The scientific rationale for the World Organisation for Animal Health standards and recommendations on avian influenza

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Summary

The World Organisation for Animal Health (OIE) prescribes standards for the diagnosis and control of avian influenza, as well as health measures for safe trade in birds and avian products, which are based on up-to-date scientific information and risk management principles, consistent with the role of the OIE as a reference standard-setting body for the World Trade Organization (WTO). These standards and recommendations continue to evolve, reflecting advances in technology and scientific understanding of this important zoonotic disease. The avian influenza viruses form part of the natural ecosystem by virtue of their ubiquitous presence in wild aquatic birds, a fact that human intervention cannot change. For the purposes of the *Terrestrial Animal Health Code* (*Terrestrial Code*), avian influenza is defined as an infection of poultry. However, the scope of the OIE standards and recommendations is not restricted to poultry, covering the diagnosis, early detection and management of avian influenza, including sanitary measures for trade in birds and avian products. The best way to manage avian influenza-associated risks to human and animal health is for countries to conduct surveillance using

recommended methods, to report results in a consistent and transparent manner, and to apply the sanitary measures described in the *Terrestrial Code*. Surveillance for and timely reporting of avian influenza in accordance with OIE standards enable the distribution of relevant, up-to-date information to the global community.

Keywords

Avian influenza – Diagnosis – Notification – OIE – Pig – Poultry – Terrestrial Animal Health Code – Wild bird – World Organisation for Animal Health – World Trade Organization.

Introduction

This report is in two parts. Part one reviews scientific information relevant to the diagnosis, pathobiology, ecology and epidemiology of avian influenza, including scientific findings and conclusions on the role played by wild birds and pigs in the circulation of avian influenza viruses. Part two relates this scientific information to the international standards set by the World Organisation for Animal Health (OIE). The OIE is an intergovernmental organisation, established in 1924, with the mandate to support international solidarity in the control and prevention of highly contagious animal diseases and transparency in reporting listed diseases. The OIE *Terrestrial Animal Health Code* (*Terrestrial Code*) contains science-based standards and recommendations on listed animal diseases and zoonoses, including avian influenza (1). In addition to supporting early detection and rapid response, application of the *Terrestrial Code* standards can be relied upon to prevent the diseases spreading via international trade. Part two specifically refers to the *Terrestrial Code*, Chapter 10.4. ('Avian influenza') and related texts, as well as Chapter 2.3.4. of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*) (2).

This report illustrates how advances in the scientific understanding of avian influenza have driven the evolution of the standards and recommendations contained in the *Terrestrial Code*. Unless otherwise

indicated, all *Terrestrial Code* citations refer to the 22nd edition, 2013.

Part 1: Diagnosis and reporting of avian influenza

Early scientific thinking on avian influenza

The first account of avian influenza dates back to 1878 when Edoardo Perroncito, a professor of pathology and parasitology at the Veterinary Faculty of the University of Torino, Italy, described a contagious disease that caused high flock mortality, affecting chickens and turkeys in northern Italy (3). In 1901 Centanni and Savonuzzi (4) showed that the agent responsible for 'fowl plague', a term that was later coined by Beaudette (5) in 1925, was an ultra-filterable agent. This eliminated the initial confusion with the acute septicaemic form of fowl cholera caused by *Pasteurella multocida*. However, Newcastle disease, which was first described in 1926 (6, 7), and is clinically similar to fowl plague in that it is capable of causing high flock mortality in susceptible poultry, continued to cause confusion in the diagnosis of fowl plague. In 1934, Burnet and Ferry (8) demonstrated that the two diseases were caused by different viruses. As explained in the review by Alexander and Brown (9), all the viruses recognised as avian influenza in the first half of the 20th Century were fowl plague virus isolates (all considered to be the same virus), belonging to what is now recognised as the H7 subtype. Outbreaks of fowl plague that were associated with imports from northern Italy were widespread across Europe during the early part of the 20th Century (10) and were made notifiable in some of these countries as early as 1903.

Scientific understanding of avian influenza after 1955

The relationship of highly pathogenic avian influenza (HPAI) virus with other influenza viruses began to emerge in 1955, when Schäfer (11) showed that 'fowl plague' viruses shared common internal antigens with influenza viruses from humans and swine. Shortly afterwards, it was demonstrated that two viruses that were shown not to kill experimentally inoculated chickens (one virus isolated from chickens in Germany in 1949 [12] and one isolated from ducklings in

Manitoba, Canada, in 1952 [13]) were also influenza viruses. In 1956, two low pathogenicity avian influenza (LPAI) viruses, which were antigenically distinct from HPAI virus, were isolated from commercial ducks with respiratory disease. These viruses, currently known as A/duck/Czechoslovokia/1956 (H4N6) and A/duck/England/1956 (H11N6), along with the German 1949 and Manitoba 1952 isolates, gave the first indications of the variable nature of influenza viruses with respect to antigenicity and virulence.

A conceptual shift in the scientific understanding of the influenza viruses that could cause HPAI began with the isolation of two viruses that were associated with fowl-plague-like disease but that were antigenically distinct from classical fowl plague virus, which belongs to the haemagglutinin antigen avian 1 or Hav1 group. The first involved a self-limiting outbreak on a small chicken farm in Scotland in 1959 and the second involved a die-off of European common terns (*Sterna hirundo*) in the Cape area of South Africa in 1961 (14). Both of these viruses, known respectively by their current nomenclature as A/chicken/Scotland/1959 (H5N1) and A/tern/South Africa/1961 (H5N3), were antigenically related to each other but did not belong to the Hav1 group. In March/April 1966 an influenza virus was isolated from turkeys in an outbreak of acute disease in an extensive turkey-breeding establishment in Ontario, Canada (15). This highly pathogenic variant, A/turkey/Ontario 7732/1966, was related serologically by surface antigens to A/chicken/Scotland/1959 (H5N1) and A/tern/South Africa/1961 (H5N3) and was later designated as A/turkey/Ontario 7732/1966 (H5N9). These three viruses were placed in the Hav5 subtype. To complicate matters further, Beard and Easterday (16) reported the isolation of A/turkey/Oregon/1971, a Hav1 Nav2 virus that was found by laboratory studies to be non-pathogenic for chickens, despite possessing the haemagglutinin of classical fowl plague virus. The existence of viruses that were of high virulence for poultry but were antigenically distinct from fowl plague viruses, as well as viruses that were antigenically related to fowl plague but were avirulent for chickens, demonstrated the need for interventions by Veterinary Authorities to be based on appropriate criteria and definitions of avian influenza viruses. At the time it was

concluded that government intervention should be limited to influenza viruses of demonstrated virulence to poultry. Reports evaluating the use of *in vivo* tests for the virulence of avian influenza virus isolates started to appear in the literature in the late 1970s (9). The first of these (17) showed that the intravenous and intracerebral pathogenicity index tests, which were normally used for Newcastle disease virus isolates, could also be used to quantitatively measure the virulence of avian influenza viruses. These tests also showed a lack of correlation between virulence and antigenic type.

