Transboundary spread of highly pathogenic avian influenza through poultry commodities and wild birds: a review

M.S. Beato & I. Capua

OIE/FAO and National Reference Laboratory for Avian Influenza and Newcastle Disease, and OIE Collaborating Centre for Infectious Diseases at the Human–Animal Interface, Istituto Zooprofilattico Sperimentale delle Venezie, viale dell'Università 10, 35020, Legnaro, Padua, Italy

Summary

The extensive circulation of the H5N1 highly pathogenic avian influenza (HPAI) virus in animals and the human health implications which it poses have led to extensive research in unexplored fields and thus a re-assessment of our understanding of this infection. Moreover, widespread infection of poultry has raised concerns about the food safety and trade implications of this infection, necessitating revised international trade regulations.

The role of wild birds has been much debated and resources have been invested to clarify the role that they may play in the spread of infection. It is now clear that wild birds may be responsible for primary introduction in a previously free area. To date it is still unclear whether HPAI infection may be maintained in wild bird populations for extended periods of time. This paper reviews existing knowledge on the transboundary spread of HPAI through poultry and poultry commodities and summarises evidence of spread through wild birds.

Keywords

Avian influenza – Poultry commodity – Wild bird.

Introduction

The unprecedented spread of H5N1 highly pathogenic avian influenza (HPAI) viruses worldwide has led to a reexamination of what is known about these viruses, including their viral ecology, epidemiology, host range and pathogenicity. One major concern, which has arisen following the H5N1 panzootic, is the negative effect of HPAI infections on the trade in poultry and associated products. The mechanisms by which avian influenza (AI) is introduced into a country and subsequently spread are extremely complex and are a result of a multifactorial process. The factors that play a role in the transmission and spread of AI include the viral characteristics (pathogenicity, resistance to chemical and physical factors, genetic constellation), the species infected and the commodities derived from them, the poultry density in the area of viral introduction, the interconnections of the poultry industry and the existence of wild bird flyways.

Before the year 2000, AI infections were considered infections of minor importance among animal

diseases. Highly pathogenic avian influenza was a sporadic disease and only direct control measures could be applied to combat it. Moreover, due to its limited occurrence little was known about the human health implications (13).

The pre-existing dogma on HPAI epidemiology has been challenged by the H5N1 HPAI panzootic. The ability of the H5N1 strain to spread between continents, the variety of species it can infect, including mammals, its ability to cause mortality in wild birds and its ability to mutate and persist in the field make H5N1 HPAI infections an extraordinary novelty in the virological era and very different from infections caused by other HPAI strains.

The unfolding of the panzootic caused by this virus has highlighted the absence of scientific information on several aspects of AI epidemiology and the urgent need to investigate them. Substantial resources (in terms of funds for surveillance, research and training) have been made available to combat this infection worldwide and the findings have stimulated the development of tailored control strategies, including vaccination.

In addition, the occurrence of H5N1 HPAI throughout Asia, Africa and Europe has raised concerns regarding the safety of poultry products for human consumption and the risk of transmission of infection to animals via swill feeding, predation and scavenging.

Foodborne transmission of infection and spread via poultry products may occur only if there is viable virus in the commodity and it is present at a concentration above the minimum infectious dose for the host.

Current European Union (EU) legislation authorises the importation of poultry products from a specified list of countries (21) and defines 'poultry' as the following: fowl, turkeys, guinea fowl, ducks, geese, quails, pigeons, pheasants, partridges and ratites (ostriches, emus, etc.) reared or kept in captivity for breeding, re-stocking or the production of meat or eggs for consumption (19).

The World Organisation for Animal Health (OIE) publishes recommendations for safe international trade in poultry and various poultry products in the Terrestrial Animal Health Code (Terrestrial Code) (41). The Terrestrial Code defines poultry as: 'all domesticated birds, including backyard poultry, used for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds, as well as fighting cocks used for any purpose'. It also defines 'commodity' as 'live animals, products of animal origin, animal genetic material, biological products and pathological material' and 'meat' as 'all edible parts of the animal' (41). Like the EU legislation, the Terrestrial Code recommends that poultry commodities can be traded safely provided they come from a country, zone or compartment shown to be free from notifiable avian influenza according to the relevant OIE criteria (41).

This paper summarises existing information on the presence of avian influenza virus (AIV) in poultry commodities (using the EU and OIE definitions of poultry) and on the role of wild birds as vehicles for the international spread of AI. In addition, areas requiring further investigation are highlighted. In Table I detailed information is presented on the studies cited.

Unvaccinated live poultry

Just as other infectious diseases of animals can be spread by the movement of infected animals, HPAI can be spread by the movement of infected poultry. Poultry movements are a documented means of transboundary spread, and thus of introduction to a previously unaffected country. Since movement restrictions apply once HPAI is diagnosed, movement of animals during the pre-clinical phase is likely to extend the spread of infection. To date, the only documented case of transboundary spread of HPAI through movement of live birds occurred in the Netherlands, Belgium and Germany in 2003.

A study of risk factors for the introduction of HPAI into poultry farms (37) showed a higher risk for poultry farms having layer hens versus farms having other poultry, based on evidence that layer hen farms have more contacts than other types of poultry farms with external objects and personnel. Alternatively, it may be because layer hen farms may have more contacts with other layer hen farms than with other poultry farms. However, the hypothesis requires further verification (18).

