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SHORT COMMUNICATION

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Prevalence of agglutinating antibodies against *Listeria monocytogenes* in chicks of the white stork (*Ciconia ciconia* Linnaeus, 1758) in Poland

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Abstract In 2002 and 2003, blood samples from white stork (*Ciconia ciconia*) chicks were examined for the presence of antibodies against *Listeria monocytogenes*. *Listeria* antibodies were detected in 121 (59%) of 205 chick samples. The probability of *Listeria* antibodies being present increased with chick age; chicks detected with *Listeria* antibodies were in better condition than those without the bacterium.

Keywords Antibodies · *Ciconia ciconia* · *Listeria* · Body condition · Chick development

Introduction

Listeria monocytogenes is a gram-positive, rod-shaped and non-spore-forming bacterium, and is widespread in the environment (i.e. soil, sewage and river water). It is a zoonosis-causing agent pathogenic to many mammalian and bird species and has increasingly been recognized as an important food-borne pathogen (Farber and Peterkin 1991). Moreover, birds potentially transfer the bacterium to humans, and therefore are of epidemiological significance (Bouttefroy et al. 1997; Vaissaire 2000). The white stork (*Ciconia ciconia*) is a bird species living in close proximity to people. The birds build nests on human-made constructions such as the roofs of farm buildings or electrical poles (Jakubiec and Guziak 1998; Schulz 1998), and sometimes have used special frames erected by people as a base for their nests. The latter is an example of directional conservation for the benefit of the Polish population of the white stork, which is also important for the world population, since about 25% of the species occur in Poland (Jakubiec and Guziak 1998; Schulz 1998). A detailed knowledge of negative factors limiting the size of the local breeding population is essential for the effective protection of the white stork population, and for improving its breeding success. Jakubiec (1991) analysed factors influencing white stork reproduction, and showed that at least some breeding losses and mortality of adult storks are due to disease. Hence, as a part of the conservation measures for this species, veterinary examination of nestlings was carried out. In this paper, we describe the incidence of antibodies to the bacterium *L. monocytogenes* in the white stork population. We consider whether chick age is related to the prevalence of *Listeria* antibodies. Moreover, we discuss the potential epidemiological role of the white stork in the transfer of *L. monocytogenes* to humans.

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Materials and methods

Field work was conducted in the breeding seasons 2002 and 2003 in two different parts of the Wielkopolska region, which has typical breeding densities of the white stork in Poland [ca. 17 breeding pairs/100 km^2 near Ostrów Wielkopolski (Dolata 2003), and ca. 4.6/100 km^2 near Poznań (Ptaszyk 1994 and unpublished data)].

The age of chicks was calculated on the basis of bill length. We measured the upper beak of the bill (tip to feathers) of all nestlings to the nearest millimeter using slide callipers. The age of each nestling was estimated

according to a table of bill development (Kania 1988). Nestlings were bled from the branchial vein, and the blood sample was collected in a plastic tube and transported to the laboratory. In total, 205 nestlings (143 in 2002 and 62 in 2003) from 75 nests were sampled. Average age of the sampled birds was 34.5 ± 11.9 days (range 6–64 days). Based on field observations of the nests, we found that all chicks survived until the fledging stage, indicating that the blood sampling procedure did not adversely affect the chicks.

The blood samples were tested for antibodies against *L. monocytogenes* by an agglutination reaction with an antigen O-V of *L. monocytogenes* (BIOVETA). Dilutions of the sera were examined in a saline solution with 0.5% phenol in a series from $1+9$, $1+19$, $1+29$, etc. (at least eight dilutions were tested). A volume of 0.5 ml of the appropriate dilution was obtained in each tube. The sera dilutions were carried out in two tube series. A volume of 0.5 ml antigen solution of serotype O-V was placed in each of the two tubes. The tubes were incubated at 37°C for 20 h and then left at room temperature for 1 h. Results were considered positive in cases where agglutination occurred. The basic serum dilution used was 1:16, and titres of greater than 16 were considered positive. In this study, according to the BIOVETA suggestion, a sample was considered a true positive if it agglutinated a second time after a 2-week interval.

From a regression line, we estimated white stork chicks' body-condition indices as residuals of body mass with age established through bill size. Data are presented as means ± SD. Data from 2 years were pooled for analyses due to small sample sizes and because there was no evidence of heterogeneity in bacterium prevalence before testing. Samples were separated into two groups: chicks with detected *Listera* antibodies and chicks without *Listeria* antibodies, and analysed by logistic regression with the chick's age as an independent value. Statistical analyses were conducted using the statistical package SPSS (Norusis 1986).