First official use of the term 'highly pathogenic avian influenza'

At the First International Symposium on Avian Influenza, held in Bethesda, Maryland, the United States of America (USA) in 1981, it was resolved that the term 'fowl plague' be abandoned and replaced with HPAI. It was agreed that HPAI virus strains should be defined by their ability to produce not less than 75% mortality within eight days in at least eight susceptible four-to-eight-week-old chickens inoculated by the intramuscular, intravenous or caudal air sac routes with bacteria-free infectious allantoic fluid or cell culture fluids (18). This definition was adopted by the World Organisation for Animal Health (OIE) in 1983. At the same symposium, Rudolf Rott presented a summary of the work that he and his co-workers had carried out, which showed that the pathogenicity of an avian influenza virus isolate was related to the proteolytic cleavage of the viral haemagglutinin (HA) into amino-terminal HA_1 and carboxy-terminal HA_2 subunits (19). They found that the haemagglutinins of non-pathogenic Hav1 strains had a cleavage site that was structurally similar to that of human influenza viruses while the haemagglutinins of the pathogenic strain had significantly more basic amino acids within the connecting peptides (19, 20). This set the stage for understanding the molecular basis of pathogenicity.

It is now understood that, in order for influenza A viruses to be infectious, the haemagglutinin precursor, HA_0, must be post-translationally cleaved by host proteases into HA_1 and HA_2 subunits

(21), thereby exposing a fusion peptide at the newly formed amino-terminal end of HA_2 (22). Pathogenicity is determined by which host protease carries out this post-translational cleavage. The HA_0 precursor of LPAI viruses has a single arginine at the cleavage site and another basic amino acid (arginine or lysine) at position –3 or –4. This cleavage site is recognised by extracellular host proteases such as trypsin that are secreted by cells that line the respiratory and digestive tracts. In contrast, the HA_0 precursor of highly pathogenic viruses contains multiple basic amino acids at its cleavage site, which is recognised by a family of intracellular proteases known as subtilisin-related proteases, of which furin is a leading candidate (23). This latter class of proteases has a much broader tissue distribution which is directly related to the ability of highly pathogenic viruses to replicate systemically. It is now generally accepted that highly pathogenic viruses evolve from low pathogenicity virus precursors (24, 25). On current evidence, this evolution appears to be restricted to viruses of the H5 and H7 haemagglutinin subtypes. In the majority of cases, this evolution appears to take place after low pathogenicity viruses have been introduced from their natural wild bird reservoir into gallinaceous poultry. The acquisition of basic amino acids at the HA_0 cleavage site, which is associated with the evolution to high pathogenicity, can occur by a number of different mechanisms, including:

– nucleotide substitution

– the duplication of purine triplets due to polymerase slippage (26), which results in the insertion of basic amino acids at the cleavage site, and

– non-homologous recombination with cellular or viral RNA (27, 28, 29).

Evolution of understanding of the origins of highly pathogenic avian influenza viruses

In April 1983, an H5N2 infection involving chickens, which presented as acute respiratory disease, declining egg production and increased mortality, was detected in Lancaster County, Pennsylvania. This virus

was initially characterised as being of low pathogenicity, based on *in vivo* tests, and so did not meet the definition of HPAI that had been proposed at the First International Symposium on Avian Influenza and subsequently adopted by the OIE. For this reason, statutory control measures were not brought into play. An additional 25 cases were diagnosed between April and October 1983 and none of the viruses isolated from these was highly pathogenic for inoculated chickens or turkeys (30). A dramatically different form of the disease appeared in early October and the virus that was isolated from these new cases was characterised as highly pathogenic, based on *in vivo* tests. Control measures were immediately introduced, including stamping out. However, diagnosis and control were complicated by the earlier and continued presence of the virus that exhibited the lower virulence phenotype. In total, the outbreak resulted in the loss of over 17 million birds in Pennsylvania and the surrounding states. Both low pathogenicity and highly pathogenic isolates possessed the identical cleavage site motif PQKKKR*GLF, typical of highly pathogenic viruses (31). It was later shown that a point mutation which resulted in a lysine replacing a threonine at amino acid residue 13 caused the loss of a glycosylation site present in the LPAI virus and led to the expression of the highly pathogenic phenotype. The carbohydrate chain associated with this glycosylation site had presumably prevented subtilisin-related proteases but not trypsin-like proteases from gaining access to the HA_0 cleavage site (32).

A subcommittee of the United States Animal Health Association was established to deliberate the problems that had been encountered in characterising the pathogenicity of the virus responsible for the 1983 epizootic. It made the following recommendations:

– to retain the *in vivo* test for pathogenicity testing but to limit it to intravenous administration, and

– that H5 and H7 viruses that do not meet the *in vivo* criterion for HPAI should have the amino acid sequence of their HA_0 cleavage site determined, and should be treated as highly pathogenic if additional basic amino acids are present (33).

These recommendations and similar specifications in the European Union (EU) legislation on avian influenza (34) were adopted by the OIE Biological Standards Commission in 1992 and published in the 14th edition of the *Terrestrial Code* in 2005.

Avian influenza epizootics with characteristics similar to those of the Pennsylvania outbreak have occurred in other locations, such as Mexico (1994) and Italy (1999). In these epizootics, a virus of low virulence, as determined by molecular and *in vivo* tests, circulated for some months before mutating to a virulent form. In an outbreak of HPAI in poultry in central Mexico, a retrospective study showed that the highly pathogenic virus was first isolated in December 1994, more than one year after the initial isolation of low pathogenicity virus (26). By the end of 1994, the outbreak had spread to involve 11 states and in January 1995 a second HPAI virus was confirmed (26, 35, 36). In Italy, an H7N1 virus of low pathogenicity spread to involve 199 farms between April and December of 1999, before mutating to a highly pathogenic form (37). The highly pathogenic virus, which had an intravenous pathogenicity index of 3.0 and the HA_0 cleavage site sequence PEIKGSRVRR*GLF, was diagnosed on 17 December 1999. Difficulties similar to those experienced in Pennsylvania in 1983 hampered control measures. However, control was eventually achieved in May 2000 – after 413 farms and nearly 14 million birds had been affected.

First use of the term 'notifiable avian influenza'

The large Italian epizootic triggered a debate about the possible need for further changes to legislative control and trade measures to be applied to avian influenza infection. Ito *et al*. (38) showed that a highly pathogenic virus could be generated by experimentally passaging an avirulent H5N3 virus of wild swan origin (A/whistling swan/Shimane/499/83) multiple times in chickens. This supported the extensive accumulated epidemiological evidence suggesting that highly pathogenic H5 and H7 viruses arise from chickens and turkeys following the introduction of virus precursors of low pathogenicity from the free-living bird reservoir. Furthermore, since the evolution to

virulence is presumably a random event, the longer that low pathogenicity H5 and H7 viruses are allowed to circulate in poultry, the greater the probability that a highly pathogenic virus will emerge. For these reasons, the EU Scientific Committee on Animal Health and Welfare in 2000 (39) and OIE *ad hoc* Expert Group Meetings in 2002 and 2004 recommended that standards and recommendations involving regulatory control and trade should be extended to all H5 and H7 viruses in poultry, regardless of their pathogenicity. A revised definition of notifiable avian influenza (NAI), including infections with H5 and H7 subtype viruses of low pathogenicity, was adopted by the OIE in May 2005. The treatment of these H5 and H7 subtypes as notifiable disease agents provided an impetus for countries to introduce enabling regulatory frameworks and obtain financial support for surveillance, reporting, stamping out and paying compensation to farmers whose flocks were depopulated because of the presence of H5 and H7 viruses of low pathogenicity. Transparency was also encouraged by minimising unjustified trade restrictions arising from the notification of strains of low pathogenicity.

