Chapter 4

ECOLOGY OF AVIAN INFLUENZA VIRUSES IN SIBERIA

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ABSTRACT

Wild aquatic birds represent major reservoirs of the influenza A virus gene pool, from which novel influenza viruses can emerge to infect other avian and mammalian species, including humans.

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These avian hosts have long been considered the natural reservoir for low pathogenic avian influenza viruses (AIVs) that, in some occasions, can evolve in poultry to become highly pathogenic (HP) AIVs posing a risk for animal and public health. However, changes in viral ecology have been recently observed with the possibility of perpetuation of HPAIVs in the aquatic bird reservoir.

Siberia is of great importance in influenza A virus ecology. After a short reproductive season, huge numbers of wild aquatic birds move from different subarctic breeding sites and congregate in pre-migration staging areas of Siberian wetlands. Major intra-and inter-continental bird flyways overlap in these breeding and/or molting grounds where water birds from different wintering regions of the world are brought together, thus providing the influenza A virus gene pool for the possible emergence of novel reassortant viruses. Moreover, in the context of the global ecology of influenza A viruses, Siberian wetlands play a crucial role in the geographical dispersal of the virus, as shown by the HPAI H5 infections, which have spread since the early 2000s via migratory birds from Asia towards Europe, Africa, Middle East and, more recently, North America.

Here we consider ecological interactions that take place within the vast region of Siberia among the avian reservoir hosts and viral populations, and the environment they utilize. We also consider ecological drivers which, in the context of the current global change, can modulate in this Asian portion of Russia the influenza A virus circulation and spill-over from wild bird reservoirs to different avian and/or mammalian hosts.

Keywords: avian influenza viruses, influenza A virus, ecology, wild birds, Siberia, Russia

INTRODUCTION

Influenza is caused by enveloped RNA viruses, members of the family *Orthomyxoviridae*. Owning the ability to infect various avian and mammalian species, influenza A viruses represent a potential risk for wildlife conservation, veterinary and public health (Webster et al., 1992; Yoon et al., 2014).

The influenza A virus genome consists of eight single stranded RNA segments of negative polarity and encodes at least 10 viral proteins: polymerase basic 2 (PB2), polymerase basic 1 (PB1), polymerase acid (PA), hemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix proteins (M1 and M2), and non-structural (NS) proteins NS1 and NEP/NS2 (nuclear export protein/NS2). In addition to the spliced products M2 and NEP/NS2

proteins, some influenza virus strains can increase the coding capacity of their genome by the use of downstream translation initiation in normal or alternative overlapping reading frames PB1-F2 and PB1 N40 proteins (Shaw and Palese, 2013). Evolutionary analyses show substantial differences among influenza A viruses obtained over several years and from different animal species, that lead to species-specific lineages for each viral protein (Webster et al., 1992).

Virus genomic RNA segments are packaged into ribonucleoprotein complexes (RNP) by the PB1, PB2, PA and NP proteins. A host cell-derived lipid envelope is just above the M1 protein layer that surrounds the 8 RNP complexes. This viral envelope harbors the HA, NA and M2 membrane proteins of the viral particle (Lamb and Krug, 2001). The major surface glycoprotein HA enables the virus attachment and internalization into the cell endocytic compartment and, together with the M2 matrix protein, the release of RNP complexes into the host cell cytoplasm. The second surface glycoprotein NA is involved in the virus maturation and enzymatic release from host cell receptors containing sialic acid (Harder et al., 2013). Both HA and NA viral proteins are major targets of the host humoral immune response, and antibodies against these primary protective antigens are key mediators of resistance to virus challenge (Wright et al., 2013).

Influenza A viruses are classified into subtypes based on antigenic properties of their surface glycoproteins, HA and NA, both spike-shaped and involved in the virus-host cell interaction (Wright and Webster, 2001). At present, 18 HA (H1-H18) and 11 NA (N1-N11) subtypes have been identified by serologic and genetic analyses, and numerous virus subtypes showing different combinations of surface proteins (e.g., H3N2, H5N1) have been detected in avian and mammalian hosts (Yoon et al., 2014). Being RNA viruses with a segmented genome, influenza A viruses are constantly evolving by two main mechanisms: the antigenic drift and the antigenic shift (De Jong et al., 2000). In the antigenic drift, accumulation of mutations over time, due to error-prone viral replication and lack of proofreading mechanisms, results in a quasispecies distribution of viral genomes, and generation and circulation of different phenotypic viral variants. In this context, a negative selection process continuously eliminates unfit genomes and favours those viral variants which may provide an evolutionary advantage. Yearly influenza epidemics in the human population are a consequence of the gradual, progressive antigenic drift that allows emerging viruses to escape the host immunity (Domingo et al., 2012). In the antigenic shift, a major antigenic change leads to the emergence of a novel and/or immunologically distinct HA and/or NA subtype within a

population. This antigenic shift can be related to direct animal-to-human transmission of a whole influenza virus, as well as to reassortment events that occur when two viruses, even from different animal species, infect hosts that may act as a "mixing vessel" (e.g., pigs). In particular, when a cell is co-infected with different influenza viruses, the segmented nature of the RNA allows genomic mixing between viruses of mammalian and/or avian origin (Medina and García-Sastre, 2011). Thus, novel viral progeny can show remarkable changes in phenotypic (HA/NA combinations) and biologic (cell tropism, host range, virulence, transmissibility) properties, enabling a sudden and widespread outbreak in immunologically naïve human population (Harder et al., 2013).

Due to antigenic shift, recurring human influenza pandemics of variable severity occur at unpredictable and irregular intervals, whereas regular seasonal epidemics occur during interpandemic periods, through the continued circulation of host-adapted and established influenza viruses. Since the beginning of the last century several influenza A virus subtypes have emerged and spread, at 10-40 years intervals, in human population. In 1918 the pandemic H1N1 virus, probably of avian-origin, caused the terrible Spanish influenza. In 1957 the pandemic H2N2 virus, arisen from reassortment between human and avian viruses, brought to the Asian influenza. In 1968 the H3N2 pandemic virus, another human-avian reassortant, caused the Hong Kong influenza. Finally, in 2009 a novel H1N1 pandemic virus, multiple reassortant of genes from North American and Eurasian swine, avian and human influenza viruses, caused the first pandemic of the 21st century, also known as "the swine flu." When they emerged, H2N2 and H3N2 pandemic viruses replaced the previously circulating influenza A virus subtype (i.e., H2N2 *vs* H1N1 and H3N2 *vs* H2N2). In 1977, a not natural event probably caused the re-emergence of H1N1 viruses responsible for several outbreaks in younger immunologically naïve people worldwide. Since then, both H1N1 and H3N2 viruses have continued to circulate in the human population to this day (Neumann and Kawaoka, 2011; Rozo and Gronvall, 2015).

Wild aquatic birds represent major reservoirs of the influenza A virus gene pool, from which novel influenza viruses can emerge to infect numerous avian species and several mammalian hosts such as humans, pigs, horses, marine mammals, dogs and cats (Webby et al., 2007). At present, sixteen HA (H1-H16) and nine NA (N1-N9) subtypes have been found in the aquatic birds of the world (Yoon et al., 2014). Within the parasite/host relationship, influenza A viruses display evolutionary stasis in these avian reservoirs, where they show limited evolution at the amino acid level and cause no or mild disease

(Webster et al., 1992). Nevertheless, the same influenza viruses can display a rapid evolution following spillover events from natural reservoir species to alternative avian and mammalian species (Domingo et al., 2012). From an ecological point of view, wild aquatic birds have long been considered the natural reservoir for low pathogenic avian influenza viruses (LPAIVs) that, in some occasions, can evolve in poultry to become highly pathogenic (HP) AIVs posing a risk for animal and public health (Webster et al., 1992). However, changes in viral ecology have been recently observed with the possibility of perpetuation, of HPAIVs in the aquatic bird reservoir (Pasic et al., 2015).

Siberia is a vast geographical region, occupying the Northern part of Asia. Its territory extends eastwards from the Ural Mountains to the watershed between the Pacific and Arctic drainage basins and stretches southwards from the Arctic Ocean to the hills of north-central Kazakhstan and to the national borders of Mongolia and China. With an area of about 13.1 million square kilometres, Siberia accounts for 77% of Russia's land area (Suslov, 1947).

The northern arctic tundra, followed southwards by the boreal forest, deciduous forest, and steppe represent the major biomes found in broad belts that cross Siberia, more or less continuously (Suslov, 1947; Yurlov, 1977, 1981). These different biomes reflect different climate conditions. Basically, a continental climate characterized by short summers and long cold winters prevails, showing more extreme conditions in the northern areas (Sinitsyn, 1959). With a very low population density (about 3 people/Km^2) Siberia is one of the most sparsely populated region of the Earth (Prokhorov et al., 1999).

Siberia is of great importance in influenza A virus ecology. After a short reproductive season, huge numbers of wild aquatic birds move from different subarctic breeding sites and congregate in pre-migration staging areas of Siberian wetlands (Gilbert et al., 2008). Major intra-and inter-continental bird flyways overlap in these breeding and/or molting grounds where water birds from different wintering regions of the world are brought together, thus providing the influenza A virus gene pool for the possible emergence of novel reassortant viruses (Li et al., 2014c; Veen et al., 2005). Moreover, in the context of the global ecology of influenza A viruses, Siberian wetlands play a crucial role in the geographical dispersal of the virus, as shown by the HPAI H5 infections, which have spread since the early 2000s via migratory birds from Asia towards Europe, Africa, Middle East and, more recently, North America (Lee et al., 2015; Li et al., 2014b; Medina and García-Sastre, 2011).

Here we consider ecological interactions that take place within the vast region of Siberia among the avian reservoir hosts and viral populations, and the environment they utilize. In particular we focus on three major

geographical zones represented by the South of Western Siberia and the two large neighboring regions of Central Asian and Far East, since they have ecological relevance for the avian influenza surveillance in wild birds. We also consider ecological drivers which can modulate, in the context of the current global change, the influenza A virus circulation and spill-over from wild bird reservoirs to different avian and/or mammalian hosts in this Asian portion of Russia.

GLOBAL ECOLOGY OF INFLUENZA A VIRUSES IN WILD BIRD POPULATIONS

Natural Reservoirs of Avian Influenza Viruses

Although avian influenza viruses (AIVs) have been isolated from a wide host range of free-living wild bird species including water birds, galliforms, passerines, doves and woodpeckers (Stallknecht and Shane, 1988), most of them are reported from over 100 aquatic bird species, mainly included in the orders *Anseriformes* (particularly ducks, geese and swans) and *Charadriiformes* (particularly gulls, terns and shorebirds) (Olsen et al., 2006). Extensive scientific evidence indicates that wild aquatic birds belonging to the above taxonomic orders are the natural reservoir of the influenza A virus gene pool, perpetuated by these avian hosts in wetland habitats (Webster et al., 1992). All the known surface glycoproteins characterizing AIVs (from H1 to H16 and from N1 to N9) and a large number of HN possible combinations have been found in the aquatic birds of the world (Yoon et al., 2014).

In particular, wild ducks play an important role in the ecological cycle of influenza A viruses which, by means of these avian reservoirs, are perpetuated in a dynamic fashion accounting for complex interactions among the host and viral populations and the environment they utilize (Brown et al., 2009; Delogu et al., 2010; Hoye et al., 2010). Several ecological strategies enable influenza A virus maintenance within the wild duck reservoir, in which levels of virus circulation are strictly related to the age, immune status and population size of birds (Delogu et al., 2003). At the population level, influenza A viruses can be perpetuated through the viral strategy of infecting new susceptible hosts, such as newborn ducks at the time when maternal antibodies, usually representative of the population immunity, disappear (Domingo et al., 1998; Stallknecht and

Shane, 1988). Thus, according to a natural seasonal dynamic, the AIV prevalence pattern in wild duck populations of the northern hemisphere is usually higher in late summer/early autumn (infection rate up to 60% in ducks congregating in marshalling areas before the southward autumn migration) and then decreases through time with lower values in winter, spring and early summer (infection rate less than 10%, as a possible consequence of developing immunity and dispersal of duck populations) (De Marco et al., 2004; Hinshaw et al., 1985; Webster et al., 1992). As almost all HA and NA subtypes have been detected in dabbling ducks (*Anas* spp.), these birds are presumably the major reservoir of LPAIVs. Several longitudinal studies have shown that different HA and NA subtypes can dominate in different duck populations and, in particular, viruses of the H3, H4, H6 antigenic subtypes are frequently isolated from the common and widely distributed Mallard (*Anas platyrhynchos*). For the NA gene, N2, N6, N8 subtypes predominate in ducks (Krauss et al., 2004; Webby et al., 2007; Wright et al., 2013). Variations in AIV subtypes detected in the wild duck reservoir have been observed in different geographic areas and bird flyways (De Marco et al., 2003a; Ito et al., 1995; Süss et al., 1994; Wallensten et al., 2007) as well as spatial and/or temporal changes in prevalence and virus diversity have been detected in several areas in waterfowl populations (De Marco et al., 2014a; Wilcox et al., 2011). The co-evolution of the host/pathogen system has favored, by natural selection, a well adapted duck/virus relationship in which LPAIVs preferentially cause asymptomatic or paucisyntomatic enteric infections, and spread by the fecal-oral transmission route (Latorre-Margalef et al., 2009; Olsen et al., 2006). *Albeit at a lower extent,* AIVs also replicate in cells of the respiratory tract, and certain HA subtypes *(e.g., H3 and H4) can be recovered* from both cloacal and respiratory samples collected during surveillance of wild ducks, with three HA-NA subtype combinations (H3N5, H3N6, H4N5) found exclusively in respiratory tract samples (Krauss et al., 2013). Infectious LPAIVs have been recently detected on feathers of wild ducks in Europe (Delogu et al., 2010), America (Lebarbenchon et al., 2013) and Asia (De Marco et al., 2014a). Considering that AIV particles in water are attracted and concentrated on body surface by preened feathers (Delogu et al., 2010), uninfected or recovered birds carrying viruses on their plumage might play an active role in spreading AIVs in nature and trigger new infections, possibly delayed in time, by a self- or allo-preening mediated ingestion of viruses (Delogu et al., 2012).

According to serologic and virologic findings obtained from North American populations of *Branta canadensis*, wild geese seem to play a minor,

if any, role as reservoir hosts of LPAIVs (Harris et al., 2010). More recently, surveillance activities for influenza A virus antibodies showed a frequent exposure to AIV in *B. canadensis* populations sampled in Minnesota, New Jersey, Pennsylvania, and Wisconsin, where H3, H4, H5, and H6 subtypes predominated (Kistler et al., 2015a). High NP and H5 antibody prevalences were found in North American mute swans (*Cygnus olor*), before the recent introduction and evolution of H5 HPAIVs in the USA (Kistler et al., 2015b). With regard to Eurasia, no AIVs were isolated from 3,245 wild geese belonging to 9 different species sampled in areas of Russia and Tibetan Plateau (Sivay et al., 2011) whereas in Europe a low prevalence of LPAIV infection (ranging from 0.7% to 2.4%) was detected by analysing over 7,000 samples collected from 11 species of wild geese and swans (Munster et al., 2007). As shown by van Gils et al. (2007) natural infections with LPAIVs appear to negatively affect foraging and migratory performance in Bewick's swans (*Cygnus columbianus bewickii* Yarrell) trapped in The Netherland.

