The lions were fed commercial food and poultry meat. In addition, they are given carcasses of animals that died in the park Tnat were left outside at the feeding area.

A pony that died in the SZC was left at the feeding area and was eaten by the lions during the following two days. No recordings are available as to which lion ate from the carcass. On the following day, the two 6 months old littermate cubs, one male and one female, showed signs of weakness and ambulatory tetraparesis that progressed to a non-ambulatory severe state of tetraparesis within the following 48 hours when only slight voluntary movements could be observed. One day later, the older littermates demonstrated mild ambulatory tetraparesis, with the female being more severely affected..Complete blood count and biochemical profiles were within the normal values for lions at our laboratory. Serology for FIV, VeLV, toxoplasma and neospora were all negative. Based on the anamnesis, the physical and neurological findings and the blood results, a tentative diagnosis of botulism intoxication was made. The paralyzed animals received supportive therapy, antibiotics (amoxicillin-clavulonic acid), ketoprofen, vitamin supplements

(B1, B12) and selenium and were hand fed during the first two weeks. The 12 months old male was the first to recover and was normal again 7 days after the onset of clinical signs. The young female started walking ten days after the onset, and both females gradually improved over the following 4 weeks. The younger male was the last to show signs of improvement but was back to normal within the following 4-6 weeks. During the entire period the paralyzed animals were separated from the rest of the prey and were fed manually.

LABORATORY EXAMINATION

Sera and feces for bacteriological examination were taken from the paralyzed animals 3 days after the onset of clinical signs. By the time of the bacteriological sampling no remains of the pony's carcass were available for examination. Superficial soil samples were therefore taken by scraping from the site where the carcass had been exposed, in a corner of the lions' feeding area. Following positive findings (see below) at this site, 4 further samples were taken from three 90° arches distanced 15, 30 and 45 meters from the focal point (a total of 12 samples). It is noteworthy that carrion leftovers, especially fowl, were scattered throughout the feeding area. In addition, nine fresh fecal specimens from the floor of the building in which the healthy lions were kept overnight were taken twice (6 on the first occasion and 3 on the second). The samples were not in direct contact with the floor. Fecal samples from 5 ponies living together with the pony that died were examined also.

The presence of botulinum toxin in the sera was determined by the mouse toxicity test as previously described (1) and found negative. Fecal specimens (about 50 grams each) and environmental samples (about 100 grams) were examined for the presence of toxigenic *Clostridium botulinum* by culture and mouse toxicity tests as previously described (1). Toxin type was determined by specific neutralization (1). The fecal specimens were submitted to general bacteriological examinations (1) but pathogenic bacteria were not isolated. Blood cholinesterase levels were measured in two of the paralyzed lions by Michel's method (3).

RESULTS

Botulinum toxin was not found in the sera. *C. butulinum* type C was cultured from the fecal samples of the young male and the 12 months old female; that of the younger female was negative. Of the 9 fecal samples taken off the floor, one was positive for *C. butulinum* type C. No other pathogenic bacteria were cultured from the samples.

C. butulinum type C was also cultured from the soil sample taken from the site where the pony's carcass was exposed but not from any of the other 12 environmental samples. In addition, *C. butulinum* type B was cultured from 3 of the 4 samples taken from the arc at a distance of 45 meters from the pony carcass. The fecal sample pool of the live ponies was negative for *C. butulinum.*

Cholinesterase levels were found to be comparable to values obtained previously in healthy young lions.

DISCUSSION

Acute onset of neurological abnormalities at the same time in a group of young animals is always suspicious of an infectious or toxicological origin. Neurological evaluation of three of the four lions described here revealed a lower motor neuron type of lesion indicating a peripheral neuropathy or a junctionopathy. No infectious agent is known to cause such an abnormality in lions. Among toxins that can affect the neuromuscular junction, chronic intoxication with organophosphates are the most common. Serology for all the common infective agents that cause nervous system changes was negative and the cholinesterase blood level was within the normal limits.

Serology for botulism toxin was negative in all lions presented here. The unequivocal proof of botulism through toxin demonstration in the serum of affected animals is often unsuccessful and thus the use of other aids such as fecal cultures is needed. The isolation of C. botulinum from feces cannot provide an unequivocal diagnosis of botulism since carriage of *C. botulinum* spores cannot be excluded. In addition, information on the prevalence of carrier animals in the entire population may provide the background with which the findings can be compared. Another important aspect in suspected cases of botulism is the connection between the afflicted animals and an established source of the toxin. Again, preferably confirmed by presence of the toxin, but if this is not possible then by the presence of toxigenic *C. botulinum.*

In the this report the suspected source of toxin was no longer available at the time of investigation, thus soil samples were obtained from 13 different areas within the lions' yard to look for possible toxigenic bacteria. Based on the results of these tests it seems that carriage of *C. botulinum* in the animal population of concern in this report is very low (1/9) compared to the isolation rate from the afflicted lions (2/3). In addition, the type of *C. botulinum* isolated from the paralyzed lions (type C) was found only at the site of the suspected source of intoxication, i.e., the pony, was exposed.

The higher rate of environmental contamination with *C. botulinum* type B spores rather than those of type C is noteworthy since poultry spoils were abundantly scattered through the feeding area, whereas carcasses of equids (ponies, zebras), usually linked to *C. botulinum* type B, were fed to the lions only rarely. This might be the result of differences in the resistance of the spores of various *Clostridium botulinum* types to environmental conditions (4), especially considering the hot, dry climate of the Israeli summer when this episode occurred. Another topic of interest is the source of contamination of the pony's carcass: *C. botulinum* type B would be expected, but type C was isolated from the lions. Thus the contamination source might have been not the pony itself but feces of scavenging birds such as crows (hooded crow, *Corvus cornix)* that abound at the enclosures. Since the carcass was exposed for a couple of days in the hot climate, conditions were likely to be suitable for the microorganism to multiply and produce toxin.

The fact that only cubs were affected may be related to feeding behavior in the group, such that the cubs were the last to be

allowed to feed from the carcass, hence possibly being exposed to higher level of toxins. Another explanation is a combination of higher toxin levels and smaller body size and low levels of antibodies in cubs in comparison with adult lions that have been exposed previously to *C. botulinum* toxin.

The neurological presentation of the lions described here is similar to that described by Greenwood (1985). In his report six circus lions *(Panthera leo)* showed neurological signs that included ataxia, hind limb paralysis and recumbency (2). The two lions examined neurologicaly in this report showed inability to swallow and fixed dilated pupils. No description of spinal reflexes for the six circus lions is available. Diminished spinal reflexes of all four limbs are the hallmark of botulism intoxication of the nervous system. The toxin affects the nervous system by preventing the release of acetylcholine to the synaptic cleft thus preventing the neuromuscular signal transmission. This is a self-limiting condition and the synapse recovers with time. Weak to diminished spinal refiexes as well as weak jaw and tail tones were recorded in our lions accompanied by a weak palpebral reflex. These peripheral neurological signs in a group of young animal fed on an unusual carcass are highly suggestive of botulism intoxication. Conservative treatment including fluid supplementation, hand feeding and antibiotics is recommended. Additional treatment with type C antitoxin and anabolic steroids as given to some of the six lions in that report did not seem to improve the outcome. None of the lions in this incident developed respiratory paralysis and all have made a complete recovery within 4 to 6 weeks after the appearance of clinical signs.

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