The above clearly shows the progressive changes that have been made to the *Terrestrial Code*. These changes have been science-based and driven by advances in our scientific understanding of the pathobiology, ecology and epidemiology of avian influenza. The leadership of the OIE in promoting the global control of avian influenza viruses of low pathogenicity has been crucial. This has helped many countries to justify and obtain resources for the control of such viruses, and thus to prevent serious outbreaks of disease due to infection with highly pathogenic viruses.

Virulence determinants of avian influenza viruses

Although the polybasic HA_0 cleavage site is considered to be the prime virulence determinant of highly pathogenic viruses, it may not by itself be enough to bestow high pathogenicity on a virus isolate. This was initially demonstrated with the early low pathogenicity A/chicken/Pennsylvania/1983 virus isolates and subsequently observed with a number of other viruses that have been isolated from

field outbreaks or generated in the laboratory. A/chicken/Texas/298313/04 (H5N2), isolated from a broiler chicken flock in Gonzales, Texas, in February 2004, was the first reported highly pathogenic virus that possessed an HPAI HA_0 cleavage site motif (PQRKKR*GLF) but was not virulent in *in vivo* tests (40). This HPAI designation complied with the OIE definition of HPAI that had been in the *Terrestrial Code* since 2005. Discordant results between molecular and *in vivo* tests used to characterise virus pathogenicity were retrospectively recognised for two other H5 field-virus isolates (41), as well as for non-H5/H7 viruses that had been engineered to contain polybasic HA_0 cleavage sites (42, 43, 44). Stech and co-workers (43) showed that inserting a polybasic cleavage site derived from HPAI A/chicken/Italy/8/98 (H5N2) into the haemagglutinin of the LPAI A/duck/Ukraine/1/63 (H3N8) virus was not sufficient to immediately transform it into a highly pathogenic virus, despite the fact that it was able to replicate in tissue culture in the absence of exogenous trypsin. This implied that the evolution of highly pathogenic viruses from H5/H7 viruses of low pathogenicity involved other changes, in addition to a polybasic HA_0 cleavage site. In contrast, Veits and co-workers (44) reported that avian influenza viruses with H2, H4, H8, and H14 haemagglutinins could support a highly pathogenic phenotype after acquiring a polybasic HA_0 cleavage site. This approach has yielded different results for H6 viruses. While the insertion of a polybasic cleavage site into A/mallard/Sweden/81/02 (H6N1) (45) supported a highly pathogenic phenotype *in vivo*, the insertion of a polybasic cleavage site into A/turkey/Germany/R617/07 (H6N2) did not (44), indicating that other 'cryptic' virulence determinants are also involved in the expression of a highly pathogenic phenotype *in vivo*. The involvement of 'cryptic' virulence determinants has been supported by the results of a number of studies (41, 42, 46). These experiments provoke important questions on the apparent restriction of the highly pathogenic phenotype to viruses of the H5 and H7 subtypes. Are H5 and H7 viruses somehow uniquely predisposed to acquiring polybasic HA_0 cleavage sites? Does a barrier exist in nature that prevents other HA subtypes from acquiring polybasic cleavage sites? It remains to be seen if a polybasic HA_0

cleavage site will ever be found in a non-H5/H7 field isolate from poultry.

In this vein, the phenomenon of non-homologous recombination as a mechanism by which H7 viruses could acquire a polybasic HA_0 cleavage site first came to light as a result of laboratory experiments (47, 48) that were carried out more than a decade before the first reports that this same mechanism was responsible for the evolution to highly pathogenic virus in the field (27, 28, 29). Taking into account the developments described above, current scientific evidence supports the recommendations of the *Terrestrial Code* (1).

The role of wild birds as a reservoir of avian influenza infection for poultry

The exposure of gallinaceous poultry to low pathogenicity H5/H7 viruses of wild bird origin does not appear to be a rare event (49). Many, if not the majority, of such exposures result in subclinical, transient infections with no spread to other flocks, which are often only recognised after the fact on the basis of flock seroconversion (49). The reason that these viruses fail to persist in poultry may be due to the fact that they are poorly adapted to their new host and are unable to replicate and be transmitted efficiently. The 50% minimum infectious dose (MID_{50}) has been used to evaluate virus adaptation to a particular species (50, 51). These studies have shown that wild-bird-origin H5/H7 viruses of low pathogenicity are generally not well adapted to chickens, requiring 100 to 1,000 times more virus to infect chickens when compared with poultry-adapted viruses. However, experimental studies have shown that, by repeatedly passaging viruses of wild bird origin through poultry, adaptation, as demonstrated by increased levels of virus replication and improved chicken-to-chicken transmission, can occur (52, 53). The adaptation process is unpredictable and appears capable of following many paths.

Wild birds in the orders Anseriformes (ducks, geese and swans) and Charadriiformes (gulls, terns and waders) are the reservoir of influenza A viruses in nature. However, reports of HPAI virus infection in wild birds without any involvement of poultry are very

rare. Two such reports include a die-off of approximately 1,300 European common terns (*Sterna hirundo*) in the Cape area of South Africa in 1961 (14) and a report of a highly pathogenic virus isolated from two wild duck species during the surveillance of free-living waterfowl in Nigeria (54). The latter report found coexisting but genetically distinguishable avian influenza viruses with a highly pathogenic viral genotype in two co-habiting species of wild waterfowl, with evidence of non-lethal infection in at least one species, and without evidence of prior extensive circulation of the virus in domestic poultry. This finding suggested that some strains with a potentially high pathogenicity for poultry could be maintained in a community of wild waterfowl (54).

Scientific evidence collected in experimental studies and during the observation of real-life disease outbreaks due to HPAI overwhelmingly concludes that these disease outbreaks originate in domestic poultry (e.g. chickens, ostriches) that are raised in intensive management systems.

Nonetheless, epidemiologic investigations and phylogenetic analysis imply that the wild bird reservoir is the original source of H5/H7 viruses of low pathogenicity that give rise to highly pathogenic viruses, and that the latter viruses do not form a separate or unique phylogenetic lineage or lineages in waterfowl (24, 25). Although outbreaks of Eurasian H5N1 HPAI in wild birds have, at times, resulted in mass mortality events, like the large die-off of wild waterfowl at Lake Qinghai, in the People's Republic of China in May to June 2005 (55), extensive testing of hundreds of thousands of apparently healthy wild birds has either failed to detect or rarely detected these H5N1 viruses. In summary, the available scientific evidence supports the hypothesis that HPAI never, or at worst very rarely, emerges in the free-living wild bird reservoir and that these viruses do not persist when introduced into this reservoir.