Although less studied, *Charadriiformes* species included in the shorebirds (e.g., sandpipers and lapwings) and seabirds (e.g., gulls and terns) groups have also been found to be natural reservoir of LPAIVs. A 16-year study carried out in North America demonstrated that shorebirds and gulls had high virus isolation frequencies during their northern migration to arctic breeding grounds, overall showing a broad range of AIV subtypes (Krauss et al., 2004). Further research conducted in North America, at Delaware Bay, has indicated that the shorebird/LPAIV system represents a source of virus subtype diversity, also suggesting that the Ruddy Turnstone (*Arenaria interpres*) is a primary host for avian influenza infection hotspots and virus reassortment in this study area (Barton et al., 2014). Low to negligible AIV prevalence results reported in Europe and Oceania indicated a spillover role of shorebirds in these continents (Curran et al., 2014; Munster et al., 2007). However, recent studies found a low but widespread circulation of AIVs in 69 shorebirds species sampled in 25 countries across western Eurasia and Africa, thus suggesting that these aquatic birds could play a role in the epidemiology of AIVs in the Old World, as in the Americas (Gaidet et al., 2012). Several HA subtypes (including H1, H2, H5, H7, and H9-H12) in combination with different NA subtypes have been found to circulate in shorebirds populations, possibly involved in maintenance mechanisms of influenza A viruses in nature (Wright et al., 2013).

The considerable amounts of LPAIVs detected in gulls have shown a wide virus diversity in this seabird group, but the actual role played by gulls in the perpetuation and transmission of influenza A viruses is poorly understood. At

present, viruses of the H13 and H16 subtypes appear both to circulate almost exclusively in gull populations and to replicate efficiently in these avian hosts, unlike observed in duck and galliform species. As also shown by experimental infections, gulls excrete LPAIV via the oropharynx and cloaca for several days (Brown et al. 2012; Costa et al., 2011; Fereidouni et al., 2014; Tønnessen et al., 2011). For the NA gene detection in *Charadriiformes*, N6 and N9 subtypes are prevalent in shorebirds and gulls (Wright et al., 2013). Similarly to information obtained from gull studies, the knowledge on influenza A virus ecology in tern populations is relatively few. Recent studies performed on seabirds sampled on islands of the Western Indian ocean showed that terns represent a major host for LPAIVs, harboring seabird-related virus subtypes (e.g., H16) as well as those usually detected in wild and domestic ducks (H3, H6, H9, H12 subtypes) (Lebarbenchon et al., 2015).

Environmental Persistence Strategy of Avian Influenza Viruses

As previously stated in this chapter, influenza A viruses usually cause an acute enteric infection in aquatic bird reservoir species, which shed high fecal viral loads in the environment (Olsen et al., 2006; Webby et al., 2007). For this reason, virus survivability outside host is extremely important to permit an environment-mediated transmission of AIVs. In particular, viral infectivity is retained in fecal material for 30-35 days at 4°C and for 7 days at 20°C. Cool and moist environmental conditions increase virus survival up to 105 days as observed, during the wintertime, in liquid manure from infected poultry farms (Easterday et al., 1997; Webster et al., 1978). In wetland ecosystems, water has a central ecological role in connecting susceptible hosts, enhancing AIV transmission by an indirect fecal-oral infection route in which depth and turnover of water can affect the host/parasite interaction (Delogu et al., 2003). Environmental factors such as water pH, salinity and temperature can influence both virus survival and transmission (Stallknecht et al., 1990a, 1990b, 2010). Usually AIVs are most stable at a slightly basic pH (7.4-8.2), low temperatures (<17 degrees C), and fresh to brackish salinities (0-20,000 parts per million) (Brown et al., 2009). Basically, the viral tenacity is inversely related to water temperature. As shown under experimental conditions, some LPAIVs can persist for months in distilled water at 4, 17, 28°C, and particular virus strains retaining infectivity over a year at 4°C are quickly inactivated at 37°C (Brown et al., 2009; Stallknecht and Brown, 2009). Analogous experiments also showed that, under different temperature and salinity

conditions, H5N1 HPAIV survivability in distilled water is in general comparable to that observed for LPAIVs but variable between HP virus strains (Brown et al., 2014). The presence of AIVs in environmental water samples has been documented in the context of avian influenza surveillance activities carried out in North America (Halvorson et al., 1985; Hinshaw et al., 1980a). More recently, field and laboratory studies have been carried out to assess AIV tenacity in environmental samples. Long-term detection of infectious viruses was obtained from lake water, collected in Alaska when the most ducks had left for autumn migration to south (Ito et al., 1995), whereas experimentally inoculated lake sediments, duck feces and duck meat, incubated at 30, 20, 10, 0°C, maintained at low temperatures long-term infectivity of various LPAIV subtypes. In particular, the time required for 90% loss of virus infectivity was highest in lake sediments (from 66 to 394 days at 0°C), followed by feces and finally meat (Nazir et al., 2011). Additional experiments, aimed to combine the effect of abiotic (e.g.. temperature, pH, salinity) and biotic (e.g., microorganisms) factors, were performed using three different types of water showing that AIVs persisted longest in distilled water, followed by filtered surface water and finally intact surface water (Keeler et al., 2013). Moreover, to evaluate the viable persistence of AIVs in extreme environmental conditions, fresh, brackish and salt water, taken in Japanese wetlands, were seeded with AIVs, frozen at -20°C and -30°C and then monitored for both long term virus infectivity (for one year, on a monthly basis) and viral resistance to freezing-thawing cycles: all examined viruses showed marked cryostability under the above mentioned experimental conditions, presumably indicative of intrinsic environmental survivability of AIVs in extreme cold weather conditions (Shoham et al., 2012). In conclusion, as also shown through results generated by a computational model, the influenza A virus persistence in aquatic habitats represents a crucial determinant for virus transmission dynamics in waterbird populations (Roche et al., 2014).

Avian influenza Virus Circulation Dynamics in Reservoir Hosts

The natural ecology of influenza A viruses accounts for a dynamic system in which a balanced host/parasite relationship is well integrated within aquatic ecosystems. With regard to the major natural reservoir of AIVs, represented by wild ducks populations, host's biological traits such as the highly gregarious behavior outside breeding season certainly enhances the virus transmission among birds congregating in marshalling and/or molting areas as well as in

wintering sites, thus enabling continuous virus circulation during seasons (e.g., late winter) characterized by low virus prevalence in reservoir species (Stallknecht and Shane, 1988). Moreover, the concurrent presence of multi-species aggregations of waterbirds in these concentration areas may also allow AIV transmission between both allopatric homospecific subpopulations and heterospecific groups (Delogu et al., 2003). In the context of the above scenario, reassortment events that may occur in the intestinal tract of ducks coinfected with different AIVs (Hinshaw et al., 1980b) represent an important viral evolutionary strategy to increase the diversity of influenza A viruses emerging in nature (Hatchette et al., 2004). The virus spread in nature is also intrinsically linked to the movements of birds, and mostly their migratory behavior (Hill et al., 2012a; Jourdain et al., 2007). According to regular seasonal movements, migratory avian species move to satisfy biological needs vital for their survival. Although species- and population-related differences exist, in the Holoarctic Region migratory birds basically move between the northern breeding areas and southern wintering grounds connected by flight paths called flyways, sometimes spanning over continents (Boere et al., 2006). Spatial and temporal segregations among wild aquatic bird populations, strictly related to the main bird flyways, breeding and overwintering grounds, have led the evolution of phylogenetically distinct evolutionary groups of influenza A viruses. At present influenza A viruses belonging to sixteen HA subtypes (from H1 to H16) can be separated into major geographic groups represented by two principal Eurasian and North American clades (Gorman et al., 1990) and two additional possible clades recognized in South America and Australia (Hansbro et al., 2010; Pereda et al., 2008). However this separation is not absolute, and gene segment reassortment between AIVs of different geographical lineages have been reported in North America (Makarova et al., 1999; Wahlgren et al., 2008), Europe (Wallensten et al., 2005) and Asia (Zhu et al., 2014). Gulls and other seabirds sampled in the Old and New World appear to play a relevant role in intercontinental reassortment of AIVs (Hall et al., 2013; Huang et al., 2014; Van Borm et al., 2012; Wille et al., 2011a).

Interestingly, LPAIVs of entirely American lineage and Eurasian lineage, as well as reassortant viruses, were found in aquatic birds sampled in Iceland in 2010-2011 period, showing the importance of the North Atlantic as a corridor for the movement of AIVs between Europe and North America (Dusek et al., 2014). Subsequently, following the polar system of intercontinental bird migration through Beringia (Alerstam et al., 2007), the wholly Eurasian 2.3.4.4 H5N8 virus emerged in December 2014 in North

America, giving rise to reassortant HPAI H5 viruses of Eurasian and North American origin and evolution (Torchetti et al., 2015).

The innovative implementation of intrinsic markers (e.g., stable isotope ratios, trace elements and genetic markers) for the study of avian migrations represents a complementary tool to bird marking techniques such as the conventional ringing and the modern satellite tracking of wild birds (Coiffait et al., 2009). Recently, stable isotope chemistry (Gunnarsson et al., 2012; Hill et al., 2012b) and genetic analyses (De Marco et al., 2014b; Winker et al., 2007) have been used to study AIV dynamics in mallard populations at a large scale, in a flyway perspective, as well as at a local scale in wintering sites. To examine the role of migratory tendency and species simpatry on the movements of Eurasian LPAIV genes into North America, Pearce et al. (2011) utilised a 50-year ring recovery dataset to characterize migratory patterns of mallards and northern pintails (*Anas acuta*). Moreover, to evaluate epidemiological links between areas of HPAI H5N1 persistence in South/Southeast Asia and pathways for virus long-distance movement, satellite tracking data of two waterfowl species (*Anser indicus* and *Tadorna ferruginea*) have been included in a geospatial risk analysis, according to which H5N1 persistence areas appear to be linked to domestic ducks whereas rare transmission events occur through migratory waterfowl (Gilbert et al., 2010).

Influenza A Virus Spillover from the Wild Bird Reservoir

As previously stated in this chapter, wild waterfowl populations have long been considered to play an important ecological role in perpetuating the influenza A virus gene pool in nature. However, highly divergent virus subtypes have been recently discovered in bats that, according to phylogenetic analyses and seroprevalence studies, could represent an additional influenza A virus reservoir, ecologically separated from the avian one and to date poorly understood (Mehele, 2014). Numerous evidences indicate that periodically influenza A viruses can be transmitted from the aquatic bird reservoir to a broad (and increasing) range of alternative avian and mammalian species (Figure 1), in turn potentially able to trigger the start of further epidemiological chains of transmission (Keenliside, 2013; Yoon et al., 2014).

Basically, the various determinants enabling the "host jump" of avian influenza A viruses are complex and intimately related to viral and host factors, whose interaction can lead to a change in the host tropism of the virus.

During virus adsorption, HA glycoproteins bind to the host cells' sialic acids, most commonly represented by the N-acetylneuraminic acid (NeuAc) with α,2,3-linkage (NeuAcα2,3 Gal) and α,2,6-linkage (NeuAcα2,6 Gal) to galactose. Quantity and distribution of these cell receptors differ in avian and mammalian species, in which influenza A viruses show a variable tissue tropism. As a consequence, human influenza A viruses preferentially recognize NeuAcα2,6 Gal, predominant in the human respiratory tract, whereas most avian influenza viruses preferentially recognize NeuAcα2,3 Gal, predominant in the intestinal and respiratory tracts of birds. Both NeuAcα2,3 Gal and NeuAcα2,6 Gal are expressed on pig trachea cells, as well as on respiratory and intestinal cells of quail, and other land-based birds, making these species potential players in the virus adaptation to new hosts and/or viral gene reassortments (Perez et al., 2003; Wright et al., 2013). As the receptor recognition is the first step for the virus/cell interaction, the HA surface antigen is the major viral determinant of host range restriction. Thus, a shift in the receptor-binding specifity of the virus is mainly linked to mutations in this viral surface antigen (Shi et al., 2014). Beside the lack of infectivity of the virus in new hosts, other possible barriers to frequent interspecies transmissions of influenza A viruses are represented by diversity in the ecology of host species and protective immunity in susceptible hosts (Gorman et al., 1992).

The virus replication into spillover (i.e., non-natural reservoir) species (Figure 1) can lead to different epidemiological scenarios, strictly related to the degree of adaptation of the virus in the new colonized hosts, with outcomes of infections ranging from sporadic and self-limiting cases to the establishment of influenza lineages characterized by efficient transmissibility (Urbaniak et al., 2014; Yoon et al., 2014).

AIVs have been isolated from a wide host range of free-living birds belonging to the *Anseriformes*, *Charadriiformes*, *Gaviiformes*, *Podicepediformes*, *Procellariiformes*, *Pelecaniformes*, *Ciconiiformes*, *Galliformes*, *Gruiformes*, *Columbiformes*, *Piciformes*, and *Passeriformes* orders (Stallknecht and Brown, 2008). However with the exception of the reservoir role played by *Anseriformes* and *Charadriiformes* birds, the epidemiological role of many other wild species found positive to AIVs is not well known yet (Olson et al., 2014). For instance, despite a number of AIVs including both LP and HP strains has been reported in passerine species and terrestrial wild birds, the AIV detection in these avian hosts appears to be usually associated with infected domestic poultry or other virus hosts (Ellis et al., 2004; Kim et al., 2012). Similarly, raptors or corvids found to be AIV

positive could have contracted the infection from other avian species, as a consequence of the respective ecological roles of predators at the top of food pyramid and scavenger species (De Marco et al., 2003b).

All reared species of poultry are susceptible to AIVs (Alexander, 2000), but obviously domestic birds included in the *Anseriformes* order, especially when reared under poor biosecurity conditions, represent an ideal "ecological bridge" for the transmission of AIVs between natural and anthropic ecosystems. In influenza A ecology, domestic waterfowl may represent an important AIV reservoir, harbouring numerous virus subtypes (Shortridge 1982). From an ecological point of view, endemically infected ducks provide a further perpetuation system of both LP (Alexander 1995; Shortridge 1995) and HP (Hosseini et al., 2013; Hulse-Post et al., 2005; Sturm-Ramirez et al., 2005) AIVs worldwide, with the probable exception of the H14 and H15 subtypes harboured in gull populations (Yoon et al., 2014).

Land-based birds such as chickens and turkeys are highly susceptible to avian influenza. However, despite their ability to efficiently replicate AIVs, only few virus subtypes (e.g., H6N1 and H9N2 in chickens) have established stable lineages in these galliform species (Yoon et al., 2014). Moreover, unlike what occurs in influenza A virus-adapted *Anseriformes* species, in gallinaceous hosts H5 and H7 virus subtypes can more easily evolve into HP strains causing severe systemic disease. As a consequence of these transmission events, divergent evolution of viruses in non-adapted avian hosts increases the viral genetic variability thus improving the global fitness of AIVs in bird populations.

