

Chapter 1

General Introduction

1.1 Background Analysis

Birds are amongst the few terrestrial vertebrates that share with humans the peculiarity of travelling in a few hours across national and intercontinental borders (Hobson 2002). The record for distance covered in a single year belongs to the arctic tern, which travels over 50,000 km between Antarctica and northern Scandinavia. Overall, billions of birds travel between continents twice a year in only a few weeks. Migration is critical in the life cycle of a bird, and without this annual journey many birds would not be able to raise their young. More than 5000 species of birds manage annual round-trip migrations of thousands of miles, often returning to the exact same nesting and wintering locations from year to year. Birds migrate to find the richest, most abundant food sources that will provide adequate energy to nurture young birds. If no birds migrated, competition for adequate food during breeding seasons would be fierce and many birds would starve. Instead, birds have evolved different migration patterns, times and routes to give themselves and their offspring the greatest chance of survival. Birds gauge the changing of the seasons based on light level from the angle of the sun in the sky and the amount of daily light. When the timing is right for their migrating needs, they will begin their journey. Several minor factors can affect the precise day any bird species begins its migration, including available food supplies, poor weather or storms and air temperatures and wind patterns. While these factors may affect migration by a day or two, most bird species follow precise migration calendars. Those calendars vary widely for different species, however, and while autumn and spring are peak migration periods when many birds are on the move, migration is actually an ongoing process and at any time of the year, there are always birds at some stage of their journeys. The distance the birds must fly, the length of time it takes to mate and produce a healthy brood, the amount of parental nurturing young birds receive and the location of birds' breeding and wintering grounds all affect when any one species migrates to stay alive.

During these yearly migrations, birds have the potential of dispersing microorganisms that can be dangerous for human as well as animal health. For instance, birds are believed to be responsible for the wide geographic distribution of various pathogens, including viruses [e.g., West Nile, Sindbis, Highly Pathogenic Avian Influenza (HPAI), Newcastle disease], bacteria (e.g., *Borrelia*, *Mycobacterium*, *Salmonella*), and protozoa (e.g., *Cryptosporidium*). An insight into the ecology of bird populations is necessary to understand fully the epidemiology of bird-associated emerging diseases. Furthermore, data about avian movements might be used to improve disease surveillance or to adapt preventive measures. However, the links between bird ecology and livestock and human disease have yet to be completely understood so there is a need to increase knowledge of avian migration patterns and infectious diseases to help predict future outbreaks of emerging diseases.

Animal influenza viruses threaten animal health, livestock productivity and food security in poor countries, but they can also evolve into dangerous human pathogens. This has been seen with the emergence of HPAI. Its main impact has been on domesticated poultry, with over 300 million birds killed or destroyed, but of considerable public health concern is the transmission of the virus from birds to humans, resulting in over 500 occurrences of disease in which over half of the infected individuals have died.

The threat from animal influenza viruses makes it essential for animal health professionals to take the lead in detecting and monitoring the occurrence of the viruses and sharing the information with the international community. Since avian influenza appears to be associated with migratory bird movements (over 1000 reported AI outbreaks since 2006, involving 25 species of wild birds in EU alone) surveillance would need to focus on detection of HPAI in both wild birds as well as domesticated poultry and it will also be necessary to establish the migratory pathways of wild birds to increase the capacity to assess their risk in spreading the virus.

In March 2010 the International Scientific Task Force on Avian Influenza and Wild Birds, led by FAO and the United Nations Environment Programme – Convention on Migratory Species, reported that waning attention to HPAI was reducing opportunities for surveillance and research, thereby affecting efforts to understand the epidemiology of the disease. The disease continues to be a major problem in Egypt and parts of Asia and outbreaks have occurred in poultry in Romania and in wild birds in Russia, China and Mongolia (Fig. 1.1). One of the most important issues that need addressing is the surveillance of wild bird populations to improve understanding of the role that they play in the dissemination of infection. Although various methods have been used to track the migration of birds there is increasing interest in utilizing methodologies that would enable tracing of migratory movement based on the birds' stable isotope signatures. A small number of stable isotopes are involved in important biological and ecological processes and there is a strong correlation between levels of these isotopes in the environment and the concentration of the same isotopes in avian tissues. Of most interest are stable hydrogen and oxygen ratios in tissues that accurately reflect those in lakes, rivers, oceans and in groundwater, along the wild bird flyways. Using stable isotopes to characterize a population involves examining the isotopic signatures of a few