This is in stark contrast to the situation in domestic poultry. The reason(s) for this difference are not known; however, Lebarbenchon *et al.* (56) have proposed that the different selective pressures which are

present in natural ecological systems (wild birds) versus artificial ecological systems (intensive poultry farming, live bird markets, etc.) may explain why HPAI viruses do not emerge or persist in natural ecosystems.

Differences in the pathobiology of avian influenza in wild birds and in domestic poultry

Influenza A virus replication in waterfowl, which takes place in the intestinal tract, favours faecal-oral and potentially faecal-faecal routes of transmission. In contrast, airborne transmission, which is most important in domestic poultry, is associated with virus replication within the respiratory tract. Lebarbenchon *et al.* (56) further suggested that the evolution of H5/H7 viruses from low to high pathogenicity, which occasionally follows the introduction of a wild bird virus into poultry, may not be solely due to a change in host species or the higher rates of pathogen transmission that may indirectly select for higher levels of virulence (low virulence may be a selective advantage when host-host contacts are infrequent). These authors suggested that the process may also be driven by a larger set of ecological parameters which are encountered in artificial ecosystems. These include a more uniform age structure, lower genetic diversity and more constant environmental conditions, all of which could contribute to the selection of more virulent virus variants. At this stage, scientific evidence does not provide a clear basis on which to predict which LPAI H5/H7 viruses will remain of low pathogenicity and which will mutate to become highly pathogenic. As discussed above, in addition to a polybasic HA_0 cleavage site, cryptic virulence determinants can also influence the pathogenic phenotype of a virus. The rate at which an H5/H7 low pathogenicity virus acquires the necessary genetic changes to become highly pathogenic is determined in large part by the error rate of the viral polymerase, which is approximately 10^{-5} mutations per site, per genome replication. The emergence of a highly pathogenic virus from a precursor of low pathogenicity can take several months, as observed in Pennsylvania in 1983 (~5 months involving 25 outbreaks), Mexico in 1995 (~13 months and present in 11 states), Italy in 1999 (~8 months and 199 outbreaks), and as has

recently been determined for A/turkey/Ontario/7732/1966 (~3 months) (57). The shift towards virulence can also occur relatively quickly, as observed with the Chile H7N3 outbreak in 2002 (~1 month), the British Columbia H7N3 outbreak in 2004 (<1 month) and the Saskatchewan outbreak of H7N3 in 2007 (~1 month). In general, the longer that an H5/H7 virus of low pathogenicity is allowed to circulate in poultry, particularly in areas of high poultry density, the greater the chances that a highly pathogenic virus will emerge. This highlights the need for early warning, based on detection and reporting in accordance with the OIE standards.

The significance of wild birds in relation to H5N1 avian influenza

The first indication that wild birds might be important in the spread of H5N1 avian influenza involved outbreaks in wild waterfowl and captive wild birds in Hong Kong in late 2002 (58). However, it was not until the large outbreak among wild birds on Lake Qinghai in May 2005 that concern over the involvement of wild waterfowl in the spread of H5N1 avian influenza came to the forefront. This outbreak, which affected a number of avian species, including bar-headed geese (*Anser indicus*), brown-headed gulls (*Larus brunnicephalus*), great black-headed gulls (*L. ichthyaetus*) and great cormorants (*Phalacrocorax carbo*) (55, 59), preceded the rapid spread of the virus to north-western Asia, Europe, the Middle East and eventually Africa, which occurred in late 2005 and 2006. Of the 23 introductions of H5N1 avian influenza into Europe, 20 were associated with migrating wild birds. In Europe, mortalities involving mute (*Cygnus olor*) or whooper (*C. cygnus*) swans were usually the first indication of the presence of H5N1 avian influenza (60, 61). This was especially true during the winter of 2005 to 2006, when spatial and temporal analysis of the outbreaks indicated that they were associated with cold weather and the congregation of waterbirds along the 0°C isotherm (62). In June and July 2007, mortalities in waterbirds associated with H5N1 avian influenza were again reported in Germany, France and the Czech Republic. Although swans and some other species that develop serious disease in response to H5N1 avian influenza virus infection

are viewed as excellent sentinel species, there is no scientific evidence that they play any significant role as long-distance vectors of the virus. Studies using wild ducks that have been experimentally infected with H5N1 viruses have demonstrated species variability in virus excretion and susceptibility to debilitating disease (63). After careful study, the potential role of the mallard (*Anas platyrhynchos*) as a long-distance vector of H5N1 avian influenza has been largely discounted (64, 65).

Large-scale surveillance to detect the presence of H5N1 avian influenza in wild birds was initiated in 2006 but has since been reduced. In its place, targeted surveillance, which focuses on sick or dead wild birds, is ongoing in many parts of the world, with the objective of alerting Veterinary Services to the possible exposure of free-ranging poultry to H5N1 avian influenza in countries that are currently free from these viruses. The reduced number of mass mortality events reported in wild birds may be related to a reduced prevalence of H5N1 avian influenza in wild birds after 2006 and/or to a reduced exposure of poultry to infected domestic ducks in enzootic countries. The true prevalence and persistence of H5N1 avian influenza in wild bird populations throughout the world remain to be elucidated. The hypothesis that best fits the accumulated scientific evidence is that infected poultry, especially domestic ducks, provide a reservoir of infection for wild waterfowl, and not the converse. Multiple studies of H5N1 viruses in the live poultry markets of China and Vietnam support the hypothesis that 'backyard' poultry and small-scale poultry farms are the main reservoir of infection for H5N1 avian influenza.

The significance of pigs in relation to H5N1 avian influenza

H5N1 avian influenza was first isolated from pigs during routine surveillance carried out in Fujian Province in southern China in 2001 and 2003 (66). This raised concerns that pigs might serve as an intermediate host in which the virus could eventually adapt to humans. Follow-up surveillance, involving 25 medium-to-large-scale pig farms in Fujian Province, was carried out in 2004 and again in 2007. Of the 499 sera from 2004 and 908 sera from 2007, none tested positive for

antibodies to H5 as determined by haemagglutination-inhibition test and confirmed by the micro-neutralisation test. More recently, the testing of 1,107 nasal swab samples collected from apparently healthy four-to-six-month-old pigs in Jiangsu Province of eastern China during the period from October 2008 to May 2009 yielded only two H5N1 viruses, giving an isolation rate of 0.18%. The two isolates, A/swine/Jiangsu/1/2008 and A/swine/Jiangsu/2/2009, were from clades 7 and 2.3.4, respectively (67).

In Vietnam, serological evidence for exposure to H5N1 avian influenza in pigs has also been reported, albeit at a very low prevalence (68). Out of a total of 3,175 pig sera that were collected in Vietnamese slaughterhouses between September 2003 and June 2004, only 0.25% of the sera were positive for H5 antibodies as determined by virus neutralisation test and western blot analysis.

