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Authors: Steinman, Amir, Millet, Neta, Frenkel, Chana, King, Roni, and Shpigel, Nahum Y.

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Presence of Antibotulinum Neurotoxin Antibodies in Selected Wild Canids in Israel

Amir Steinman,[1] Neta Millet,[1] Chana Frenkel,[2] Roni King,[3] and Nahum Y. Shpigel[1,4] [1] Koret School of Veterinary Medicine, Faculty of Agricultural, Food, and Environmental Quality Sciences, The Hebrew University of Jerusalem, PO Box 12, Rehovot 76100, Israel; [2] Department of Soil and Water Sciences, Faculty of Agricultural, Food, and Environmental Quality Sciences, The Hebrew University of Jerusalem, PO Box 12, Rehovot 76100, Israel; [3] Israel Nature and Parks Authority, 3 Am Ve'Olamo Street, Jerusalem 95463, Israel; [4] Corresponding author (email: shpigeln@agri.huji.ac.il)

ABSTRACT: Serum samples from 35 golden jackals (*Canis aureus syriacus*), eight wolves (*Canis lupus*), and four red foxes (*Vulpes vulpes*) from various regions of Israel were collected during the years 2001–04 and tested for antibodies to *Clostridium botulinum* neurotoxin (BoNT) types C and D. Antibodies against BoNT types C and D were detected in 10 (29%) and in 3 (9%) of 35 golden jackals, respectively, using enzyme-linked immunosorbent assay. This report describes detection of anti BoNT antibodies in wild canids other than coyotes (*Canis latrans*) for the first time and demonstrates that *C. botulinum* type C is prevalent in Israel.

Key words: Botulism, *Canis aureus syriacus*, *Canis lupus*, *Clostridium botulinum*, golden jackal, serology, *Vulpes vulpes*, wild canids.

Botulism is a potentially fatal disease characterized by muscular paralysis caused by neurotoxins produced by *Clostridium botulinum* (BoNTs). Birds, horses, cattle, sheep, minks, and ferrets are sensitive to BoNTs, whereas certain species of carrion-eating birds and mammals such as turkey vultures (*Cathartes aura*) and dogs have been reported to be resistant to intoxication (Barsanti et al., 1978; Lindstrom et al., 2004). High levels of BoNTs can be found in carcasses of small mammals and birds that were carrying the bacteria in their intestinal tract. It is probable that resistant species are able to feed on such carcasses and develop an immune response with no apparent ill effects (Ohishi et al., 1979; Lindstrom et al., 2004).

The extent of exposure of wild canids to BoNTs, except for coyotes (*Canis latrans*) (Ohishi et al., 1979), is unknown and is usually based on clinical reports of intoxication, which are rare. The presence of BoNT antibodies indicates earlier exposure, which could be the result of response to either ingested preformed toxin or toxin produced by *C. botulinum* in the animal's intestinal tract (Ohishi et al., 1979). Detection of a humoral immune response to BoNTs was previously used as an aid in the diagnosis of botulism in cattle (Jubb et al., 1993) and recently in a dog (Bruchim et al., 2006).

The golden jackal (*Canis aureus syriacus*) is one of the most prevalent wild canids in Israel (Shamir et al., 2001). Jackals are known to be omnivorous scavengers, consuming small mammals, chickens, carrion, insects, vegetables, and fruits (Shamir et al., 2001). Given known consumption of carrion, detection of anti-BoNT antibodies in free-ranging jackals and other wild canids might indirectly indicate the presence of *C. botulinum* in the study area. Carcasses containing *C. botulinum,* when incorporated into farm animal feed crops in the preparation of silage or hay, might cause botulism. During the years 2002–05, the incidence of cattle botulism in Israel increased; however, exposure to BoNTs by detection of a humoral immune response to the toxins cannot be determined because of immunization of the entire Israeli cattle herd. This study is part of extensive ecological research aimed at understanding the ecology and epidemiology of botulism in cattle in Israel.

Sera were collected from 35 wild golden jackals, eight wolves (*Canis lupus*), and four red foxes (*Vulpes vulpes*) caught in Israel during the years 2001–04 as a part

of the rabies control program. Serum samples from 30 dogs referred to the Koret School of Veterinary Medicine–Veterinary Teaching Hospital for reasons not related to botulism during 2005 were used as negative controls. Two beagle dogs that were born and raised in an animal laboratory were inoculated with a series of three doses of commercial-type C and D bivalent toxoid vaccine (Botulism vaccine, Prondil S.A., Montevideo, Uruguay). After collection, sera samples were separated by centrifugation and kept at −80 C until analyzed. The study was approved by the ethics committee of the Koret School of Veterinary Medicine, The Hebrew University of Jerusalem.

The levels of specific anti-BoNT type C (BoNT/C) and anti-BoNT type D (BoNT/D) antibodies in the sera were determined using enzyme-linked immunosorbent assay (ELISA) as described previously in cattle (Steinman et al., 2006) and in a dog (Bruchim et al., 2006), with minor modifications. Formalin-inactivated BoNT/C (1.6 mg/10 ml) or formalin-inactivated BoNT/D (1.7 mg/10 ml) (Prondil S.A., Montevideo, Uruguay) was used as the coating antigens. Alkaline phosphatase-conjugated affinity purified rabbit anti-dog IgG (Jackson Immuno Research Laboratories, West Grove, Pennsylvania, USA) was used as the conjugate antispecies antibody.

Serologic results were normalized to ELISA units (EUs), which were calculated using the formula [optical density (OD) test sample − OD negative control] divided by [OD positive control − OD negative control] (Dijkastra et al., 2003). A sample was considered positive if its EU value was higher than the positive/negative cutoff value, which was defined as the mean EU value plus 2.6 times the standard deviation of the control group (99% confidence interval) (Shamir et al., 2001).

Ten of 35 (29%) jackals had detectable levels of anti-BoNT/C antibodies, and three (9%) of these 10 also had detectable levels of anti-BoNT/D antibodies. Anti-BoNT type C or D antibodies were not detected in any of the foxes and wolves. Jackals carrying anti-BoNT/C antibodies were detected in three geographic areas (31°44′N, 35°00′E, 31°44′N, 34°44′E, and 32°20′N, 34°51′E). All three jackals carrying anti-BoNT/D antibodies were caught in the same area (31°44′N, 35°00′E).

The results of this study suggest that *C. botulinum* type C is prevalent in Israel based on the seroprevalence of type C BoNT antibodies detected in jackals. The lack of anti-BoNT type C or D antibodies detected in the foxes and wolves might be a result of the low number of animals tested or the result of different feeding habits of these species in the study area.

Clostridium botulinum type C is mainly found in intestinal contents and carcasses of birds, whereas the most common source of *C. botulinum* type D is carcasses of small mammals such as rodents (Popoff, 1995). Poultry litter, which is an important source of cattle botulism, frequently contains carcasses of dead chickens in which *C. botulinum* type C has multiplied and produced its toxins (Livesey et al., 2004). It is therefore possible that jackals are exposed to BoNT/C when feeding on carcasses of dead chickens. Small mammals, such as rodents, on the other hand, are either less frequently eaten or are caught and eaten fresh before the bacterium can multiply and produce toxin. It is also possible that jackals are carriers of the bacteria, as was suggested previously (Ohishi et al., 1979). However, this can be demonstrated only by fecal cultures.

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