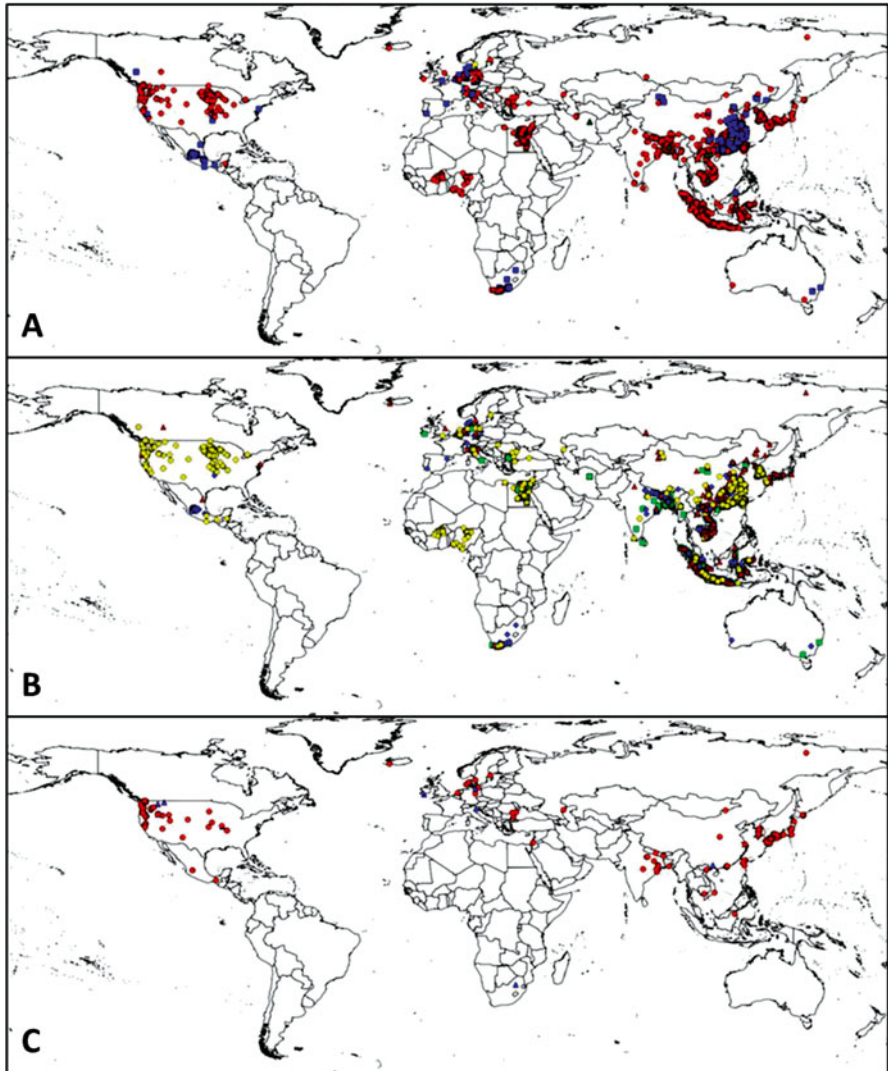


Fig. 1.1 Characteristics of the reported outbreaks of AI in domesticated poultry and wild migratory birds in the period from 1 January 2011 until 13 May 2015 (Source: FAO 2015).