In Indonesia, virological and serological surveillance carried out during January to February 2005, October 2006 to February 2007 and November 2008 to April 2009 demonstrated H5N1 avian influenza in pigs in 2005 to 2007 but not in 2008 to 2009 (69). In 2005, a total of 35 H5N1 viruses were isolated from five out of seven private or commercial farms located in the Tangerang district of Banten Province. All of the positive farms had poultry on site and phylogenetic analysis showed that all of the viruses clustered with chicken isolates.

In 2006 to 2007, a total of 17 H5N1 avian influenza viruses were isolated from private, commercial or government farms as well as slaughterhouse specimens collected in Banten, East Java, North Sumatra and South Kalimantan Provinces. Similarly to the 2005 surveillance period, poultry were either found on site or within a kilometre of the affected site. These results could suggest poultry-to-pig transmission. However, they should be interpreted with caution, as the evidence is based on results from a single laboratory and no other laboratory has been able to reproduce these results. The findings of Löndt *et al.* (70) argue against the hypothesis of poultry-to-pig transmission. In this study, pigs that were co-housed with ducks or

chickens infected experimentally with the H5N1 clade 2.1.1 virus A/turkey/Turkey/1/2005 failed to become infected as determined by polymerase chain reaction (PCR) testing of nasal swab samples and haemagglutination-inhibition assay of sera collected at seven and 14 days post contact.

Setting aside the questions about poultry-to-pig transmission, the fact that viruses with nearly identical genes were isolated from pigs on the same farms in Indonesia did imply pig-to-pig transmission. Two experimental studies (68, 71) have confirmed the susceptibility of pigs to H5N1 avian influenza. In both studies, pigs were inoculated with 10^6 50% egg infectious dose (EID_{50}) of H5N1 virus by the intranasal route. Nasal swab specimens collected from these pigs demonstrated that virus shedding occurred from 1 to 5 days post-inoculation and that virus titres were modest, ranging from ~1 to 4 $\log_{10}$ EID_{50}/ml of swab sample media. In one study (71), virus was found only in the tissues of the respiratory tract (nasal turbinates, tonsils, trachea and lungs), with no evidence of systemic involvement. In the other study (68), virus was also found in the liver, in addition to tissues of the respiratory tract, despite the fact that viraemia was not detectable. In the study that did address the question of pig-to-pig transmission (68), there was no evidence for transmissibility, although the small numbers of infected and in-contact animals that were used reduced the statistical power of the experiments.

In summary, pigs are susceptible to infection with H5N1 avian influenza, with virus replication appearing to be restricted to the respiratory tract. This is associated with a brief period of virus shedding and the infection manifesting itself either subclinically or as a mild respiratory disease. Sporadic field cases may have been the result of poultry-to-pig transmission but more evidence is needed to substantiate this hypothesis. Evidence for pig-to-pig transmission is scant at present. Although one plague-purified clone of an Indonesian swine H5N1 isolate (A/swine/Banten/UT3062/2005) was shown to possess an A134S substitution within the 130-loop of the receptor binding pocket that is responsible for human-type receptor recognition, and could bind to avian-type and human-type sialic acid

receptors (69), a fully swine-adapted virus has yet to appear. Based on current scientific evidence, pigs are considered to be dead-end hosts for H5N1 avian influenza.

Because live pigs and porcine products present no significant risk of transmitting H5N1 avian influenza viruses either to humans or to poultry, the *Terrestrial Code* does not make recommendations regarding the implementation of health measures for pigs and pig products in relation to avian influenza (1).

Diagnosing avian influenza

The standards for diagnostic tests, including pathogenicity testing of influenza A virus isolates from poultry, are described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*) (2). The prescribed test for agent identification for the purposes of international trade involves inoculating the allantoic cavity of specific-pathogen-free embryonating fowl eggs that are at between nine and 11 days of incubation. The presence of influenza A virus-group-specific-antigen can be confirmed by an immunoassay, such as the agar gel immunodiffusion test, or lateral flow devices, which detect influenza A nucleoprotein or matrix protein. Alternatively, influenza A virus nucleic acid can be detected by the use of reverse-transcription PCR (RT-PCR), using either nucleoprotein-specific or matrix-protein-specific primers (72). Antigenic subtyping of an influenza A virus isolate should be carried out by haemagglutination-inhibition and neuraminidase-inhibition tests, using highly specific reference antisera. The presence of H5 and H7 subtype influenza A viruses can also be determined by RT-PCR using H5- and H7-specific primers (72). The pathogenicity of a virus isolate can be determined by *in vivo* and molecular-based methods. Using *in vivo* methods, an HPAI virus is defined as: ‘any virus that is lethal for six, seven or eight of eight 4-to-8-week-old susceptible chickens within ten days following intravenous inoculation with 0.2 ml of a 1/10 dilution of bacteria-free, infective allantoic fluid’ or ‘any virus that has an intravenous pathogenicity index (IVPI) greater than 1.2’ (2). For all H5 and H7 viruses that have been determined to

be of low pathogenicity in chickens, using one of the *in vivo* methods described above, the amino acid sequence of the connecting peptide of the haemagglutinin must be determined. If the sequence is similar to that observed for other HPAI virus isolates (i.e. it contains a polybasic HA_0 cleavage site), the isolate is then considered to be highly pathogenic.

The detection of infection with an avian influenza virus can be based upon isolating and characterising the virus, as described above, or by identifying the presence of viral RNA that is specific for a virus of low or high pathogenicity. The latter can be achieved using a number of different molecular-based methods. Advances in molecular-based diagnostic techniques have allowed avian influenza infections to be detected more rapidly than can be achieved with methods that depend on virus isolation. This can significantly reduce the high-risk period, which is defined as the time interval between the introduction and the detection of a pathogen, which is important for the rapid and effective implementation of control measures. Identifying viral RNA that is specific for avian influenza can be achieved by a number of different methods, including PCR-based amplification methods that can be coupled with either Sanger sequencing or one of the next-generation sequencing methods (reviewed in 73 and 74). As an example, and in response to the westward spread of H5N1 avian influenza, Hoffmann *et al*. (75) developed an H5-specific, real-time RT-PCR assay designed to amplify a 150 nucleotide region of the H5 gene which incorporates the HA_0 cleavage site. This assay uses two probes; one targeting a sequence that is reasonably conserved among various H5 viruses, and a second that is specific for the cleavage site of Qinghai-lineage H5N1 viruses. Another example is a pan-haemagglutinin RT-PCR developed by Gall *et al*. (76). This universal primer set targets the HA_0 cleavage site of all influenza A virus subtypes; the products of which can then be used in sequencing reactions.

