The role of the legal and illegal trade of live birds and avian products in the spread of avian influenza

T. van den Berg
Head of Avian Virology and Immunology, Veterinary and Agrochemical Research Centre (VAR), Groeselenberg 99, B-1180 Brussels, Belgium

Summary
The panzootic of the H5N1 strain of highly pathogenic avian influenza has become an international crisis. All parts of the world are now considered at risk due to trade globalisation, with the worldwide movement of animals, products and humans, and because of the possible spread of the virus through the migration of wild birds. The risk of introducing notifiable avian influenza (NAI) through trade depends on several factors, including the disease status of the exporting country and the type of products. The highest risk occurs in the trade of live birds. It is important to assess and manage these risks to ensure that global trade does not result in the dissemination of NAI. However, it is also important that the risk of infection is not used as an unjustified trade barrier. The role of the regulatory authorities is thus to facilitate the safe trade of animal products according to international guidelines.

Nevertheless, the balance between acceptable risk and safe trade is difficult to achieve. Since the movements of poultry and birds are sometimes difficult to trace, the signature or ‘identity card’ of each isolated virus can be very informative. Indeed, sequencing the genes of H5N1 and other avian influenza viruses has assisted greatly in establishing links and highlighting differences between isolates from different countries and tracing the possible source of introduction. Recent examples from Asia, Europe and Africa, supported by H5N1 molecular fingerprinting, have demonstrated that the sources of introduction can be many and no route should be underestimated.

Keywords

Introduction
When outbreaks of highly pathogenic avian influenza (AI) occur in new places, analysing the possible route of entry is often complicated by:

– the limited capacity of some countries to investigate disease
– the scarcity of information on illegal movements of poultry or poultry products
– delays in reporting outbreaks when they first occur.

As a consequence, in many countries, the index case is not the first case of infection. In addition, highly pathogenic AI (HPAI) can occur in smallholder or village poultry without being diagnosed because mortality in village flocks occurs regularly from other causes, such as Newcastle disease (ND), which is endemic in many parts of the world. Newcastle disease is particularly endemic in Africa and
Asia, where H5N1 is presently entrenched. These deaths are not always reported and, even if local authorities have been informed, there is no guarantee that all cases will be investigated or that further reporting will occur. This under-reporting is most evident in areas where human cases have occurred in the absence of reported avian infections (‘human sentinels’) (84). Indeed, sick poultry is often sold by farmers and villagers as soon as it begins to show signs of disease. However, delays in notification can also occur on large commercial chicken farms, even though it is usually difficult to hide such cases for extended periods, because the number of infected birds increases rapidly. For instance, belatedly reported outbreaks of H5N1 in Japan in 2004 have not only demonstrated that HPAI can easily spread beyond national borders, but also show the importance of early reporting and tracing the source of introduction (59). This failure to report disease promptly must be taken into account when analysing outbreaks and likely sources of introduction. In addition, effective trace-forward and trace-back activities are complicated by the rapid changes that can occur in poultry production and complex interactions between the different poultry sectors. Therefore, marketing systems should be studied and monitored regularly and frequently by the regulating Veterinary Services (50).

In a recent study, Kilpatrick et al. attempted to predict the pathways by which H5N1 had and could spread between countries (40). The authors integrated data on phylogenetic relationships of virus isolates, migratory bird movements and trade in poultry and wild birds to determine the pathway for 52 individual introductory events into various countries, and predict future spread. Assigning relative probabilities to the trade in poultry and wild birds, but also to natural movements of wild birds, the study suggested that most introductions of H5N1 into Europe probably came through the natural migrations of wild birds, whereas the spread through Asia and Africa involved both migratory birds and the poultry trade. The main indicator used for risk from the poultry trade was the legal trade in live poultry from infected countries, much of which involves day-old chicks. However, the legal and illegal trade in wild birds was also identified as an important potential pathway, unless all imported birds are quarantined and tested for AI. Using H5N1 as a model, Kilpatrick et al. concluded that determining the pathways by which HPAI is spread has crucial implications for predicting and preventing future outbreaks, by targeting controls at the main sources of introduction.

An assessment of the role of wild birds in the dissemination of HPAI is given elsewhere in this volume. All available information on the ability of wild birds to spread H5N1 viruses was also reviewed in a European Food Safety Authority (EFSA) risk assessment but there was considerable uncertainty about the conclusions (25). This is also true for the role of trade. Indeed, although the legal and illegal trade of live birds and bird products (with an emphasis on the specific role of poultry) may play a major role in the spread of HPAI, even over large distances, there is much speculation about the mode of entry of the virus into unaffected countries. During previous epizootics of HPAI subtypes H5 and H7, it was shown that the expansion of these viruses was due to human activities, in particular, movements of poultry or their products (1, 98, 99). Although the epidemiology is more complex, the same mechanisms have played a crucial role in the dispersal of the recent Asian HPAI H5N1 panzootics (55, 56). The authors will attempt to evaluate this situation in terms of the risk of spreading HPAI virus (HPAIV), with a focus on the recent H5N1 crisis in Asia.

**Definition of trade**

**Legal trade**

Poultry is the fastest-growing livestock industry of all, benefiting from efficiency gains in production and associated lower prices, as well as from public health considerations, since the crisis in beef sales due to bovine spongiform encephalopathy. Both per capita consumption and the poultry share of the meat market have shown a consistent increase in most countries. Projections are for these trends to continue, as consumer preference, as well as price, continues to favour poultry. Every day, millions of live poultry are moved around the world by ground, air and sea transport, and could potentially carry HPAI to fresh areas. Poultry production uses intensive housing and management systems to maximise the main advantages of poultry as a food source. Such intensification is accompanied by an increase in the incidence of disease. Since the poultry industry is a worldwide activity, using similar genetic stock in comparable holdings, all the major poultry-producing nations can expect similar disease problems.