Vaccinated live poultry

Vaccination using appropriate vaccines and respecting biosecurity management guidelines prevents clinical signs in most species, but may not prevent shedding of the virus. Thus, vaccinated/exposed birds must be considered a potential source of infection. In theory, to overcome this, it should be possible to differentiate vaccinated birds from birds exposed to field strains of virus through the application of an appropriate monitoring system based on DIVA (differentiating infected from vaccinated animals) (12, 14). In practice, this has never been applied in the field and is very complex from a management point of view. Unless absence of viral circulation can be demonstrated, vaccinated poultry should not be traded, as they may carry infection.

Highly pathogenic avian influenza viruses in commodities other than live birds

Meat

The majority of the literature published on the presence of AIV in skeletal muscle of birds has dealt with the histopathological lesions caused by the infection. Although experiments reporting the detection of AIV by immunohistochemical techniques were published at the end of the 1980s, only later, with the emergence of H5N1, was poultry meat perceived as a public health risk, leading to targeted research in this area.

The ability of duck isolates of H5N1 HPAI virus to colonise muscle tissue was assessed in four-week-old specific-pathogen-free (SPF) chickens (39). Following intranasal infection, breast muscle was collected and found positive on days 2 and 3 post infection (p.i.). Another two experimental reports showed the presence of H5N2 and H5N3 HPAI

Table I
Summary of data available in literature on the presence of highly pathogenic avian influenza viruses in poultry commodities
Modified from Beato et al. (4)

Commodity	Species	Strains	Infection	Infectious dose	Titres (log10 EID ₅₀ /g) in commodity
Meat	Chickens	A/duck/Anyang/AVL-1/01 (H5N1)	Е	10 ⁶ EID ₅₀ /0.1 ml	5.3 to 5.5
		A/chicken/Pennsylvania/1370/1983 (H5N2)	Е	107EID ₅₀ /0.1 ml	2.2 to 3.2
		A/tern/South Africa/61 (H5N3)	E	10 ⁶ EID ₅₀ /0.1 ml	>4
	Turkey	A/turkey/ltaly/4580/99 (H7N1)	E	10 ⁶ EID ₅₀ /0.1 ml	4.38
	Duck	A/duck/Anyang/AVL-1/01 (H5N1)	E and N	106EID ₅₀ /0.1 ml	3 to 4
		A/goose/Vietnam/3/2005 (H5N1)	Е	106EID ₅₀ /0.1 ml	3
		A/Vietnam/1203/2004, A/ThailandPB/6231/2004	Е	106EID ₅₀ /0.1 ml	4 to 6 (2-week-old birds)
		A/crow/Thailand/2004, A/egret/HK/757.2/2002 (H5N1)	Е	106EID ₅₀ /0.1 ml	
		A/egret/HK/757.2/2002 (H5N1)	Е	10 ⁵ EID ₅₀ /0.1 ml	>2 (5-week-old birds)
		A/duck/Vietnam/12/2005 (H5N1)	Е	107EID ₅₀ /0.1 ml	1.5
Eggs	Turkey	A/turkey/Ontario/7732/66 (H5N9)	Е	Not reported	Not reported
		H5N2 (Virginia/1985)	Ν	Not reported	Not reported
	Chicken	H5N2 (Virginia/1985)	Ν	Not reported	Not reported
	Duck and geese	H5N1 (strain not reported)	Ν	Not reported	Not reported
	Quail	H5N1 (strain not reported)	Ν	Not reported	4.6 to 6.2
Feathers	Chicken, turkey, quail, guinea fowl	A/chicken/Yamaguchi/7/2004	Ε	10 ^{5.8-6.2} EID ₅₀ /0.1 ml	Not investigated
		A/chicken/Miyazaki/K11/2007			
		A/chicken/Hong Kong/220/1997			
	Duck	A/chicken/Yamaguchi/7/2004	Е	108EID ₅₀ /0.1 ml	Not investigated
		A/chicken/Miyazaki/K11/2007			
	Turkey	H5N1 (strain not reported)	N	Not available	Not investigated
Liver	Duck	A/chicken/Vietnam/12/2005 (H5N1)	Е	10 ⁷ EID ₅₀ /0.1 ml	Not reported
Blood	Chicken	A/tern/South Africa/61 (H5N3)	Е	10 ⁷ EID ₅₀ /0.1 ml	4
		A/chicken/Pennsylvania/1370/1983 (H5N2)	Е	10 ⁶ EID ₅₀ /0.1 ml	Not reported
	Pigeon, geese	A/turkey/Ontario/7732/66 (H5N9)	Е	108EID ₅₀ /0.5 ml	Not recovered
	Turkey	A/turkey/ltaly/4580/99 (H7N1)	Е	10 ⁶ EID ₅₀ /0.1 ml	1 to 5.8
		A/turkey/Ontario/7732/66 (H5N9)	Е	10 ^{8.7} EID ₅₀ /0.5 ml	2.7 to 3.7
	Duck	A/chicken/Vietnam/12/2005 (H5N1)	Е	10 ⁷ EID ₅₀ /0.1 ml	Not reported
		A/turkey/Ontario/7732/66 (H5N9)	Е	108EID ₅₀ /0.5 ml	Not recovered

E: experimental EID: egg infective dose N: natural

viruses in the meat of three- to four-week-old SPF chickens (26, 36).

Only one experimental study has been published on the presence of AIV in turkey meat (38). Vaccinated and unvaccinated turkeys were infected with H7 HPAI and low pathogenic avian influenza (LPAI) viruses. None of the samples obtained from birds vaccinated and challenged with either HPAI or LPAI strains contained viable virus. As expected, meat obtained from unvaccinated HPAI-challenged birds contained significant amounts of virus.

