

ECOLOGICAL FEATURES OF INFLUENZA A VIRUS INFECTION IN WILD BIRDS

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Summary

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The relationship between the biological distinctive features of wild migratory birds and the ecology of influenza A virus in nature is revealed. On the basis of literature data and personal research, the importance of viral carriers with regard to the prevalence of the virus among the wild birds species, the seasonal variations, age-rated susceptibility, subtype distribution and the transmission in wild and domestic birds are reviewed.

Key words: avian influenza virus, subtypes, epidemiology, wild birds

Since the isolation of the first avian influenza viral (AIV) strains, a number of questions related to their persistence in ecosystems, the transmission mode among wild, domestic birds and mammals have appeared (Stallknecht, 1997). Some of them are already solved, others are yet unexplained.

BIOLOGICAL FEATURES OF WILD BIRDS AND THEIR RELATIONSHIP TO AIV

A part of the large group of wild birds (more than 8600 species), are migratory, thus traveling to shorter or longer distances and carrying pathogenic microorganisms, including AIV. The different avian species move along different flyways, most of them parallel to lines of longitude and some of them partially overlap (Fig. 1). Some of birds live and migrate in large groups (flocks) with close contact among them. Another part forms flocks only prior to migration. Many of

migratory birds are waterbirds. They live in and move in regions with large water areas (Hinshaw *et al.*, 1986; Stallknecht, 1992).

AIV are isolated from migratory and non-migratory birds, inhabiting various ecosystems from different regions of the world – Europe, Asia, Australia, North and South America, from penguins in Antarctica (Hinshaw *et al.*, 1981; Stallknecht & Shane, 1988; Stallknecht, 1992; Stallknecht, 1997). This demonstrates that AIV are not geographically limited.

As genetically unstable organisms (due to the segmented genome), influenza viruses, including AIV, could be transferred to and become accustomed to domestic birds and mammals, including man (Liu *et al.*, 2003).

ECOLOGICAL CIRCULATION OF AIV

Studies from the 1980-ties have evidenced not only the uneven geographical distribution of AIV but also the different rates of

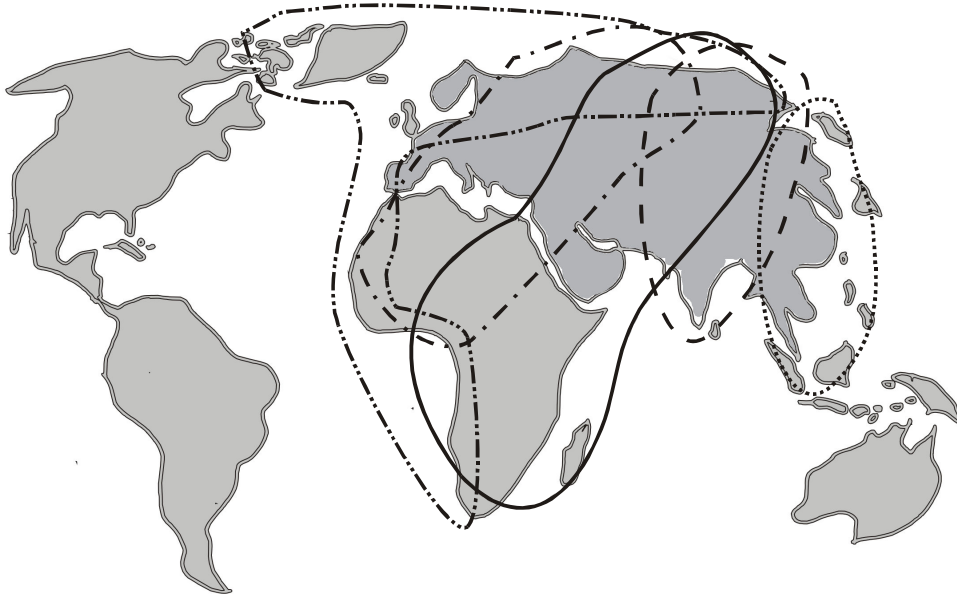


Fig. 1. Main migratory flyways of wild birds: — East Africa/West Asia flyway; West Asia flyway; - - - Central Asia flyway; - - - East Atlantic flyway; - . - Black Sea/Mediterranean flyway.

infection of susceptible avian species. Up to now, AIV isolates from 105 avian species, belonging to 12 orders are recognized (Björn *et al.*, 2006). Furthermore, the sensitivity among the susceptible species is different.