Avian influenza has become a symbol of this new age of vulnerability. The globalisation of food trade, facilitated by the liberalisation of world trade, offers many benefits and opportunities, but also represents new risks for disease transmission, as infectious agents can be disseminated from the original points of production, processing or packaging to locations thousands of kilometres away on the same day (18). As an illustration of the importance of the poultry sector, in 2005 the United States of America (USA) imported more than 16.8 million day-old chicks and other live poultry, with similar figures for hatching eggs (8).
Increasing globalisation of the poultry-meat supply chain has led to the consolidation and evolution of transnational companies by vertical or horizontal integration, and the development of business ‘clusters’. There are significant benefits when transnational companies incorporate large parts of the food processing chain, from slaughterhouse to processing plant, into their enterprises; in particular, improved purchasing power and greater intellectual, technological and production resources for organisations to draw upon to provide products that meet different customer needs (52). Agro-industry, later called agribusiness, can be defined as the social production process that conditions, preserves and/or transforms raw materials whose origins are in agricultural, livestock or forestry production. Agro-industry plays a vital role within social production because it fills the seasonal and geographical gaps in the supply and distribution of agricultural produce, allowing the relatively constant consumption of agricultural products in the great population centres. In some instances, agro-industry transforms agricultural goods, modifying their particular characteristics to adapt them for consumption, which diversifies the ways the goods are consumed, and creates new commodities and products (9). This extensive diversification makes control of the poultry product chain increasingly difficult.

Twenty years ago, it was suggested that integrated pig-duck agriculture, an efficient food production system traditionally practised in certain parts of the People's Republic of China, puts these species into close contact and provides opportunities for creating new influenza recombinants that can be the starting point for a pandemic (77). More recently, it has been pointed out that, with high-intensity agriculture and the movement of livestock across borders, suitable conditions might exist not only in China, but also in Europe and elsewhere (46).

Commercial circuits can also play an important role in the dissemination of AI within a country. In their analysis of the environmental factors contributing to the spread of H5N1 in mainland China, Fang et al. (28) concluded that the transportation of poultry and their products along the highways contributed significantly to the long-distance spread of the disease. They established a predictive risk map for identifying the areas where surveillance, vaccination and other preventive interventions should be targeted. More recently, a supermarket bargain offer for frozen ducks in October 2007, in cities in Brandenburg county, was the origin of a silent spread of H5N1 virus in Germany (36). The insidious incursion of HPAIV H5N1 into the food chain was made possible by the absence of clinical signs in infected ducks, making syndromic surveillance useless. These findings strongly suggest the need for regular virological monitoring of duck flocks, as serology is often poorly indicative in this species.

Small-scale trade and live bird markets

Live bird markets are found in many parts of the world. They serve as a source of poultry meat for local populations who prefer to buy fresh poultry to eat. These farmers’ markets may be a source of AI infection in which healthy or even sick live birds carry the virus. In the USA, live bird markets have been recognised as a significant reservoir of AI virus (AIV) for poultry since 1986, when an epidemiological link was made between low pathogenic AIV (LPAIV) H5N2 infections in commercial poultry and the presence of the same lineage of LPAIV H5N2 in the live bird markets in the north-east of the USA (78, 80). Since that time, markets have been routinely monitored for the presence of AIV. In 1994, LPAIV H7N2 was introduced into the north-eastern live bird markets. This virus has persisted in the markets, despite efforts to eradicate it. Genetic analysis of isolates gathered over time shows that the H7N2 virus has been accumulating mutations (additional basic amino acids near the cleavage site of the H protein) that favour the emergence of a highly pathogenic virus. Since 1996, the lineage of the LPAI H7N2 virus found in live bird markets has been linked to at least eight LPAI outbreaks in commercial poultry, resulting in the destruction of millions of birds and an economic loss of millions of dollars to the poultry industry, including the costs of eradication and loss of export markets (80).

In Asia, live bird markets were the source of the HPAIV H5N1 that was transmitted to 18 people in Hong Kong, and killed six (81). This central role has also been shown in Hanoi, Vietnam, in 2001, where HPAIV H5N1 was detected in domestic birds in a live bird market (58), and in China (99). These so-called ‘wet markets’ are widespread in Asian countries and countries to which Asian people have migrated, and they are recognised as important reservoirs of H5N1 viruses, if the markets use a continuous flow system and especially if poultry are allowed to remain for longer than 24 hours on site (81). Mixing different species of domestic poultry (terrestrial poultry and waterfowl) in live bird markets is a common practice in most South Asian countries. This facilitates virus dissemination and evolution. Finally, these markets also represent a perfect interface where domestic and wild-caught birds are kept in close proximity, posing a high risk of cross-contamination. Live bird markets are therefore blamed for maintaining and spreading AIV and pose major challenges to veterinary and public health authorities. This is exemplified by recurrent findings of HPAIV H5N1 in bird markets, such as the Hong Kong market in June 2008, where the first outbreak of H5N1 in five years was declared, despite the implementation of ‘down periods’, when the bird markets are closed, and extensive controls (43, 71, 81). Similarly, although the last outbreak of H5N1 in Nigeria occurred in October 2007, at least four new incursions were recorded.
... if birds turn out to be responsible for entry of HPAI H5N1 into the Western Hemisphere, illegal import of an infected bird or bird product seems the most likely mode of entry. We base this conclusion on the fact that illegally imported birds, unlike infected, free-flying migrants, are provided food and water ad libitum and protected from predators, greatly increasing their chances of survival in an infectious state. Furthermore, these birds often end up in close association with other, similarly protected birds, sharing the same food or water, a situation that provides ample opportunity for viral transmission.