Field observations and experimental investigations have been reported on the possible recovery of H5N1 HPAI virus from duck (*Anas platyrynchos*) meat (39). The presence of H5N1 HPAI in poultry meat was detected in a batch of duck meat shipped from the People's Republic of China (China) to South Korea in 2001 (39). These studies showed that only H5N1 HPAI strains of duck origin were able to colonise skeletal muscle and that virus was isolated at an early stage of infection (39), although Li *et al.* (27) reported that a goose isolate of H5N1 HPAI subtype could

also be detected in duck muscle. Colonisation of meat in ducks infected experimentally has been shown to be related to the age of infection. In this respect, Pantin-Jackwood *et al.* (30) tested four different H5N1 viruses in two- and five-week-old Pekin ducks (*A. platyrhynchos*). The viruses used in their study could be recovered from the skeletal muscle of two-week-old birds on day 2 p.i. By contrast, only one strain was able to replicate in muscle tissue of five-week-old Pekin ducks and was present at a lower titre (indicating an age-dependent variability).

High titres of an H5N1 HPAI strain were detected in muscle tissues of call ducks (*A. platyrhynchos* var. *domestica*) reared for meat consumption in Japan and infected experimentally (17). In another laboratory investigation on the efficacy of whole inactivated AIV vaccine in waterfowl, the viral colonisation of meat of experimentally infected Pekin ducks was assessed using an H5N1 HPAI strain (5). Viable virus was detected in 100% of meat samples collected from unvaccinated birds on days 3 and 4 p.i., and in none of the samples collected from the vaccinated birds.

Eggs

The most likely route of viral infection in eggs is thought to be the contamination of the shell by faecal material following oviposition. However, the presence of AIV in the egg contents also may occur by vertical transmission. Samadieh and Bankowski (35) assessed the vertical transmissibility of AIV in turkeys with two virus strains, for which no information on the subtype and pathogenicity was reported. This study showed that embryonated eggs infected with influenza viruses survived the infection and poults developed haemagglutination-inhibition titres.

The first report of the presence of AIV in chicken eggs dates back to 1985. It documents the presence of an H5N2 HPAI virus in the yolk, albumen and shell surface of eggs collected from chicken flocks infected naturally during the 1984 HPAI outbreak in Pennsylvania and Virginia (11). The shell, albumen and yolk were sampled from eggs obtained from commercial layers, broiler breeders and turkey breeder flocks. Most of the eggs sampled were of market quality, with a low percentage (10%) having lesions attributable to AI infection. The outer egg shells were sampled by wiping them with sterile swabs. The internal contents of the eggs (yolk, albumen and yolk and albumen together) were sampled after the egg shell had been disinfected. Avian influenza virus was recovered from the yolk, albumen and shell surface of chicken eggs only. In general, virus recovery was higher from albumen than from yolk or from albumen and yolk together. In addition, virus was recovered at a lower rate from eggs laid from chicken flocks not showing clinical signs.

There is only one report on the presence of AIV in turkey eggs. Narayan *et al.* (29) isolated an H5N9 HPAI virus from eggs laid by turkeys infected experimentally. Virus was detected in the yolk after disinfection of the shell. However, during an outbreak caused by an H5N2 HPAI strain in the United States, no virus was recovered from turkey eggs laid by affected flocks (11).

The isolation of H5N1 HPAI virus from shell washes of duck and goose eggs has been reported (27). The eggs were confiscated at Guangzhou airport in China from travellers coming from Vietnam in 2005.

A single study has documented the presence of H5N1 HPAI in quail eggs (33). The contents of the eggs (a mixture of albumen and allantoic fluid) of naturally infected Japanese quails (*Coturnix coturnix japonica*) contained high titres of the H5N1 virus.

Feathers

Very few studies have been published on the presence of AIV in feathers. The virus may localise by means of contamination through faecal material and contaminated dust. Other routes of contamination linked to the systemic nature of this infection are also possible. H5N1 HPAI was detected on day 1 p.i. in the feather follicle epithelium of chickens, Japanese quails, turkeys and guinea fowl infected by the intranasal route (31). H5N1 HPAI was detected in feathers of call ducks, in which many types of feathers, especially the developing ones, exhibited necrosis of the epithelium (from the epidermal collar to the pulp cap) and of the developing rachis and barb ridge on days 3 to 7 p.i. In the necrotic epithelium of the feathers AIV was detected by immunohistochemistry (43). The same author has also reported the identification of AIV of the H5N1 HPAI strains in the feathers and skin of domestic ducks (A. platyrhynchos var. domestica) and geese (Anser cygnoides var. domestica) of four weeks of age (44). Virus isolation attempts from skin samples of ducks and geese yielded positive results after experimental infection. The isolation of H5N1 HPAI virus from turkey feathers was reported during the 2007 H5N1 epidemic in the United Kingdom (UK) (personal communication, M.J. Slomka, European Reference Laboratory for Avian Influenza and Newcastle Disease, Veterinary Laboratories Agency, UK).

Avian influenza virus may persist in feathers for significant periods if storage conditions are favourable. When feathers were detached from the body during the active phase of viral replication, the infectious virus was recovered from the stored feather tissue at +4°C and +20°C for 160 days and 15 days, respectively (45).

The identification of AIV in feathers detached from infected birds has major commercial implications due to its interference with the international trade in feathers used for duvets and down jackets.

Other commodities

Cultural diversity is often reflected by different eating habits. The consumption of a vast variety of poultry products prepared in different ways in different geographical locations means that what is considered normal in some countries may not be considered the same way in others. Thus, complete control of cross-border trade becomes problematic but critical, as it affects animal and public health, especially in those countries where raw poultry products are consumed (for example, blood pudding).

