

## LETTER TO THE EDITOR

## AVIAN INFLUENZA VIRUSES DETECTED IN EUROPEAN MAGPIE NESTLINGS

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Influenza A viruses are negative-strand RNA viruses with segmented genome belonging to the family *Orthomyxoviridae*. They are classified into subtypes (H1-H16 and N1-N9) according to the characteristics of their surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). Ecological studies have established that all influenza A viruses are derived from strains circulating in wild birds, where they usually appear to be apathogenic (1). Transmission of avian influenza viruses (AIVs) from wild birds to domestic poultry and their subsequent circulation in the poultry can result in an increase of pathogenicity and transfer of the gene segments to human influenza viruses by reassortment (2). In May 2007, WHO reported that H7N2 viruses caused outbreaks in poultry farms in England. These viruses were transmitted also to the humans and four individuals were infected in Wells and north-west England (3). European magpie (*Pica pica*), a medium-size passerine bird from the family *Corvidae* is a common resident breeder evenly distributed in urban, rural, and open landscapes. It prefers nesting near towns, villages, and recently in strips of green along the highways. The species from the family *Corvidae* are usually associated with the transfer of West Nile virus (4, 5, 6) and St. Louis encephalitis virus (7, 8).

These viruses can be transferred to humans through feces-contaminated water or through vectors like mosquitoes and ticks that are carried by the wild birds.

Up to now, the susceptibility of the European magpie to AIV infection has not been studied. The passerine birds are not regarded as an important reservoir of AIV. Therefore, we studied the prevalence of AIV among European magpie and especially the possible transmission of AIV to their nestlings.

In April 2007, the oropharyngeal and cloacal samples were collected from 30 nestlings, 1–9 days old, in the nests around Trnava. The nests were 0.5–2 km far from each other. The collected swabs were extracted in 2.5 ml of PBS and 100 µl aliquots of the extracts were used for purification of RNA that was used in nested RT-PCR as described previously (9, 10). The rest of the aliquots were inoculated into embryonated eggs for virus isolation. AIV-positive samples were used in nested RT-PCR with primers specific to each HA and NA subtype. The designed primers were specific for conserved regions of each subtype of HA and NA, respectively. The sequences of the primers are available upon request.

The results (Table) showed that at least 50% of nestlings from each nest except nest 6 were AIV-positive. A total of 11 oropharyngeal samples and 7 cloacal samples were found as AIV-positive. Samples taken from both oropharynx and cloaca of the same nestling were AIV-positive only in one case. Subtype H7N2 was identified in all infected nestlings. However, there were two nests where different subtypes were

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**Abbreviations:** AIVs = avian influenza virus(es); HA = hemagglutinin; NA = neuraminidase

Nest (No.)	No. of tested nestlings	No. of positive samples	Subtypes of AIV	
			Oropharynx	Cloaca
1	5	3	H7N2	–
			H7N2	H7N2
			–	H2N2
2	3	2	H7N2	–
			H9N1	–
3	3	1	H7N2	–
4	5	4	H7N2	–
			H7N2	H7N2
			–	H7N2
5	5	3	–	H7N2
			–	H7N2
			H7N2	H7N2
6	4	0	–	–
7	5	3	H7N2	–
			H7N2	–
			H7N2	–

found together, H2N2/H7N2 and H9N1/H7N2 in the nest 1 and 2, respectively. Despite all effort, we did not succeed in an isolation of AIVs from the sampled material.

We confirmed the high prevalence of AIV in nestlings. However, we cannot provide the information about prevalence of AIV in parental birds, because it is difficult to trap the adult birds. We assume that the nestlings were infected from the parents during the feeding. This suggestion was supported by the fact that only one, occasionally two different subtypes were found in each nest. The viruses were detected in oropharynx, but also in cloaca. However, we cannot exclude the possibility of AIV transfer from a hen into eggs, but the experimental proof of such transmission is still missing. In our opinion, it is more likely that the nestlings were infected by feeding from their parents infected by different AIV subtypes.

The most surprising was the finding that only three different subtypes (H2N2, H7N2, and H9N1) of AIV were detected in examined nests. Our previous studies showed a high diversity of AIVs circulated in wild birds at one locality even in one particular species (T. Betáková, unpublished data). The low incidence of circulating subtypes of AIV in the population of European magpie could be affected by their social life and specific life style, the small

number of tested samples, and/or relatively small distance among the nests.

Prevalence of AIV in passerine birds has previously been reported as being particularly low (11, 12, 13, 14). As the main AIV reservoir are usually considered wild birds belonging to the orders *Anseriformes* (ducks, geese, and swans) and *Charadriiformes* (gulls, terns, and waders) (1). However, AIV was isolated from other wild bird species, but not enough attention was paid to the evaluation of their role as prospective hosts for AIV. Our results confirmed that the European magpie was susceptible to AIV infection and was able to spread the virus in the occupied environment.

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