Map A symbols: red circle [●]=H5 AI; blue square [■]=H7 AI; green triangle [▲]=H9 AI; grey star [☆];

Map B symbols: AI outbreaks reported during 2011 (grey star [☆]), 2012 (green square [■]), 2013 (blue square [■]), 2014 (red triangle [▲]) and 2015 (yellow circle [●]);

Map C symbols: AI outbreaks in wild (red circle [●]) and captive birds (blue triangle [▲])

individuals that are representative of the entire population. The hydrogen and oxygen isotope composition of environmental water varies spatially across the globe and because it is a constituent of many biosynthetic pathways, the isotopes' pres-

ence is relayed to animal tissues, providing the means to link data on groundwater isoscapes with isotope levels in biological tissues such as feathers. This isotope data would reveal migration patterns and enable identification of the breeding areas of birds sampled at non-breeding grounds and disease outbreak sites.

1.2 Migratory Birds and HPAI

To date, only a small number of migratory birds have been tracked by satellite transmitters to establish links with disease outbreaks in domestic poultry, with little evidence so far of direct correlation with HPAI. Furthermore, surveillance of 750,000 “healthy” wild birds has not revealed many infected individuals. Circumstantial evidence suggesting that the spread of the H5N1 virus to new areas can be facilitated by migratory wild birds has come from studies on ducks in Asia marked with satellite transmitters (Yamaguchi et al. 2010) that were tracked during an outbreak of highly pathogenic H5N1 avian influenza virus.

The satellite transmitters were attached to northern pintail ducks several months before the H5N1 virus was discovered in dead and dying whooper swans at wetlands in Japan. Twelve percent of marked pintails used the same wetlands as infected swans and the pintail ducks were present at those sites on dates the virus was discovered in swans. During the first week after they become infected with H5N1 virus, ducks such as pintails can shed the virus orally or in their faeces, contributing to the virus’ spread. Some of the marked pintails migrated 700 miles within 4 days of leaving the outbreak sites; marked pintails ultimately migrated more than 2000 miles to nesting areas in eastern Russia. The discovery that northern pintails made long-distance migrations during the period when an infected duck would likely shed the virus offers insight into how H5N1 could be spread by wild birds across large areas (Yamaguchi et al. 2010).

In another study, the movements of bar-headed geese marked with GPS satellite transmitters at Qinghai Lake, China were traced in relation to virus outbreaks and disease risk factors. A previously undocumented migratory pathway between Qinghai Lake and the Lhasa Valley of Tibet where 93 % of the 29 marked geese overwintered was discovered. From 2003 to 2009, 16 outbreaks in poultry or wild birds were confirmed on the Qinghai-Tibet Plateau, and the majority were located within the migratory pathway of the geese. Spatial and temporal concordance between goose movements and three potential H5N1 virus sources (poultry farms, a captive bar-headed goose facility, and H5N1 outbreak locations) indicated that ample opportunities existed for virus spill over and infection of migratory geese on the wintering grounds. Their potential as vectors of H5N1 was supported by rapid migration movements of some geese and genetic relatedness of H5N1 virus isolated from geese in Tibet and Qinghai Lake. This study was the first to compare phylogenetics of the virus with spatial ecology of its host, and the combined results suggest that the geese play a role in the spread of H5N1 in this region (Prosser et al. 2006; Zhou et al. 2011).

Defining the migratory behaviour of animals is fundamental to understanding their evolution and life history; much of the effort to study migration in birds, has been driven by the needs for conservation in changing habitats. The advances in understanding of migration, particularly in North America and Europe have been aided considerably by stable isotope techniques. The methods have not yet been applied to study the migratory birds and the dissemination of HPAI, but this is likely to be remedied in the near future. This manual provides background information on Stable Isotope Analysis (SIA) for scientists investigating HPAI from Member States engaged in Collaborative Research Projects or Technical Contract Projects and wish to understand how the disease is disseminated to provide information that would enable better risk assessment and more effective control and prevention measures.

In order to follow the global distribution of animal diseases, as well as to enable rapid information sharing, FAO has established an Animal Disease Information System (EMPRES-i), (FAO 2015) which enables for filtering disease records according to different predefined criteria. Specifically for AI, record-sets can be generated, which enable for correlation according to the species of origin of the outbreak (domesticated or wild birds), period of the observation/reporting of the outbreak and the location of the outbreak. The data on the evolution of the AI globally, obtained from EMPRES-i system over the period of 5 years (January 2011–May 2015) is shown on Fig. 1.1 and Table 1.1.