On the basis of the frequency of isolation, existing studies have demonstrated that the principal carriers and transmitters of AIV in nature are wild waterfowl (Stallknecht & Shane, 1988; Stallknecht, 1992; Stallknecht, 1997; Björn *et al.*, 2006), whose species-related sensitivity is different. Most frequently, AIV isolates have been obtained from birds of the Anseriformes order that are worldwide distributed. From the 149 species of this order, AIV isolates were detected in only 30 species (about 5%). The highest number of isolates was present in the Anatidae family. Within this family, the most af-

ected birds were those from the *Anas* genus, and among the belonging species – *Anas platyrhynchos* (Hinshaw *et al.*, 1986; Stallknecht & Shane, 1988; Zarkov *et al.*, 2006) (Table 1). Another order of wild birds important with regard to AIV susceptibility is Charadriiformes (shorebirds and relatives) that are also encountered on a global scale. The distribution of affected species within this order is also irregular – most frequently, AIV is isolated from gulls and other shorebirds (common terns etc).

Other important facts about the ecological behaviour of AIV in wild birds are:

1. In them, all AIV antigen combinations have been proved (Fouchier *et al.*, 2003).

2. In wild birds, the infection is most commonly asymptomatic (with minor ex-

Table 1. Studied samples from wild birds and AIV isolates in Bulgaria

Order	Family	Genus	Species	Samples	Isolates
Gaviformes	Gaviidae	Gavis forst	<i>Gavia arctica</i>	1	0
Anseriformes	Anatidae	Anser	<i>Anser albifrons</i>	59	0
			<i>Anser anser</i>	48	0
			<i>Anser erythropus</i>	5	0
		Branta scop.	<i>Branta ruficollis</i>	4	0
			Anas	<i>Anas clypeata</i>	1
		<i>Anas crecca</i>		1	0
		<i>Anas platyrhynchos</i>		107	1
		<i>Anas penelope</i>		2	0
		<i>Anas querquedula</i>		7	0
		<i>Anas platyrhynchos</i>		107	1
		Tadorna flem.	<i>Tadorna tadorna</i>	1	0
		Aythya boie	<i>Aythya fuligula</i>	28	0
		Mergus	<i>Mergus merganser</i>	8	0
Charadriiformes	Laridae	Larus	<i>Larus melanocephalus</i>	4	0
			<i>Larus argentatus</i>	52	0
Pelecaniformes	Phalacrocoracidae	Phalacrocorax briss	<i>Phalacrocorax pygmaeus</i>	2	0
			<i>Phalacrocorax carbo</i>	12	0
Gruiformes	Rallidae	Fulica	<i>Fulica atra</i>	33	0
Total: 5	5	10	18	281	1

ceptions in South Africa in 1961 and nowadays with the H5N1 subtype). This is assumed to be due to the thousand-year old adaptation of AIV in them (Gorman *et al.*, 1990). This also permits the more prolonged persistence of the virus in the organism of these birds.

3. The transmission of the virus is faecal-oral, and in waterfowl – faecal-water-oral. Infected ducks shed a considerable amount of AIV – up to $10^{8.7}$ EID₅₀/g faecal mass (Webster *et al.*, 1978). For AIV with low pathogenicity, the duration of viral shedding is no less than 30 days (17 days for H5N1 – Webster & Govorkova, 2006). In the faecal-water-oral transmission, the preservation of viral infectivity in water is also important, with major effects of chemical composition of water, its temperature and pH (Zarkov, 2006).

4. More than one AIV subtype could occur in one bird. The intestinal tract of birds is a convenient place for genetic change (including reassortment) and alteration of the biological properties of the virus.

