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A CASE OF DEATH DUE TO *SALMONELLA* TYPHIMURIUM IN A WHITE-TAILED EAGLE *HALIAEETUS ALBICILLA* (LINNAEUS, 1758)

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ABSTRACT: Infections induced by *Salmonella*, including *S. enterica* Typhimurium, are rarely registered in free-living birds despite the wide range of animal species susceptible to infection by this serotype. *Salmonella* Typhimurium frequently causes infections in birds associated with aquatic environments, although it is cases occurring in birds on industrial farms that are most often registered. Cases of *S.* Typhimurium in endangered raptor species are practically never noted, and in these rare cases the pathogen is usually isolated from birds living in zoos. The isolation of *S.* Typhimurium from a White-tailed eagle *Haliaeetus albicilla* from a natural environment is an interesting case that may explain one of the causes of the reduction in the population of this species.

Key words: Salmonella Typhimurium, *Haliaeetus albicilla*, free-living birds infections.

Introduction

The White-tailed eagle *Haliaeetus albicilla* (Linnaeus, 1758) belongs to the Accipitridae family. White-tailed eagles in Poland are resident birds. The total population of the species in Poland is estimated at about 700 breeding pairs; this is the largest refuge for this bird in Europe. Like most free-living raptors, the species is strictly protected and is on the IUCN Red List of Threatened Species.

Currently no monitoring is conducted in Poland of microorganisms that are potentially pathogenic for these birds. Testing of birds, usually post mortem in government veterinary laboratories, is done incidentally and is usually limited to autopsy. Microbiological cultures are rarely performed because in most cases death is caused by mechanical injuries or chemical poisoning. Nevertheless, the literature reports cases of isolation of such bacteria as *Salmonella* (Reche et al. 2003), *Campylobacter* (Molina-Lopez et al. 2011), *Enterococcus* (Marrow et al. 2009) and *Clostridium* (Clare McW et al. 2009) from free-living raptors, but these cases are very rare and mainly noted in birds living in zoos or other forms of captivity.

Material and methods

Over the last 10 years three autopsies of White-tailed eagles have been performed at the laboratory of the Institute of Veterinary Hygiene in Lublin. Cause of death was determined based on examination of internal organs during the autopsy and in some cases additional tests. In two birds the cause of death was found to be injury – in one case due to a gunshot wound and in the other to perforation of the digestive tract following ingestion of a foreign body. In the third case the autopsy found no signs of mechanical injury. The autopsy revealed a number of changes that led to the suspicion of bacterial infection.

The bird (a female White-tailed eagle, body weight 4.9 kg) was delivered for examination in March 2013. The autopsy revealed inflammatory lesions in the mucous membrane of the intestines, digesta in the stomach, severe inflammatory congestion of the intestinal mucous membrane, with faeces of a liquid, diarrheal consistency which had left traces on the bird's feathers. The only change observed in the internal organs was a slight swelling of the liver, with no perceptible pathological changes in the remaining organs. A substantial loss of elasticity in the muscle tissue and skin due to dehydration was observed. Because of the nature of the changes observed during the autopsy, parasite testing was carried out, as well as diagnostic PCR of DNA samples isolated directly from the faeces to test for the presence of *Salmonella* spp., *Campylobacter* spp. and *Clostridium* spp.

Parasite testing was carried out by the flotation method using a Fecalyzer and Fecasol kit (Vetoquinol, France) according to the producer's instructions, and by examining the intestinal digesta under a stereoscopic microscope.

DNA material was isolated from faecal samples collected from three different sections of the intestine (the jejunum, ileum and cecum) using the commercial kit Gene MATRIX Stool DNA Purification Kit (EurX, Gdańsk, Poland) according to the producer's instructions. PCR was carried out in a TPersonal thermocycler (Biometra, Germany), separately for each group of bacteria. In each reaction 5µl of the experimental sample was used, and DNA samples of the following strains as positive controls: *Salmonella* Typhimurium ATCC13311, *Campylobacter jejuni* ATCC29428 and *Clostridium perfringens* ATCC13124. The total volume of the reaction mixture was 25 µl and consisted of 2.5 µl of 1x reaction buffer (100 mM Tris-