Liver

Avian influenza virus was detected in the livers of Pekin ducks infected with an H5N1 HPAI virus (5). Liver samples collected on days 3 and 5 p.i. were positive by virus isolation. This has important implications for trade

and, potentially, for human health in those areas where liver is eaten raw or undercooked.

Blood

In certain countries avian blood is consumed uncooked and thus may represent a source of infection for animals and potentially for humans. As far as the authors are aware, fresh or clotted blood is not traded internationally and thus the risk of introducing HPAI to a country through the trade or illegal importation of fresh or clotted blood is very low. However, HPAI infection includes a viraemic phase, which explains the detection of HPAI in the blood of chickens (H5N2), turkeys (H5N9) and ducks (H5N1) (36).

There have been two reports of the detection of viable virus in chicken blood. Kishida *et al.* (26) reported significant titres of H5N3 subtype AIV in the blood of chickens on day 1 p.i. Viraemia was also detected on days 1 to 3 p.i. in fourweek-old SPF chickens infected with the H5N2 HPAI strain (36). H5N9 HPAI virus was isolated at 16 h and 24 h p.i. from the blood of 24-week-old turkeys infected oro-nasally (29). Viraemia was detected in turkeys infected experimentally with an H7N1 HPAI isolate (from 1 to 5.8 log median egg infectious dose) (38). After the experimental infection of vaccinated and unvaccinated Pekin ducks with an H5N1 HPAI virus at seven weeks of age, viraemia was demonstrated on days 3 and 4 p.i. in unvaccinated birds only (5).

H5N1 in wild birds

The risk of outbreaks in Europe of HPAI associated with wild birds has been reviewed comprehensively (3). Wild water birds are considered the natural reservoirs of LPAI viruses (1), but HPAI viruses responsible for high mortality in domestic birds do not have recognised wild bird reservoirs. H5 and H7 HPAI viruses are generally considered to emerge from LPAI precursors once introduced and adapted to gallinaceous poultry populations, and not to occur in wild birds (1, 2). The 16 haemagglutinin subtypes of AIV cause unapparent or mild disease in wild birds, with rare exceptions. Prior to 2002, HPAI virus had only rarely been isolated from freeliving water bird populations. There had been a few isolated cases associated with AIV-infected poultry flocks (15) and one large mortality event associated with an H5N3 HPAI infection in common terns (Sterna hirundo) in South Africa in 1961 (6). The ecology of HPAI changed with the emergence and spread of the Asian H5N1 HPAI virus. The spread of this virus over Asia, Europe and Africa led to mortalities in more than 75 wild bird species in 38 countries (22). The isolation of the virus in dead

migratory birds indicated a potential role for those bird populations in the dissemination of HPAI.

The unprecedented situation that occurred in Asia resulted in the spillover of infection to naive populations of wild birds. The role of wild birds in the geographical spread of HPAI has been debated extensively. The behavioural ecology of birds drives the epidemiology of influenza infection in novel directions by influencing the ability of the virus to spread. The risk of a wild bird species introducing, spreading or maintaining an AIV to a given area is linked to a number of factors, including:

- the species of susceptible animal
- the number and age of target individuals
- the characteristics of the geographical area of origin and destination
- the local (seasonal) abundance of that species
- the gregariousness of the species during the breeding, migration and non-breeding seasons (3).

Because most of the wild birds from which Asian H5N1 HPAI has been isolated to date were either dead or dying, the incubation period of this disease for most Asian migratory birds is unknown. Thus, the real potential for virus introduction can only be estimated because of the extensive variability among target species. For example, complete data on the ability of any species to fly significant distances while infected are lacking.

In an attempt to begin to define the situation, several studies have been conducted on the susceptibility of wild birds to HPAI infections and their ability to continue migration and thus carry H5N1 HPAI over considerable distances. Experimental infections show that many species of birds can be infected and that a range of clinical statuses can be recorded. Some bird species may survive infection and shed H5N1 HPAI without apparent disease (10, 16, 24), or with clinical signs of limited duration and intensity, while others may succumb (23, 36, 37). Experimental studies also indicate that there is a high variation in the innate susceptibility of avian species, even within the same order (24, 31, 32). In addition, little is known about the immune response and duration of immunity of wild birds.

The spread of H5N1 in Europe, where infected wild birds were found in several countries that had not reported outbreaks in poultry, suggests that wild birds can carry virus to areas previously unaffected (8). Based on the analysis of the spread of H5N1 HPAI, 9 of 21 introductions of the virus into Asia were most probably caused by movements of infected poultry. Three further incursions were most likely related to wild birds. In Europe, 20 of 23 introductions were associated with migrating wild birds (23). Despite extensive global wildlife surveillance efforts, the detection of infection with H5N1 HPAI is rare (9, 34). It therefore seems that most wild birds that carry infection

will develop a clinical condition, which implies that they spread infection only during the pre-clinical or early clinical phases. In addition, the number of H5N1 isolations from wild birds sampled between 2006 and 2009 declined and it may be postulated that wild birds are incapable generally of sustaining an endemic cycle of H5N1, but are infected as a result of spilling over of infection by certain virus subtypes in domestic birds.

A detailed study of the movements of birds infected with H5N2 HPAI in Nigeria illustrates the difficulties in extrapolating from experimental data on wild bird infection. Healthy infected birds (white-faced whistling ducks [Dendrocygna viduata] and spur-winged geese [Plectropterus gambensis]) were tracked by satellite telemetry. The study determined that they survived infection with the HPAI virus. This is a rare finding of infection with HPAI genotype virus in healthy wild birds (22). However, it would seem that there may be extensive variability among subtypes, as one of the birds tracked in this study travelled vast distances and was still found to be HPAI-positive 18 days after first testing positive for the virus, whereas the duration of illness and viral shedding recorded generally in waterfowl inoculated with H5N1 HPAI viruses is less than 7 days.