One of the routes of evolution of AIV subtypes is their adaptation from one to another avian or mammalian species. Thus, the phylogenetic analysis of viral amino acid composition made clear that all mammalian strains originated from avian strains (Gammelin *et al.*, 1990; Gorman *et al.*, 1990). This event is most commonly observed in Asia, where most reassortants were detected, including the last H5N1 strain (Liu *et al.*, 2003). This is due to a number of predisposing factors, such as the big density of the population, frequent contacts between the virus and various avian and mammalian species that

are reared together. This regional factor is further supported by the structure of the agriculture in China, permitting an active circulation of AIV among the susceptible residents:

- rearing of many ducks (the principal AIV carriers) that are an important part of human food in China;
- the river deltas are ideal for duck rearing, the farms being usually in open-air;
- the river deltas are freely visited by wild waterfowl too;
- often, other domestic birds and mammals, especially pigs, are reared near the duck farms.

All that provides excellent conditions for AIV adaptation from wild to domestic birds or mammals and vice versa. Wild birds frequently move to other regions and thus, distribute the various AIV subtypes and assist to their preservation and diversity in ecosystems. An example for such a rapid distribution is AIV H12. For the first time, it is isolated in Canada from wild ducks and 4 years later, it is recovered from domestic ducks in China.

SEASONAL PATTERNS AND AGE-RELATED SUSCEPTIBILITY OF BIRDS TO AIV

The distribution of AIV with regard to the age and season in wild migratory birds is not uniform. It is known that migration of birds occurs twice – in spring and autumn. It happens when the new generation, hatched and grown at residence sites, is ready to migrate together with its parents. In the period prior to migration (formation of flocks), young birds are in close contact with many other arriving from various regions, some of them AIV carriers. As a result, young non-immune birds become infected. Although AIV is isolated all the

year round, the localization of the virus at that time is mainly in adolescent birds. In Canada, 60% of isolates have been obtained from young birds (Hinshaw *et al.*, 1986). Similar results are reported in the USA as well. In the state of New York, isolates are predominantly from young wild birds (Deibel *et al.*, 1985). The continuous observations in Alberta, Canada have shown that AIV were isolated in 22 % to 65 % of samples of young birds vs 6% to 37% from those of adults (Hinshaw *et al.*, 1985). In Pennsylvania, 17% of samples of young birds were AIV positive, vs 6.2% of adult birds' samples (Nettles *et al.*, 1986). In 1998–2000, the isolates from young birds in Minnesota were 8–10 times more numerous (17 % in adolescent vs 2.1% in adult birds) (Hanson *et al.*, 2003). Similar results in adult wild ducks are reported in north Europe and Siberia – 6.5% and 8% respectively (Björn *et al.*, 2006). After the migration, flock immunity is developed and the percentage of AIV isolation decreases.

The seasonal pattern in the distribution of AIV in wild waterfowl is often detected by means of sentinel domestic susceptible birds. They are left at places, visited by wild birds. The experiments in the Baltic region show that there is a peak of AIV isolation in wild ducks in late summer. In Minnesota (USA), the peak is July–November with decrease in May–June (Stallknecht & Shane, 1988; Stallknecht, 1992). The data reported by Nettles *et al.* (1986) in Pennsylvania demonstrate the highest frequency of isolation in August (54% of isolates) and September (45% of isolates), and reduction in isolation rates in October and November.

Whereas the investigations on seasonality in AIV prevalence in wild ducks are based upon a more prolonged research, such data became recently available in

gulls and shorebirds (order Charadriiformes). A peak in AIV isolation in these species was observed in spring. A second peak was recorded after the end of migration for migratory species (Stallknecht, 1992).

DISTRIBUTION OF AIV SUBTYPES AMONG THE SUSCEPTIBLE WILD BIRD SPECIES

The AIV subtypes are irregularly distributed among the different orders and species of wild birds. In the Anseriformes order, prevailed isolates with haemagglutinin 4 (H4) – 28.5 %, followed by H3 with 18 %, H6 with 16 % and H11 with 14 % (Stallknecht, 1992). Less numerous were isolates with H1, H2, H10, H12 (Björn *et al.*, 2006) and the least had H5, H7 and H9. A part of the last three types are causing highly pathogenic infections in domestic birds.