Part 2: Preventing the spread of avian influenza through international trade in birds and avian products

Introduction to the World Organisation for Animal Health

The OIE is an intergovernmental organisation, established in 1924, with the goal of supporting international solidarity in the control and prevention of highly contagious animal diseases, including through the promotion of transparency in reporting listed diseases. In 1968, the OIE published the first edition of the *International Animal Health Code*, now called the *Terrestrial Animal Health Code* (*Terrestrial Code*) (1). In addition to setting standards for the improvement of animal health and welfare and veterinary public health worldwide, the *Terrestrial Code* sets sanitary standards to ensure safe international trade in terrestrial animals and their products. The *Terrestrial Code* specifies both general ('horizontal') and disease-specific health measures to be used by the Veterinary Authorities of importing and exporting countries to avoid the transfer of agents that are pathogenic for animals or humans, while at the same time avoiding unnecessary barriers to trade (1).

The OIE Terrestrial Animal Health Standards Commission (Code Commission) is the elected commission responsible for updating the *Terrestrial Code* each year, based on scientific information and inputs provided by Member Countries and relevant organisations. The Code Commission is supported in this activity by two other elected commissions, the Scientific Commission for Animal Diseases and the Biological Standards Commission.

In 1995, with the signing of the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) (77), the OIE was mandated as a reference organisation to set health standards for trade in animals and animal products. As of October 2014, the OIE has 180 Members.

Background on international trade in birds and avian products

Poultry is the fastest-growing livestock industry. Global broiler meat production is forecast at 82.2 million tons for 2012 (78). The top exporters of chicken meat in 2012 in decreasing order were: Brazil, the USA, the EU, Thailand, China, Argentina, Turkey, Canada and Chile. The top importers of chicken meat in 2012 in decreasing order were: Japan, Saudi Arabia, the EU, Mexico, Russia, Iraq, Hong Kong, Vietnam, the United Arab Emirates and Angola. Trade in live poultry is also very significant, involving hatching eggs, day-old chicks and older poultry. There is also a significant global trade in pet and hobby birds and birds for zoological collections.

The risks associated with the trade of live birds and poultry products in the spread of avian influenza were reviewed in 2009 (79). This study found that 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, including over large distances. Based on findings in other papers, the review indicated that illegal poultry movements are extensive in South-East Asia, as is the illegal trade in fighting cocks, wild birds (particularly birds of prey) and exotic 'pet' or companion animal birds. In view of the largely illegal nature of cock fighting and the movement of these birds, this trade represents a particular risk for introducing avian influenza, as has occurred in Thailand and Laos. The review also reported that the movement of fighting cocks was associated with the spread of Newcastle disease virus in western states of the USA between 2002 and 2003, showing the potential importance of such birds in spreading avian influenza (79).

Relevant definitions in the *Terrestrial Code*

As with all normative publications, it is important to establish clear and unambiguous definitions of key terms and concepts. The *Terrestrial Code* contains several definitions relating to avian influenza, including those for notifiable avian influenza, poultry, zoning, and compartmentalisation (1). At the 81st General Session in

May 2013, the General Assembly agreed to a number of amendments of the text in Chapter 10.4. ('Avian influenza'). These modifications did not significantly change the requirements; rather the objective was to present them more clearly.

Definition of poultry

In the *Terrestrial Code*, ***poultry*** is defined 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'. Birds that are kept in captivity for reasons other than those stated above, including birds that are kept for shows, races, exhibitions, competitions or for breeding or selling these categories of birds, as well as pet birds, are not considered to be poultry. For example, hobby pigeons do not qualify as poultry, based on this definition. So-called 'backyard poultry' and fighting cocks were included in the *Terrestrial Code* definition of poultry following the meeting of the Code Commission of October 2006.

The OIE definition of poultry is most commonly used in connection with birds in the super-order Galloanserae, which includes the order Galliformes and the family Anatidae, within the order Anseriformes. The most important species, from a commercial viewpoint, are chickens (*Gallus gallus*), turkeys (*Meleagris gallopavo*), ducks (*Anas platyrhynchos domesticus*), geese (*Anser anser var. domestica*) and pigeons (*Columba livia*). Based on their use for the production of meat, eggs or feathers, many other avian species may fall within the OIE definition of poultry, including Indian peafowl (*Pavo cristatus*), guineafowl (*Numida meleagris*), Japanese quail (*Corturnix coturnix japonica*), the common pheasant (*Phasianus colchicus*), emus (*Dromaius novaehollandiae*) and ostriches (*Struthio camelus*) in the order Struthioniformes, and rheas (*Rhea americana*) in the order Rheiformes. All these species are susceptible to infection with influenza A viruses and can thus participate in virus amplification and spread. In addition to domesticated birds used for commercial or

production purposes, the *Terrestrial Code* definition includes fighting cocks, which have been implicated in the spread of H5N1 avian influenza, including to humans, in South-East Asia.

Definition of avian influenza

Prior to May 2013

Before May 2013, NAI was defined in the *Terrestrial Code* (1) as an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any avian influenza virus with an IVPI greater than 1.2 (or, as an alternative, with at least 75% mortality), as described below.

The 'NAI viruses' were divided into highly pathogenic notifiable avian influenza ('HPNAI') and low pathogenicity notifiable avian influenza ('LPNAI'), which were defined as follows:

'HPNAI viruses have an IVPI in six-week-old chickens greater than 1.2 or, as an alternative, cause at least 75 percent mortality in four-to eight-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75 percent mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin molecule (HA_0); if the amino acid motif is similar to that observed for other HPNAI isolates, the isolate being tested should be considered as HPNAI;

'LPNAI are all influenza A viruses of H5 and H7 subtype that are not HPNAI viruses.'

Modifications adopted at the 81st General Session, May 2013

In May 2013, the title of the chapter was changed to 'Infection with avian influenza viruses', for consistency with the approach used throughout the *Terrestrial Code*. Article 1 was deleted and the definition was modified to the following:

'For the purposes of the *Terrestrial Code*, avian influenza is defined as an infection of poultry caused by any influenza A virus of the H5 or

H7 subtypes or by any influenza A virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75 percent mortality) as described below. These viruses are divided into high pathogenicity avian influenza viruses and low pathogenicity avian influenza viruses.'

The text was modified at several points throughout the chapter to reflect the amendment of the definition.

The occurrence of infection was redefined, as follows:

'The virus has been isolated and identified as such or specific viral RNA has been detected in poultry or a product derived from poultry.'

The requirement to report HPAI viruses in birds other than poultry was not changed. The amended text reads:

'Infection with influenza A viruses of high pathogenicity in birds other than poultry, including wild birds, should be notified according to Article 1.2.3. However, a Member should not impose bans on the trade in poultry commodities in response to such notification, or other information on the presence of any influenza A virus in birds other than poultry, including wild birds.'

Complementing this amendment, Article 1.2.3. was modified to clarify that 'infection with avian influenza viruses as well as infection with influenza A viruses of high pathogenicity in birds other than poultry' were included on the OIE list of diseases for the purposes of notification, according to the requirements set out in Chapter 1.1.

In summary, these amendments did not change the requirements for safe trade in any significant way. Rather, the presentation of the chapter was improved and clarified.

Other relevant definitions

Further relevant definitions are as follows (1):

Establishment: means the premises in which animals are kept.