Pet and hobby birds

In addition to wild bird movements, trade in domestic poultry and poultry products and the release of exotic and/or game birds have played a role in the spread of H5N1 HPAI from continent to continent (25).

Two isolated incursions of H5N1 HPAI into Europe occurred in 2004 and 2005 and are examples of the influence of humans on the spread potential of AI viruses. The first was detected when eagles smuggled from Thailand and confiscated at Brussels airport were shown to be infected with H5N1 virus, similar genetically to isolates in Thailand (40). The second, when investigations of deaths in captive caged birds imported from Chinese Taipei and held in quarantine in the UK showed that the birds were infected with H5N1 HPAI. H5N1 HPAI virus has also been reported in Houbara bustards (*Chlamydotis undulata macqueenir*): the birds showed clinical signs after importation into the Kingdom of Saudi Arabia (28).

Conclusions

Transcontinental spread of HPAI had never occurred prior to the H5N1 HPAI panzootic. Transboundary spread has occurred in the past, although the extent of the recent ongoing episode overshadows previous outbreaks. The

principal reasons for this are that HPAI infections have never become endemic, having been controlled through stamping out policies, and spillover to waterfowl or to wild birds has occurred only sporadically.

Although sporadic infections have occurred previously in wild birds, the introduction of HPAI to a disease-free country or region had never occurred by this route prior to the H5N1 epidemic.

It appears that the introduction of infection to wild or domestic bird populations has occurred through migratory waterfowl in a significant number of instances. Although HPAI readily infects a variety of animals and, because of its systemic nature, is found in many poultry products, reported cases of transboundary spread through live birds or products are few. Even so, the presence of AI has led to import bans being imposed on products from infected countries. This has been exacerbated where exporting countries have been unable to identify zones or compartments that are free from the infection. Furthermore, in under-resourced countries which are already experiencing widespread infection, the occurrence of outbreaks linked to legal or illegal trading of poultry products or wild birds is likely to be under-reported.

Extensive global wildlife surveillance efforts have detected infection with H5N1 HPAI viruses in healthy wild birds on rare occasions (9, 22), but the significance of wild birds as a source of infection and their influence on the epidemiology of H5N1 HPAI viruses is yet to be established fully. So far, wild bird surveillance has given an insight into the role of migratory pathways in the global spread of avian influenza viruses. Its use as a real-time tool to establish the risk of introduction into a country is unproven. Whatever the risk of introduction of certain HPAI viruses by wild bird movements, there is evidence suggesting that they may play an important role in transmission and spread. In addition, the changing genetic and antigenic profiles of H5N1 HPAI make it reasonable to expect that our understanding will evolve following the emergence of a new virus with different characteristics.

Current knowledge concerning the spread of H5N1 in Europe, where infected wild birds have been found in several countries that have not reported outbreaks among poultry, suggests that wild birds can indeed carry the virus to areas unaffected previously (8), without this necessarily resulting in spread to commercial poultry.

Data collated in this paper indicate that AIV may be recovered from a variety of poultry commodities. The presence of AIV in poultry products is influenced by the characteristics of the viral strain, including its pathogenicity and tropism, and thus its ability to colonise particular body tissues.

Although it appears that HPAI viruses are detectable in the commodities discussed above, data are still fragmentary and further studies should be carried out in a more extensive and coordinated manner in order to properly assess the risk of introduction to a given area and/or host by poultry products.

Highly pathogenic avian influenza strains are able to colonise poultry meat (26, 36, 39), so infection with these viruses poses a risk of spread and transmission through this commodity. More information is available on the presence of H5N1 HPAI in skeletal muscles of avian species, especially ducks (5, 27, 30, 43). The presence of the H5N1 HPAI strain in skeletal muscle is not related necessarily to its ability to cause disease in waterfowl. Thus the absence of clinical signs does not exclude the recovery of viruses from muscle tissues.

The rates of detection of HPAI viruses in poultry meat are higher during the early stages of infection, indicating an elevated risk linked to the trade of meat collected in the pre-clinical or early clinical phases of the disease.

Two experimental studies are available on the efficacy of vaccination in preventing viraemia and viral colonisation of meat in ducks and turkeys (5, 38). This is relevant because vaccination has been introduced among the control strategies for AI (20, 29). The results of these two experiments indicate that no virus could be recovered from meat or viscera of vaccinated birds. However, since this information was generated under laboratory conditions, the presence of virus in meat obtained from vaccinated birds under field conditions cannot be excluded.

With regard to eggs, HPAI viruses were detected in the internal contents of eggs and from swabs or washes of the egg shell of chickens, ducks and quails (27, 29, 33). Avian influenza virus is present in eggs either via contamination with infected faeces and/or by vertical transmission. It is important to state that published studies on vertical transmission of AIV are few.

With reference to other commodities, untreated blood, viscera and feathers have also been shown to harbour virus under natural or experimental conditions. Further studies are necessary to assess the persistence of AIV in other commodities.

In conclusion, the paucity of data, combined with the evolving eco-epidemiological situation of H5N1, requires continued attention and targeted surveillance in wild and domestic birds. This is also necessary because HPAI viruses may emerge unpredictably in poultry once they are introduced from their low pathogenic progenitors circulating in wild birds. The application of rigorous biosecurity measures in the poultry industry is essential to prevent transmission of AIV from the wild to the domestic host, which is a prerequisite to further spread within the poultry industry. In addition, compliance with the international standards of the OIE will continue to reduce the risk of HPAI, including viruses of the H5N1 subtype, being introduced through trade into a free country or region.