Responsible for huge economic losses in the poultry industry worldwide, HPAIVs have also shown the ability to directly infect humans, sometimes causing fatal cases (Herfst et al., 2014). Among emerging pathogens, H5N1 HPAIVs of Asian origin caused, from late 2003 to the 20th of January 2016, 846 human infections including 449 fatal cases reported in Eurasia, Africa and America (only a single travel-related case in Canada) (WHO, 2016a), thus representing a paradigmatic example of feared avian zoonosis. In addition to the above-mentioned HPAIVs, several LPAIV subtypes recently emerged in Asia (e.g., H6N1, H10N8, H7N9) have provided evidence of direct bird-to-human transmission frequently associated to a severe outcome in human hosts, despite the mild symptoms caused in birds (Gao et al., 2013; To et al., 2014; Wei et al., 2013). In this context, major and increasing concern is caused by the H7N9 LPAIV, that since the first occurrence in China, in March 2013, was found in patients showing lower respiratory tract lethal infections. This novel reassortant virus, whose genes are entirely of avian origin, has possibly

become enzootic in chickens by the establishment of multiple regionally distinct lineages and reassortant genotypes (Lam et al., 2015). A total of 571 human infections with the H7N9 virus, including 212 deaths, have been laboratory-confirmed as of 23 February 2015 (WHO, 2016b). With regard to the HA antigenic subtypes that have shown the ability to cross the species barrier and infect humans, rapid evolution events are occurring in H5, H7 and H9 AIVs. Interestingly, enzootic H9N2 AIVs play a crucial role as gene donors of internal genes to several emerging zoonotic influenza viruses in China (Gu et al., 2015).
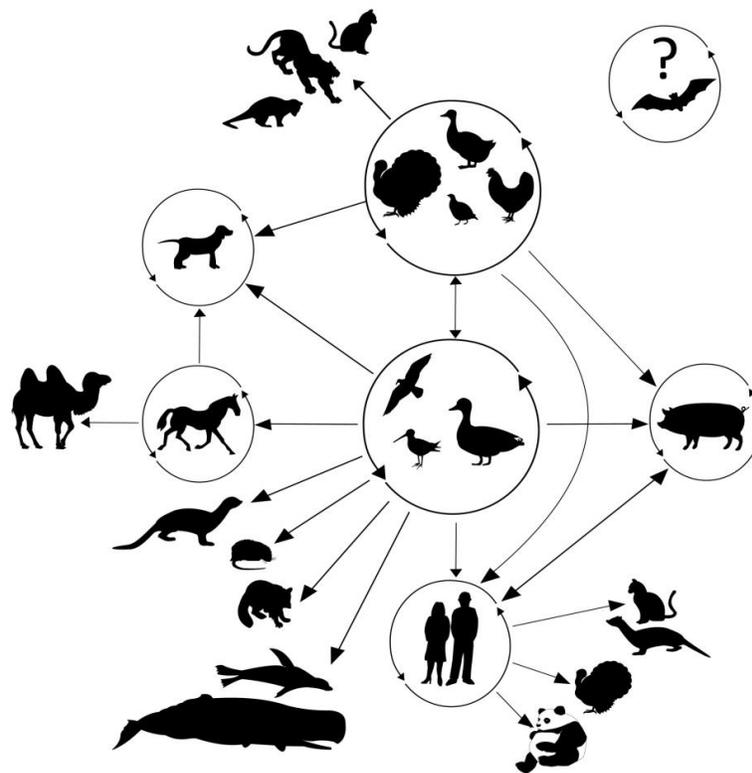

Figure 1. Examples of Influenza A Virus Interspecies Transmission. Simple arrows indicate the sporadic spillover of avian influenza viruses from wild (aquatic bird) and/or domestic (poultry) reservoir species to new colonized hosts that show different virus adaptation degrees and in turn enable the trigger of further chains of transmission. Double-headed arrows indicate a possible bidirectional virus transmission. Circular arrows include species in which stable, species-adapted, virus lineages circulate. Bats could represent an additional influenza A virus reservoir, whose ecological role is poorly understood to date.

Including the HP avian H5N1 virus, which has shown a broad host range in mammalian hosts (Kaplan and Webby, 2013), sporadic infections caused by emerging influenza A viruses of avian origin, characterized by highly variable severity, have been reported in mammalian hosts, such as humans (Herfst et al., 2014), pigs (Urbaniak et al., 2014), horses, marine mammals, mustelids (Parrish et al., 2015), felids, canids (Harder et al., 2013), viverrids (Kaplan and Webby, 2013), procyonids (Root et al., 2014), rodents (De Marco et al., 2014a), ursids (Li et al., 2014a), camelids (Yondon et al., 2014). However only a limited number of virus subtypes are able to circulate as stable lineages in mammals (Figure 1). As known for a long time, species-adapted virus lineages circulated/circulate in human (H2N2/H1N1, H3N2), pig (H1N1, H1N2, H3N2) and horse (H7N7/H3N8) populations, in which they caused/cause predominantly respiratory disease of different severity degree (Yoon et al., 2014). More recently, additional lineages of influenza A viruses, showing an avian (H3N2) and equine (H3N8) origin, emerged and established themselves in dog populations of South Korea, China, and the U.S.A (Harder et al., 2013).

Avian Influenza Virus Surveillance

Although sporadic AIV infections were detected in wild birds prior to the 1970s, it was not until then that systematic surveillance studies revealed the widespread distribution of influenza A viruses in wild avian populations (Webster et al., 1992). Since then, numerous AIV surveillance activities have been carried out in wild birds worldwide, especially by focusing the attention on the aquatic bird reservoir species (Gaidet et al., 2012; Grillo et al., 2015; Krauss et al., 2004; Lebarbenchon et al., 2015; Wallensten et al., 2007). Systematic AIV surveillance studies in wild birds particularly increased after the HPAIV (H5N1) emergence in Asia (Bevins et al., 2014; Subbarao and Katz, 2000).

As recently reviewed by Hoye et al. (2010), 191 published reports of surveillance in wild birds addressed the following 4 major lines of investigation: i) early detection of HPAIVs; ii) ecology and epidemiology of LPAIVs in avian populations; iii) AIV diversity and evolution within wild bird species; iv) AIV detection as part of broader healthy surveys in wild birds.

The use of surveillance systems based on virological diagnosis of avian influenza generally predominates, however seroprevalence studies can represent additional useful tools to provide indirect evidence of AIV circulation in wild bird populations (De Marco et al., 2004).

In the context of AIV surveillance, virus prevalence detected in wild birds may be affected by several factors, such as season and area of sampling, bird species examined and methods of sample collection and analysis (Ip et al., 2008). Recent studies used an innovative and more sensitive approach to AIV surveillance, obtained by integrating the conventional cloacal swab collection, aimed to show the fecal virus shedding, with the harvesting of samples from birds' plumage, aimed to detect viral particles concentrated onto birds' bodies (De Marco et al., 2014a; Delogu et al., 2010; Lebarbenchon et al., 2013). A particular sampling method, based on the collection of swabs rubbed over feathers located on the bird's breast and flanks, allows the detection of AIVs concentrated from aquatic environment to birds' plumage (Delogu et al., 2010). Whereas analyses of cloacal and/or oropharyngeal swabs reveal viruses shed by infected ducks, viruses isolated from feathers may not have infected the sampled individual yet and just come from the environment. As previously reported by Delogu et al. (2010), virological examination of feather samples can reveal the presence of birds testing "false-negative" by conventional AIV surveillance system (virus isolation negative from cloaca and/or oropharynx) but carrying infectious virus on their plumage.

Avian influenza in poultry can represent a notifiable animal disease. In fact, according to the OIE Terrestrial Animal Code (OIE, 2015) avian influenza has been recently defined as

" *... an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any influenza A virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described   ...*"

Both high and low pathogenicity avian influenza viruses can match these classification criteria, and several AIV surveillance plans are mandatory, under national and international laws, in captive-reared birds worldwide.

THE WILD BIRD NATURAL SYSTEM OF AVIAN INFLUENZA VIRUS CIRCULATION IN SIBERIA

Siberia's Terrestrial Biomes

Extending over the polar, boreal and temperate domains, Siberia includes three large-scale biomes[1]: tundra, taiga, and steppe (Crawford, 2013). Admitted that there are not clear boundaries among them, in the following paragraphs the main features of the three biomes are presented.

Tundra

The Siberian tundra (from the Finnish word *tunturi*, meaning treeless plain) is located in the north-eastern part of Russia, between 60° to 80° North latitude, and 70° to 180° East longitude. The climate here is extremely harsh and its severity intensifies from West to East Siberia and is temperated at the Arctic-Pacific coast. The temperature of the coldest month vary from −23 to -35°C in West, -29 to -40°C in East Siberia and from -20 to -34°C in the Beringiiskaja tundra. Summers are cool with temperatures of 3 to 12°C. Precipitation is only 150 to 260 mm per year including melted snow, but everywhere significantly exceeds evaporation and humidity of soils is usually high. Growing period (number of days with t > 10°C) is from about 0 in the extreme North to 50 days in the South. Wind in tundra ranges from 50 to 100 km per hour.

The plants of the Siberian tundra include fungus, grasses and shrubs growing low to the ground to be sheltered from the wind and the cold. Plant roots spread out on the surface to take in water. They don't grow deep because the soil is always freezing and thawing which breaks up roots. One of their adaptations to the cold climate is tiny hairs on their leaves to make possible keep in air moisture. The animals of the tundra comprises fish, birds, mammals and insects. The animals survive with extra fat and thick fur to keep them warm. Most animals are low to the ground and their arms, legs, tails and ears are small to keep from losing heat. Hundreds of types of birds stay for the summer only.

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[1] A biome, or vegetation zone, includes all areas where the vegetation and animal life are broadly similar. Biomes are shaped by climate, together with the local rocks and soil type.

Tundra is typically divided into three latitudinal belts: arctic tundra, northern (archetypal) tundra and southern (shrub) tundra. In Siberia we can also distinguish two terrestrial tundra ecoregions: Northeast Siberian coastal tundra and Taimyr-Central Siberian tundra. They contain a wide range of mountainous and coastal tundra habitats in a relatively intact condition. Northeast Siberian coastal tundra is a narrow strip of tundra on the coast of the East Siberian Sea, extending east of the Lena Delta to the marshy lowland at the mouth of the Kolyma River. It is located along the coast of the Eastern Siberian and Laptev Seas, between the Yana and Kolyma Rivers and it represents some of the most productive arctic tundra wetland areas in northeastern Russia. The climate of the area is under the influence of both ocean and continental processes. Vegetation features of the region are typical for dwarf shrub arctic and typical tundra, but some sparse patches of larch forest are present along the southern border of the region. Permafrost underlies the entire region and plays a large role in landscape formation and botanical distribution. Common plant species include cotton grass (*Eriophorum* spp.), sedges (*Carex* spp.), dryas (*Dryas punctata*), willows (*Salix* spp.), crowberry (*Empetrum* spp.), cranberry (*Vaccinium vitis-idea*), and mosses. These tundra regions are favoured by numerous species of birds that migrate along Nearctic and Palearctic flyways to breed here. The large river deltas are some of the most important breeding areas and host between 40 and 60 breeding species each summer (Goryachkin et al., 1994; Pearce et al., 1998b). The area between the Yana and Indigirka rivers is the breeding ground for a large majority of the world's population of Steller's and spectacled eiders (*Polysticta stelleri* and *Somateria fischeri*) (Pearce et al., 1998a); both listed in the Red Data Books of Russia and listed as threatened on the U.S. Endangered Species List. Other characteristic birds for this region are Red-throated Loon (*Gavia stellata*), Brant Goose (*Branta bernicla*), Curlew Sandpiper (*Calidris ferruginea*), Sharp-tailed Sandpiper (*Calidris acuminata*), Broad-billed Sandpiper (*Limicola falcinellus*), Ross' Gull (*Rhodostethia rosea*), Ruff (*Philomachus pugnax*), Willow Grouse (*Lagopus lagopus*), and Snow Bunting (*Plectrophenax nivalis*).

Taimyr-Central Siberian tundra corresponds to the montane, lowland and arctic desert in the Taimyr tundra vegetation province (Kurnaev, 1990). The Taimyr reindeer herd, a migrating tundra reindeer (*Rangifer tarandus sibiricus*), is the largest reindeer herd in the world. Home to the largest migrating herd of wild reindeer in Asia, the Taimyr and Siberian Coastal Tundra also provides nesting areas for approximately 50 bird species, of which nine are endangered - including Bewick's swan and the bean, red-

breasted, and lesser white-fronted geese (*Anser fabalis, Branta rufucollis, Anser erythropus*). The region's freshwater systems support nearly 60 species of fish and are important spawning grounds for certain species.

Taiga

Russians have given the name taiga to the subarctic, marshy, lands of Eurasia with their extensive coniferous forests. The term has come to indicate the circumpolar coniferous forest belt, of the cold, subarctic region, that lies between the tundra to the north and temperate forests to the south. The taiga (1,224 million hectares) is the second world's largest terrestrial biome, or about 31% of the world's forest cover (FAO, 2015). In Eurasia, it covers most of Sweden, Finland and Norway territories, some lowland/coastal areas of Iceland, much of Russia, from Karelia in the west to the Pacific Ocean, and northern Kazakhstan land, northern Mongolia, and northern Japan (on the island of Hokkaidō). The main tree species are spruce (*Picea* spp.), pine (*Pinus* spp.), larch (*Larix* spp.) and fir (*Abies* spp.), which are all coniferous, but a few deciduous species such as birch (*Betula* spp.), aspen and poplar (*Populus* spp.) also occur, especially in the early stages of forest succession.

The Siberian taiga is the largest forest in the world, stretching some 5,800 kilometers, from the Pacific Ocean to the Ural Mountains, and some 1,000 km from north to south. This taiga region was completely glaciated, or covered by glaciers, during the last ice age. The soil beneath the taiga has low level of nutrients and often contains permafrost—a layer of permanently frozen soil—making it difficult for many plants to take root. In other areas, a layer of bedrock lies just beneath the soil. Both permafrost and rock prevent water from draining from the top layers of soil. This creates shallow bogs known as muskegs. Muskegs can look like solid ground, because they are covered with moss, short grasses, and sometimes even trees. However, the ground is actually wet and spongy. It is a biome characterized by coniferous forests consisting mostly of pines, spruces and larches. The dominant tree species varies depending on several factors, namely growing season and summer temperatures. Russian taiga has spruces, pines and larches depending on the region, while the Eastern Siberian taiga is a vast larch forest. Taigas have few native plants besides conifers.

The East Siberian taiga ecoregion is a very large bio-geographic region in eastern Russia. It is located in the heart of Siberia, stretching over 20° of latitude and 50° of longitude (52 degrees north to 72 degrees north, and 80 degrees east to 130 degrees east). The climate in the East Siberian taiga is subarctic and displays high continentality, with temperature extremes ranging

from 40°C to −62°C. Winters are long and very cold, but dry, with little snowfall due to the effects of the Siberian anticyclone. Summers are short, but can be quite warm considered such a northerly location. Precipitation is relatively small, ranging from 600 to 200 millimetres, decreasing from east to west. The topography of this ecoregion is varied, consisting of wide, flat plains and areas of karst topography. In contrast to the neighbouring West Siberian taiga, large bogs and wetlands are conspicuously absent. Some trees also shed their leaves annually, a characteristic of deciduous forests.

The southernmost latitudinal belt within the subzone of southern taiga in West Siberia is the ecoregion Western Siberian hemiboreal forests (Ermakov et al., 1991, 2000; Hämet-Ahti, 1963). North to south, the width of this forest belt is only about 150 km. Although there are no endemic plant species, the area contains the highest level of biodiversity in western Siberia. It includes mixed small-leaf-coniferous, small-leaf and coniferous grass forests. This forest evolved from the ancient Pliocene coniferous-broadleaf forest in situ. In its original composition, it consisted of *Pinus* spp. with a submixture of other coniferous species such as *Picea* spp., *Abies* spp., and *Larix* spp.; broadleaf species such as *Castanea* sp., *Quercus* sp., *Tilia* spp., *Fagus* spp., *Carpinus* spp. and *Ulmus* spp.; and small-leaf species such as *Populus tremula* and *Betula* spp. After the glacial period, some of the broadleaf species did not survive. Those that survive include: *Populus nigra*, and in the west of the region *Tilia cordata*, and in the east of the region *Tilia sibirica* (Lesa SSSR, 1969).

