Case Report Rapport de cas

Botulism in 2 urban dogs

Ane Uriarte, Jean-Laurent Thibaud, Stéphane Blot

Abstract — Two dogs from the same owner were referred for ascending weakness and paresis of 2 to 3 days duration. Electromyography and electroneurography determined that there were normal F-waves, decreased compound action potential, and decreased activity on repetitive nerve stimulation. These findings were valuable in diagnosing botulism in the dogs.

Résumé — Botulisme chez 2 chiens urbains. Deux chiens appartenant au même propriétaire ont été référés pour une faiblesse croissante et une parésie d'une durée de 2 ou 3 jours. L'électromyographie et l'électroneurographie ont déterminé qu'il y avait des ondes F normales, un potentiel d'action acquis réduit et une activité réduite lors d'une stimulation répétitive des nerfs. Ces constatations ont été utiles pour le diagnostic du botulisme chez les chiens.

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Disorders of the neuromuscular junction have a wide range of clinical presentations, which frequently pose a diagnostic challenge to clinicians. Botulism is a rare disease of the neuromuscular junction caused by *Clostridium botulinum,* an anaerobic ubiquitous bacterium. Food-borne botulism in humans can be fatal, and requires mechanical ventilatory support in over 60% of patients (1). Carrion eaters and some carnivores, including dogs, are resistant to botulinum toxin (BoNT) (2), but a few clinical cases have been described in dogs and cats (2, 3). Definitive diagnosis is often difficult (4), but electrophysiologic assessment is a useful tool for diagnostic screening of an ascending lower motor neuron (LMN) paresis, which can be an emergency situation. Two papers characterize its utility in dogs intoxicated by *C. botulinum* (2,5) with the last one published 23 y ago (5). This report describes clinical signs, electrodiagnostic findings and successful recovery of 2 dogs with suspected *C. botulinum* intoxication.

Case descriptions

Two dogs from the same owner were referred for an ascending weakness and paresis that intensified over 2 to 3 d. The dogs had been fed expired canned food and had been allowed to roam freely, with the possibility of eating carcasses.

Case 1

A 2-year-old cross-breed 25-kg female dog had become worse over the past 2 d. Upon presentation, the dog was not able to stand but was in good body condition and the body temperature, pulse, and respiratory rate were unremarkable. A neurological examination demonstrated paresis affecting all 4 limbs, with normal mentation and satisfactory cranial nerve function. The withdrawal reflexes were absent in the front and hind limbs. The patellar and cranial tibial reflexes were absent in both hind limbs and the dog was unable to urinate. Pain perception was intact and there was no muscle atrophy or hyperesthesia.

Based on clinical history and examination, the differential diagnosis for causes of acute quadriparesis with diffuse LMN dysfunction is: polyradiculoneuropathy, coral snake poisoning, tick paralysis, lasalocid-induced toxicosis, and botulism. Other causes that usually result in normal reflexes with progressive weakness on exercise, such as polymyositis and myasthenia gravis (MG) were also considered. The dog was hospitalized and received supportive treatment with the administration of intravenous fluids and the placement of a urinary catheter. Twenty-four hours after admission the dog improved gradually and regained ambulatory paresis. Withdrawal reflexes were present in the 4 limbs but they were weak. Thoracic radiographs were unremarkable and 72 h after admission the dog could stand and urinate normally.

In order to characterize the LMN paresis, an electrophysiological test was performed under general anesthesia, 7 d after the first symptoms appeared. At that time, the dog had a mild ataxia and weak spinal reflexes. The electrophysiologic study was done with a Nicolet Vicking IV D (Nicolet Biomedical, Viasys Healthcare, Hong Kong) and the reference range was determined

Unité de Neurologie, École Vétérinaire d'Alfort, 7 Av Général de Gaulle, 94700, Maisons-Alfort, France.

Address all correspondence to Dr. Ane Uriarte; e-mail: auriarte@ndsr.co.uk

Dr. Uriarte's current address is North Downs Specialist Referrals, Friesian Buildings 3 & 4, Brewer Street Dairy Business Park, Brewer Street, Bletchingley, Surrey, United Kingdom RH1 4QP.

by examination of 15 normal dogs between 25 and 40 kg. The dog was induced with propofol (Rapinovet; Mallinckrodt Veterinary, Levallois-Perret, France), 4 mg/kg, and diazepam (Valium; Roche laboratoire, Neuilly-sur-Seine cedex, France), 0.2 mg/kg and was intubated and maintained on oxygen and fluothane (Forene; Zeneca Pharma, Cergy, France). Electromyography (EMG) to determine the spontaneous electrical activity of muscle, and electroneurography (ENG) that measures the action potentials of the peripheral nerves were carried out. In a normal anesthetized dog, once brief insertional activity has disappeared, any activity should be recorded. The EMG study of the 4 limbs did not elicit any spontaneous activity except that insertional activity increased in duration.