Le point sur la dissémination du virus de l'influenza aviaire hautement pathogène par les produits aviaires et les oiseaux sauvages

M.S. Beato & I. Capua

Résumé

L'extension de la circulation du virus H5N1 de l'influenza aviaire hautement pathogène (IAHP) dans les populations aviaires et ses effets sur la santé humaine ont conduit les scientifiques à explorer et à approfondir des champs d'investigation jusque là inconnus et à réévaluer leur perception de cette infection. En outre, les inquiétudes suscitées par la distribution mondiale de l'infection chez les volailles et par ses conséquences pour la sécurité sanitaire des aliments et la sécurité des échanges internationaux ont imposé de revoir les réglementations internationales applicables au commerce.

Le rôle de l'avifaune a fait l'objet d'examens approfondis et des ressources ont été investies pour élucider le rôle que peuvent jouer les oiseaux sauvages dans la propagation de l'infection. Il est désormais établi que l'avifaune peut être à l'origine de la première introduction de la maladie dans une zone précédemment indemne. On ignore encore si l'infection due au virus de l'IAHP peut persister longtemps dans les populations d'oiseaux sauvages. Les auteurs font le point sur l'état actuel des connaissances concernant la propagation transfrontalière de l'IAHP par les volailles et les produits aviaires et résume les éléments qui démontrent le rôle joué par les oiseaux sauvages dans la propagation du virus.

Mots-clés

Avifaune – Influenza aviaire – Produit aviaire.

Estudio de la diseminación transfronteriza de la influenza aviar altamente patógena a través de mercancías aviares y aves salvajes

M.S. Beato & I. Capua

Resumen

La amplia circulación de la cepa H5N1 del virus de la influenza aviar altamente patógena (IAAP) en los animales y sus consecuencias para la salud humana han llevado a investigar a fondo una serie de temas hasta ahora inexplorados y a reconsiderar todo lo que sabemos de esta infección. Además, el contagio generalizado de aves de corral ha generado inquietud acerca de los efectos de esta infección sobre la inocuidad de los alimentos y el comercio, lo que ha llevado a revisar las reglas que presiden las transacciones comerciales internacionales.

También se ha hablado mucho de la función de las aves salvajes y se han invertido recursos en dilucidar cuál puede ser su papel en la propagación de la infección. Ahora está claro que a veces son responsables de la introducción primaria de la enfermedad en una zona hasta entonces libre de ella. De momento no se sabe a ciencia cierta si la IAAP puede o no subsistir durante largos periodos de tiempo en poblaciones de aves salvajes. Los autores pasan revista a lo que hasta ahora se sabe de la propagación transfronteriza de la IAAP por las aves de corral y sus derivados, y resumen los datos que demuestran la transmisión de la enfermedad a través de aves salvajes.

Palabras clave

Ave salvaje – Influenza aviar – Mercancía de origen aviar.

References

- 1. Alexander D.J. (2000). A review of avian influenza in different bird species. *Vet. Microbiol.*, **74** (1-2), 3-13.
- Alexander D.J. (2007). An overview of the epidemiology of avian influenza. Vaccine, 25 (30), 5637-5644.
- 3. Artois M., Bicout D., Doctrinal D., Fouchier R., Gavier-Widen D., Globig A., Hagemeijer W., Mundkur T., Munster V. & Olsen B. (2009). Outbreaks of highly pathogenic avian influenza in Europe: the risks associated with wild birds. *In* Avian influenza (T. Mettenleiter, ed.). *Rev. sci. tech. Off. int. Epiz.*, 28 (1), 69-92.
- 4. Beato M.S., Capua I. & Alexander D.J. (2009). Avian influenza viruses in poultry products: a review. *Avian Pathol.*, **38** (3), 193-200.
- Beato M.S., Toffan A., De Nardi R., Cristalli A., Terregino C., Cattoli G. & Capua I. (2007). – A conventional, inactivated oil emulsion vaccine suppresses shedding and prevents viral meat colonisation in commercial (pekin) ducks challenged with HPAI H5N1. Vaccine, 25 (20), 4064-4072.
- Becker W.B. (1966). The isolation and classification of tern virus: influenza virus A/Tern/South Africa/1961. *J. Hyg.* (London), 64 (3), 309-320.
- 7. Boon A.C., Sandbulte M.R., Seiler P., Webby R.J., Songserm T., Guan Y. & Webster R.G. (2007). Role of terrestrial wild birds in ecology of influenza A virus (H5N1). *Emerg. infect. Dis.*, **13** (11), 1720-1724.
- 8. Boyce W.M., Sandrock C., Kreuder-Johnson C., Kelly T. & Cardona C. (2009). Avian influenza viruses in wild birds: a moving target. *Comp. Immunol. Microbiol. infect. Dis.*, **32** (4), 275-286.
- 9. Bragstad K., Jorgensen P.H., Handberg K., Hammer A.S., Kabell S. & Fomsgaard A. (2007). First introduction of highly pathogenic H5N1 avian influenza A viruses in wild and domestic birds in Denmark, Northern Europe. *Virol. J.*, 4, 43.
- Brown J.D., Stallknecht D.E., Beck J.R., Suarez D.L. & Swayne D.E. (2006). Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. *Emerg. infect. Dis.*, 12 (11), 1663-1670.
- 11. Cappucci D.T. Jr, Johnson D.C., Brugh M., Smith T.M., Jackson C.F., Pearson J.E. & Senne D.A. (1985). Isolation of avian influenza virus (subtype H5N2) from chicken eggs during a natural outbreak. *Avian Dis.*, **29** (4), 1195-1200.
- 12. Capua I. (2007). Vaccination for notifiable avian influenza in poultry. *In* Animal vaccination Part 1: development, production and use of vaccines (P.-P. Pastoret, M. Lombard & A.A. Schudel, eds). *Rev. sci. tech. Off. int. Epiz.*, **26** (1), 217-227.

