

## Avian botulism at a sugar beet processing plant in South Moravia (Czech Republic)

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**ABSTRACT:** An episode of mortality in waterbirds occurred on a sedimentation reservoir with effluents from the sugar beet processing plant at Hrusovany n.J. (South Moravia, Czech Republic) in summer 2003: tens of black-headed gulls (*Larus ridibundus*), several lapwings (*Vanellus vanellus*), one little ringed plover (*Charadrius dubius*), one ruff (*Philomachus pugnax*), one wood sandpiper (*Tringa glareola*), and two avocets (*Recurvirostra avosetta*) died. One of the two avocets and the plover, local breeders, were examined and found positive for *Clostridium botulinum* type C toxin by use of the toxin-neutralization test. Avocet is classified as a rare bird species according to Red Book data and is listed as critically endangered species in the Czech Republic, and is therefore protected. Avian botulism can occasionally cause deleterious effects to waterbird fauna and its protection.

**Keywords:** *Clostridium botulinum*; free-living birds; Charadriiformes; avocet; *Recurvirostra avosetta*; *Charadrius dubius*; *Larus ridibundus*

From 19 June to 27 August of 2003, mortality of waterbirds occurred on a sedimentation reservoir with effluents (disposal water and sludge) from the sugar beet processing plant at Hrusovany n.J. in the district of Znojmo, South Moravia, Czech Republic (48°51' N, 16°25' E). The bird species affected were black-headed gull, *Larus ridibundus* L. (several tens of carcasses); lapwing, *Vanellus vanellus* (L.) (several carcasses); little ringed plover, *Charadrius dubius* Scop. (one carcass: adult bird); ruff, *Philomachus pugnax* (L.) (one carcass); wood sandpiper, *Tringa glareola* L. (one carcass); and, most notably, avocet, *Recurvirostra avosetta* L. (two carcasses: one adult and one juvenile bird). Except for wood sandpiper and ruff, all four other species bred here successfully in 2003.

The aim of this study was to examine dead birds from this epornitic for the presence of botulinum toxin, as clinical symptoms of moribund birds were suggestive of botulism (Wobeser, 1997; Friend and Franson, 1999). In addition, the birds were also examined virologically for arboviruses.

### MATERIAL AND METHODS

Two birds found dead on the sedimentation reservoir on 19 June and 10 July 2003 respectively were supplied for laboratory examination:

(1) Avocet, *Recurvirostra avosetta* L. A fresh carcass of a juvenile bird with the body in good condition, with no blow fly larvae present. Samples of the blood, liver, heart, and the contents of gizzard and intestine were taken for virological and toxicological studies, and maintained at –60°C until examination.

(2) Little ringed plover, *Charadrius dubius* Scop. A decaying carcass of an adult bird, with about 40 blow fly larvae (maggots: *Lucilia* sp.) in peritoneal cavity, and only a part of visceral organs remaining (liver). Samples of liver and maggots were taken for toxicological examination, and maintained at –60°C.

Virological examination: 10% (w/v) homogenates from the liver and heart tissue were prepared in cooled phosphate-buffered saline (PBS, pH 7.2)

with 0.7% of bovine serum albumin and antibiotics (penicillin 500 i.u./ml, streptomycin 200 µg/ml), and centrifuged at 3 000 rpm at 4°C for 20 minutes. The supernatant was inoculated intracranially (0.02 ml) into SPF suckling (2–3 days old) ICR mice (Velaz Prague, Czech Republic) which were observed for a period of 3 weeks.

Toxicological examination was only aimed at botulism diagnosis: 10% (w/v) homogenates in PBS with antibiotics but without bovine serum albumin were prepared from both the organ samples and maggots, and centrifuged at 3 000 rpm at 4°C for 20 minutes. The supernatant was distributed in 0.8 ml volumes in tubes and mixed with either botulinum type C antiserum IMUNA (final concentration 40 units/ml) or PBS (control), incubated at laboratory temperature in the dark for 30 min, and then inoculated (0.4 ml) intraperitoneally into adult male ICR mice. Native blood with heparin was also tested. Bacterial sterility of each sample was checked in meat-peptone broth and thioglycolate broth incubated at 37°C.

## RESULTS

Virological examination of the organs (liver and heart tissues) of the avocet was found to be negative: none of the 11 suckling mice inoculated with the heart homogenate was moribund and/or died; although four of the 10 suckling mice inoculated with the liver suspension died on days 2 and 3 post inoculation, the passage of the brain homogenate from these dead mice did not kill the intracerebrally inoculated suckling mice. The cause of death in the first passage was obviously a bacterial contamination of the sample. The decayed liver sample from the plover was not examined virologically.