Results

L. monocytogenes antibodies were detected in 121 (59.0%) of 205 white stork chicks analysed. In 28 (37.3%) nests, all the chicks had *Listeria* antibodies, in 35 nests (46.7%) all chicks were uninfected, and in 12 nests (16.0%) chicks that were positive and chicks that were negative for *Listeria* antibodies occurred together. The three groups of nests did not differ significantly in the number of chicks per nest (Kruskal-Wallis ANOVA, $H_2 = 3.78$, $P = 0.151$, $n = 75$).

The presence of *Listeria* antibodies increased with chick age (logistic regression, $\chi^2_{\text{Wald}} = 55.92$, $\text{df} = 1$, $P < 0.0001$, Fig. 1).

White stork chicks with *Listeria* antibodies were in better condition (expressed by the body condition index) than uninfected chicks (Mann-Whitney *U*-test, $Z = -2.11$, $P = 0.035$, Fig. 2).

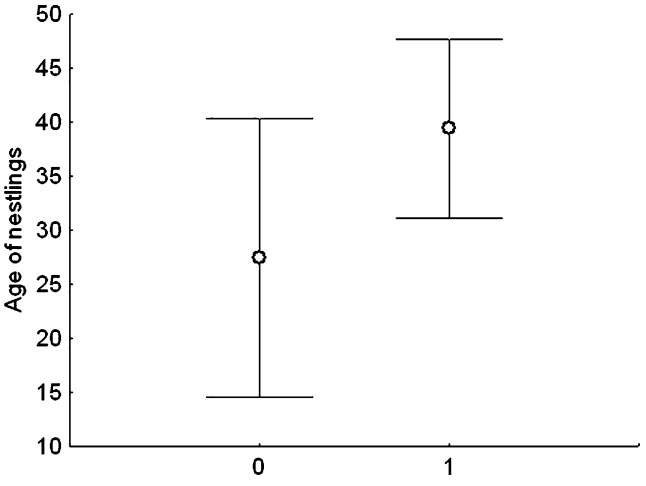

Fig. 1 Differences in white stork chicks' ages (in days) between those uninfected (*0*) and infected (*1*) with *Listeria*. Data are presented as means ± SD

Discussion

L. monocytogenes is a bacterium detected in several wild bird species (Ralovich and Domjan-Kovacs 1996; Bouttefroy et al. 1997; Yoshida et al. 2000). The method used by us for the detection of *Listeria* infections (agglutination test) was rather robust. However, the results are not necessarily comparable with those obtained by more quantitative techniques (cf. Pelisser et al. 2001).

To the best of our knowledge, this is the first documentation of *Listeria* antibody occurrence in the white stork. Infection is thought to occur at local foraging areas through food sources and to be transported to the chicks by their parents during feeding. This would explain the positive correlation between chick age and the occurrence of *Listeria* infection. In white storks, older chicks are fed with small mammals, whereas younger chicks are fed mainly with earthworms and insects

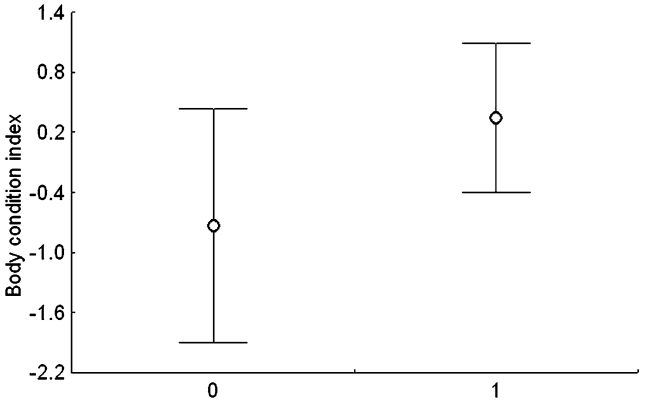

Fig. 2 Differences in condition index (standarised by age and body mass; for details see Materials and methods) between white stork chicks uninfected (*0*) and infected (*1*) with *Listeria*. Data are presented as means ± SD

(Profus 1986; Schulz 1998; Antczak et al. 2002). In Poland, Jasińska (1961) found *Listeria* in the common vole (*Microtus arvalis*) and the common shrew (*Sorex araneus*) from sewage farms, which are important foraging sites for storks (Pinowska and Pinowski 1989; Tryjanowski and Kuźniak 2002).

The feeding success of chicks can also explain the lack of any negative influence of *Listeria* infection on the chicks' condition. We observed that chicks in better condition, which were positive for *Listeria* antibodies, received better food (small mammals versus invertebrates) from their parents than chicks negative for *Listeria* antibodies.

In conclusion, it is suggested that white storks act as reservoirs and/or carriers of *L. monocytogenes*. It would be useful to examine the possible transfer of *Listeria* from white storks to domestic animals and human food, for example through faeces (Bouttefroy et al. 1997; Andrzejewska 2001).

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