- 13. Capua I. (2008). Avian influenza in the EU. *Zoonoses public Hlth*, **55** (1), 1.
- 14. Capua I. & Marangon S. (2003). The use of vaccination as an option for the control of avian influenza. *Avian Pathol.*, **32** (4), 335-343.
- 15. Capua I. & Mutinelli F. (2001). Mortality in muscovy ducks (*Cairina moschata*) and domestic geese (*Anser anser* var. *domestica*) associated with natural infection with a highly pathogenic avian influenza virus of H7N1 subtype. *Avian Pathol.*, **30** (2), 179-183.
- 16. Chen H., Li Y., Li Z., Shi J., Shinya K., Deng G., Qi Q., Tian G., Fan S., Zhao H., Sun Y. & Kawaoka Y. (2006). Properties and dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in western China. *J. Virol.*, **80** (12), 5976-5983.
- 17. Chen H., Smith G.J., Li K.S., Wang J., Fan X.H., Rayner J.M., Vijaykrishna D., Zhang J.X., Zhang L.J., Guo C.T., Cheung C.L., Xu K.M., Duan L., Huang K., Qin K., Leung Y.H., Wu W.L., Lu H.R., Chen Y., Xia N.S., Naipospos T.S., Yuen K.Y., Hassan S.S., Bahri S., Nguyen T.D., Webster R.G., Peiris J.S. & Guan Y. (2006). Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. *Proc. natl Acad. Sci. USA*, **103** (8), 2845-2850.
- 18. De Jong M.C., Stegeman A., van der Goot J. & Koch G. (2009). Intra- and interspecies transmission of H7N7 highly pathogenic avian influenza virus during the avian influenza epidemic in the Netherlands in 2003. *In* Avian influenza (T. Mettenleiter, ed.). *Rev. sci. tech. Off. int. Epiz.*, **28** (1), 333-340.
- 19. European Union (EU) (1990). Council Directive of 15 October 1990 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs (90/539/EEC). Off. J. Eur. Communities, L 303, 6-28.
- 20. European Union (EU) (2006). Commission Decision 2006/148/EC of 24 February 2006 on introducing preventive vaccination against highly pathogenic avian influenza H5N1 and related provisions for movements in France. *Off. J. Eur. Union*, **L** 55, 51-57.
- 21. European Union (EU) (2006). Commission Decision 2006/696/EC of 28 August 2006 laying down a list of third countries from which poultry, hatching eggs, day-old chicks, meat of poultry, ratites and wild game-birds, eggs and egg products and specified pathogen-free eggs may be imported into and transit through the Community and the applicable veterinary certification conditions, and amending Decisions 93/342/EEC, 2000/585/EC and 2003/812/EC. Off. J. Eur. Union, L 295, 1-76.

- 22. Gaidet N., Cattoli G., Hammoumi S., Newman S.H., Hagemeijer W., Takekawa J.Y., Cappelle J., Dodman T., Joannis T., Gil P., Monne I., Fusaro A., Capua I., Manu S., Micheloni P., Ottosson U., Mshelbwala J.H., Lubroth J., Domenech J. & Monicat F. (2008). Evidence of infection by H5N2 highly pathogenic avian influenza viruses in healthy wild waterfowl. PLoS Pathog., 4 (8), e1000127.
- 23. Ghedin E., Sengamalay N.A., Shumway M., Zaborsky J., Feldblyum T., Subbu V., Spiro D.J., Sitz J., Koo H., Bolotov P., Dernovoy D., Tatusova T., Bao Y., St George K., Taylor J., Lipman D.J., Fraser C.M., Taubenberger J.K. & Salzberg S.L. (2005). Large-scale sequencing of human influenza reveals the dynamic nature of viral genome evolution. *Nature*, 437 (7062), 1162-1166.
- 24. Keawcharoen J., van Riel D., van Amerongen G., Bestebroer T., Beyer W.E., van Lavieren R., Osterhaus A.D., Fouchier R.A. & Kuiken T. (2008). Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerg. infect. Dis.*, **14** (4), 600-607.
- 25. Kelly T.R., Hawkins M.G., Sandrock C.E. & Boyce W.M. (2008). A review of highly pathogenic avian influenza in birds, with an emphasis on Asian H5N1 and recommendations for prevention and control. *J. avian Med. Surg.*, **22** (1), 1-16.
- Kishida N., Sakoda Y., Eto M., Sunaga Y. & Kida H. (2004). Co-infection of Staphylococcus aureus or Haemophilus paragallinarum exacerbates H9N2 influenza A virus infection in chickens. Arch. Virol., 149 (11), 2095-2104.
- 27. Li Y., Lin Z., Shi J., Qi Q., Deng G., Li Z., Wang X., Tian G. & Chen H. (2006). Detection of Hong Kong 97-like H5N1 influenza viruses from eggs of Vietnamese waterfowl. *Arch. Virol.*, **151** (8), 1615-1624.
- 28. Monne I., Fusaro A., Al-Blowi M.H., Ismail M.M., Khan O.A., Dauphin G., Tripodi A., Salviato A., Marangon S., Capua I. & Cattoli G. (2008). Co-circulation of two sublineages of HPAI H5N1 virus in the Kingdom of Saudi Arabia with unique molecular signatures suggesting separate introductions into the commercial poultry and falconry sectors. *J. gen. Virol.*, 89 (Pt 11), 2691-2697.
- 29. Narayan O., Lang G. & Rouse B.T. (1969). A new influenza A virus infection in turkeys. IV. Experimental susceptibility of domestic birds to virus strain turkey-Ontario 7732-1966. *Arch. ges. Virusforsch.*, **26** (1), 149-165.
- 30. Pantin-Jackwood M.J., Suarez D.L., Spackman E. & Swayne D.E. (2007). Age at infection affects the pathogenicity of Asian highly pathogenic avian influenza H5N1 viruses in ducks. *Virus Res.*, **130** (1-2), 151-161.
- 31. Perkins L.E. & Swayne D.E. (2001). Pathobiology of A/chicken/Hong Kong/220/97 (H5N1) avian influenza virus in seven gallinaceous species. *Vet. Pathol.*, **38** (2), 149-164.