1.3 Using SIA to Understand the Dissemination of HPAI – The Way Ahead!

Most elements consist of one or more stable isotopes – elements having the same number of protons, but differing in the numbers of neutrons. Stable isotopes are those isotopes of an element that do not decay through radioactive processes over time. For instance, the element carbon (C) exists as two stable isotopes, ^{12}C and ^{13}C , and the element hydrogen (H) exist as two stable isotopes, ^1H and ^2H .

Stable isotope contents are expressed in ‘delta’ notation as δ values in parts per thousand (‰), where:

$$\delta R\text{‰} = (R_s / R_{\text{Std}} - 1) \times 1000$$

and R_s and R_{Std} are the ratios of the heavy to light isotope (e.g. $^{13}\text{C}/^{12}\text{C}$) in the sample and the standard, respectively. The stable isotope ratios of hydrogen, carbon, nitrogen, oxygen and sulphur are denoted in delta notation as $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\delta^{34}\text{S}$, respectively. R values have been carefully measured for internationally recognized standards. The standard used for both H and O is Standard Mean Ocean Water (SMOW), where ($^2\text{H}/^1\text{H}$) standard is 0.0001558 and $^{18}\text{O}/^{16}\text{O}$ is 0.0020052. The original SMOW standard is no longer available and has been replaced by a new International Atomic Energy Agency (IAEA) standard, VSMOW. The international

Table 1.1 Quantitative information on the reported outbreaks of AI in domesticated poultry and wild migratory birds in the period from 1 January 2011 until 13 May 2015 (Source: FAO 2015)

Species category	Pathogenicity (HP or LP) – H subtype of the AIV	Number of outbreaks/number of cases affected during the outbreak						Sub total
		2011	2012	2013	2014	2015		
Captive	HP-H5	1/1			1/	8/5	10/6	
	LP-H5		2/8				2/8	
	LP-H7			1/4			1/4	
	Total captive	1/1	2/8	1/4	1/	8/5	13/18	
	HP-H5	1927/763,007	551/666,346	489/306,795	891/520,219	1614/2,359,345	5472/4,615,712	
	HP-H7	1/10	48/2,699,685	86/780,143	4/3	3/139	142/3,479,980	
	HP-NoInfo	1/10					1/10	
Domestic	LP-H5	2/40	24/1584	37/6071	25/8179	24/15,526	112/31,400	
	LP-H7	24/4369	1/	40/7186	34/3039	6/2373	105/16,967	
	LP-H9	39/45,003	69/3	35/6	8/	4/	155/45,012	
	LP-NoInfo	6/26		1/1		2/	9/27	
	NoInfo-			34/2519			34/2519	
	NoInfo							
	Total domestic	2000/812,465	693/3,367,618	722/1,102,721	962/531,440	1653/2,377,383	6030/8,191,627	

Species category	Pathogenicity (HP or LP) – H subtype of the AIV	2011	2012	2013	2014	2015	Sub total
Wild	HP-H5	63/101	25/1183	4/2	37/35	70/217	199/1538
	HP-H7		2/11	1/5		1/8	4/24
	HP-NoInfo					1/	1/
	LP-H10				1/		1/
	LP-H5	1/			2/		3/
	LP-H7			1/	1/		2/
	LP-H9				1/		1/
	Total wild	64/101	27/1194	6/7	42/35	72/225	211/1562
	Grand total	2126/812,567	757/3,368,820	921/1,102,732	1376/531,475	2066/2,377,613	7246/8,193,207
	NoInfo	HP-H5	59/	34/	38/	39/	147/
HP-H7			1/				1/
LP-H10				1/	2/		3/
LP-H7				152/	329/	185/	666/
LP-H9		2/		1/	1/		5/
Total NoInfo		61/	35/	192/	371/	333/	992/