The Northeast Siberian ecoregion is one of the largest unaltered boreal forest areas in the world. This forest serves as a significant carbon sink, helping to lessen the impact of global climate change. Basin areas of Yana and Indigirca rivers serve as important breeding areas to the rare Little Curlew (*Numenius minutus*) and Siberian Crane (*Grus leucogeranus*). Much of the area lies within the Arctic Circle and all of it is covered by permanently frozen ground (or permafrost), which greatly influences the region's ecology and limits forests to this ecoregion. A vast area of taiga forest makes up the majority of this region. Larch (*Larix cajanderi*) tree stands accompanied by *Betula pendula* are found throughout the region. Forests with *Ledum palustre*, *Vaccinium uliginosum*, lichens and mosses in ground cover dominate in ecoregion. Forests of *Populus suaveolens* and *Chosenia macrolepis* occur in the valleys of the rivers (Shcherbakov, 1975; Sochava, 1980).

One of the characteristics of the taiga is the usual presence of podzol soils. Podzols typically seize a surface humus layer of slowly decomposing coniferous litter, underlain by a light grey or nearly white mineral layer,

which, in turn, is underlain by a brownish or reddish layer. The humus is typically strongly acid. The light grey horizon results from the massive leaching of nutrients; they are deposited lower down, in the reddish layer. When a podzol is ploughed, planted and exposed to air and rain the grey layer frequently coalesces into a "hard-pan" and is quite impermeable to water, and it may then remain in such a condition for many years.

Many kinds of animals live in the taiga. All animals have to be well-adapted to the cold. Birds native to the taiga usually migrate south during the freezing winter months. Small animals, mostly rodents, live close to the floor. Many birds of prey, such as owls and eagles, hunt these animals from the trees of the taiga. Moose (*Alces alces*), the largest type of deer in the world, is able to live in the cold taiga. Like all deer, moose are herbivores. They favour the aquatic plants growing on the taiga's bogs and streams. Few large carnivorous animals live in the taiga. Bears and lynx (*Lynx lynx*) are fairly common. The largest cat in the world, the 300-kilogram (660-pound) Siberian tiger, is a native taiga species. Siberian tigers (*Panthera tigris altaica*) live in a small part of eastern Siberia. They hunt moose and wild boars (*Sus scrofa*).

Steppe

Steppe is an ecoregion in the montane grasslands and shrublands and temperate grasslands, savannas, and shrublands biomes, characterized by treeless grassland plains, apart from those near rivers and lakes. The prairie (especially the shortgrass and mixed prairie) is an example of a steppe, though it is not usually categorised as such. It may be semi-desert, or covered with grass or shrubs or both, depending on the season and latitude. The term is also used to denote the climate encountered in regions, too dry to support a forest but not dry enough to be a desert. The soil is typically of chernozem type.

Steppes are usually characterized by a semi-arid and continental climate. The mid-latitude steppes can be summarised by hot summers and cold winters, averaging 250–510 mm (10–20 in) of precipitation per year. Precipitation level alone is not what defines a steppe climate; potential evapotranspiration must also be taken into account.

Two types of steppe can be recorded: i) temperate steppe (the "true" steppe), found in continental areas of the world; ii) subtropical steppe: a similar association of plants that can lodge in the driest areas with a Mediterranean-like climate; it usually has a short wet period. Peculiar types of steppe include shrub-steppe and alpine-steppe.

The world's largest steppe region, often referred to as "the Great Steppe," is found in Eastern Europe and Central Asia, and neighbouring

countries stretching from Ukraine in the west through Russia, Kazakhstan, Turkmenistan and Uzbekistan to the Altai, Koppet Dag and Tian Shan ranges.

Siberia's Ecological Regions

Siberia is an extensive geographical region in the North-Eastern part of Eurasia, bounded to the West of the Ural Mountains from the far Eastern regions of Russia, from the North Arctic Ocean, from the South border with the neighboring Kazakhstan, Mongolia and China. With an area of 13.1 million km^2 (5.1 million square miles) Siberia takes up roughly 77% of Russia's total territory and covers almost 10% of Earth's land surface (148,940,000 km^2). This wide region is divided into Western Siberia (including Khanty-Mansi and Yamal-Nenets autonomous districts; Tyumen, Kurgan, Novosibirsk, Omsk, Tomsk and Kemerovo regions, Altai Krai, Altai Republic) and Eastern Siberia (including the Krasnoyarsk and the Zabaikalye territories, Amur and Irkutsk regions, republics of Khakassia, Tuva, Buryatia and Yakutia). This Russian area can also be divided into Southern Siberia (mountainous part), North-Eastern Siberia and Central Siberia (Suslov, 1947).

In this section we focus on three major geographical zones represented by the South of Western Siberia and the two large neighboring regions of Central Asia and Far East. These territories are of great interest for the ecology of both avian hosts and pathogens, thus having significant relevance for the avian influenza surveillance in wild birds in Northern Eurasia. In particular we consider the basic geographic and climatic characteristics as well as ecological interactions occurring between the avifauna and influenza A viruses in these three interrelated areas.

Western Siberia

Taiga is the main vegetation zone of West Siberia, with a tundra belt on the northern fringe, and a temperate forest zone in the south. The climate of Siberia is continental and varies dramatically between short summers (about one-month-long on the North Coast/North of the Arctic Circle) and long winters of very cold climate. Temperature in January is from -15°C to -40°C; in July from +15°C to + 35°C (Slyadnev, 1972). North to south, Western Siberia is divided into 5 zones: tundra, forest-tundra, forest, forest-steppe and steppe. This huge variety of zone and intrazonal landscapes could not fail to affect the abundance and diversity of fauna of these places. There are more than 40 species of trees, of which the most common is the Siberian cedar, pine,

birch, aspen, poplar, willow. There are nearly 500 vertebrate species, including 99 wild mammals, 350 birds, 9 reptiles, and around 60 fish species (Zykov, 1960).

Huge amounts of rivers and lakes are in the South of Western Siberia territories, located on the migration routes of many bird species and providing nesting habitats for large numbers of species ecologically associated with wetlands. The largest water body of this region is the Chany Lake (55°05'-54°28'N, 76°42'-78°25'E), a group of lakes and ponds of various sizes and types located on the territory of Barabinsk lowland relief, characterized by the alternation of low, gently sloping, and relatively narrow hills extending from North-East to South-West, with shallow wide depressions. This relief determines a unique placement of water and vegetation, which in turn form the habitat of wild birds. A characteristic feature of these water reservoirs is the shallow depth of the lake basins, which are usually flat, muddy, and with sloping banks on the bottom. The magnitude and types of water bodies vary from large lakes to small wetlands, and the entire water column in summer warms up fully (Yurlov, 1977). Most lakes are freshwater with water and emergent vegetation. The most characteristic plant, both in water and on the banks, is the reed. Many ponds are rich in invertebrates, the most common of which are aquatic beetles, water mites, leeches, larvae and adult beetles, larvae of mayflies and dragonflies. In these habitats characterized by high ecosystem productivity and high host density, the largest animal biomass is represent by freshwater zooplankton (Yurlov, 1977, 1981).

Central Asia

Located in the South, the Central Asian Region includes the territories of Siberia, Kazakhstan, Mongolia, China and other Central Asian countries. The most interesting in this regard is the territory of the Altai-Sayan basin, which is located on the border of Russia and Mongolia and characterized by several depressions, with numerous rivers and lakes. The diversity of lakes in this territory includes small salt lakes (e.g., Dus-Khol and Khol lakes), salt lakes (Uvs-Nuur and Hadyn lakes) and large freshwater reservoirs in the North-East of Tuva. The Great Lakes basin is a broad intermountain reservoir bordered by mountains of Mongolian and Gobi Altai to the South and West and located on the boundary of the Republic of Tuva and Mongolia. Its western part includes the largest water body of Mongolia, that is the saline Uvs-Nuur Lake which represents an important breeding and stopover area for the different migratory waterfowl.

In winter, weather conditions are characterized by very cold temperatures, clear skies, little wind, and little snow. In summer, warm and even hot conditions, with various humidity degree, depend on territories. The climate is continental, dry, with significant seasonal and daily fluctuations in temperature. The average January temperature in the plains is from -10 to -25°C, ranging in July between +20 to +25°C (around +10°C on the Tibetan Plateau). The annual amount of precipitation on the plains does not usually exceed 200 mm, and areas (such as the Taklimakan Desert, Gashunskaya Gobi, Tsaidam, Changtang plateau) receive less than 50 mm. The greatest amount of precipitation falls in the summer. The ridge rainfalls are of 300-500 mm per year and in the South-East, under the effect of the summer monsoon, up to 1,000 mm per year. Central Asia dry climate is characterized by strong winds, abundance of sunny days (240-270 per year) and low water content in several closed drainage basins (Sinitsyn, 1959).

Far East Region

This is the eastern part of Russia, which includes the area of basins of rivers flowing into the Pacific Ocean, as well as the island of Sakhalin, Kuril Islands, Wrangel Island, Commander Islands and Shantarsky Island (Gvozdetskii and Mikhailov, 1978). The region is bordered by China to the south, by the Democratic People's Republic of Korea to the extreme south, by Japan to the south-east and by the USA in the Bering Strait in the far north-east.

Very contrasting situations characterize the Far East Region climate, which varies from the arctic and subarctic in the north of Yakutia and Kamchatka, in Magadan and Chukotka to monsoon in Sakhalin, in Jewish and Amur regions, and continental in Primorsky and Khabarovsk regions. Most parts of Yakutia and the north-west of the Amur region are dominated by sharply continental climate whereas a maritime climate occurs in the Kamchatka Peninsula and the Kuril islands. These extreme differences are due to the huge length of the Far East Region territory from north to south (nearly 4,500 km) and from west to east (2,500-3,000 km), as well as to the interaction between the Pacific Ocean and land in North Asia. In the cold season, especially in winter, the Far East is dominated by westerly winds carrying dry frosty air of Siberia as anticyclones. During warmer months, the wind begins to blow in from the ocean, bringing cyclones and, consequently, cloudy weather and rain. Summarizing, air masses circulate according to a monsoon climate (wind blowing from the mainland and ocean in winter and summer, respectively). Based on the above characteristics of the movement of air flows,

seasonal variability in rainfall is expected. For example, in Khabarovsk about 470-490 mm of rain fall from December to March and only 50-60 mm from June to September. In general, rainfalls in the Far East are distributed unevenly even within the same or neighboring territories, as occur in the Chukotka Autonomous Okrug, where rain precipitation ranges from 150 to 600 mm per year, and in the Kamchatka Territory and Magadan region, where annual precipitation ranges from 400 to 800 mm. Relatively larger rainfall events (an average of 500 to 1,000 mm per year) characterize the remaining territories.

The temperature regime of the Far East Region is characterized by a sharp cold increase during the cold season as moving away from the coast inland. For example, on the east coast of the Kamchatka Peninsula, the average January temperature ranges from -4 to -6°C, while in the center of the peninsula drops to values from -16 to -22°C. Similarly, on the coast of the Chukotka Autonomous Region, Magadan and Khabarovsk Krai the average January temperature ranges from -16 to -20°C, while on the border with Yakutia drops dramatically (from -30 to -35°C). Again, in Primorye, the average temperature in winter months is relatively high (from -6 to -8°C) on the banks, while on the border with China drops to values from -20 to -24°C. In the Amur and Jewish autonomous regions the average January temperature ranges between -20 and -26°C. Unlike what happens in the cold season, during the warm time of the year the variation of the average temperature is greatly reduced. This, again, is one of the features of the monsoon climate. While average July temperatures in the far north of the Chukotka Autonomous Okrug can range from +3 to + 8°C and from -2 to -1°C, in almost all the rest of the Far East Region, the average July temperature is between about +10 and + 15°C. Only in the southern part of the region, on the border with China, during summer months the average temperature is between +17 and + 21°C (Gvozdetskii and Mikhailov, 1978).

Russian Natural Ecosystems Involved in Avian Influenza Ecology

Epidemiology, genetics and evolution of pathogens are inseparably connected with the diversity of the environmental factors, especially with respect to the bird ecology. Biology of migratory birds is one of the principal drivers of avian influenza virus (AIV) ecology. Crossed by main flyways connecting China, Southeast Asia, and Russia, Siberia is a key region for research on influenza virus spreading via migratory birds (Marchenko et al.,

2012; Veen et al., 2005). In the Palearctic Region, the North-West Russia and Siberia are major breeding areas for many migratory waterfowl of the family *Anatidae*. More in general Siberia has its own specific avifauna, including migratory bird species crossing Europe, Asia and Africa in the spring. In this context, the territory of the West Siberian lowland is of particular importance, covering an area of 2,745,000 km^2 (Gilbert et al., 2006).

Western Siberia wetlands serve intensively as habitat for a large number of birds during nesting and seasonal migrations. Overall, 241 avian species from 45 families, included into 16 bird groups, can be found in the Chany Lake system, and during migration period the number of some migratory bird species increases of 2-2.5 times. Species number, abundance and migration routes of the Southwest Siberian waterbirds were reviewed by Ryabitsev (2008), who described 446 species of birds by examining the wider area including steppe and forest-steppe subzones, encompassing the Administrative regions of Kurgan, partly Tyumen, Omsk, Novosibirsk and partly Altay, Urals and its surroundings. In particular twenty-six waterbird species are considered abundant (i.e., species whose number is systemically more than 10 individuals per square kilometer in any type of landscape) through the region during at least one stage of the annual cycle, and all of them are breeding species, except the Ruff which is really abundant during spring migration (Toropov, 2008). As population density and species composition of bird communities depend on the different types of zones, it is obvious that viral reservoirs have a relevant ecological significance in the enzootic and/or epizootic circulation of AIVs. So, the steppe zone is characterized by the lowest total biomass of birds, and here, as in forest-steppe, dominate only synantropic species as Common Magpie (*Pica pica*) and Rock Dove (*Columba livia*) (Yurlov, 1981). Several lake systems of Western Siberia represent key ornithological territories (Yurlov, 2006). One of them includes the large flow-through lake Sargol (2,700 ha) and a 23,700-hectare area with a few small salt lakes and adjacent wetlands. This area enables favorable conditions for the waterbird habitat in different seasons and in years characterised by different moisture conditions. For examples, in wet years birds concentrate in flooding of brackish ponds and marshes. In the summer-autumn period, the total number of waterbirds in this area is almost always greater than 20,000 individuals. This lake system is of international importance for Whooper Swan (*Cygnus cygnus*: over 500 individuals), Greylag Goose (*Anser anser*: 3,500-5,000 individuals) and Common Crane (*Grus grus*: 3,500-5,800 individuals) and, in particular, concentrations of geese, cranes, and ducks increased in recent year during autumn migration (Vartapetov et al., 2003).

According to a recent report (Savchenko, 2009) the modern avifauna of Central Asia, includes 20 orders of birds, with 383 species which are migratory or sedentary (n. 309), alien species (n. 31), vagrant (n. 38) and wintering only (n. 5).