In ducks (bird species with the largest part of isolates), a total of 63.8 % of AIV had H3, H4 and H6, as reported in Minnesota, USA. Moreover, they are detected during the entire year (Hanson *et al.*, 2003). In Canada, only two subtypes (H3N8 and H4N6) are evidenced in all 44 duck isolates during the past 8 years (Thorsen *et al.*, 1980; Deibel *et al.*, 1985). Kawaoka *et al.* (1988) observed that out of 12.2% AIV isolates, 8% had H5, 2.6% – H9 and 1.6% – H7. Up to present, strains with H13, H14, H15 and H16 have not been isolated from ducks (Björn *et al.*, 2006).

With regard to the prevalence of neuraminidase (N) in ducks, 24 % of isolates had N2, 23.4 % – N8 and 17.5 % – N6 (Schafer *et al.*, 1993).

In the Charadriiformes order, the most commonly detected AIV were those with H9 and H13 (Kawaoka *et al.*, 1988). In

Maryland, USA, 52 % of gull isolates were H13 (Garnett, 1986). Less frequently, AIV with H14, H15 and H16 were encountered (Björn *et al.*, 2006).

Among the other orders of birds (another 10 being susceptible to AIV), isolates are few and their role in the ecological circulation of AIV is less essential.

TRANSMISSION OF AIV AMONG SPECIES

The literature data demonstrate that in wild and domestic birds, the susceptibility to the same AIV strains is variable depending on the stage of adaptation of the strain to the particular avian species. This is best illustrated after experimental infection of bird species, other from the original species of the isolate (Bahl & Pomeroy, 1977; Zarkov, 2007) and observations during outbreaks. The first AIV isolates from diseased or dead ostriches did not infect other species (Becker, 1967). The experiments with guinea fowl, pheasants, pigeons, ducks and turkeys, infected with a pathogenic A/turkey/Ontario/7733/66 H5N9 isolate showed a various immune response in the different species, various periods of virus shedding and different mortality rates (Slemons & Easterday, 1972). In another experiment, gulls and ducks infected with a turkey AIV strain, exhibited a different virus shedding period (24 days in gulls and only 6 days in ducks). Antibodies were detected in gulls but not in ducks (Bahl & Pomeroy, 1977). The research of Homme & Easterday (1970) made clear that Canada geese were not sensitive to a turkey isolate, but the virus was isolated from other avian species – pheasants and ducks (in the latter, no antibody response was present as well).

Purposeful studies to determine the

pathogenicity of AIV isolates to other avian species and for the preservation of the virus in the ecosystem were performed after the outbreak in hens caused by a highly pathogenic H5N2 strain in Pennsylvania in 1983/84. The strain was found to produce only mild clinical signs of illness in pheasants, gulls and none in Peking ducks. In pheasants, the virus was regularly shed up to the 15th day of the experiment, whereas only one of 12 ducks became infected (Wood *et al.*, 1985). The data of Nettles (1986) showed an adaptation of the strain to birds from the Galliformes order (hens, turkeys, quails, pheasants).

The performed large screening studies of wild birds (during the quarantine in Pennsylvania in 1984) aimed to establish whether HPAIV strain H5N2 was disseminated among them. From the numerous other isolates, only one subtype was H5N2, but it was different in pathogenic, antigenic and genetic aspects. The results showed no dissemination of the highly pathogenic H5N2 from susceptible domestic to wild birds. Also, strains with the same haemagglutinin and neuraminidase configuration have been isolated from wild ducks 4 years prior to the outbreak, suggesting that the non-pathogenic subtype was present in the ecosystem and was spread among wild birds and turkeys (isolates were repeatedly recovered from turkeys) (Hinshaw, 1986). A similar finding is observed now with the highly pathogenic H5N1 strain. In 2004, a strain from the same serotype was isolated from wild birds in Siberia. The detailed genetic investigations of the strain showed that it was not identical to the virus that caused the outbreak in Asia, Europe and Africa (Swayne, 2004).

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