32. Perkins L.E. & Swayne D.E. (2003). – Comparative susceptibility of selected avian and mammalian species to a Hong Kong-origin H5N1 high-pathogenicity avian influenza virus. *Avian Dis.*, **47** (3 Suppl.), 956-967.

- 33. Promkuntod N., Antarasena C., Prommuang P. & Prommuang P. (2006). Isolation of avian influenza virus A subtype H5N1 from internal contents (albumen and allantoic fluid) of Japanese quail (*Coturnix coturnix japonica*) eggs and oviduct during a natural outbreak. *Ann. N.Y. Acad. Sci.*, 1081, 171-173.
- 34. Salzberg S.L., Kingsford C., Cattoli G., Spiro D.J., Janies D.A., Aly M.M., Brown I.H., Couacy-Hymann E., De Mia G.M., Dung do H., Guercio A., Joannis T., Maken Ali A.S., Osmani A., Padalino I., Saad M.D., Savic V., Sengamalay N.A., Yingst S., Zaborsky J., Zorman-Rojs O., Ghedin E. & Capua I. (2007). Genome analysis linking recent European and African influenza (H5N1) viruses. *Emerg. infect. Dis.*, 13 (5), 713-718.
- 35. Samadieh B. & Bankowski R.A. (1971). Transmissibility of avian influenza-A viruses. *Am. J. vet. Res.*, **32** (6), 939-945.
- 36. Swayne D.E. & Beck J.R. (2005). Experimental study to determine if low-pathogenicity and high-pathogenicity avian influenza viruses can be present in chicken breast and thigh meat following intranasal virus inoculation. *Avian Dis.*, 49 (1), 81-85.
- 37. Thomas M.E., Bouma A., Ekker H.M., Fonken A.J., Stegeman J.A. & Nielen M. (2005). Risk factors for the introduction of high pathogenicity avian influenza virus into poultry farms during the epidemic in the Netherlands in 2003. *Prev. vet. Med.*, **69** (1-2), 1-11.
- 38. Toffan A., Serena Beato M., De Nardi R., Bertoli E., Salviato A., Cattoli G., Terregino C. & Capua I. (2008). Conventional inactivated bivalent H5/H7 vaccine prevents viral localization in muscles of turkeys infected experimentally with low pathogenic avian influenza and highly pathogenic avian influenza H7N1 isolates. *Avian Pathol.*, 37 (4), 407-412.
- 39. Tumpey T.M., Suarez D.L., Perkins L.E., Senne D.A., Lee J.G., Lee Y.J., Mo I.P., Sung H.W. & Swayne D.E. (2002). Characterization of a highly pathogenic H5N1 avian influenza A virus isolated from duck meat. *J. Virol.*, 76 (12), 6344-6355.
- 40. Van Borm S., Thomas I., Hanquet G., Lambrecht B., Boschmans M., Dupont G., Decaestecker M., Snacken R. & van den Berg T. (2005). Highly pathogenic H5N1 influenza virus in smuggled Thai eagles, Belgium. *Emerg. infect. Dis.*, 11 (5), 702-705.
- 41. World Organisation for Animal Health (OIE) (2010). Terrestrial Animal Health Code, 19th Ed. OIE, Paris.

- 42. World Organisation for Animal Health (OIE)/Food and Agriculture Organization of the United Nations (FAO)/Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) (2007). Vaccination: a tool for the control of avian influenza. Recommendations of an OIE/FAO/IZSVe Scientific Conference co-organised and supported by the European Union, 20-22 March, Verona. OIE, Paris. Available at: www.oie.int/eng/info_ev/Other%20Files/A_Guidelines% 20on%20AI%20vaccination.pdf.
- 43. Yamamoto Y., Nakamura K., Kitagawa K., Ikenaga N., Yamada M., Mase M. & Narita M. (2007). Severe nonpurulent encephalitis with mortality and feather lesions in call ducks (*Anas platyrhyncha var. domestica*) inoculated intravenously with H5N1 highly pathogenic avian influenza virus. *Avian Dis.*, **51** (1), 52-57.
- 44. Yamamoto Y., Nakamura K., Okamatsu M., Yamada M. & Mase M. (2008). Avian influenza virus (H5N1) replication in feathers of domestic waterfowl. *Emerg. infect. Dis.*, **14** (1), 149-151.
- 45. Yamamoto Y., Nakamura K., Yamada M. & Mase M. (2010).
 Persistence of avian influenza virus (H5N1) in feathers detached from bodies of infected domestic ducks. *Appl. environ. Microbiol.*, 76 (16), 5496-5499.