Wetlands of Central Asia are reproductive and stopover sites of a wide range of ducks species. Gadwall (*Anas strepera*) is widely distributed in this region. The Northern Pintail, Northern Shoveler (*Anas clypeata*), Eurasian Wigeon (*Anas penelope*), Common Teal (*Anas crecca*), Common Pochard (*Aythya ferina*) and Tufted Duck (*Aythya fuligula*) are the main representative migratory species of the family *Anatidae*. Their distribution area covers a significant part of Eurasia, and some ringing recovery data show that birds nesting on the territory of Central Asia, migrate over long distances from breeding sites to their wintering grounds in Western Europe, Africa, Mediterranean areas and India. In particular, Common Pochard, one of the most common duck species, migrates to main wintering grounds in Iran, India, Pakistan and southern China.

Over two-thirds of the 800 species representative of the avifauna of Russia are found in the Far East Region, and the stability of populations of about half of them is exclusively supported by reproduction in local habitats. Even the Maritime Region, area that accounts for the 0.75% of the territory of Russia, houses 360 species of birds. The full list contains 557 species from 75 families and 22 orders, including rare species such as the Far Eastern White Stork (*Ciconia boyciana*), Black-necked Crane (*Grus nigricollis*), Japanese Crane (*Grus japonensis*), Mandarin Duck (*Ayx galericulata*), Red-legged Kittiwake (*Rissa brevirostris)*, Glaucous-winged Gull (*Larus glaucescens*), Red-faced Cormorant (*Phalacrocorax urile*) (Nechaev and Naumova, 2009). About 100 species of birds of the Far East Region occupy the northern boundary of their distribution, including colonial birds. Khanka Lake (the largest freshwater reservoir in this region) and its wetlands, comprising extensive marshes and lakes, represent a unique natural complex of international importance, which has no analogues throughout the Far East. This ecological system provides a suitable habitat for 333 species of birds including white storks (*Ciconia ciconia*), Japanese and black cranes, herons, ducks, geese. One of the branches of the East Asian Flyway crosses this area and during the peaks of spring and fall migrations around 2 million birds visit these places (Nechaev, 1971, 1974).

To emphasize the pivotal role played by the East Asia Region in the biodiversity conservation, it is worth noting that some of the more than 100 autochthonous species of mammals — e.g., Amur Tiger, *Panthera tigris*

altaica; Far Eastern Leopard, *Panthera pardus orientalis*; Amur Leopard Cat, *Prionailurus bengalensis*; Spotted Deer, *Axis axis*; Asian Black Bear, *Ursus thibetanus*; Wildcat, *Felis silvestri*s; sea otters, *Enhydra lutris*; fur seals (*Callorhinus ursinus*)— and several reptile and fish species are endangered wildlife species.

Bird Migrations and International Wetlands of Siberia

As shown in Figure 2, recent research has identified eight major pathways for migratory birds worldwide. These include the East Atlantic, the Black Sea/Mediterranean, the East Asia/East Africa, the Central Asia, the East Asia/Australasia Flyways, and three flyways in the Americas (Boere et al., 2006). Five major migration routes of wild birds cross the territory of Russia, but the following are of particular importance for the territory of Siberia and Far East Region: the Black Sea/Mediterranean, the East Asia/East Africa, the Central Asia, the East Asia/Australasia Flyways (Figure 2).
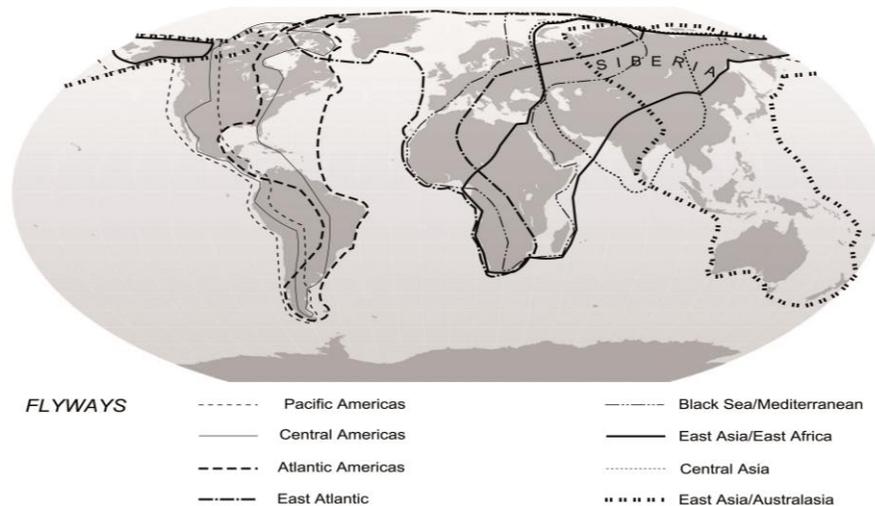

Figure 2. Main General Flyways used by Migratory Shorebirds. Five out the eight main global migration routes used by wild birds cross the territory of Russia. Global map of the World was obtained, on March 16 2016, from site http://earthobservatory.nasa.gov/Images/category.php?cat_id=2, maintained by the NASA Earth Observatory. Information used to depict global flyways was obtained, on March 30 2016, from site http://www.birdlife.org/worldwide/programme-additional-info/migratory-birds-and-flyways, maintained by Birdlife International.

An analysis of the territorial distribution of the main migration routes allow us to distinguish several regions playing an important role in the epidemiology of AIVs in Siberia and the Russian Far East. In particular, the territory of the Chany Lake system in West Siberia, and wetlands of the Central Asia and Russian Far East are characterized by the presence of a large number of water bodies, which attract significant numbers of wild water birds from various locations within their migratory routes (Veen et al. 2005). Therefore, avian influenza surveillance activity in wild birds of the above regions most likely can allow timely detection of avian influenza viruses in natural habitats and their spread among wild birds in wetlands. Migratory birds wintering in different regions of the world, such as North-Western and Central Europe, the coast of Black, Mediterranean and Caspian seas, the Middle East, West, Eastern and Southern Africa, South-West and Central Asia, the Indian subcontinent, South, East and Southeast Asia, as well as migrants nesting in the northern part of Russian regions, converge in the South of Western Siberia, characterized by the presence of numerous wetlands that represent important nesting areas for these avian populations. Overall, millions of wild birds find suitable habitats in this area, crossed by three migratory routes, including the main Central Asia route (Figure 2) (Veen et al., 2005; Yurlov, 1977).

The South of Western Siberia is crossed by river valleys (Tobol, Ishim, Irtysh, Ob rivers), and is occupied by lakes, salt-marshes, wet meadows and other wetland types. Birds' migration routes overlapping in South Western Siberia bring birds from many regions of Eurasia and Africa indirectly into contact. With regard to the migratory routes towards the wintering areas, Western and South-Western directions are the prevalent ones, while a less bird number travel to the South and South-East (Atkinson-Willes, 1976; Savchenko, 2009; Veen et al., 2005; Yurlov, 1977). Being Southwestern Siberia a crossroads of flyways (Veen et al., 2005) birds involved in such a wide migration dispersion belong to at least three different zoogeographic populations (Atkinson-Willes, 1976; Vartapetov et al., 2003). However, less information is available for flyway connecting Siberia and Central China, considered crucial for the spread of the HPAI H5N1 virus. Very little information is limited to sporadic data obtained from both bird ringing recoveries in different regions of South East Asia and Western Siberia, and satellite tracking of avian species in North West regions of the PRC, Tuva and Mongolia (Prosser et al., 2009).

Data on bird migrations are obtained by the method of banding (with the aim of resampling) wild birds nesting in the territory of the South of Western

Siberia, where mass migrations occur from late March to the first half of June and from the second half of July to mid-October. In certain habitats mass accumulations of birds are periodically observed from spring to fall. At the time of breeding birds can congregate in mono- or multi-species colonies with sometimes several thousand individuals. In post-breeding periods and during migration local migration clusters of birds can include up to 50 avian species and a total of more than 20 thousand individuals at a time. Obviously, these mass aggregations of birds can also enhance interactions among birds of different species and populations, thus creating favorable conditions for the transmission and spread of various infectious diseases (Vartapetov et al., 2003). Results obtained from long-term banding research carried out on waterbirds in South-West Siberia are reported by Veen et al. (2005), which have shown that different species of wild birds use different migration routes. Species such as Gray Heron (*Ardea cinerea*), Grayleg Goose, Bean Goose, Greater White-Fronted Goose (*Anser albifrons*) and wood sandpipers (*Tringa glareola*) migrate to wintering areas in Northwest Europe. Various species of wild ducks, such as Mallard, Gadwall, Northern Pintail, Northern Shoveler, Eurasian Wigeon, Common Teal, Common Pochard, Tufted Duck, use three to five wintering grounds, the most important of which are the Caspian Sea, southwest Asia, Eastern and southern Africa and South Asia. Among wild duck species, Tufted Duck migrates to less remote areas. Most species of shorebirds, except the Northern Lapwing (*Vanellus vanellus*), migrate to South Asia; however, some species migrate towards the Caspian Sea, southwest Asia, Eastern and southern Africa. All kinds of gulls and the Coot (*Fulica atra*) winter at the North of the Caspian Sea, in South and South-West Asia, Eastern and southern Africa. The Black Tern (*Clidonias niger*) migrates in a westerly direction to the shores of the Caspian, Black and Mediterranean seas and the Atlantic coast of West Africa, in the territory which intersects the East-Atlantic migratory route (Veen et al., 2005). In particular, data obtained from banding of wild water birds show that numerous migration routes intersect and overlap in the area of the South of Western Siberia, which represents a crossroads of flyways (Veen et al., 2005). In addition, many species of these birds can use different flyways and/or wintering grounds. All this creates favorable conditions for the annual "mixing" of different populations of wild birds nesting and wintering in remote geographic regions, thus contributing to the spread of various pathogens on the territory of many countries. It is therefore evident the importance of carrying out epidemiological surveillance

in Western Siberia, namely in the territory of the Chany lake system that provides seasonal staging areas for a great diversity of water bird species.

Central Asia is a unique place where numerous lakes provide suitable habitats for resting and breeding of a great variety of bird species migrating from South and South-East Asia to Russia (Atkinson-Willes, 1976; Savchenko, 2009). Some wild bird species migrate along the Central Asian Flyway and breed in lake ecosystems of China, Mongolia and Russia (Kitson, 1978), where H5N1 outbreaks occurred (Bi et al., 2015; Chen et al., 2005; Marchenko et al., 2011, 2012; Sakoda et al., 2010; Sharshov et al., 2010b). Climatic and eco-geographical conditions have led to the richness and/or peculiarity of the fauna. Thus, physical-geographical characteristics of Central Asia create favorable conditions for the presence, during migration and/or breeding seasons, of a large number of species of wild birds. In particular, wetlands of this area provide seasonal staging areas for a broad range of water birds, which play a key role in the epizootic emergence. In June 2006 an outbreak of HPAI H5N1 was reported in wild birds at the Uvs Nuur Lake in Central Asia, Russia, and the affected species were: Great Crested Grebe (*Podiceps cristatus*), Little Grebe (*Tachybaptus ruficollis*), Black-headed Gull (*Larus ridibundus*), Common Pochard, and Spoonbill (*Platalea leucorodia*) (Sharshov et al., 2010a). In June 2009, another HPAI H5N1 outbreak was reported in wild birds in Mongolia (Doitiin Tsagaan Lake and Doroo Lake) and at the Uvs Nuur Lake (Sakoda et al., 2010). The bird species involved in this outbreak were the same as in 2006. In this context, Sharshov et al. (2010b) hypothesized that water bodies like the Qinghai Lake and the Uvs Nuur Lake might play an important role in the circulation of HPAIV. Thus, they enhanced the existing surveillance program in this area, by sampling activities of both live and dead birds during breeding and migration periods. The above hypothesis was confirmed in June 2010, when an outbreak of HPAI H5N1 virus was again reported at Uvs Nuur Lake (Marchenko et al., 2011).

The Central Asia migration route covers a large area of Eurasia from the Arctic to the Indian Ocean (Figure 2). This flyway includes several routes of wild waterfowl, most of which migrate from breeding sites in Northern Russia to wintering grounds in South and South-East Asia. Some species of sandpipers make annually a very long trans-equatorial migration; during summer in the Northern hemisphere, they breed in the high Arctic and then in the fall or winter move to more favorable average or South latitude, down to South America, South Africa and Australia (Veen et al., 2005). More in general (Figure 2), crossing the borders of many countries, the Central Asia

flyway covers the territory of 30 countries in North, Central and South Asia. For example, Veen et al. (2005) have shown that the main populations of Common Teal breeding in southwest Siberia migrate within the Central Asian Flyway. This is confirmed by the data of ringing recoveries from India, Pakistan, Afghanistan, Kazakhstan and other countries of Central and South Asia. The annual migration of wild birds from South-East Asia to Russia shows the importance of the AIV Surveillance in Central Asia, since a significant part of the avifauna occurring in the area is represented by species which are the main natural hosts of AIVs.

The Far East, which borders with the Western Siberia region, also represents one of the most important areas in ecology of birds and their pathogens (Kuo et al., 2013; Manin et al., 2010; Okazaki et al., 2000; Ozawa et al., 2015). The territory of Russian Far East is crossed by the three Central Asia, East Asia/Australia, and Pacific Americas waterbird flyways, combining bird populations of Asia, Oceania, and North America (Alaska Peninsula) (Figure 2). Thus, geographic and ecological features of Russian Far East Region create favorable conditions for AIVs distribution, evolution and persistence. A large part of these territories covers the East Asia/Australasia migration route (Figure 2), which extends to the South of the Russian Far East and Alaska, through East and South-East Asia, to Australia and New Zealand, encompassing the territories of 22 countries. Wetlands included in this migration route provide suitable habitats for more than 50 million migratory waterbirds of more than 250 different populations, including 28 species threatened with global extinction (Flint et al., 1983). Russian Far East and Bering Strait area (Beringia) are known to be an avenue of AIV gene pool spreading between the Old and the New Worlds. Possibility of gene segment reassortment here is very high, especially in consideration of the bird population ecology (Manzoor et al., 2008; Wahlgren et al., 2008). Australian surveillance for avian influenza viruses in wild birds showed, in that region, different shorebird and gull AIVs having a high chance of transhemispheric transmission (Hurt et al., 2006).

The Russian Far East is unique in terms of area occupied by small and big lakes, multiple rivers and wetlands, and it is an ideal habitat and nesting ground for water birds (Sivay et al., 2012). Studies on seasonal bird migrations in southern Primorye, West Chukotka, South Kuril Islands, Western Kamchatka, and Eastern Mongolia have shown the presence of significant difference in species composition of migrants in different geographical areas. By identifying the main wintering areas of birds of various territories in the

East of the Asian part of Russia it was seen that the most important species winter in South-East Asia. Therefore, the territory of the Russian Far East is one of the places where there are mass concentrations of wild waterfowl during migration and nesting. For this reason, the natural geographic conditions of the Far East are favorable for the transmission and dissemination of influenza viruses in populations of various species of wild birds.