**Risks of introduction through trade**

The risk of introducing HPAI through trade depends on several factors, including:

- the ability of the importing country to demonstrate freedom from notifiable avian influenza (NAI) through adequate surveillance and diagnostics
- the type of NAI present (LPAI versus HPAI)
- the type of products traded
- the use of any treatment for virus inactivation.

Thomas and Swayne (88) have recently proposed a classification from highest to lowest risk, according to the specific product, as follows:

- a) live poultry (older than day-old)
- b) live birds other than poultry
- c) day-old poultry
- d) hatching eggs
- e) eggs for human consumption
- f) egg products; products derived from poultry, such as semen, raw meat and other untreated products
- g) products derived from poultry which have been treated to inactivate influenza viruses.

In general, live birds constitute the highest risk for transmission as the virus is replicating in the bird and shed into the environment. In addition, the risk of indirect dissemination through contaminated materials, supplies, transport and people (e.g. clothes, shoes, car tyres, etc.) is very real.
Risk of introduction through trade in live birds

Live poultry (other than day-old poultry)

All species of poultry can potentially be infected by every AIV subtype, including H5/H7 (20). Thus, live infected poultry are theoretically effective potential carriers for the introduction of AIV. In most poultry species, HPAIV infections induce clear clinical signs but could also evolve without clinical signs, depending on the virus strain and other factors, such as the age and species of poultry, and its immune status (2, 3, 12, 20). Clinical examinations conducted on live poultry before export, although essential, may miss HPAIV infection in a species that does not show clear clinical signs, such as ducks, or in birds that are incubating the infection. Indeed, domestic ducks may be asymptomatic carriers of NAI (12, 31, 33, 34, 61, 85, 86). Similarly, LPAI infections may induce no or only mild clinical signs in affected poultry and thus remain undetected (11). Finally, vaccinated birds, which are free from clinical signs but could still be infected by a field strain, might pose a risk of AIV introduction. All these situations may result in apparently healthy birds harbouring and spreading the infection (25).

Hatching eggs and day-old poultry

Avian influenza viruses are generally lethal for poultry embryos and infected eggs would probably not hatch in the hatchery of the exporting country. Day-old chicks might also be exposed to AIV, albeit for a more limited time than older birds, and are therefore also likely to be infected. It must be noted that eggs would have been in the incubator in the country of origin for 21 days, which means the infection should probably have become apparent in the parent flock before export took place, which would have led to export prohibitions (25, 27).

Highly pathogenic AIV have been found on the surface of and inside eggs laid by infected hens. Hatching eggs put into the incubators are fumigated with formaldehyde or subjected to other disease control measures, in accordance with European Union (EU) legislation (22) and the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code (Terrestrial Code) 2007 (103), to ensure that AIV are removed from their surface.

The risk of introducing LPAI infection into a country which imports hatching eggs from a country not known to be free from LPAI is mainly related to faecally contaminated materials (e.g. trays, packaging materials, etc.) which may be re-used in the importing country (106). However, legal requirements for fumigation and egg packaging are likely to reduce these risks to negligible levels (27).

Live wild or ‘exotic’ birds

The global movement of animals for the companion animal trade has been estimated at some 350 million live animals, worth approximately US$20 billion, per year (39). Approximately one-quarter of this trade is thought to be illegal, hence not inspected or tested (Fig. 1). Disease outbreaks resulting from trade in wildlife have caused hundreds of billions of dollars of economic damage globally (39). Before the 1970s, the lack of control on bird imports resulted in the introduction of so-called ‘exotic’ avian diseases, such as ND and AI, into the USA.

The outbreak of ND in poultry in Southern California between 1972 and 1974 (62) was linked to the uncontrolled movement of exotic birds from Central America into the USA. This outbreak caused the destruction of nearly 12 million chickens and the cost of the eradication campaign was estimated at US$56 million (57). It led to increased quarantine and testing requirements for importing birds into the USA (1974), UK (1976) and other countries (88). This decision was reinforced by the detection in the same region of 15 type A influenza viruses in 15 cases, involving 12 different species of exotic birds, including some that had already been distributed to various locations (83). In view of the enormous trade in captive birds caught in the wild, many countries imposed quarantines on imports during the 1970s. As a result, many more AIV from both passerine and psittacine species have been isolated since that time. Quarantine procedures include isolation in indoor, air-filtered cages and standard testing for common poultry diseases, including AI. This is not available everywhere in the world. Despite the implementation of these control measures, AIV have been isolated from smuggled or illegally imported birds on several occasions.