Compartment: means an animal subpopulation contained in one or more establishments under a common biosecurity management system with a distinct health status with respect to a specific disease or specific diseases for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade.

Zone/region: means a clearly defined part of a territory containing an animal subpopulation with a distinct health status with respect to a specific disease for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade.

Veterinary Authority: means the Governmental Authority of an OIE Member, comprising veterinarians, other professionals and para-professionals, having the responsibility and competence for ensuring or supervising the implementation of animal health and welfare measures, international veterinary certification and other standards and recommendations in the *Terrestrial Code* in the whole territory.

OIE requirements for reporting avian influenza

In the *Terrestrial Code*, the requirements for reporting disease events to the OIE are provided in Chapter 1.1. and the OIE-listed diseases in Chapter 1.2. According to Article 1.2.3., infection with avian influenza viruses (defined as an infection of poultry – see Chapter 10.4.), as well as infection with influenza A viruses of high pathogenicity in birds other than poultry, are listed by the OIE (1). The *Terrestrial Code* requires the notification of highly pathogenic influenza in all birds. In addition, findings of H5/H7 LPAI viruses in poultry should be reported to the OIE. These requirements are intended to encourage Members to report avian influenza virus infection in wild birds without running the risk of losing international markets due to the imposition of trade bans that are not based on science.

OIE policies on recognising the status of a country, zone or compartment

For certain diseases the OIE has, since 1995, provided standardised procedures for the official recognition of the disease status of Member Countries. Taking effect from May 2014, this procedure applies to four ruminant diseases, one equine disease and one disease of pigs. In 1998, the WTO confirmed the OIE mandate to recognise disease-free areas based on the SPS Agreement. Official recognition of disease-free status provides significant market access benefits.

As of 2014, the OIE does not grant official recognition for avian influenza. However, OIE Members may make a self-declaration on the freedom of the entire country or of a zone or compartment within the national territory. Self-declarations must be based on sound evidence demonstrating that the OIE requirements, particularly those on surveillance, for the disease in question have been satisfied. The declaration is made under the full responsibility of the Member Country concerned. The OIE may publish information relevant to self-declarations but it does not accept responsibility for shortcomings in the information provided, nor does it make any -undertaking regarding the maintenance of the declared health status.

Provisions on country, zone or compartment freedom from avian influenza

In Article 10.4.3., the *Terrestrial Code* makes provision for considering a country, zone or compartment as being free from avian influenza. This must be based on documented evidence that there has been no infection with avian influenza viruses in poultry for at least 12 months. The *Terrestrial Code* also contains provisions for regaining disease-free status after the occurrence of infection with an avian influenza virus of high or low pathogenicity.

Article 10.4.4. contains provisions for considering a country, zone or compartment free from infection with avian influenza viruses of high pathogenicity, based on documented evidence showing:

– the absence of infection in poultry with HPAI viruses during the last 12 months, although its status with respect to LPAI viruses may be unknown, or

– based on surveillance in accordance with Articles 10.4.27. to 10.4.33., the country, zone or compartment does not meet the criteria for freedom from avian influenza but any virus detected has not been identified as highly pathogenic.

A key concept in the *Terrestrial Code* is the use of surveillance to demonstrate the absence of virus circulation. Articles 10.4.27. to 10.4.33. specify the key parameters for effective surveillance to demonstrate the absence of virus circulation. These parameters depend on historical and geographical factors, industry structure, population data and proximity to recent outbreaks.

Surveillance should be under the responsibility of the Veterinary Authority, should include active and passive surveillance and, where applicable, targeted surveillance, and should utilise clinical, virological and serological surveillance methods.

With respect to the detection of H5 or H7 subtype antibodies in the absence of virus, the *Terrestrial Code* states that, when antibodies to H5 or H7 subtype avian influenza viruses are detected in poultry and are not a consequence of vaccination, an immediate and thorough epidemiological and laboratory investigation into their source should be initiated. This should not be considered as an occurrence of infection if further investigation fails to isolate virus or detect viral RNA.

OIE standards and recommendations on trade in birds and avian products

All of the following text is based on the 22nd edition of the *Terrestrial Code* (2013).

Findings of avian influenza in birds other than poultry

According to Article 1 in Chapter 10.4., infection with HPAI viruses in birds other than poultry, including wild birds, should be notified according to Article 1.1.3. However, a Member Country should not impose bans on the trade in poultry commodities in response to such notification, or other information on the presence of any influenza A virus in birds other than poultry, including wild birds.

The rationale for this article is that wild birds are considered to be the natural reservoir for influenza A viruses globally, and the control of influenza A viruses in the wild bird population is not possible. Therefore, all countries have some risk with regard to the introduction of avian influenza viruses to poultry. Control of this risk is feasible through the effective separation of and reduction of transmission between wild and domestic populations. Reports of avian influenza viruses in wild birds are useful for the purpose of global surveillance and should not result in trade restrictions. Trade bans following such reports do not help to prevent the spread of avian influenza. In fact, such actions discourage reporting, hinder global surveillance and, therefore, increase the risk of disease spread.

Risk pathways for the entry of avian influenza viruses

The spread of avian influenza viruses of highly pathogenic and low pathogenicity subtypes is associated with human activities involving the movement of infected birds, their products or contaminated fomites.

Risk analysis can be used to classify commodities into four groups, based on the relative likelihood of virus transmission (79). These are:

1) live poultry and other birds

2) genetic material, including one-day-old chicks, hatching eggs and semen

3) commodities for human consumption, such as eggs and meat, and

4) other commodities (e.g. feathers, feather meal and poultry meal).

The *Terrestrial Code* recommendations and scientific rationale for each of these groups are set out below. The definitions of freedom from avian influenza in the *Terrestrial Code* are explicitly linked to the requirements for surveillance – a fact that underpins the safeguards provided by the measures described below.

Risk management: live poultry and other birds

Since infected birds are actively shedding virus, their movement represents the greatest risk for introducing virus to a farm, region or country. The incubation period (the time between exposure to the virus and the first appearance of clinical signs) is variable and depends on a number of factors, including the virus isolate and its adaptation to a particular host species, the immune status of the host, and environmental stressors, as well as the dose and route of exposure. Under natural conditions, the incubation period for individual birds can be as short as three days and for infected flocks as long as 14 days (80). For the purposes of the *Terrestrial Code*, the incubation period for avian influenza is defined as 21 days. This definition reflects the longest period that may elapse between the introduction of the pathogen into the animal and the occurrence of the first clinical signs of disease. This provides an important added safety margin in reducing the risk of introducing the virus through trade. In cases of infection with HPAI virus, severe clinical signs are normally found at the individual bird and flock levels. This is particularly applicable to gallinaceous poultry but may not apply to ducks and geese. In contrast, infection with LPAI viruses may give rise to a range of clinical presentations from subclinical to severe. The more severe clinical presentations usually occur when complicated by the presence of other pathogens. For this reason, the 'infectious period' (which is the time between the first detection of virus in bodily secretions or excretions and the absence of detectable virus) is more relevant than the 'incubation period' in determining the period required for the application of control measures to prevent transmission. The infectious period can be longer than the incubation period, i.e. virus

may be shed in secretions and excretions before the onset of clinical signs and after clinical signs have abated. The infectious period typically lasts seven to ten days but can be as long as 21 days.