The compound muscle action potential (CMAP) is the summation of nearly synchronous muscle fiber action potentials recorded from a muscle after stimulation of the nerve supplying the muscle (6). The amplitudes of the CMAP reflect the number of the motor units activated. The amplitudes of the CMAP were decreased in the posterior and anterior interosseous, tibialis cranialis, and extensor carpi radialis muscles. Nevertheless, motor nerve conduction velocity (MNCV), which is the speed of propagation of an action potential along a nerve, was normal in the tibial, peroneal, ulnar, and radial nerves. In order to prevent a decrease in velocity induced by hypothermia (7), the dog's temperature was maintained between 37°C and 37.5°C using a heating carpet (Gaymar Industries, Orchard Park, New York, USA). The reduced CMAP amplitude without spontaneous activity and normal MNCV was interpreted as a blockage of neuromuscular transmission.

Late waves were recorded in the ulnar and tibial nerves (H reflex), and in the peroneal and radial nerves (F reflex). The H reflex, when present, is a compound action potential with a consistent evoked latency at low intensity (6). The F reflex is a compound action potential evoked intermittently by a supramaximal electric stimulus. The presence of normal amplitudes and latencies of the late waves rule out a proximal dysfunction.

Repetitive nerve stimulation (RNS), performed at 0.7, 2, 3, 5, 10, 20, and 50 Hz, is routinely used to assess the integrity of neuromuscular transmission by repeated supramaximal stimulation of a nerve. At 3 Hz a decrement of 20% was recorded. These findings, along with the neurological examination, and the fact that 2 dogs from the same owner were symptomatic, supported our tentative diagnosis of botulism. The dog was discharged from the hospital 8 d after admission following a normal neurological examination.

Case 2

A 1-year-old female cross-breed dog weighing 23 kg from the same owner was also referred for an ascending weakness and paresis that deteriorated over 3 d. Medical history and neurological examination were similar except for the presence of cranial nerve deficit. The cranial nerve motor responses were affected causing reduced ear, eye, and lip mobility, and reduced gag reflex leading to excess salivation. Additionally, the dog showed a mydriasis with sluggish pupillary light response with no other abnormality on ophthalmic examination.

The dog was hospitalized and received the same supportive treatment as the dog in case 1, including the placement of a urinary catheter. Electrophysiological diagnosis was planned 1 wk after the first appearance of symptoms. Twenty-four hours after admission there was further deterioration and voluntary movements disappeared. Because of polypnea and thoracic murmurs a thoracic radiograph was taken that revealed a megaesophagus and bronchopneumonia. Cephalosporin (Rilexine; VIRBAC Suisse SA Europastrasse, Glattbrugg, Switzerland), 15 mg/kg, BID was prescribed and a gastric feeding tube was put in place. Due to the respiratory complication, the electrophysiologic examination was not carried out.

Eight days after admission there was complete recovery of the spinal reflexes. Although the dog was still in sternal recumbence, it was discharged from the hospital due to the clinical amelioration leading to a good prognosis. Twenty-five days after the first symptoms occurred, the dog recovered normal ambulation. Thoracic radiographs revealed no signs of bronchopneumonia or megaesophagus, so the gastric feeding tube was withdrawn. Clinical history, neurologic examination, and recovery without specific treatment in 25 d supported our tentative diagnosis of botulism.

Discussion

In the last 15 y, within the 3 referral neurological veterinary practices in Paris, 5 other dogs have been suspected as having suffered botulism [Blot S (École vétérinaire d'Alfort), Thibaud JL (ENVA), Cauzinille L (CHV Fregis), Gnirs K (Adventia), personal communication, 2009]. This low frequency underlines the interest in our cases and reminds us of the necessity to keep botulism in mind when parasympathetic signs are found together with generalized, progressive and extensive paralysis.