HCl, pH8.8, 500 mM KCl, 100 mM $(NH_4)SO_4$, 1% TritonX-100), 2mMol $MgCl_2$ (1 µl), dNPT mix (2.5 µl), thermostable *Pwo* DNA polymerase (1 U) (DNA Gdańsk, Poland) and 10 pM of each primer (Genomed Warszawa – Poland). The primers used were specific for *Salmonella* spp. – ST11, ST15 (Aabo eta al. 1993), *Campylobacter* spp. – Camp f, Camp r (Denis et al. 2001) and *Clostridium* spp. – Clos58-f, Clos780-r (Amit-Ramach et al. 2004). The PCR reactions were carried out in the following conditions: 94°C – 3 min.; 35 repetitions of the following cycles: 94°C – 1 min., 52°C – 1 min. (*Salmonella* spp. and *Campylobacter* spp.) and 54°C – 1 min. (*Clostridium* spp.), 72°C – 1 min; and a final extension (72°C – 7 min.). The amplified products were separated in 2% agarose (Conda, Spain), documented, and analysed using Gel Doc 2000 software (BioRad). The PCR test was performed twice.

Based on the positive PCR results for *Salmonella*, a full microbiological culture was conducted according to norm PN-EN ISO 6579:2003 to test for *Salmonella* spp. In a culture grown on differential-selective media (Xylose-Lysine Deoxycholate Agar, Brilliant Green Agar – Oxoid) growth typical of *Salmonella* sp. was obtained. Five characteristic colonies were collected for further identification. Following purification of the strains according to the instructions in the norm, they were confirmed to belong to the genus *Salmonella* based on their biochemical properties, using the commercial test ENTEROtest 24 (Erba Lachema, Czech Republic), and 100% agreement for *Salmonella enterica*. subsp. enterica was obtained. Serological type was determined based on the White-Kauffmann-Le Minor scheme – edition 2007.

Results and discussion

No adult parasites, parasite eggs or protozoan oocysts were identified, which ruled out internal parasites as a cause of the diarrhoea.

A positive result for *Salmonella* spp. (a product of 429 bp) was obtained in the samples collected from the ileum and cecum. No products characteristic of *Campylobacter* or *Clostridium* were identified. Further identification tests (cultures, biochemical and serological tests) showed that all of the Salmonella strains isolated belonged to the serotype Typhimurium (04; i;1,2).

The result obtained allows us to put forth the hypothesis that *Salmonella* Typhimurium was the direct cause of death. *Salmonella* Typhimurium infections in adult domestic birds often have no symptoms and the birds become carriers; adult birds rarely die as a result of these infections (Henderson et al. 1999). Cases of death from *Salmonella* in wild birds are almost never registered, and cases of infection are usually without symptoms. In the case of latent infection by *Salmonella* sp., full manifestation of clinical symptoms is usually influenced by a sharp decrease in resistance. This is likely to have occurred in the case described here, because the bird died in the winter when a thick layer of snow made it difficult to find food, resulting in the malnourishment visible during the autopsy. White-tailed eagles feed on prey or carrion; their main source of food is animals permanently or temporarily associated with water, particularly aquatic birds, which are one of the main reservoirs of *Salmonella* Typhimurium (Hoelzer et al. 2011). In this case the intestines of the eagle were probably colonized by *Salmonella* when the bird ingested a carrier of the bacteria. External environmental factors and long-term starvation led to a drastic reduction in the eagle's

resistance, resulting in a full manifestation of disease symptoms. This is probably not an isolated case, but data are lacking because microbiological cultures are not obligatory in the case of deaths of free-living birds, including endangered species.

In conclusion, *Salmonella* bacteria may be a significant factor reducing the population of birds of prey.

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Streszczenie

Przypadek stwierdzenia Salmonella Typhimurium u bielika zwyczajnego Haliaeetus albicilla

Zakażenia wywołane przez pałeczki Salmonella, w tym *S. enterica* Typhimurium, są rzadko rejestrowane u wolno żyjących ptaków pomimo szerokiego zakresu gatunków zwierząt podatnych na zakażenie tym serotypem bakterii. Salmonella Typhimurium często powoduje infekcje u ptaków związanych ze środowiskiem wodnym, chociaż najczęściej zdarzają się przypadki występujące u ptaków w gospodarstwach i hodowlach drobiu. Przypadki S. Typhimurium u zagrożonych gatunków drapieżnych praktycznie nigdy nie są odnotowywane, a w rzadkich przypadkach patogen jest zwykle izolowany od ptaków żyjących w ogrodach zoologicznych. Wyizolowanie S. Typhimurium od bielika *Haliaeetus albicilla* (L.) pochodzącego ze środowiska naturalnego jest interesującym przypadkiem, który może wyjaśnić jedną z przyczyn zmniejszenia populacji tego gatunku.