In the USA, Panigrahy and Senne (60) noted that the majority of AIV from caged birds came from passerine species. It may well be that passerines are the natural reservoir for these viruses (37), and that the spread to psittacine species occurs at holding stations or during transit. Another possible source of introduction is hunting for game with falcons and other trained raptors, which is practised in a number of countries around the world. The birds have close contact with humans and are highly domesticated, and yet the purpose for which they are kept means they also have contact with wild birds. In this context, it is important to note that the first incursion of the deadly Asian HPAIV H5N1 into the EU occurred in October 2004, with two illegally imported Thai eagles. In this case, a pair of crested hawk eagles (Spizaetus nipalensis), smuggled in hand luggage from Thailand to Belgium, were found to be carrying the virus (Fig. 2). Although they had shown no clinical signs, upon necropsy one bird presented bilateral pneumonia and H5N1 HPAIV was isolated from the lung material (95). Although these
birds were detected and quarantined, they serve as an example of how such imports could spread the virus. There have been two recent reports of AI infections of falcons. Manvell et al. (53) reported the isolation of an HPAIV of the H7N3 subtype from a peregrine falcon (*Falco peregrinus*), dying in the United Arab Emirates. The virus showed close homology with H7N3 viruses responsible for outbreaks in Pakistan four years earlier (6). During the HPAI outbreaks in Italy in 2000, an H7N1 virus was isolated from a saker falcon (*F. cherrug*) that died three days after normal hunting activity (51). There was a single isolation of the H5N1 HPAIV in Hong Kong in January 2004 from a peregrine falcon that was found dead (64).

In view of the largely illegal nature of such birds and their movement, fighting cocks represent a particular risk for
introducing AIV, as shown by HPAIV H5N1 isolations in Thailand (92) and Laos (70), where cock fighting is very popular. This resulted in the issuing of official disease control passports in Thailand (Fig. 3). The potential role of fighting cocks has been emphasised in the propagation and spread of ND virus (NDV) in western states of the USA between 2002 and 2003, and indicates how important such birds could be in the introduction and spread of AI (78, 79).

Risk of introduction through bird products

Eggs for consumption and egg products

Highly pathogenic AIV have been reported as being present on the surface and in the contents of eggs laid by infected hens (10, 88, 89). H5N1 HPAIV has also been isolated from washing the shells of waterfowl eggs confiscated from travellers entering China from Vietnam (45). There is no report of any natural infection of laying
birds with LPAIV that has resulted in eggs which contain the virus (48, 88), although LPAIV has been isolated from the oviduct (106).

Egg products are frequently obtained from eggs which have been downgraded from table eggs, often due to cracked shells. As a result, these products may be considered to have a greater risk of contamination with faeces/virus than intact table eggs, if they have not been treated in a way that would reduce the likelihood of virus survival to an acceptable level. The OIE Terrestrial Code contains guidelines for the inactivation of AI in eggs, egg products and meat (103). Swayne and Beck (89) conducted a series of experiments aimed at assessing the heat inactivation of an H7N2 LPAIV, an H5N2 HPAIV, two low-virulence NDV and a virulent NDV in various egg products at commercial temperatures. They considered that the industrial standard of 54.4°C for seven days for dried egg white would be inadequate for the acceptable heat inactivation of an HPAIV but adequate to inactivate LPAIV and NDV. The standard of 67°C for 15 days would be sufficient to inactivate HPAIV.

**Fresh poultry and meat products**

It is well recognised that HPAIV may be detected in the muscle tissues of infected birds (49, 63, 88, 92, 93, 94). Particular consideration should be given to fresh duck meat. Ducks usually remain healthy when infected with HPAIV, although they become viraemic and the virus may be isolated from their internal organs (94, 100). For instance, HPAIV H5N1 has been isolated from duck meat imported into Korea (93, 94). On the other hand, there have been very few reports in which the presence of LPAIV in meat has been investigated in either experimental or field infections of poultry. Kishida et al. (42) reported the isolation of LPAIV H9N2 from imported chicken meat and were able to demonstrate the presence of the virus in the muscles of chickens which had been experimentally infected with the isolated virus. However, other studies failed to detect the virus in skeletal muscles or meat (88).

Poultry meat products undergo different forms of treatment, mostly heat treatment, which is likely to reduce the potential level of contamination with viable virus to an acceptable level. Influenza viruses are usually considered to be heat labile. The figures generally quoted are that influenza viruses are inactivated by heat-treating for 15 min at 56°C or for 5 min at 62°C (20, 41). Two studies (90, 91) suggest that the standard cooking temperature of 70°C would inactivate AIV and NDV in infected poultry meat within a few seconds. However, other factors, such as the level of contamination and the probability of virus survival in some quantities of the product, still need to be assessed (25).

**Semen, feathers and down and waste**

Although it has never been reported, there is a theoretical risk that HPAIV may be present in the semen of infected viraemic poultry. Using standard artificial insemination procedures, Samadieh and Bankowski (76) were able to show that turkey hens became infected when given semen experimentally contaminated with AIV. There is also some international trade in and movement of semen from birds other than poultry, usually zoo birds or endangered species. The risk of semen being infectious is probably slightly higher than for poultry, because of the uncertainty of the status and susceptibility of the donor birds to AI infection (27).

Feathers and down from poultry, especially ducks and geese, are used as a filling for duvets, pillows, thermal clothing and other textiles. The highly pathogenic H5N1 strain has been isolated from the skin of experimentally inoculated ducks and geese and, although this has not been demonstrated for other AIV so far, the detection of viral antigens and observation of the virus in the feather epidermis raise the possibility of feathers as a source of infection (104). In addition, as the feathers are removed from the carcasses after slaughter, or harvested from live birds, i.e. down feathers from the breasts of geese, they can be contaminated with infectious faeces or other body fluids.