Clostridium botulinum produces 7 potent neurotoxins (1), but only 1 type (type C, BoNT/C) has been implicated in botulism in dogs (4). Food-borne botulism results from ingestion of foods that contain BoNT. The toxin is absorbed by the intestine and is transported by the blood to the muscles. The neurotoxins inhibit acetylcholine release into the neuromuscular junction by binding irreversibly to presynaptic nerve endings, and causing enzymatic cleavage of cholinergic vesicle shuttle proteins (1). BoNT has a generalized effect on the neuromuscular junction involving both striated and smooth muscles. As recorded in our cases, landmarks of botulism are rapid, progressive, symmetric, LMN paresis accompanied by cranial nerve abnormalities and disruption of the parasympathetic system (8) without sensory deficits.

Botulism was suspected from the history and clinical signs, especially when both dogs shared the same environment. However, definitive diagnosis is based on finding the toxin in serum, feces, vomitus, or samples of the ingested food. BoNT can be detected using various techniques, including enzyme-linked immunosorbent assays (ELISAs), paramagnetic bead-based electrochemiluminescence detection, or mouse inoculation. In 2006, Bruchim et al (4) described the first report of canine botulism intoxication that was confirmed by testing for serum antibody titers to BoNT. Some authors claim that it is often difficult to make a definitive diagnosis of botulism because circulating toxin levels are often low, source material

Table 1. Pathophysiological, clinical, and electrophysiological comparison between botulism, acute polyradiculopathy, myasthenia gravis, and lasalocid poisoning.

	Botulism	Idiopathic acute polyradiculopathy	Myasthenia gravis	Lasalocid
Pathophysiology	Inhibit acetylcholine release into the neuromuscular junction by binding irreversibly to presynaptic nerve endings and causing enzymatic cleavage of cholinergic vesicle shuttle proteins.	Peripheral neuropathy where the nerve roots and the ventral root components of spinal nerves develop varying degrees of axonal degeneration, paranodal and segmental demyelination, and leukocytic infiltration.	Depletion of the nicotinic acetylcholine receptors in the neuromuscular junction.	Suggested to induce changes in the membrane potential by influencing the permeability of neuronal cell membranes to cations.
Neurologic examination	— Decreased reflexes — Rapidly pogressive tetraparesis/plegia — Respiratory compromise — Para-sympathetic symptoms — Megaesophagus	— Decreased reflexes — Rapidly progressive tetraparesis/plegia — Facial weakness — Dysphonia — Respiratory compromise — +/− hyperesthesia	— Normal reflexes — Muscular weakness and excessive fatigability — Megaesophagus	— Decreased reflexes — Rapidly tetraparesis/plegia — Respiratory compromise — Dysphonia — Cranial nerves abnormalities — Hyperthermia
EMG	Normal/subnormal	Abnormal	Normal	Unknown
ENG	— Decreased CMAP amplitudes — Normal F-waves — RNS decrement at low Hz (and increment at high Hz in humans)	— Decreased CMAP amplitudes — Absent F-waves — Usually normal MNCV	— Usually normal CMAP amplitudes — RNS decrement at low Hz	Unknown

EMG — electromyography, ENG — electroneurography, CMAP — compound muscle action potential, RNS — repetitive nerve stimulation, MNCV — motor nerve conduction velocity.

may be absent, and available analytical methods lack sensitivity (9). Furthermore, any toxin in samples may be rapidly degraded (10). These circumstances underscore the value of complementary diagnostic techniques such as electrodiagnostic testing.

Because the vectors of tick paralysis and coral snake poisoning have never been present in France, idiopathic acute canine polyradiculoneuropathy (ACP) and lasalocid poisoning were the remaining possibilities. Clinical signs in botulism can be similar to those of ACP, with rapidly progressive tetraparesis/plegia, facial weakness, dysphonia, and respiratory compromise (11). Nevertheless, the presence of parasympathetic signs and the fact that the 2 dogs from the same owner were symptomatic at the same time can exclude this diagnosis.

Lasalocid is a carboxyl ionophore antibiotic that is used for the prevention of coccidiosis in chickens and turkeys, and as a growth promoter in ruminants (12). There are 2 reports of lasalocid poisoning in dogs (12,13). As in our case report, LMN quadriparesis, salivation, and dyspnea were the major symptoms in the dogs intoxicated with lasalocid. However, clinical signs appeared less than 12 h after eating a commercial food contaminated with lasalocid (13) and the neurological symptoms were accompanied by hyperthermia. Body temperature was normal in our dogs, clinical symptoms progressed over 2 to 3 d, and the dogs were not fed a commercial food. Accidental poisoning is unlikely as there are no chicken, turkey, or ruminant farms near the owner's kennel.

