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BOTULISM IN WILD WATERFOWL IN SOUTHERN MORAVIA (CZECHOSLOVAKIA)

Frequent deaths of waterfowl on the ponds of Southern Moravia in the vicinity of Lednice, Pohořelice and Hodonín have been reported in some years during the last decade (particularly in 1972, 1973, 1974, 1976 and 1981) (Macháček P., Vertebrat. zprávy (Brno) No. 1: 53—54, 1973, Hájek V., Zprávy Morav. ornitol. sdruž., pp. 96—98, 1977). Most affected were ducks (particularly *Anas platyrhynchos* and *A. crecca*, less frequently *Aythya* spp.), coots (*Fulica atra*), and some shorebirds (*Calidris* spp., *Tringa* spp., *Actitis hypoleucos*), less frequently black-headed gulls (*Larus ridibundus*), geese (*Anser anser*), swans (*Cygnus olor*) and other water birds on the muddy shores of the ponds. Mass deaths accompanied by partial to complete paralysis of muscles prior to death occurred mostly in late summer and were correlated with higher temperatures of water and mud (e.g., still on September 19, 1973, the temperature of both water and mud was 15 °C in the pond Hlohovecký, pH of water being 9.0—9.5) and with the decrease of water level.

The examinations carried out by various laboratories since 1975 did not result in any exact diagnosis, but some causes, as chemical intoxication from the environment, parasitic infections, viral infections and others, were reliably excluded. Botulism, intoxication of birds by the toxin of *Clostridium botulinum*, was considered in 1973, but examinations of a teal (*Anas crecca*) and a dunlin (*Calidris alpina*) found dead on the pond Hlohovecký at Lednice on 15th September 1973 were negative. Nevertheless, due to the symptoms of dying birds and with regard to the experiences from other European countries, botulism was still suspected as a possible cause of these deaths. We have therefore made use of the epizooties in 1981 for further investigations.

In September 1981, waterfowl was dying among others also on the pond Starý near Pohořelice (Břeclav district). We examined three dead mallards (*Anas platyrhynchos*) and one ill tufted duck (*Aythya fuligula*) from this locality. The duck healed spontaneously and only its blood serum was examined by intraperitoneal inoculation of mice. The autopsy of the three dead mallards revealed cachexia, medium to very strong diarrhea of yellow-green or brown-green colour, haemorrhagia of intestinal tract and hyperemia of liver. Most conspicuous was this finding in the mallard in which intoxication was later demonstrated. The light yellow-green diarrhea was observed also in the tufted duck. Suspensions from heart, liver, and contents of stomach and intestines were prepared from the dead mallards at the approximate ratio of 1 : 2 in phosphate buffer solution (PBS), pH 7.4. The suspensions were left in dark for 2 h at 20 °C, then centrifuged for 15 min at 1,000 × *g*, divided in three portions of 1.5 ml each, placed into tubes and mixed with 0.4 ml of either PBS (control) or diagnostic antitoxin serum SEVAC against botulism (Imuna Šarišské Michalany, No. 010280). Polyvalent antiserum against A, B, C, D, E types (à 20 i.u./ml) was added in one tube and antiserum against C type (100 i.u./ml) in the other. After incubation in dark at 20 °C for 30 min, 0.6 ml from each tube were inoculated intraperitoneally to three males of randombred SPF mice of ICR strain (Velaz Praha) weighing 22 g.

Botulism was not demonstrated in one of the mallards (male) (not even the control mice died). In case of the other mallard (female), the control mice died within 48 h only after inoculation of blood coagulum from heart, but the examination could not be repeated due to the small amount of suspension. In case of the third mallard (male), however, all control mice died within 48 h after inoculation of the suspensions from liver and contents of stomach and intestine. However, suspensions of these materials heated for 2 min at 100 °C or incubated with antiserum against botulism, either polyvalent or of C type, did not cause the death of any mouse till day 7 p.i. (duration of the experiment). Consequently, the death of this mallard was caused by botulotoxin of C type. The examination of serum from the tufted duck for botulotoxin was negative. Since our studies were directed at the diagnosis of bird intoxication, supplementing examinations of samples of organs, mud or water by cultivation were not carried out.

This is the first record of botulism in wild waterfowl in Czechoslovakia. Similar cases have been reported from other European countries (Sweden, Denmark, Great Britain, Netherlands, Spain, GDR), America, South Africa, Australia and New Zealand (see Haagsma J. et al., Neth. J. vet. Sci. 5: 12—33, 1972, Martinovich D. et al., N. Z. vet. J. 61—65, 1972, Smith G. R., Wildfowl 27: 129—138, 1976, Köhler B. et al., Monatsh. Vet. Med. 32: 178—182, 1977). In almost all cases, the birds were intoxicated by botulotoxin of C type.

In 1979, botulism of C type was recorded in a flock of domestic geese on a pond near Nové Hrady in Southern Bohemia (Dr. K. Marjánková, personal communication 1980).

In summer 1982, the epornitic of botulism re-appeared in the locality "Starý" pond at Pohořelice: three dead *A. platyrhynchos* and one *L. ridibundus* were examined in July and all of them were found intoxicated by botulotoxin (serotype C).

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