Birds infected with HPAIV excrete virus particles in their faeces, which can remain infectious for several weeks.
inside organic matter (47). The transportation of droppings or waste to fertilise fish farms in Asia, Eastern Europe and Africa could also be a major route of dispersal (30, 56). Indeed, the widespread practice of using poultry faeces (chicken, duck and other poultry) or wild bird manure (‘guano’) in agriculture and aquaculture as fertiliser (77), and in untreated form as food for pigs and fish, would need more consideration as manure provides a potential new source of infection.

International, national and regional measures and regulations

Checking imports at border inspection posts is one of the main safeguards for ensuring that imports of live poultry and avian products meet the required legal standards. In the EU, information on import requirements is laid down in Council Directive 90/539/EEC (22). Live animals and products of animal origin must undergo veterinary checks, which include a ‘documentary check’ of the accompanying certificates and an ‘identity check’ to ensure that the animals are as described in the documentation, and may include a ‘physical check’ to ensure the animals do not show any signs of poor health. However, these measures cannot avoid the introduction of AIV-infected poultry if they do not exhibit clinical signs (23). There are no specific measures in force at present to avoid the introduction of AIV by poultry species that are clinically far less susceptible, and thus show few or no signs, such as ducks. For instance, in Europe, the Commission approves border inspection posts when the Member State in which the border inspection post is located has given adequate guarantees that the post conforms with EU requirements (see list in 24). Border inspection posts are operated under the authority of the individual Member State and that State is responsible for implementing the proper import controls. To ensure that border inspection posts are functioning efficiently and uniform rules are applied, the Food and Veterinary Office (FVO) conducts inspections. The frequency of these inspections is determined by:

a) assessing the potential risks for animal and public health in the EU

b) the quantitative and qualitative patterns of trade, including:

– the type and species of animals or products of animal origin concerned

– relevant information on possible illegal imports and the potential risk of disease introduction

– data collected by the TRACES system (TRAde Control and Expert System), which monitors the movement of animals and animal products within the EU (24)

– the history of previous inspections.

The conclusion reached in the inspection reports of the FVO is that these safeguards are not 100% effective in ensuring that everything which enters the EU ‘legally’ conforms to EU animal and veterinary public health requirements (23, 25, 27).

When importing live exotic birds other than poultry, quarantine procedures include isolation in indoor, air-filtered cages and standard testing for common poultry diseases, including AI. However, these measures are not practised everywhere in the world. According to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the EU has been the major importer of wild birds, with around 800,000 birds imported each year from 1999 up until 2005 (see below). A large proportion of the birds imported into the EU were transported over considerable distances within the EU from the border inspection post before arriving at the final quarantine station. Therefore, it is recommended that the distances that birds are transported between the border inspection post and quarantine station should be reduced to a minimum. In 2005, as HPAIV H5N1 was shown to be endemic in several regions of Asia and Africa, an EU-wide ban on the importation of exotic birds was declared, as were stricter rules on the private ownership of these birds. The EFSA Panel on Animal Health and Welfare adopted, during its meeting of 26 to 27 October 2006, a scientific opinion on the animal health and welfare risks associated with the import of wild birds, other than poultry, into the EU. This opinion emphasises, in particular, the fact that data on the imports of such birds are scarce and identifies possible tools and options to reduce any identified animal health risk due to these imports (26). The temporary ban became permanent after the adoption of the opinion and led to a new regulation on controls conducted in third countries that export birds, other than poultry, to the EU (23). Improvements at the point of export should have the most impact in reducing the probability of infected birds entering the Community and import conditions should be laid down to ensure that only third countries which are authorised to import such birds into the EU can do so.

Two main international agencies, the Food and Agriculture Organization of the United Nations (FAO) and the OIE, play an essential role in promoting the safe trade of livestock and animal products at regional, national and international levels. To this end, programmes and activities have been developed to aid in controlling transboundary and emerging diseases (18). Regional regulations, such as those of the EU and the United States Department of
Agriculture (USDA) etc., must conform to the requirements of these international bodies. The recommendations from various international bodies, including the OIE, FAO, EU and USDA, are addressed elsewhere in this volume.

The best method of ensuring fair and safe trade is to import birds and bird products from NAI-free countries only. Exporting countries must prove the freedom of their country, zone or specific compartment from NAI by both passive and active surveillance, according to the recommendations on AI in the OIE Terrestrial Code 2007 (88, 103).

Illegal importation is, by its nature, extremely difficult to control. Illegal importation may be attempted at recognised international entry points by incorrect and/or misleading documentation or simply by concealment, without any attempt to make a declaration. This may occur at either a border inspection post or other recognised international border entry point, including those for land, sea and airport entry. Detection then relies upon the vigilance of the enforcement authorities and the development of specific detection systems, such as x-rays and detection dogs (Fig. 4). A detection dog is trained to use its senses (almost always that of smell, so they are often called ‘sniffer dogs’) to detect substances such as explosives, illegal drugs or blood. Some receive specific training to identify illegal imports of live birds, cooked and raw poultry meat and eggs that may carry the H5N1 virus, and are used by customs officers for secret checks on flights from affected areas. For instance, sniffer dogs are being used in the UK at Heathrow, Gatwick and Manchester airports to screen passengers and baggage from China, Russia, Egypt, Thailand and Vietnam for possible smuggled food (21).

Finally, illegal importation may be attempted by avoiding border entry points altogether. For instance, small boats or aircraft may try to enter at places other than ports or international (customs) airports, and vehicles may try to cross borders secretly, well away from border posts. Detection then relies upon the vigilance of the enforcement authorities. However, no information is currently available on the probability or frequency of smuggling by these routes, although it can be estimated to be considerable.

