LETTER TO THE EDITOR

DETECTION OF AN INFLUENZA VIRUS IN WILD WATERBIRDS MIGRATING THROUGH SLOVAKIA IN AUTUMN 2004

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Slovakia is a country, through which one of the dominant European north-south birds' migratory routes crosses. Twice a year millions of birds of various species fly over this territory. Migratory waterbirds – most notably wild ducks – are a natural reservoir of avian influenza viruses, and they are also most resistant to influenza infection (*1*). Migratory birds may be dangerous to human population by spreading diseases, which have an ability to break-out to epidemic because of lack of immunity of the population. The surveillance of avian influenza (AI) viruses has never been done in Slovakia until autumn 2004, when we performed this task in waterbirds migrating through Slovakia. Out of 96 samples examined only 2 were positive for the influenza A virus. The hemagglutinin of both isolates was of H7 subtype.

AI is a highly contagious viral disease, which occurs in poultry and other birds. It was first identified in Italy in the early 1900s. There are various AI virus strains, among which high pathogenic strains reach almost 100% fatality rate. Wild birds are often carriers of low pathogenic strains causing no disease symptoms. A contact of domestic flocks with wild migratory birds has taken place at the origin of many epidemics in poultry. AI may be occasionally transmitted to humans and other animals, usually following a direct contact with infected birds (*2, 3*). Recently, the poultry industry world-wide has suffered serious losses due to AI epidemics. Since 2003, the particularly virulent H5N1 strain has caused more than 125 million birds to die or to be destroyed in South-East Asia. AI is still endemic in this region and its eradication appears to be extremely difficult. Outbreaks of avian flu occurred also in the USA, Canada and South Africa in 2004. In Europe, recent major outbreaks of AI occurred in Italy (1999–2000) and the Netherlands and to a lesser extent in Belgium and Germany (2003). The outbreak in the Netherlands led to the destruction of around 30 million birds.

In most cases, AI viruses do not infect humans. However, these viruses have a tendency to mutate and may occasionally spread to other animals and humans. In particular, there have been cases of humans infected with certain high pathogenic AI virus strains due to direct contact with disease-carrying birds. The 2003 outbreak of the disease in the Netherlands resulted in 1 human death and numerous mild human infections (*4*). In South-East Asia, more than 50 people have died from AI during an outbreak (*5, 6*). There is now a firm evidence that these high pathogenic strains actually originate from low-pathogenic ones as a result of virus mutation(s) (*7, 8*). Low pathogenic strains can be transmitted to domestic poultry from wild birds such as ducks and geese. Low pathogenic strains cannot be eradicated from wild birds, but the infection of domestic poultry can be effectively controlled and thus the mutation of a low pathogenic strain into a high pathogenic one can be prevented.

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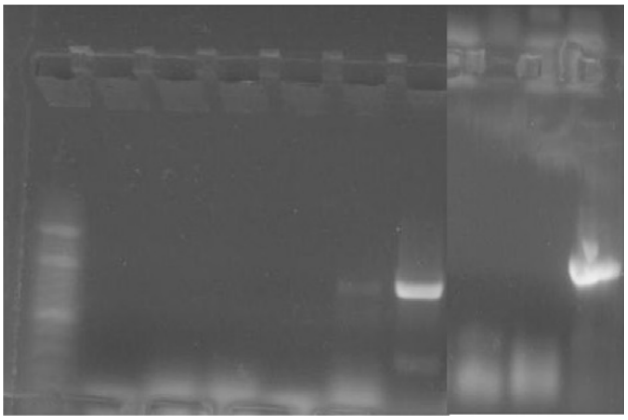
Abbreviation: AI = avian influenza



Another, but really a major concern is that a possible genetic change in an AI virus strain circulating in Asia could lead to a new strain capable of human-to-human transmission.

Cloacal and crop swabs were collected from 96 captured birds in several weekly intervals. The specimens were taken from the wild waterbirds migrating through Slovakia, namely near ponds in Eastern Slovakia. They were frozen in liquid nitrogen and transferred to the laboratory. The swabs were extracted in 3 ml of phosphate-buffered saline. An aliquot (100 µl) of the extract was used for RNA extraction by using the RNeasy Mini kit (Qiagen), while the rest, after filtration through a 0.45 µm filter, was inoculated into MDCK cell cultures or 10-day-old chicken embryonated eggs for virus isolation. The presence of an influenza virus was assessed by a hemagglutination test with 1% rooster red blood cells.

The extracted RNA was subjected to RT-PCR. cDNAs s were synthesized by reverse transcription of viral RNAs by using random oligonucleotide primers. cDNAs were amplified by PCR using the primers F1M (5'-CAAGACC AATCCTGTCACCTCTG-3') and R1M (5'-CAAAATGA CCATCGTCAACATC-3') covering the conserved region of M gene. The PCR was performed in 35 cycles of 94°C/30 secs (denaturation), 55°C/30 secs (annealing) and 72°C/1 min (extension). Specific PCR products of 821 bp were identified by agarose (1.5%) gel electrophoresis and ethidium bromide staining. The PCR products of 821 bp were sequenced and the sequences were compared with those available in GenBank.

The virus was detected by RT-PCR in the specimens from two bird species, namely a common snipe (*Gallinago gallinago*) and a long-eared owl (*Asio otus*). An example of detection of the virus by RT-PCR and subsequent agarose gel electrophoresis is given in the figure. While the lane 7 shows a positive result, the lanes 2–6 and 8–9 prove negativity. A positive control was in the lane 10. As regards the virus isolation experiments, despite three blind passages, they were not successful.

By comparing the obtained two sequences (about 99% identity) with those available in GenBank, we found a 96.7% identity with the strain A/FPV/Dobson/27 H7N7 (Acc. No. M63526), a 91.6% identity with the strain A/duck/Hong Kong/610/79 H9N2 (Acc. No. AF523494), and a 90.2% identity with the strain A/duck/Hong Kong/698/79 H5N3 (Acc. No. AF098565) (*9–11*). We can conclude that the M genes of our two isolates of influenza A virus are closely related to H7 subtype. This finding suggests that these H7 strains might not have a strong pandemic potential compared to H5 strains. On the other hand, the strains that are less pathogenic for poultry have a greater opportunity to become widespread since they permit their hosts to survive unharmed. Thus, they can continue to reassort and are more likely to have opportunity to find the best gene constellation (*12, 13*) that would permit infection of humans and facilitate further person-to-person transmission. Avian H7 strains were apparently responsible for the outbreak of AI in Europe. It is estimated that at least 1,000 individuals were infected with the H7N7 strain with one fatal case during the epidemic in the Netherlands in 2003 (*4, 14*).

The remaining question is why we have detected the influenza virus in two birds only. It is possible that (i) we have sampled the birds in the season and region where influenza viruses were not prevalent and/or (ii) the method used for detection of the virus was not efficient enough. Possibly, a nested PCR with another pair of primers could increase the sensitivity of the method and detect the virus in more samples. Despite the low virus incidence, our preliminary results show that influenza virus may be present not only in the birds migrating through Slovakia, but also in the wild birds living in this area. The inter-species barriers among the birds have become much more permeable than previously anticipated. Increasing the heterogeneity of influenza virus strains in these hosts' results in an enlarged and dynamic influenza gene pool in continuous flux. The diversity of genotypes, gene constellation, and host receptor specificities provide influenza A virus with multiple options of hosts (*13, 15, 16*). Therefore, it is very important to continue the surveillance and characterization of influenza virus strains in the birds in our territory. Early detection of low pathogenic strains in birds is a key factor to prevent development of high pathogenic strains and severe forms of the disease.

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