Confirmed AI outbreaks with no record on the total number of cases and the species affected (Source: FAO 2015)

carbon standard is the PDB, where ($^{13}\text{C}/^{12}\text{C}$) is 0.0112372 and is based on a belemnite from the Pee Dee Formation. As with SMOW, the original PDB standard is no longer available, but the IAEA provides Vienna-PDB with a similar R value. Atmospheric nitrogen is the internationally recognized standard with an R value ($^{15}\text{N}/^{14}\text{N}$) of 0.0036765. Lastly, the internationally recognized standard for sulfur is CDT, the Canyon Diablo Troilite, with a value ($^{34}\text{S}/^{32}\text{S}$) of 0.0450045. Typically, during most stable isotope analyses, investigators would not use IAEA standards on a routine basis. Instead, laboratories establish secondary reference materials to use each day that are traceable to IAEA standards and that bracket the range of isotope ratio values anticipated for the samples.

Although ecologists refer to “isotope signatures or isotope fingerprints” the values obtained for a sample do not provide a unique fingerprint but a distinctive profile; a more neutral notation is isotope value, rather than signatures.

The δ notation is derived as follows:

$$\delta\text{H}(\text{‰}) = \left(\frac{\text{Isotopic ratio sample}}{\text{Isotopic ratio standard}} - 1 \right) \times 1000$$

The right side of the equation is the measure of the light to heavy isotope ($^2\text{H}/^1\text{H}$) and that ratio is multiplied by 1000 transform the values into whole numbers. The isotope reference points were established many years ago and results reported can be negative or positive (‰) relative to the accepted international standard. Thus, a δH value of +150 (‰) means the sample has 150 parts per thousand (15 %) more deuterium in it than the standard; while if the value were negative it would be 150 times less deuterium than the standard. Primary reference material is limited in quantity so laboratories tend to use local standards calibrated against a reference standard. The primary isotopic reference standard for hydrogen and oxygen is Vienna Standard Mean Ocean Water (VSMOW).

It will be necessary to develop suitable procedures for monitoring wild birds to detect HPAI, determine their origin and migratory pathway to estimate the duration of their stay at the place of capture. This will require the application of detailed standard operating procedures for feather and tissue collection, data recording, sample storage, and designation of a laboratory to carry out the SIA. Since tissue samples could come from birds suspected of carrying avian influenza virus it will be necessary to install safeguards to prevent possible infection. Soft tissues could be freeze-dried and both these and keratinaceous samples heated to 100 °C for 20 min to destroy any viruses. Alternative methods would be to irradiate specimens at a central location before making them available for study. Various innovative methods for virus detection should also be considered, including environmental sampling to detect H5N1 virus in water, or faeces (Khalenkov et al. 2008; Cheung et al. 2009; Dovas et al. 2010) in locations where wild birds congregate. Other procedures that will be useful could include identification of bird species using DNA barcoding from feather or faeces (Lee et al. 2010) to provide better links to identifying those birds that are truly implicated in dissemination of HPAI.

There are already available data on variation in stable isotope ratios across the globe and how this is reflected in tissue samples from birds inhabiting different regions. It might be necessary however to collect environmental samples from different locations if there is not sufficient data already available. Collection of wild birds is a specialized procedure and it will be necessary to liaise with wildlife groups skilled in this task, who are also able to provide basic data on the ecology of different bird species. The FAO Wildlife Unit can provide inputs in this area through its links with various wildlife organizations in order to carry out sampling with greater efficiency.

Consideration needs to be given to which isotopes to analyse. While stable hydrogen can be used to obtain information on the wider geographical location, further details might be inferred on the local habitat by analysing carbon, nitrogen and sulphur. Samples from keratinaceous tissues e.g. feathers and claws, will provide an isotope ratio of the place when they were grown. In blood, stable isotope ratios could be used to determine timing of arrival on breeding or wintering grounds. Isotope ratios in the blood relate to those in the current environment hence a difference between isotope ratios in blood and the environment would indicate newly arrived birds compared with birds that had been staying in a particular location for some time. If infected birds were to be found it should be possible to establish where the disease was acquired – at the site or in a previous location. By understanding migratory movements it will be possible to predict risk and derive models to show the spread of HPAI and identify areas that pose a significant potential for being locations where the dissemination of HPAI is greater due to the particular congregation of wild birds, H5N1 virus and domestic poultry.

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