Since wildlife markets are networks with major hubs, these trading points provide opportunities for control measures with maximum effect (17). Focusing efforts on wildlife markets, to regulate, reduce or, in some cases, eliminate the trade in wildlife altogether, could provide a cost-effective approach to lessening disease risks for humans, domestic animals, wildlife and ecosystems (39).

**Molecular fingerprinting for tracing sources of introduction of avian influenza viruses**

Since the movements of poultry and birds are often difficult to trace, the signature or ‘identity card’ of each isolated virus can be very informative. Indeed, sequencing...
the genes of HPAIV H5N1 and other AIV has assisted greatly in establishing links and differences between H5N1 virus isolates from different countries, and tracing possible sources of introduction. The first two incursions of HPAIV H5N1 into Europe, which occurred in 2004 and 2005, were rapidly traced with the help of their molecular signature. The first was detected when eagles smuggled from Thailand and confiscated at Brussels Airport in Belgium were shown to be infected with an HPAIV H5N1 strain genetically similar to those isolated in Thailand (95). The second was uncovered when investigations into the deaths of captive parrots in quarantine in the UK, ostensibly from Taiwan, showed the cause of death to be HPAI H5N1 infection. Since Taiwan was free of H5N1, it is believed that the parrots were infected by other birds in quarantine and, indeed, the virus was genetically closest to viruses isolated in China (66).

In some cases, geographically distant viruses can be shown to be closely related at the molecular level, as in the case of an H5N1 outbreak on a turkey farm in Suffolk, UK, in 2007. Sequencing data indicated the likely origin by uncovering more than 99% homology with a contemporary isolate present in domestic waterfowl in Hungary (38). Likewise, based on sequence data, it has been suggested that some strains of H5N1 HPAIV in Vietnam may have originated in China (16, 97). Similarly, progenitors of Indonesian H5N1 viruses have been identified in Hunan Province in southern China (96). The poultry trade may be responsible for introducing the virus into Vietnam, while the transmission route from Hunan to Indonesia remains unclear. Strains of H5N1 HPAIV isolated in Japan and the Republic of Korea in 2003 and 2004 are very closely related, suggesting a common (but unknown) origin (54). The most closely related virus to these was detected in poultry in southern China. On the other hand, viruses introduced to these countries in 2006 and 2007 were closely related to each other, but not to the 2003 and 2004 viruses (104). This indicates new incursions of virus rather than persistence of the earlier strain.

In other cases, molecular data demonstrated the independent introductions of viruses in different countries. Sequencing of the viruses isolated in France and Germany, during the incursions of HPAIV H5N1 into Europe during 2006 and 2007 (32, 87), indicated multiple but separate introductions of the virus. Moreover, molecular investigations suggest initial introductions of separate clades of H5N1 HPAIV into Thailand (clade 1) and Indonesia (subclade 2.1), followed by disease spread within each country (16, 84). Additional incursions have subsequently been detected in Thailand (84) but not Indonesia, where only the initial strains have persisted and evolved into further subclades. The precise origin or mode of entry of these viruses is not known. The most closely related viruses are those isolated in China in 2003 (102). Genetic data also suggest multiple incursions of H5N1 HPAIV into Vietnam (84) and Hong Kong, with the latter exposed to many different genotypes since 1997. This reflects the broad genetic diversity of H5N1 viruses in the region over this period, starting with the original goose viruses (46).

Molecular studies have also demonstrated that the evolution of HPAIV H5N1 is continuing, as shown by the constant emergence of new virus sublineages. For example, viruses belonging to the new subclade 2.2 were detected in wild birds in Qinghai Province in China in 2005 (15, 16, 105), and later found in Europe and Africa. Other new lineages that have been detected in the last few years include subclade 2.3 viruses detected in south-east China and Southeast Asia since 2005, clade 4 viruses in Guiyang Province, and clade 7 viruses in Shanxi Province in China in 2006 (99).

Molecular typing of other AIV subtypes circulating in the infected region also proved very useful in studying the evolution and dynamics of H5N1, since many viruses appeared to be reassortants. Indeed, the 2003 and 2004 epidemics in Southeast Asia were due not to the introduction and spread of a single virus, but to multiple viruses which were genetically linked to the goose/GD/96 lineage via the haemagglutinin gene (16, 82). Similarly, phylogenetic analysis of H5N1 subtypes isolated from Vietnam during 2005 and 2007 showed that multiple sublineages were present. Clade 2.3.4 viruses have replaced clade 1 viruses in northern Vietnam, whereas clade 1 viruses are still detected in southern Vietnam. Furthermore, reassortment between these two sublineages has occurred (58). Similar evolution has also been recently described in Indonesia (44). Phylogenetic classification of these reassortant viruses can provide clues about their genetic, temporal and geographic origins.

Accumulating and sharing gene sequences has thus become a priority. This is the aim of the Global Initiative on Sharing Avian Influenza Data (GISAID), a project which aims to expand and complement existing efforts with the creation of a global consortium (http://gisaid.org), which would foster the international sharing of AI isolates and data (7, 13, 102). These findings have already shown how whole-genome analysis of influenza (H5N1) viruses is instrumental in a better understanding of the evolution and epidemiology of this infection, which is now present on the three continents that contain most of the population of the world (75). These and related analyses will fingerprint each H5N1 virus, as well as other subtypes, not only allowing their unequivocal identification but also tracing their origin and adding them to the watchlist for future epidemiological surveillance. This should help in understanding the dynamics of infection between wild and
domesticated bird populations, as well as the role of trade and human activities in the dissemination of AIV, which, in turn, should promote the development of more targeted control and prevention strategies.