Toxicological examination of the avocet, using the toxin-neutralization test, detected botulinum type C toxin in the blood (diluted twofold with PBS) and liver: the suspensions killed the mice, whereas the same suspensions with antitoxin neutralized the toxicity. Homogenates of the heart and the contents of gizzard and intestine were negative for the toxin. The samples containing toxin (blood, liver) were also exposed at 100°C for 2 min, and after that processing they did not kill mice when inoculated intraperitoneally.

Examination of the plover by the toxin neutralization test demonstrated type C botulinum toxin in both the liver homogenate and in the maggots

removed from the peritoneal cavity of the bird. Titration of the 10% suspensions revealed presence of the toxin even in dilution of  $10^{-4}$  in the liver, and up to  $10^{-3}$  in the maggots.

## DISCUSSION

Botulism of free-living waterbirds was first laboratory diagnosed in South Moravia in 1981 (Hubalek et al., 1982), although several epornitics, suggestive of botulism, were observed in previous years here (Hudec and Pellantova, 1985). The birds most seriously affected with botulism in southern Moravia have been ducks, swans, coots, lapwings, sandpipers, other waders and black-headed gulls. Sometimes individuals of rare and endangered bird species succumbed to botulism, e.g. during a big epornitic in 1988 (Hubalek et al., 1991) spoonbill (*Platalea leucorodia*), curlew (*Numenius arquata*), black-tailed godwit (*Limosa limosa*) or redshank (*Tringa totanus*). A small outbreak of type C botulism occurred among waterbirds (several black-headed gulls, one wood sandpiper) on Mlynsky pond at Lednice in summer of 2002: botulism was laboratory confirmed in one gull kindly supplied by Ing. V. Vyhnalek (unpublished). However, botulism was not described in avocets previously, at least in the Czech Republic. Nonetheless, one of the authors (Z.H.) observed a number of moribund and dead avocets, stilts (*Himantopus himantopus*) and other waterbirds on the shallow Mandra lake and nearby salinas with brackish water close to the shore at Burgas, eastern Bulgaria, on 24 June 1974. According to the clinical symptoms of the affected birds (wing and neck paralysis etc.), this morbidity was probably due to botulism (although no laboratory examination was carried out). In southern Saskatchewan, the American avocet (*Recurvirostra americana*) belongs to the bird species most commonly affected by botulism (Adams et al., 2003). Avocets thus seem to be very susceptible to this intoxication. It is possible that the lately recorded decline of avocet populations in Bulgaria, Ukraine and southern Russia (Hagemeyer and Blair, 1997) could have been caused, or affected, by botulism.

In Europe, the avocet breeds locally in salt and brackish marshes and pools, estuaries, also by shallow fresh water and fishponds at a low water table, from southern Sweden to southern Spain (most breeding pairs are in the Netherlands) along the coasts of the Atlantic Ocean in western Europe,

sporadically in south and southeastern Europe from Italy to Ukraine and south Russia (Hagemeijer and Blair, 1997). Regular breeding in Central Europe is limited to Hungary and east Austria (Neusiedler See). Breeding of avocet in the Czech Republic since beginning of the 20th century was irregular and very rare (Hudec and Stastny, 2005). It included two regions: (1) South Bohemia, with 1–6 pairs breeding on fishponds in the years 1943, 1946, 1947, 1990, 1991, 1993, 1995, 1998, 2000, 2001, and 2002; (2) South Moravia, with 1–9 pairs breeding on fishponds in 1948–1958, 1961, 1995, and 2003. The avocet is classified as a “Rare” bird species according to Red Book data in Czech Republic (Sedlacek et al. 1988), while even as “Vulnerable” in Slovakia (Danko et al., 2002). The species is also included in the Red Lists in Austria, Germany, Poland and Russia. It is protected as “Critically Endangered” according to Nature Conservation Act 114/92 Sb. and Ordinance of the Ministry of Environment 395/92 Sb. in the Czech Republic. Moreover, the avocet is protected by Bonn Convention, Bern Convention, European Birds Directive 79/409/EC, and included in the Agreement on the Conservation of African-Eurasian Migratory Waterbirds. European Threat Status is “Secure”, and European Species Conservation Status is category SPEC 4/3, i.e. the species whose global population is concentrated largely in Europe (Hagemeijer and Blair, 1997; BirdLife International, 2004).

It is obvious that botulism can occasionally bring severe damage to waterbird fauna (especially to Anseriformes and Charadriiformes) and its protection in both Eurasia and North America, and it deserves an increased attention of wildlife biologists and veterinarians. During episodes of avian botulism, a very important conservation strategy is the timely removal of bird carcasses, as blow fly larvae appear in them very rapidly during hot summer days. In epornitic foci, these maggots contain large concentrations of botulinum toxin (for instance, see the plover in this study) and present a huge danger for other birds feeding on them, sometimes even up to early spring the following year (Hubalek and Halouzka, 1991).

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