Even if MG commonly results in normal reflexes and does not show autonomic signs, it is a fairly frequent disease and should be included in the tentative diagnoses for this clinical presentation. Myasthenia gravis produces a depletion of functional nicotinic acetylcholine receptors. Muscular weakness and excessive fatigue may affect ocular, facial, oropharyngeal, and oesophageal or limb muscles (14). Although muscle weakness is the hallmark of botulism, autonomic dysfunction is the underlying clinical sign that could differentiate it from MG. The cholinergic receptors of smooth and cardiac muscle have different antigenicity than skeletal muscles so are not affected by MG (Table 1). Nevertheless, Gajanayake et al (15) described a dog with autoimmune myasthenia gravis and dysautonomia. However, this is an uncommon presentation, and the fact that 2 dogs from the same owner were affected exclude this possibility. Moreover, the course of disease differs completely from botulinum intoxication.

Electrodiagnostic descriptions of canine botulism have only rarely been published (2,5). These reports showed normal (2) or abnormal spontaneous activity (5), decreased CMAP amplitudes with normal MNCV (2,5) and decrement after RNS at 3 Hz (5). In our case, the decreased CMAP amplitudes with normal MNCV agree with the findings recorded previously (2,5). As in the study of Barsanti et al (2), fibrillation potentials were not recorded in our dog. This differs from the results of Van Nes et al (5). At any rate, electromyographic abnormalities in botulism are secondary to the functional denervation caused by limited release of ACh (acetylcholine) and can be present or not, depending on the severity of the disease (6) and the chronicity of the symptoms. The absence of spontaneous activity may be explained by the mild signs and the short duration of the disease before the EMG study.

Similarly, electrophysiological study in cases of human botulism may reveal fibrillation potentials, decrease of the motor amplitudes, and abnormalities in RNS. Repetitive nerve stimulation serves to "stress" diseased neuromuscular junction by

depleting the stock of readily releasable acetylcholine (16). In adult dogs, reference values for RNS at ≤ 3 Hz have been documented with no decrement of more than 10% (5). A stimulation rate of 2 to 3 Hz is fast enough to deplete the immediately available stored ACh, but slow enough to avoid neurosecretory mechanisms that may actually enhance neuromuscular transmission. In our case, a decrement of 20% was recorded at 3 Hz, showing a diseased neuromuscular junction.

In human patients, a substantial postactivation facilitation after brief isometric exercise or rapid-rate stimulation (20 to 50 Hz) is common in children, but is present in only 60% of adults. Neurosecretory mechanisms actually enhance neuromuscular transmission by liberation of ACh (accumulation of calcium in the nerve terminal) (16) and recruitment of muscle fibers not activated by the first stimulus (6). On the contrary, this postactivation facilitation has never been described in botulism in dogs. In normal dogs a decrement of the CMAP is recorded after high RNS (5,17,18). In our case, a decrement was recorded at 10 Hz RNS. Results obtained for RNS at 20 to 50 Hz were not conclusive, likely due to a technical problem.

The electrophysiological findings differed from those of the other potential causes. Acute canine polyradiculoneuropathy is a peripheral neuropathy where the nerve roots and the ventral root components of spinal nerves develop varying degrees of axonal degeneration, paranodal and segmental demyelination, and leukocytic infiltration (19). Spontaneous activity changes, markedly decreased CMAP amplitudes and absence of F-waves are the earliest and most reliable indicators of ACP (19). F-waves were present in our dog. Decremental response to RNS characterized in our dog could lead to misdiagnosis with MG; however, decreased CMAP amplitude is not usually reported in MG (14). There is no electrophysiological description of lasalocid poisoning. An acute myopathy has been suggested to be the result of the poisoning (12). In this situation abnormal spontaneous activity can be expected.

Severe ascending generalized LMN tetraplegia can be life threatening. Management must be performed in intensive care facilities, as ventilatory support could be required if weakness of the intercostal muscles or diaphragm is suspected or if severe dysphagia is reported with possible aspiration pneumonia. Correct diagnostic hypotheses should be rapidly established in order to start treatment promptly and give a survival prognosis to the owner as soon as possible. Botulism can be lethal, and is always considered a medical emergency. Due to new end plate formation, acetylcholine release is restored without treatment and dogs usually recover in 2 to 3 wk with only supportive therapy.

In conclusion, botulinum intoxication should be suspected when an ascending LMN paresis with parasympathetic signs appears in dogs. Electrophysiological testing can help with the diagnosis of botulism, especially when only 1 dog is affected or laboratory testing is inconclusive or not easily available. CVJ

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