Case studies of H5N1: examples from China, the United Kingdom and Nigeria

China

The range of genotypes and considerable variability within individual genotypes demonstrate the presence of a large pool of H5N1 HPAIV circulating in China before and during the 2003 to 2004 epizootic (16, 99). Genetic characterisation of isolates from south-east China, Vietnam, Thailand and Indonesia has shown that H5N1 HPAIV was introduced into various regions of Southeast Asia from southern China between 2001 and 2005 (16), but few detailed studies have been conducted on the mode of introduction. Evidence from Hong Kong demonstrated that the trade in live poultry could be a potential source of infection. Between 1999 and 2001, geese and ducks sent from mainland China to a central slaughtering facility in Hong Kong were repeatedly found to be infected on arrival (14, 35). The large volume of trade in live chickens and other poultry between Guangdong Province in China and Hong Kong was also implicated as a source of infection from 2001 to 2003. During this period, the H5N1 virus genotypes found on farms in Hong Kong were less varied than those detected in live poultry markets (35, 46). These markets were supplied with poultry from farms in Hong Kong and Guangdong Province, suggesting that local farms were not the only source of infection for poultry markets.

United Kingdom

Although wild birds are now accepted as having played an important role in the introduction of H5N1 into Europe, one explanation for the relatively few outbreaks noticed during 2006 and 2007 might be the generally high biosecurity standards of European poultry holdings. The UK represents one example of a well-prepared country that encountered three different probable sources of introduction:
- wild birds
- exotic birds
- the (illegal) trade in poultry meat.

The UK first reported HPAIV H5N1 in early 2006, when one wild mute swan washed up on the shores of Scotland and tested positive for the presence of antibodies against the virus. Later, in early 2008, HPAIV H5N1 was found to have caused the deaths of mute swans at Abbotsbury Swannery, a sanctuary located nine miles from Weymouth. The source of introduction was attributed to migrating birds (73). Before that, in 2005, an outbreak of HPAIV H5N1 at a bird quarantine station had been attributed to smuggled birds 'launed' (i.e. concealed among legitimate imports) in a legally imported consignment of birds (67). Then, in February 2007, an outbreak of HPAIV H5N1 occurred on a single turkey farm in Suffolk, and the trade in poultry meat was implicated as the most likely source of infection. Indeed, the source of the virus was not clear but epidemiological investigation, including sequence analysis, indicated that the introduction probably came from imported fresh turkey meat from a subclinically infected flock in Hungary. As a result, the company, Bernard Matthews Foods, ceased selling turkeys raised outside the UK, a move designed to address concerns that the outbreak of 'bird flu' in 2007 may have been caused by infected birds imported from sub-contractors in Hungary (38, 73).

Nigeria

The situation in Africa is less clear. Nigeria was the first country in Africa to report the emergence of this highly pathogenic virus. The outbreaks in Nigeria in early 2006 may have been caused by a supply of infected live poultry, including day-old chicks, from different sources, including East Asia and Turkey. Sampling of 5,000 wild water birds in African wetlands supported this view, since no evidence of HPAIV H5N1 was found, indicating that wild birds probably played a relatively minor role in the spread of AI in this case. Instead, the considerable movement of poultry and poultry products into Nigeria points to trade as the more likely source of introduction (33). The spread of the virus to other countries in West Africa may well have occurred through the movement of poultry and poultry products, or the spread of fomites, but this, too, remains speculative. The Nigerian authorities, however, immediately focused on the poultry sector, one of the largest and most industrial in sub-Saharan Africa. Avian influenza broke out again on a single farm with over 40,000 birds. Nevertheless, first analyses of H5N1 sequences in poultry from two different farms in Lagos showed that three H5N1 lineages were independently introduced, and were more similar to strains previously identified in Europe, South Asia and Egypt (19). As these introductions appeared through routes that coincided with the flight paths of migratory birds, this pathway was also considered.

Recent phylogenetic analyses of 35 newly isolated viruses suggest that Nigeria was infected by multiple sources of the H5N1 virus, classified into six sublineages (29). This study...
points out that the West African sub-region operates as a free trade zone, with poor quarantine and border controls, and it is very likely that the trans-border movements of humans, along with trade in poultry and poultry products and weak biosecurity, played a significant role in the spread of a large cluster of diverse viruses from infected African countries. In conclusion, the authors stated that H5N1 introductions into Nigeria were linked to commercial poultry and not wild birds (29). Along with these findings, in August 2008, laboratory results from Nigeria and the OIE Reference Laboratory of Padua in Italy showed that a newly discovered virus strain (H5N1, clade 2, subclade EMA3) is genetically different from the strains that circulated in Nigeria during earlier outbreaks in 2006 and 2007. This new strain has never before been reported in Africa and is more similar to strains previously identified in Italy, Afghanistan and Iran in 2007 (72).

As with many countries, it is still not known whether the first cases of infection detected in Nigeria were, in fact, the first cases to occur. Moreover, despite the fact that molecular data suggest that wild birds cannot be ruled out, trade seems to have played a significant role in H5N1 epidemiology in Africa.

Conclusions

Trade in poultry and poultry products occurs on a global scale. While this means that the best breeding and commercial stock are available to producers, and offers consumers increasingly greater choice, it has potential drawbacks in terms of the spread of poultry diseases between trading partners. It is important to assess and manage these disease risks to ensure that global trade does not result in the dissemination of poultry diseases. However, it is also important that the risk of disease should not be used as an unjustified trade barrier. The role of the regulatory authorities is to facilitate the safe trade of animal products, according to international guidelines. However, a delicate balance must be found between the risks of disease and the ensuring of safe trade.

