LETTER TO THE EDITOR

MONITORING OF INFLUENZA VIRUSES IN WATERFOWL AND TERRESTRIAL BIRDS IN EASTERN SLOVAKIA

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Wild birds belonging to the orders *Anseriformes* and *Charadriiformes* are considered to be a natural reservoir of all 16 hemagglutinin and 9 neuraminidase subtypes of influenza A viruses and are the major known biological source from which avian influenza virus (AIV) can be introduced to poultry (*1–3*). AIV have been isolated from the migratory birds that were usually sick or dead suggesting that they would have limited potential for carrying the viruses over long distances unless subclinical infections were prevalent (*4*). However, there is a strong circumstantial evidence that the wild birds can become infected from domestic poultry and potentially can exchange viruses sharing the same environment (*4, 5*). Prevalence of AIV in passerine birds is known to be particularly low (*6–9*).

In this study, we used a nested PCR to detect the presence of AIV in the samples of migratory waterfowl and terrestrial birds in eastern Slovakia. Moreover, we focused on detection of H5 viruses in this population of birds, since H5N1 virus represented a serious threat to birds and humans in Asia in 2005.

In May and October 2005, we collected oropharyngeal, cloacal, or fecal samples form 187 birds represented by 9 orders and 38 species (Table). Birds were sampled in the National Park – Senianske ponds in eastern Slovakia that represents one of the most significant localities for breeding and resting waterfowl in Slovakia. All orders includes waterfowl except the orders *Passeriformes*, *Strigiformes*, and *Cuculiformes,* which represented forest dwelling birds or birds in other terrestrial habitat.

Collected samples were thoroughly blended and extracted in 3 ml of PBS and 100 µl aliquot was used for purification of RNA, using the RNeasy Mini kit (Qiagen) following manufacturer's protocol. cDNAs of viruses were synthesized from purified RNA by reverse transcription using random oligonucleotide primers. The reverse transcription step and nested PCR was done by using the primers against conserved region of M gene (*10, 11*). For the identification of H5 subtype was used RT-PCR using PCR kit Avian influenza virus multiplex (Genekam Biotechnology AG) specific for H5 gene.

The majority of AIV positive samples were obtained from the oropharynx. We have never found positive oropharyngeal, cloacal, or fecal sample collected from the same bird. In May 2005, 32 birds (29%) carried the AIV. Interestingly, 34.5% of AIV positive birds represented waterfowl, while only 11% were terrestrial birds. In October 2005, 58% of tested birds were positive for AIV. Remarkable was the similarity of the percentages of AIV positive waterfowl and terrestrial birds (58.5 and 54.5%).

The RNA from the AIV positive samples was subjected for further testing using the PCR kit specific for H5 subtype. Despite all effort, the H5 subtype was not identified in any of the collected samples.

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Abbreviation: AIV = avian influenza virus

Table 1. Detection of AIV in the samples collected from birds in May and October 2005