Thus, our analysis of results suggest the relevance and importance of avian influenza surveillance in wild birds of the South of Western Siberia, Central Asian and Far East of Russia, areas having suitable climate and geographical characteristics for a rich avifauna. As epizootological monitoring of avian influenza in natural conditions is based on the survey of aquatic and semi-aquatic biocenotic complexes, studies should be primarily focused on wetland habitats, occupied by areas of bird concentration and/or nesting and/or seasonal migrations along intercontinental flyways. It is important to stress that the territory of Russia is crossed by five major bird migration flyways, and of these the Black Sea/Mediterranean, the East Asia/East Africa, the Central Asia, the East Asia/Australasia Flyways are of particular importance for Siberia and Far East of Russia. Numerous studies have shown the importance of various groups of wild birds in the AIV circulation and the wide diversity of viruses circulating among wild birds in Siberia. This information is presented in the next section of this chapter.

AVIAN INFLUENZA VIRUS SURVEILLANCE AND SPILLOVER EVENTS FROM RESERVOIR HOSTS IN SIBERIA

Avian Influenza Surveillance Program in Siberia

Western Siberia wetlands are an important sanctuary for wild aquatic birds representing major reservoirs of influenza A virus gene pool, from which novel influenza viruses can emerge to infect other avian and mammalian species, human beings included (Webster et al., 1992). Human and animal influenza surveillance studies in Russia started as far back as the 1970s, but increased dramatically after the first highly pathogenic H5N1 outbreak in the Novosibirsk Region of Western Siberia (Lvov et al., 2004).

Table 1. Different combinations of Hemagglutinin (HA) and Neuraminidase (NA) subtypes detected in avian influenza viruses isolated in Siberia (2002-2015)

		NA subtype									
		N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
HA subtype	H1	■	■								
	H2	■	■								
	H3	■	■				■	■	■		
	H4	■					■		■		
	H5	■	■	■					■		
	H6	■	■				■				
	H7	■	■								
	H8				■				■		
	H9										
	H10						■	■			
	H11						■		■	■	
	H12										
	H13		■				■		■		
	H14										
	H15				■						
	H16			■							
	H17										

Since 2002, large-scale virological surveillance in wild birds was sistematically carried out in West Siberia (mainly represented by the Chany lake system), Far East and Central Asia (Russia, Mongolia and China) (De Marco et al., 2014a; Marchenko et al., 2010, 2011, 2012, 2015; Razumova et al., 2005, 2006; Sayfutdinova et al., 2012; Sharshov et al., 2014; Sivay et al., 2011, 2012, 2013, 2014). These long-term studies, mainly focused on waterfowl species, have revealed considerable diversity of AIV subtypes in wild birds' populations, and the opportunity to collect a large number of AIV isolates from wild and domestic birds, to better understand the mechanisms and molecular features related to virus spreading and interspecies transmission. More than 25,000 samples from wild birds, poultry and mammals have been analyzed between 2002 and 2015 (De Marco et al., 2014a; Marchenko et al., 2010, 2011, 2012, 2015; Razumova et al., 2005, 2006; Sayfutdinova et al., 2012; Sharshov et al., 2014; Sivay et al., 2011, 2012, 2013, 2014). All animals were handled in compliance with animal

welfare and in accordance with the ''Guidelines to the Use of Wild Birds in Research'' (Fair et al., 2010), issued by the Institutional Animal Care and Use Committee (IACUC). All influenza A virus surveillance activities were approved by the Committees on Biomedical Ethics, Research Institute of Experimental and Clinical Medicine, Russian Academy of Sciences, Novosibirsk. Specimens were tested for AIVs by standard methods, and the virus isolates were characterized by using virological assays and molecular-biological methods, according to WHO and OIE manuals (OIE, 2015; WHO, 2002, 2011). In this context, the Influenza Research Team working at the Novosibirsk University and Research Institute of Experimental and Clinical Medicine isolated more than 400 AIVs of 34 subtypes (Table 1).

Avian Influenza Virus Prevalence in Siberian Birds

The comparative analysis of avian influenza surveillance data obtained from wild birds in Western Siberia shows that the total isolation rate ranged from 3.4% to 12.5%. Most of theses viruses were found in *Anseriformes* species whereas a lower number of isolations, mainly from gull species, was obtained from *Charadriiformes* (De Marco et al., 2014a; Marchenko et al., 2010, 2012, 2015; Razumova et al., 2006; Sayfutdinova et al., 2012; Sharshov et al., 2014; Sivay et al., 2012, 2013, 2014). These data agree with the variability of the isolation rate, which is known to be related to the sampling season and taxonomy/ecology of the group of birds investigated. For instance, De Marco et al. (2014a) reported prevalences of LPAI virus isolation ranging from 10.2% (17/166), when calculated on the overall sample of wild birds, to 14.4% (17/118), when calculated on wild *Anseriformes* species, only. The study, carried out in 2012, evaluated the dynamics of circulation and/or introduction of AIV strains in populations of wild bird species ecologically linked to aquatic biotopes as well as in captive-reared waterfowl species representing a potential domestic reservoir of AIVs (De Marco et al., 2014a). In particular, a new and more sensitive approach to AIV surveillance, obtained by integrating the conventional cloacal swab collection with the harvesting of samples from birds' plumage, was used in natural and anthropic ecosystems representing a possible wildlife/domestic interface (Delogu et al., 2010; Lebarbenchon et al., 2013). This innovative approach to AIV surveillance, based on the detection of viral particles concentrated onto birds' bodies after their fecal shedding, did not show any evidence of environmental circulation of H5N1 HPAIV in the study area. Whereas comparable proportions of wild

Anseriformes tested virus isolation positive from cloaca and feathers (5.9% *vs* 8.5%, respectively) were detected, the overall prevalence of virus isolation was 2.4 times higher than that obtained only from cloacal swab examination (14.4% *vs* 5.9%, respectively). These data emphasize the usefulness of the combined cloacal/feather sampling approach, which is able to both improve the virus detection sensitivity of conventional AIV surveillance systems and discriminate birds with active virus replication and shedding from ''false-negative'' birds that carry infectious virus passively on their plumage (Delogu et al., 2010). During the period between the virus adhesion to the bird's body and the infection (possibly due to self-and/or allo-preening), uninfected birds could move in nature and spread AIVs, which may include HP AIVs, by a mechanism that certainly does not affect the fitness of the host (Delogu et al., 2010). De Marco et al. (2014a) have used for the first time this new surveillance approach in the Western Siberia, which is considered a geographic area at risk for the emergence of AIVs, including the HPAIVs.

Additional systematic monitoring programs of AIVs were also carried out in the neighboring regions of Western Mongolia, Kazakhstan, and South of Central Siberia (Krasnoyarsk Territory, the Khakass Republic) (Marchenko et al., 2010). Biological material for these activities was usually collected in autumn in the following territories (and sites) representative of the Central Asian flyway: Mongolia (Uvs Nuur, Achit Nuur, and Har Us Nuur lakes), Kazakhstan (Alakol Lake) and Russia (Uvs Nuur Lake, several small lakes of the Khakass Republic, and south of Krasnoyarsk Territory). As a result, 2,604 samples were collected from birds and analyzed. Altogether 102 species of wild birds from 14 orders were examined for evidence of influenza virus, and most of them belonged to the *Anseriformes*, *Charadriiformes*, and *Passeriformes* orders, with comparable percentages of bird specimen number from these orders (23.2%, 26.3%, and 25.9%, respectively). Eleven and half percent of birds belonged to storks (*Ciconiiformes*). Other 10 orders accounted for 13% percent of birds. Seventeen influenza virus strains were isolated from analyzed material. Fourteen LPAI strains (84%) were isolated from birds belonging to the *Anatidae*, *Laridae*, *Podicipedidae*, and *Phalacrocoracidae* families. These results showed that different groups of water birds are involved in AIV circulation in Central Asia. *Anseriformes* species such as Tufted Duck, Velvet Scoter (*Melanitta nigra*), Common Merganser (*Mergus merganser*), Mallard, Ruddy Shelduck (*Tadorna ferruginea*), Common Pochard, Common Teal, and Northern Shoveler accounted for 53% of all isolated viruses. Another 29.4% of isolates was obtained from *Charadriiformes* (Herring Gull, *Larus argentatus*), *Podecipediformes* (Great

Crested Grebe), and *Pelecaniformes* (Great Cormorant, *Phalacrocorax carbo*). The most of these species migrate through considerable distance in Eurasia. For instance, common pochards sampled migrate to coastal regions of the Mediterranean and Black seas, Eastern Africa, the Caspian seashore, Iran and India, whereas Eastern populations migrate to the east and southeast, reaching Southeastern Asia and Japan (Veen et al., 2005). The nesting area of Common Teal populations is very extensive and covers almost all of Western Europe and most of Middle Asia and Iran, northwestern Mongolia, Manchuria, the northern half of Japan, and the western part of North America up to the Great Lakes. Teals overwinter in Western Europe, in Mediterranean areas, and in southern Asia. The study performed by Marchenko et al. (2010) has revealed that AIVs circulate actively among different species of wild birds in Central Asia, where the large number of lakes and diversity of avifauna play an important role in AIV perpetuation and spread throughout the Asian continent. Avian influenza viruses were also isolated from the Marsh Harrier (*Circus aeruginosus*) and from species that are not intimately linked to aquatic habitats, such as the Common Magpie and the Isabelline Wheatear (*Oenanthe isabellina*). The involvement of the latter two species in influenza ecology is poorly described and occurs at random. Some cases of influenza virus isolation from raptor and perching birds have been described in the literature and both LPAI as well as HPAI H5N1 viruses have been isolated from birds of these orders. However, there are no data on the spread and further transmission of influenza virus by these bird species (Kwon et al., 2005; Marjuki et al., 2009), that are probably an evolutionary dead end in influenza virus spread.

Russian Far Eastern territory is an ideal habitat and nesting ground for birds moving along the East Asian/Australasia flyway. Surveillance activities have been carried out by analyzing of 6,091 samples collected from 2007 to 2012 in the Russian Far East from 171 bird species belonging to 36 different families (Sayfutdinova et al., 2012). Forty influenza virus strains of different subtypes were isolated and characterized, such as H3, H4, H6, H10, and H11 HA subtypes and N1, N2, N6, and N8 NA subtypes in different combinations. The overall LPAI isolation rate was 0.65% and phylogenetic analysis revealed that Russian AIVs belong to Eurasian avian-like virus and gull-like virus clades and are related to viruses isolated in Southeast Asia. Previous serological analyses of birds sampled revealed a common serum reactivity to the same HA subtype, thus suggesting a stability of the viruses circulating in birds of this region (Lvov, et al., 2004). Although the LPAIV isolation rate detected by Lvov, et al. (2004) were significantly higher than that obtained by Sayfutdinova et al. (2012) (i.e., 3.5-5.7% vs 0.65%) this substantial difference

can be partly explained by the sampling of different bird species. The virus isolation rate from *Anseriformes* was 1.86% (Sayfutdinova et al., 2012) whereas studies performed in Western Siberia (De Marco et al., 2014a) showed a LPAIV isolation rate in wild ducks equal to 14.4%, with the most abundant H4N6 virus subtype. Other studies aimed to monitor AIVs, including HPAI H5N1 viruses, in wild birds of the Russian Far East (2003-2011 period) have shown a wide diversity of AIV isolation rates and subtypes (De Marco et al., 2014a; Marchenko et al., 2010, 2011, 2012, 2015; Razumova et al., 2005, 2006; Sayfutdinova et al., 2012; Sharshov et al., 2014; Sivay et al., 2011, 2012, 2013, 2014), that correlate with different areas of the territory and underline the importance to control various regions of the country during AIV surveillance activities.

The case of a highly pathogenic variant of H5N1 virus isolation in poultry, was reported in 2008 in Russian Far East (Lvov et al., 2008). The geographic nearness of Russian Far East territory to Japan islands contributes to the possibility of spillover event of Asian HP viruses to the Russian regions. Since 2004, several outbreaks of HPAI H5 viruses were reported in Japan in 2004, 2007-2011 (H5N1 subtype) and in 2014-2015 (H5N8 subtype). The outbreaks occurred in both wild birds and poultry populations (Abdo et al., 2014; Gamoh et al., 2016; Sakoda et al., 2012; Uchida et al., 2012; Yamamoto et al., 2011). Phylogenetic analysis showed that these Japanese AIVs were related to Asian HPAI H5 viruses, detected in China, Korea, and Taiwan. In addition, the Far East region is very interesting because of the high probability of emergence of reassortant Eurasian and American viruses as a result of transcontinental gene mixing (Wille et al., 2011b) and broad cross-population contacts between wild birds living in Eurasia (Bianki and Dobrynina, 1997). The current M gene phylogenetic tree can be divided into two clades: the Eurasian classical avian-like AIVs (including viruses isolated in other Asian countries) and that grouping the gull-like AIVs (involving H13 and H16 virus subtypes from gulls and one of the Russian viruses detected in wild duck). The vast majority of the Russian AIV strains belong to the Eurasian classical avian-like clade. It also should be noted that the existence of several influenza virus lineages, which circulate in wild birds and in which the strains do not group according to place or time, were also shown for the North American continent (Spackman et al., 2005). The urgency of AIV monitoring study in migrating waterbirds along the East Asian/Australian flyway has increased in view of new LPAIV H7N9 subtype which has been identified in Eastern China. AIV H7N9 mounted the species barrier and caused fatal cases among humans (Chen et al., 2013).

Long-term surveillance activities showed that isolation rate from *Anseriformes* was significantly higher than that obtained from *Charadriiformes* and cranes. Duck species found to be most commonly infected by AIV in Siberia were the Common Teal, Northern Shoveler, Common Pochard, Mallard. With regard to *Charadriiformes*, the Black-headed Gull, Mew Gull (*Larus canus*), Herring Gull, Slaty-backed Gull (*Larus schistisagus*) species were found to be positive for H5N1, H10N6, H13N2, H13N6, H13N8, H16N3 subtypes. These data emphasize the importance of the gull group of Asia as important reservoir for different AIVs.

It is known that passerine birds such as sparrows (e.g., *Passer domesticus, P. montanus*) or magpies could represent a potential ecological bridge between the captive-reared birds and wild bird reservoirs of AIVs, possibly attracted by the presence of ponds and canals in areas surrounding poultry farms. However, virologic surveillance of synanthropic birds sampled in the south part of West Siberia during the HPAI epizootic period in autumn 2005 provided one LP H4 subtype, isolated from a common magpie, whereas no AIVs were found during the postepizootic period (winter 2005-2006) (Sharshov et al., 2007).

In general, the overall isolation rate was different between seasons. Interestingly, no isolates were obtained from spring sampling in Siberian wetlands, whereas most viruses were detected in the autumn (September) and predominantly from juvenile individuals (De Marco et al., 2014a; Marchenko et al., 2010, 2012, 2015; Razumova et al., 2005, 2006; Sayfutdinova et al., 2012; Sivay et al., 2012, 2013, 2014).

Sivay et al. (2011) conducted a unique study in the extreme Northern territories of Siberia (Taimyr Peninsula), breeding areas of wild geese in the tundra during spring migration. A total of 3,245 samples obtained from wild goose species in Siberia (i.e., Bean Goose; Grater White-fronted Goose; Graylag Goose; Lesser White-fronted Goose; Brant Goose; Red-breasted Goose; Snow Gooose, *Anser caerulescens*; Bar-headed Goose, *Anser indicus*) were analyzed and tested virus negative, thus suggesting the lack of AIV circulation in wild birds (geese) at the Russian Arctic North in spring time. Unlike dabbling ducks that feed on water surfaces, geese and certain swan species graze in pastures and agricultural fields. This difference in feeding could potentially preserve these animals from efficient virus transmission and may explain the low prevalence or absence of influenza A viruses in geese and swan species, as observed in northern Europe (Munster et al., 2007). The low overall prevalence of AIVs and the limited number of HA subtypes previously detected in geese (Munster and Fouchier, 2009) are in agreement with this hypothesis. While wild geese seem to play a marginal role in the natural

ecology of influenza A viruses, it is very important to keep in mind the severe HP H5N1 outbreak that in late April-early May 2005 affected thousands of bar-headed geese at the Qingai Lake (China) (Sivay et al., 2011, 2012).