Conclusions on the source of infection are often based on a process of elimination, or on associations and probabilities, rather than definitive evidence. Disease investigations are time consuming and require adequate resources and appropriately trained investigators. When the disease outbreak occurs, the focus should preferably be placed on investigation rather than solely on control. Even in developed countries, control and eradication of the disease have taken priority over investigation (5). Unfortunately, in countries with limited veterinary resources, investigations are often relegated to a secondary role or even neglected entirely, due to the cost of control programmes. This situation is improving in many countries, as a result of the increased funding of Veterinary Services, and it is expected that the quality of data on disease outbreaks and the prevalence of infection will improve in the future (81).

Improvements in the quality of data collection and reporting, including molecular fingerprinting and tracing, are crucial in understanding general patterns in outbreaks, possible routes of transmission and the potential impacts on migratory bird populations. This information can be used to target contingency efforts, predict future outbreaks and guide effective policy to reduce the economic and conservation impacts of AI. There are several ways in which HPAI can be spread within and between countries. Three major potential routes are the movements of infected poultry (and poultry products), movements of caged wild birds through trade, and natural movements of wild birds (see 65 as a pertinent example). Effective responses in controlling disease through strong veterinary scrutiny and effective management of trade need to focus on all three of these possible methods of spread if they are to be successful.

Le rôle du commerce légal et illégal de volailles vivantes et de produits aviaires dans la propagation de l’influenza aviaire

T. van den Berg

Résumé

La panzootie d’influenza aviaire hautement pathogène due à la souche virale H5N1 a pris la proportion d’une véritable crise internationale. Toutes les régions du monde sont désormais considérées à risque, du fait de la mondialisation des échanges qui se traduit par des mouvements planétaires d’animaux, de produits
et d’êtres humains, mais aussi en raison des risques de propagation du virus par les migrations d’oiseaux sauvages. Le risque d’introduction de l’influenza aviaire à déclaration obligatoire dans le cadre des échanges internationaux dépend d’un certain nombre de facteurs, dont le statut sanitaire du pays exportateur et le type de marchandise commercialisée. Le risque le plus élevé concerne les échanges de volailles vivantes. Il est essentiel de conduire une évaluation et une gestion du risque afin que le commerce mondial ne soit pas la cause d’une dissémination de l’influenza aviaire à déclaration obligatoire. Il faut aussi veiller à ce que le risque d’infection ne serve pas d’argument pour dresser des barrières injustifiées au commerce. Le rôle des organismes de réglementation est de faciliter le commerce sécurisé de produits d’origine animale, en conformité avec les lignes directrices internationales.

Néanmoins, il n’est pas aisé de trouver un juste équilibre entre le niveau de risque acceptable et la sécurisation des échanges. Étant donné que les mouvements de volailles et d’oiseaux sont parfois difficiles à suivre, il est très instructif d’établir la signature ou « carte d’identité » de chaque virus isolé. Le séquençage des gènes des virus H5N1 et d’autres virus de l’influenza aviaire a été d’un grand secours pour repérer les similarités et mettre à jour les différences entre les isolats obtenus dans différents pays, ainsi que pour remonter jusqu’à la source éventuelle d’introduction du virus. Les épisodes récents survenus en Asie, en Europe et en Afrique, et les techniques d’empreinte moléculaire appliquées aux virus H5N1 ont montré que diverses sources d’introduction sont possibles et qu’aucune voie ne doit être négligée.

**Mots-clés**


Consecuencias del comercio legal e ilegal de aves vivas y subproductos aviares en la propagación de la influenza aviar

T. van den Berg

**Resumen**

La panzootia de la cepa H5N1 del virus de la influenza aviar altamente patógena dio origen a una crisis internacional. Actualmente se considera que la enfermedad amenaza a todas las regiones del mundo debido al transporte internacional de animales, mercaderías y seres humanos consiguientes a la mundialización del comercio, así como a la posible propagación del virus por conducto de las migraciones de aves silvestres. El riesgo de introducir la influenza aviar de notificación obligatoria por medio del comercio depende de varios factores, comprendidos el estatus sanitario del país exportador y las categorías de productos adquiridos. Las transacciones de aves vivas son las que mayores peligros entrañan. Para evitar que el comercio mundial se transforme en una vía de diseminación de la enfermedad de notificación obligatoria es preciso determinar y evaluar los riesgos. Pero también es importante impedir que el riesgo de infección se convierta en una barrera comercial injustificada. La función de los organismos reglamentarios consiste en facilitar las transacciones seguras de productos animales conforme a las normas internacionales.
No obstante, es difícil alcanzar un equilibrio entre riesgos aceptables y comercio seguro. Como en algunos casos puede resultar difícil rastrear los desplazamientos de aves de corral y silvestres, la creación de una “signatura” o “documento de identidad” de cada virus aislado puede ser muy provechosa. La secuenciación de los genes del subtipo H5N1 y demás virus de la influenza aviar ha sido de gran utilidad para determinar homologías y diferencias entre las muestras de distintos países y rastrear el origen de la introducción de la enfermedad. En Asia, Europa y África, la “signatura” molecular del H5N1 permitió demostrar recientemente que la introducción puede llegar por múltiples rutas y, por consiguiente, que ninguna debe subestimarse.

Palabras clave

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