Order	Species	May		October	
		No. of tested birds	No. of positive birds	No. of tested birds	No. of positive birds
Passeriformes					
	*Acroc. Schoenobaenus (*Sedge Warbler	1	0	0	0
	*Acroc. Schoenobaenus (*Sedge Warbler	2	0	0	0
	*Delichon urbica (*House Martin)	13	1	0	0
	*Hirundo rustica (*Barn Swallow)	10	2	0	0
	*Motacilla flava (*Citrine Wagtail)	6	0	0	0
	Motacilla alba (White Wagtail)	0	0	2	1
	*Panurus biarmicus (*Bearded Tit)	1	0	0	0
	*Passer montanus (*Eurasian Tree Sparrow)	1	0	0	0
	*Phylloscopus trochilus (*Willow Warbler)	0	0	1	1
	*Riparia riparia (*Sand Martin)	1	0	0	0
	Sturnus vulgaris (Common starling)	0	0	7	4
Charadiiformes					
	*Actitis hypoleucos (*Common Sandpiper)	3	0	0	0
	*Calidris alpina (*Dunlin)	1	0	14	7
	Calidris minuta (Little Stint)	0	0	1	0
	*Calidris temminckii (*Temminck‘s Stint)	5	1	0	0
	*Charadrius dubius (*Little Ringed Plover)	2	0	0	0
	Gallinago gallinago (Common Snipe)	0	0	16	8
	*Gallinula chloropus (*Common Moorhen)	0	0	1	1
	Larus ridibundus (Black-headed Gull)	13	5	0	0
	*Limosa limosa (*Black-tailed Godwit)	1	1	0	0
	*Lymnocryptes minutus (*Jack Snipe)	0	0	9	8
	*Philomachus pugnax (*Ruff)	12	5	2	1
	*Pluvialis squatarola (*Grey Plover)	0	0	2	1
	Tringa glareola (Wood Sandpiper)	28	12	0	0
	*Tringa totanus (*Common Redshank)	2	1	0	0
Anseriformes					
	Anas crecca (Common Teal)	0	0	6	4
	*Anas querquedula (*Garganey)	2	2	4	2
	*Anas platyrhynchos (*Mallard)	0	0	1	1
	*Aythya ferina (*Common Pochard)	1	0	0	0
Podicipediformes					
	Ardea cinerea (Grey Heron)	0	0	1	0
	*Podiceps cristatus (*Great Crested Grebe)	2	1	0	0
Gruiformes					
	*Fulica atra (*Common Coot)	1	1	1	1
	Rallus aquaticus (Water Rail)	0	0	1	1
Ciconiiformes					
	Ardea cinerea (Grey Heron)	0	0	1	0
	*Nycticorax nycticorax (*Night Heron)	1	1	0	0
Coraciiformes					
	Alcedo atthis (Common Kingfisher)	0	0	2	0
Strigiformes					
	*Asio otus (*Long-eared Owl)	1	0	4	2
Cuculiformes					
	*Cuculuc canorus (*Common Cuckoo)	1	0	0	0
Total		**111 (100%)**	**32 (29%)**	**76 (100%)**	**44 (58%)**

We confirmed the high prevalence of AIV in waterfowl and terrestrial birds. The majority of positive samples were obtained from oropharynx. These results corresponded with the previous findings, where the oropharyngeal swabs were more suitable for detection of AIV in the chicken infected through the intranasal route of inoculation (*12, 13*). It was shown that AIV can be detected from oropharyngeal swabs for at least 6 days p.i., but data from cloacal swabs are missing (*14*). Our results showed that the optimal results were obtained, when oropharyngeal, cloacal, and fecal samples obtained from the same bird are tested simultaneously.

Only 1–2% of thousands tested samples obtained from wild birds in Netherlands and Sweden were found positive for AIV by using RT-PCR (*5, 7*). Previous studies reported 9.9–10.5% of AIV-positive samples collected from wild birds in Germany (*15, 16*). In 2004, only 2% of samples analyzed by RT-PCR were positive in Slovakia (*10*). In 2005, the number of AIV-positive samples raised up to 29% in spring and up to 58% in autumn. It is obvious that the nested PCR dramatically increased the sensitivity of AIV detection. The number of the positive samples depends profoundly on the sensitivity of the method used for AIV detection, the birds' species, and the season.

As it was mentioned above, the prevalence of AIV in passerine birds is assumed to be particularly low. In our case, the AIV was detected in relatively high percentage in some our passerine birds' samples (Common Starling). It is obvious, that prevalence of AIV was in general higher in waterfowl then in terrestrial birds. Further testing and focusing on passerine birds will be required in the future. The specific RT-PCR kit was used to identify the H5 subtype, but surprisingly the H5 subtype was not detected in our samples. Nevertheless, this finding corresponded with the findings of others, who very rarely identified H5 subtype in surveillance studies made in Europe in 2005 (*17, 18*).

Coordinated surveillance of influenza in humans and animals is needed, and the human and veterinary surveillance systems should be linked to exchange information, diagnostic tools and antigens. The surveillance of AIV can play a key role in the early identification and ongoing monitoring of a pandemic influenza virus as well as the annual epidemics.

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