Diversity of Avian Influenza Viruses in Siberia

As previously shown in this chapter, numerous subtypes of AIVs circulate in Siberia (table 1). Most frequently detected isolates are low pathogenic H3N8 and H4N6 AIVs (De Marco et al., 2014a; Sayfutdinova et al., 2012; Sharshov et al., 2014; Sivay et al., 2011, 2012, 2013, 2014) but, although with variable prevalence, 34 antigenic subtypes (including H1N1, H1N2, H3N1, H3N2, H3N3, H3N6, H3N8, H4N6, H5N1, H5N3, H6N2, H8N4, H8N8, H13N2, H15N4, H16N3 and some other viruses) have been isolated. With regards to the H3N8 and H3N6 AIVs, widely spread among wild bird populations in Eurasia and America, it should be noted that no highly pathogenic variants of H3 influenza viruses have ever been found, though several H3N8 virus variants adapted to mammals were described in literature. Some of these variants cause epizootics among horses with further transmission to dogs (Kirkland et al., 2010).

It is known that rare and unique avian influenza viruses (e.g., H8N8, H15N4, H16N3, H13N2, LPAI H5N1) as well as different reassortant strains circulate in Siberia (De Marco et al., 2014a; Sayfutdinova et al., 2012; Sharshov et al., 2014; Sivay et al., 2011, 2012, 2013, 2014).

Avian influenza H8 subtypes have been isolated in both hemispheres, although most of these viruses were detected in North America. There is information about 147 virus strains of the H8 subtype in the Influenza Virus Resource (Bao et al., 2008). The most common subtype combination is H8N4, which was also found in West Siberia, even though the H8 subtype was also detected in combination with N2, N3, N5, and N7 NA subtypes. The information on virus isolates of H8N8 subtype is very limited (Abenes, 1983; Daum et al., 2007). However, most of them were isolated from wild ducks and turkeys. Sivay et al. (2014) firstly isolated an AIV of H8N8 subtype from a common teal in Siberia (Chany Lake) in fall 2009. The genome analysis shows that all genes belong to AIV Eurasian lineages. The PB2 gene was similar to a Mongolian LP AIV H7N1 and a Chinese HP AIV H5N2 (Kang et al., 2011; Sivay et al., 2014).

Before the detection in Siberia, the rarely identified influenza A viruses of the H15 HA subtype have been isolated exclusively in Australia (Krauss

et al., 2007). Sivay et al. (2013) reported the isolation of an H15N4 influenza A virus (A/teal/Chany/7119/2008) in Western Siberia, showing a previously unrecognized level of genetic diversity between H15 strains and the possible formation of a separate sublineage of H15 subtype AIVs in Russia. Phylogenetic analysis shows that the internal genes of the A/teal/Chany/7119/2008 strain belong to the Eurasian clade and that the H15 and N4 genes were introduced, by migration of bird species between continents, into the gene pool of circulating endemic AIVs through reassortment events (Sivay et al., 2013). Interestingly, a new H15 AIV was detected in Ukraine in 2010, carrying the unique H15N7 subtype combination. This virus replicated efficiently in chicken eggs, and antisera against it reacted strongly with the homologous antigen, but at lower titers against the reference Australian antigen. The amino acid motifs of the HA cleavage site and receptor-binding site were different from those in the Australian viruses. The new virus, together with an H15 virus from Siberia in 2008, constitutes a new clade of H15 AIV isolates (Muzyka et al., 2016).

A double reassortant H13N8 influenza A virus was isolated from a gull in the border of Siberia and Mongolia. Complete genome sequence analysis indicated the complicated evolutionary history. The PA gene belongs to classical Avian-like lineage and more likely originated from non-gull avian virus pool (Sharshov et al., 2014). Data confirm the state of extensive geographic mosaicism in AIVs from gulls in the Northern Hemisphere.

AIVs isolated from gulls in West Siberia also included H16N3 and H13N2 subtypes whereas H10N6 and H13N6 viruses were mostly found in the Russian Far East (Sayfutdinova et al., 2012). Gull populations and species differ in the different Flyways. AIVs have been sporadically isolated from gulls in South America. Sequencing and phylogenetic characterization of an H13N9 virus from Kelp Gull (*Larus dominicanus*) suggest the presence of influenza genes unique to the Southern Hemisphere. Phylogenetic analysis of the 8 viral genes of H13N9 virus in Argentina showed that the 6 internal genes are related to the isolates from Chile and Bolivia. The analysis also indicates that a cluster of phylogenetically related AIVs from South America may have evolved independently, with minimal gene exchange from influenza viruses in other latitudes (Pereda et al., 2008). Sharshov et al. (2014) showed that the Mongolian H13 virus is antigenically distinct from H13 viruses of East Asian/Australasian Flyway. Most likely surface glycoproteins of AIVs in gulls of different flyways evolved independently but still have a chance of gene exchange. These data highlight the relevance of this study on rare influenza viruses in Central Asia, in terms of AIV evolutionary ecology in gulls, and

confirm the unique nature of the Siberian territory, which acts as a "virus incubator," enhancing virus circulation and reassortment of numerous rare viruses.

Thus, natural spillover events from wild birds to alternative hosts can be expected in Western Siberia. Several H2N2 viruses were isolated from wild birds sampled in the Chany Lake system and, at the same time and site, an influenza A virus of H2N2 subtype was detected from a wild mammal species, the Muskrat (*Ondatra zibethicus*) (De Marco et al., 2014a). Full genome sequences indicate that this virus belongs to Eurasian avian-like influenza viruses. Moreover, amino acid analysis and experimental results suggest that A/muskrat/Russia/63/2014 (H2N2) virus can replicate in mammalian cells and mice without prior host adaptation. It is assumed that this virus could have directly crossed the interspecies barrier from birds to muskrat at Chany Lake. Influenza viruses of H2 subtype are not prevailing in wild bird populations, in which they can be detected in combination with all nine NA subtypes. It is however important to emphasize that the most common H2N3 subtype has been also isolated from swine and environment (Ma et al., 2007). In 2000, H3N8 virus isolations were reported in wild birds and muskrat in western part of Eastern-Asian migration flyway (Lvov et al., 2004). For all these reasons, it is very important to investigate influenza viruses circulating in less investigated hosts, such as seals, in very close contact with aquatic birds. In 2012, research carried out at the Novosibirsk State University revealed the infection of marine mammals in the Caspian Sea region (located to the west of Central Asia) (Dr. A. Shestopalov, unpublshed data). This virus strain, isolated from the Caspian seal (*Phoca caspica*), belongs to LPAIVs of H4N6 subtype, previously found in wild ducks.

Another health risk is represented by the circulation of different avian paramyxoviruses (APMV types 1-9), which are also detected in migratory waterfowl during other monitoring programs such as those for AIVs. Similarly to several AIVs, the APMV-1 (Newcastle Disease Virus, NDV), frequently detected in migratory birds, can pose severe risks when introduced into poultry farms. As recently reported several APMVs were isolated from wild birds in Siberia. In particular, the APMVs-1 were detected in Rock Dove and Mallard (Yurchenko et al., 2015a, 2015b) whereas Sobolev et al. (2016) firstly isolated APMV-4 and APMV-6 from the Common Teal, which is one of the most numerous species sampled in wild bird AIV *hotspots* within the Asia (Chany Lake System). Because ducks are highly migratory species, crossing the Eurasia in different directions twice a year during their long migrations (Stanislawek et al., 2002; Wille et al., 2015), they represent a possible

mechanism for the global movement of these viruses. Being the Black Sea/Mediterranean Flyway the most important for teals, it is possible to assume that the most probable virus circulation in duck populations is associated with this migratory route. However, APMV-4 and APMV-6 were detected at the largest crossing points of migration routes in Eurasia, in breeding and molting sites where birds from different populations congregate. Phylogenetic analysis of these APMVs confirmed the possible virus transmission.

As suggested by the frequent isolation of several unidentified hemagglutinating agents (De Marco et al., 2014a) different APMVs and novel AIVs probably circulate in wild bird populations in Siberia. Similarly to what shown in North America and Europe (Goekjian et al., 2011; Lindh et al., 2008) the probability of co-infection with AIVs and APMVs can complicate studies on specific pathogen's effect on its host, but can also offer a valuable opportunity to explore unique biological properties of the pathogen, such as its virulence, during the co-infection (Tolf et al. 2013; Wille et al., 2015).

Surveillance of Emerging Avian Influenza Viruses in Siberia and Human Health Implications

The role that wild bird migrations may play in the spread of HPAI is still debated, although scientific evidence suggests their involvement in the intercontinental diffusion of different AIVs. The occurrence of HPAI outbreaks in nature and the transmission from poultry to humans have continued since the virus was first isolated in 1996 in Hong Kong (Chen et al., 2005). The history of HPAI H5N1 emergence and re-emergence shows the importance of Central Asian region in its maintenance (Marchenko et al., 2011; Sharshov et al., 2010a, 2010b, 2011). H5N1 viruses of Asian origin have been reported in Western Siberia and Qinghai-Tibetan Plateau (Central China) since 2005 and caused numerous outbreaks in wild birds and poultry. In spring 2005 at Qinghai Lake, an unprecedented outbreak of HPAI H5N1 caused the death of more than 6,000 migratory birds including over 3,000 bar-headed geese and in following years the virus has re-emerged in wild birds along the Central Asia flyway several times (Prosser et al., 2011). Various migrating waterbird species involved in HPAI H5N1 outbreaks use natural ecosystems of Western Siberia. Most of them move along the Central Asian Flyway and breed at the Lakes of China, Mongolia and Russia where H5N1 outbreaks occurred, and several studies have shown the correlation between

wild birds migration routes and H5N1 outbreak occurrence (Gilbert et al., 2006, 2010; Prosser et al., 2011; Sakoda et al., 2010). With high probability, bird migration is one of the possible ways of HPAI H5N1 spreading between China and Siberia. However, further scientific evidences are needed to model the potential connections concerning HPAIV H5N1 epidemiology and bird migrations between the south of Western Siberia and Qinghai Province (China). Experimental infection of waterfowl species commonly involved in H5N1 HPAI outbreaks, such as bar-headed geese and ruddy shelducks, showed that H5N1 HPAI virus can also cause neurologic disease with varying outcomes of severity, with possible implications for the role that wild waterfowl may play in the spreading and transmission of this virus in endemic regions (Nemeth et al., 2013). Migratory birds cannot fly the full distance to their annual migratory destination, and usually interrupt their migration to rest and refuel in wetland ecosystems, contributing to increase the spread of viruses in terms of abundance and territorial distribution (Fang et al., 2008). The HPAI H5N1 virus may cause a high proportion of deaths in wild bird populations (Chen et al., 2005) by infecting not only ducks, geese and gulls, but also grebes, cormorants, herons, rails and even crows, doves and falcons (Gilbert et al., 2006). The H5N1 virus was even detected in dead spoonbills in the Western Siberia in 2009 (Sharshov et al., 2010b). In Western Siberia, several ducks and gulls that were tested H5N1-positive, have been found asymptomatic and apparently in good health (Sharshov et al., 2010a). On the other hand, during H5N1 outbreaks on Qinghai Lake in 2005/2006, bar-headed geese, as well as some brown-headed gulls (*Larus brunnicephalus*), great black-headed gulls (*Larus ichthyaetus*) and great cormorants were found dead, because of the HPAIV infection (Gilbert et al., 2006, 2010; Prosser et al., 2011; Sakoda et al., 2010). As no death was reported among ducks during the Qinghai outbreak, wild ducks have been often indicated as long-distance vector, enabling the spread of the HPAI virus (Guan et al., 2012), especially between South-Eastern Asia and Western Siberia (Gilbert et al., 2006; Sharshov et al., 2010a, 2010b).

Since 1996, the HA genes of A/goose/Guangdong/1/96-like HPAI A/H5 viruses continue to rapidly evolve and 11 H5 clades actively circulating during recent years have been identified, up to 2014, by the WHO/OIE/FAO H5N1 Evolution Working Group (Smith et al., 2015) In the context of avian influenza surveillance activities carried out in Siberia, the following H5N1 clades were detected: Clade 2.2 in 2005-2007; Clade 2.3.2 in 2008-2010 and Clade 2.3.2.1c in 2014-2015 (Marchenko et al., 2011, 2016; Sharshov et al., 2010a, 2010b, 2011).

In summer 2005, the first influenza A (H5N1) outbreak in Russia was reported in the territory of Western Siberia (Lipatov et al., 2007). The virus killed thousands of poultry and subsequently spread westward across Eurasia to Europe and Africa (Lipatov et al., 2007; Sharshov et al., 2010a, 2010b, 2011). The nucleotide sequence of the HA gene showed that strains isolated in Russia in 2005 were related phylogenetically to Clade 2.2 of H5N1 virus, which has been responsible of outbreaks among wild birds at Qinghai Lake in China (Chen et al., 2005). In 2005–2007, severe influenza outbreaks among wild birds were reported throughout the whole territory of Russia (Sharshov et al., 2010a), and full genome sequences of virus isolates showed high homology to Qinghai-like influenza (H5N1) viruses. The largest epizooties among wild birds in the territory of Russian Federation, occurred in 2006, 2009 and 2010 at Uvs-Nuur Lake (Siberia), and the virus of Clade 2.3.2 affected the following bird species there: Great Crested Grebe, Little Grebe, Black-headed Gull, Common Pochard, and Spoonbill (Sharshov et al., 2010b). During May-June 2009 and 2010, several dead migratory birds were found in both the Qinghai Lake region (China) and Western Siberia (Russia) (Marchenko et al., 2011; Sharshov, et al., 2010b, 2011). Nine 2.3.2 HPAI viruses (H5N1) were isolated in 2009 and 2 were isolated in 2010 from great cormorants, brown-headed gulls, great black-headed gulls, great-crested grebes and bar-headed geese. Although there is no evidence that the H5N1 AIV was transmitted from eastern Asian (inner China or across the Himalayas) to the Qinghai Lake region, the East Asia/Australasia flyway possibly accounted for an important route of transmission. From late 2014 to 2015 a novel Clade 2.3.2.1c of H5N1 reassortant virus emerged and caused several outbreaks in wild birds (Bi et al., 2015). In particular, this virus was found in Siberia (2014-2015) in wild ducks and Rook (*Corvus frugilegus*) (Marchenco et al., 2016). As the largest outbreaks of HPAI occur in Southeast Asia (Gilbert et al., 2006; Hulse-Post, et al., 2005), the elucidation of possible migration exchange between these regions and Western Siberia is extremely important in the prevention and control of virus emergence and spreading. These regions cover East Asia/Australian flyway, and the presence of large mountain ranges to the south of Western Siberia suggests a clear separation of birds migrating along the Central Asian flyway to wintering grounds in Central and South Asia from birds migrating to winter in Southeast Asia. However, the presence of ring recoveries from different parts of China, Japan from birds ringed in Western Siberia, as well as the fast spread of HPAIV H5N1 outbreaks shows intensive migration exchange between these regions.

Recent outbreaks of avian-origin H5N8, H6N1, H7N9, H9N2 and H10N8 influenza viruses raise concern for the emergence of novel reassortant viruses in Eurasia and their potential threat to the human population. Despite the increasing risk posed to humans, H7N9, H6N1, and H10N8 viruses are usually classified as LPAIV for birds. At present, there are no reports of H6N1 and H7N9 virus isolations in Siberia. By contrast, influenza A/H5N8 virus has been isolated from a migratory eurasian wigeon in Sakha Republic of the Russian Far East. The strain A/wigeon/Sakha/1/2014 (H5N8) has been shown to be pathogenic for mammals and similar to viruses that caused outbreaks in wild birds and poultry in Southeast Asia and Europe in 2014 (Marchenko et al., 2015).

Isolations of H10N6 and H10N7 viruses have been also reported in Siberia (Marchenko et al., 2012; Sayfutdinova et al., 2012). Usually H10 influenza viruses are known to be not widespread, and previous isolations were reported in North America, Germany, Iran, Egypt, Korea, and Japan. However, human cases of infection with H10N8 viruses were identified in late 2013 and early 2014 in Jiangxi, China, and have raised concerns about the origin, prevalence, and development of these viruses in this region. By using data from 12 years of influenza surveillance in southern China, it was shown that H10 viruses were regularly introduced by migratory ducks to domestic ducks on lakes, which represent major aggregation sites of migratory birds in Asia (Ma et al., 2015). The presence of this subtype in bird populations of Siberia and Far East and possibility for gene exchange with others Asian viruses have also been reported. There are no detections of H9N2 influenza A viruses in Siberia. These viruses have undergone extensive reassortments in different host species, often providing the "backbone" for novel emerging zoonotic viruses. Several lines of evidence indicated that H9N2 viruses constantly evolve and their pathogenicity to mammals should be closely monitored to prevent the emergence of novel pandemic viruses (Bi et al., 2011).

Western Siberia is of great importance in ecology and epidemiology of influenza because of the possibility of reassortment among human and avian influenza viruses (Ilyicheva et al., 2011, 2013; Sharshov et al., 2010a; Sobolev et al., 2012). Territorial relations established by seasonal migrations of Western Siberian birds are extremely wide since this region is an intersection point of migration flows of bird wintering in different regions of the world. Reassortant influenza viruses that can cause outbreak among population might emerge in Western Siberia with high probability (Ilyicheva et al., 2013). Thus, it is extremely important to carry out widespread study on human and avian

circulating viruses, by analyzing their molecular biological properties, phylogenetic links in this region, as well as herd immunity to influenza virus serotypes with epidemic potential. In the context of the annual influenza surveillance activities implemented in Siberia, sera from subjects potentially exposed to H5N1 infected birds (possible asymptomatic virus carriers) and/or contaminated habitats (high virus tenacity at low temperatures), were tested to detect antibodies to the A/H5N1 influenza virus. Taking into account that influenza A/H5N1 viruses have been isolated from wildfowl and poultry since 2005, fortunately no human cases of H5N1 influenza have been reported so far in Russia. However there was evidence of anti-H5N1 antibodies in humans (Onishchenko et al., 2010). Twenty-six serum samples obtained from residents of Khanty-Mansiysk Autonomous Area and 2 samples from residents of Novosibirsk region were positive for antibodies to serotype A/H5. There were no clinical cases of avian influenza A/H5N1 infection in medical history of studied persons (Onishchenko et al., 2010). This emphasizes the importance to focus on detection and control of AIV transmission in animals and humans, with the goal of preventing future epidemics and pandemics.

Ecological Drivers of Avian Influenza Virus Infections in Birds: the Siberia Region in the Face of the Current Global Change

After the first report of avian influenza outbreak in wild birds, caused by a HPAI H5N3 virus responsible for the death of about 1,300 common terns (*Sterna hirundo*) in 1961 in South Africa (Becker, 1966), a growing number of LP AIVs have been isolated from a wide variety of wild birds (mostly waterbirds). As previously stated in this chapter, the perpetuation of AIVs in the aquatic bird reservoir accounts for a balanced virus/host relationship in nature (Stallknecht and Shane, 1988; Webster et al., 1992), whereas HPAIVs of H5 and H7 subtypes periodically emerge in domestic birds, in which LPAIVs of possible wild bird origin can shift to highly virulent strains (Lebarbenchon et al., 2010). After the above-mentioned mass mortality of common terns, the occurrence of outbreaks of disease caused by HPAIVs in wild birds was considered a sporadic event related to outbreaks of HPAI viruses in poultry (Capua and Marangon, 2000).

However, the Asian-origin H5N1 A/goose/Guangdong/1/1996 (Gs/GD) lineage of HPAIV, which have pandemic potential for humans, emerged in south-East Asia in 1997 and caused the so called "bird flu" incident responsible for poultry outbreaks, initially controlled by effective mass

slaughter of birds across the Hong Kong SAR. Important changes in influenza A virus natural ecology have been observed in the context of the first H5N1 "bird flu" re-emergence in poultry of South-East Asia in late 2002, when HP H5N1 AIVs were also isolated from waterfowl species (geese, ducks and swans), captive greater flamingoes (*Phoenicopterus ruber*) and other wild birds (Little Egret, *Egretta garzetta*) at two waterfowl parks, and from two dead wild grey herons and a black-headed gull in Hong Kong. These were the first reported cases of lethal influenza virus infection in wild aquatic birds since 1961 (Sturm-Ramirez et al., 2004). HP H5N1 viruses were also detected in both a dead feral pigeon (*Columba livia*) and a dead tree sparrow (*Passer montanus*) during the second outbreak (Ellis et al., 2004).

Between late 2003 and early 2004, the HP H5N1 virus started its progressive spread among domestic and wild birds throughout areas of Eastern and South-East Asia, later involving, as H5 virus clade 2.2, the Asian portion of Russia, Europe, the Middle East, and Africa (Medina and García-Sastre, 2011; Olsen et al., 2006). Morbility and mortality associated with the HPAI H5N1 infection in wild birds have provided a new scenario for evaluating the potential involvement of migratory avifauna in spreading and maintaining HPAIVs in natural habitats (Bourouiba et al., 2010).

Since 2003-2004, the H5N1 Gs/GD strain continued to evolve both genetically and antigenically (Sonnberg et al., 2013), causing repeated waves of infections characterized by a panzootic spread. Moreover, the virus ability to replicate in apparently healthy free-range ducks (Kim et al., 2009) and extensive trade of live poultry (Beato and Capua, 2011) further hindered infection control strategies. As of July 2015, endemic HPAIV H5 infections were being reported in 6 regions of the world represented by China, Vietnam, Indonesia, parts of India, Bangladesh and Egypt (ProMED-mail, 2015). Reassortant H5 viruses of the Gs/GD lineage, having N2, N3, N6 or N8 genes, recently emerged in South-East Asia (WHO, 2015). Among these, the H5N8 HPAIV (lineage H5 clade 2.3.4.4), emerged in late 2013 in China. In 2014, these strains showed a strong ability to spread to South Korea (Dalby and Iqbal, 2015; Jeong et al., 2014), then, through an unusual intercontinental diffusion, it reached North America (Ip et al., 2015) where infected both wild and domestic birds along with novel reassortant viruses (H5N1 and H5N2), which have genes from both Eurasian and North American origin. Phylogenetic analysis of isolates and the overall pattern of waterfowl flyways indicated the occurrence of intercontinental spread of three genetically distinct subgroups of this Asian-origin H5N8 virus by infected migratory birds, often found to be apparently healthy (Lee et al., 2015; Pasic et al., 2015; Torchetti et

al., 2015). In January 2015, a novel H5N1 HPAIV reassortant killed wild migratory birds in China (Bi et al., 2015). These emerging AIVs, known as Sanmenxia Clade 2.3.2.1c-like H5N1 viruses, possess the closest genetic identity to A/Alberta/01/2014 (H5N1) virus, found to be highly pathogenic in both birds and mammals (Maurer-Stroh et al., 2014).

Given this unusual involvement of wild birds in HPAIV epidemiology, a key unanswered question arises: is avian influenza ecology changing in a changing world?

The significant and ongoing transformation of the terrestrial biosphere into anthromes, strictly related to the current global change, involves anthropogenic changes such as human population growth, land use, climate change, globalization of trade, and agricultural intensification (Ellis et al., 2010; Vandegrift et al., 2010). All these interacting factors may alter the global ecology and evolution of influenza A viruses in avian hosts. For example, the increased need for proteins, strictly related with the human population growth, has lead to the development *of intensive poultry farming systems, aimed to* obtain *animal meat production at a relatively low cost. In a context where an average commercial broiler farm can generate about 500,000 birds per year (*Vandegrift et al., 2010), *high numbers and densities of birds as well as their homogeneous genetics and immune status might affect the hosts' susceptibility to AIVs and facilitate, especially in* gallinaceous species, *the evolution of* H5 and H7 virus subtypes into HP strains. Unlike wild aquatic birds, domestic waterfowl have an almost continuous population turnover and no migration limits when moving by commercial trades. In endemic countries, outdoor farming systems of domestic ducks might represent the actual epidemiological reservoir of HP H5 viruses, enabling continuous spillover events into wildfowl populations. Probably, HPAIVs are not perpetuated in the wild aquatic bird reservoir, but scientific evidence shows that HP viruses are more frequently spread by migratory birds.

The second unanswered question arising is: how can ecological drivers modulate, in the vast Siberia Region, the influenza A virus circulation in wild bird reservoirs?

In the context of the current global change, several ecological driving forces based on virus, host and other environmental traits can affect the AIV epidemiology. Virus- and host-related drivers involved in the environmental and inter-individual AIV transmission, host receptivity and susceptibility to the infection, host population immunity and dispersal (Gaidet et al., 2012) have been previously discussed in this chapter. With regard to environmental drivers, several climatic parameters such as temperature, relative humidity,

and precipitation are obviously involved in the virus/host interaction and, in particular, in the AIV epidemiology in wild birds. Klaassen et al. (2012) reviewed possible effects that global change processes (e.g., climate change, land use changes, habitat destruction and fragmentation) could have on migratory behavior of birds. As AIV spread in nature is intrinsically linked to the movements of birds, and mostly their migratory behavior, recent studies have evaluated possible consequences of climate change on avian influenza ecology (Brown and Rohani, 2012; Fuller et al., 2012; Gilbert et al., 2008). As previously stated in this chapter, Siberia is one of the most sparsely populated regions of the Earth. For this region, excluding a significant direct impact of human activities, indirect effects of climate change on bird movements could influence avian influenza ecology in the vast Siberia Region. Several human activities have enhanced the natural green-house effect by adding carbon dioxide and other greenhouse gases to the atmosphere and this is very likely causing the increase in the Earth's average temperature (Ciccarese, 2011). Climate change effects have been reported to: i) cause northward shifts of distribution of many bird species; ii) increase avian species diversity in northern latitudes; iii) anticipate spring bird migration northward (Vandegrift et al., 2012). As Siberian wetlands play a crucial role in the geographical dispersal of AIVs (Lee et al., 2015; Li et al., 2014c) all the above factors might affects influenza A virus circulation and spill-over from wild bird reservoirs to different avian and/or mammalian hosts.

CONCLUSION

Taking into account the influenza A virus characteristics, its wide host range and the presence of animal reservoirs, the influenza virus infection can impact wildlife conservation, veterinary and public health (Yoon et al., 2014).

Siberia is of great importance in influenza A virus ecology. After a short reproductive season, huge numbers of wild aquatic birds move from different subarctic breeding sites and congregate in pre-migration staging areas of Siberian wetlands (Gilbert et al., 2008). Major intra-and inter-continental bird flyways overlap in these breeding and/or molting grounds where water birds from different wintering regions of the world are brought together, thus providing the influenza A virus gene pool for the possible emergence of novel reassortant viruses (Li et al., 2014c; Veen et al., 2005). Moreover, in the context of the global ecology of influenza A viruses, Siberian wetlands play a crucial role in the geographical dispersal of the virus, as shown by the HPAI

H5 infections, which have spread since the early 2000s via migratory birds from Asia towards Europe, Africa, Middle East and, more recently, North America (Lee et al., 2015; Li et al., 2014b; Medina and García-Sastre, 2011).

The Asian portion of Russia, which includes the Western Siberia, Central Asia and Far East regions, plays a relevant role in influenza A virus ecology, representing the possible global epicenter of AIV persistence and evolution (De Marco et al., 2014; Sivay et al., 2012). In particular, wetland ecosystems of the vast Siberia Region provide suitable habitats for wild aquatic bird populations which in turn represent the main natural reservoirs of the influenza A viruses, from which novel reassortant viruses can emerge and spread to other avian and mammalian species, including humans (Marchenko et al., 2012; Sivay et al., 2012; Yoon et al., 2014). Extensive surveillance activities carried out across Siberia have shown high variability in both isolation rates and diversity of viruses circulating in wild bird reservoir populations, in which changes in circulation dynamics of LPAIV antigenic subtypes have been detected (De Marco et al., 2014a; Marchenko et al., 2010, 2011, 2012, 2015; Razumova et al., 2005, 2006; Sayfutdinova et al., 2012; Sharshov et al., 2014; Sivay et al., 2011, 2012, 2013, 2014). The results of active virological surveillance, strictly related to bird migrations and to the intrinsic mutation ability of influenza A viruses, have shown the emergence of novel and/or rare AIV strains, such as the reassortant H15N4, H5N8, H13N8 virus subtypes (Sayfutdinova et al., 2012; Sharshov et al., 2014; Sivay et al., 2013, 2014). All the above circumstances fit in the influenza A virus evolution, spillover events and potential adaptation to novel hosts, such as the Muskrat (De Marco et al., 2014a), and Caspian Seal (Dr. A. Shestopalov, unpublshed data).

Siberian wetlands play a relevant role also in the ecology of the H5N1 HPAIV of Asian origin, that after the first emergence (in summer 2005) in both poultry and wild birds of Western Siberia (Lipatov et al., 2007) spread westward across Eurasia to Europe and Africa (Lipatov et al., 2007; Sharshov et al., 2010a, 2010b, 2011). From 2005 to 2015, several HP influenza outbreaks among wild birds were reported throughout the whole territory of Russia (Bi et al., 2015; Marchenko et al., 2011, 2016; Sharshov et al., 2010a, 2010b, 2011). Whereas no HPAIV H5N1 human cases were reported in Siberia, serological evidences suggest the occurrence of virus spillover from birds to residents of Siberia (Onishchenko et al., 2010).

In the context of AIV surveillance, in-depth studies of isolates, based on bioinformatic approaches to virus molecular evolution and phylogenesis, allow to better understand the Siberian fauna's role in the global ecology of influenza A viruses and provide new insight into the distribution and evolution

of AIVs, due to ecological relationships of migratory bird populations of Russia and different Asian, European, African countries and Australia, particularly with regard to H5 and H7 viruses. This emphasizes the importance to focus on detection and control of AIV transmission in animals and humans, with the goal of preventing future epidemics and pandemics. Moreover, a better understanding of LP and HP AIV infectivity and pathogenicity in wild bird populations is critically important to preserve both public and veterinary health sectors but also to assess how, *from a wildlife conservation point of view*, AIV infection can affect the *conservation status of wildlife species.*

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