The *Terrestrial Code* provisions for the importation of poultry (including day-old chicks) take into account the status of the country, zone or compartment from which the poultry originate, and are based on veterinary attestations regarding, among other things, the source of the poultry, the absence of clinical signs of infection, and the use/non-use of vaccination.

For live birds other than poultry, Article 10.4.6. makes provision for importation regardless of the avian influenza status of the country of origin. Trade should be based on a veterinary certificate attesting to the absence of clinical signs of infection with a virus that would be considered avian influenza in poultry; a minimum of 21 days' isolation before shipment; testing of a statistically valid sample of the birds; and information on vaccination (as appropriate).

The *Terrestrial Code* definitions of freedom from avian influenza are explicitly linked to the requirements for surveillance – a fact that underpins the safeguards provided by the measures above.

Risk management: poultry meat

When chickens are infected with an HPAI virus, because of the systemic nature of the infection, virus can be found in the visceral organs, brain, skin, skeletal muscle, bone and blood, as well in respiratory secretions and alimentary tract excretions. In contrast, in chickens infected with LPAI viruses, virus is restricted to the respiratory and alimentary tracts with no systemic involvement (51). However, there is potential for the meat to be contaminated by virus from the respiratory or gastrointestinal tracts during processing of the carcasses, if the birds are in the acute infectious phase (81). For fresh poultry meat, Article 10.4.19. recommends that, in the case of importation from a country, zone or compartment free from avian influenza, or free from infection with high pathogenicity viruses in poultry, the Veterinary Authorities require an attestation that the entire

consignment of meat comes from poultry that have been kept in a country, zone or compartment free from infection with HPAI viruses in poultry since they were first hatched, or for at least the past 21 days; that they have been slaughtered in an approved abattoir in a country, zone or compartment free from infection with HPAI viruses in poultry; and that they have been subjected to ante- and post-mortem inspections in accordance with Chapter 6.2. and found free of any signs suggestive of avian influenza.

Risk management for processed poultry meat and products is covered in section 2.9.

Risk management: eggs for human consumption

As a consequence of cloacal shedding, LPAI virus can be found on the surface of eggs laid by acutely infected hens, but such virus has not been demonstrated in the internal contents of chicken eggs (82). There is one report of a (non-reportable) avian influenza virus being detected in the internal contents of eggs (83). This resulted from experimental infection of breeder turkeys with the H3N2 subtype virus A/turkey/Ohio/313053/04 (83).

No studies have demonstrated LPAI virus in the internal contents of chicken eggs and surface sanitisation of eggs is therefore considered as an effective means of managing the risk associated with eggs imported from a country or zone that is infected with such viruses. This, however, may not be the case with eggs that originate from a country or zone affected by HPAI viruses, as it has been established that such viruses can be found on the eggshell surface as well as within the internal egg contents (82). Provisions for this difference are covered in Article 10.4.15. of the *Terrestrial Code* which, in effect, recommends processing by heat treatment to destroy the avian influenza virus when importing egg products from countries/zones/compartments that are not (a) free from avian influenza or (b) free from infection with HPAI influenza in poultry.

Risk management for processed eggs for human consumption is covered in section 2.9.

Risk management: poultry feathers and derived products

Perkins and Swayne (84) detected influenza A nucleoprotein antigen in the basilar and intermediate epithelium of the feather follicles of seven gallinaceous species that were infected intranasally with A/chicken/Hong Kong/220/97 (H5N1). Yamamoto *et al*. (85, 86) reported that H5N1 HPAI can replicate in the feather epidermal cells of subclinically infected domestic ducks and later (87) showed that higher viral loads were associated with feather specimens, compared with those found in oropharyngeal or cloacal swabs. Furthermore, infectious virus could be recovered from feathers under favourable storage conditions – 15 days if stored at 20°C and 160 days if stored at 4°C (87).

There is a significant international trade in feathers used for commercial purposes. Although the transmission of avian influenza viruses by feathers has not been documented in practice, it would be valuable to have more scientific evidence to make a definitive assessment of the risks. The *Terrestrial Code* recommends processing to inactivate avian influenza viruses that may be present. Processing parameters are recommended for feather meal but details of an effective processing regime are yet to be defined for the feathers and down of poultry and other birds.

Inactivation of avian influenza viruses in poultry products

Avian influenza viruses are relatively unstable and can be inactivated by a number of physical methods, including heat, extremes of pH, hypertonic conditions and desiccation (88). The *Terrestrial Code* contains recommendations for the inactivation of avian influenza virus in eggs, egg products and meat for human consumption, based on scientific studies (82, 89, 90). The times and temperatures listed in Articles 10.4.25. and 10.4.26. for the inactivation of avian influenza virus in eggs and meat, respectively, are sufficient to achieve a 7-log kill, providing an acceptable safety margin.

Compartmentalisation: a tool to safeguard against avian influenza

As descibed in the *Terrestrial Code*, compartmentalisation is a procedure that may be used by a country for the purpose of disease control and/or international trade. A compartment is an animal subpopulation with a distinct health status, which is contained in establishments that are under a common biosecurity management system and to which surveillance, control and biosecurity measures have been applied. The concept of a defined subpopulation of animals with a 'higher health status' also applies to zones. While a compartment is defined primarily by management and husbandry practices that relate to biosecurity, a zone is primarily defined on a geographical basis, with reference to natural, artificial or legal boundaries. In practice, spatial considerations and good management play important roles in the application of both concepts. In both cases, the Veterinary Authority has authority over the definition and approval of the subpopulation. The compliance of livestock producers and associated industries with the rules established by the Veterinary Authority is paramount to the successful maintenance of a compartment or zone.

In many countries, commercial poultry production takes place in 'industrial', vertically integrated production systems, where all inputs and outputs are under the control of a company or consortium of companies. This type of production system is well suited to compartmentalisation. At the request of Member Countries, the OIE is providing advice to help to implement this concept.

Vaccination against avian influenza

The OIE does not recommend the widespread use of vaccination for the prevention of avian influenza in general but the *Terrestrial Code* does contain recommendations on vaccination in outbreak situations to prevent the spread of the virus and to manage the risk of human exposure. Where vaccination is used, the *Terrestrial Code* outlines considerations relevant to achieving a satisfactory level of flock immunity and makes recommendations on surveillance in vaccinated

flocks. The *Manual* contains standards and recommendations on vaccines and diagnostic tests.

Conclusions

Both avian influenza and the poultry industry have undergone significant changes since the establishment of the OIE in 1924. Coincident with these changes, the global trade of birds, poultry and poultry products has increased substantially. The standards in the *Terrestrial Code* are based on scientific information and risk assessment, consistent with the principles of the WTO SPS Agreement. Application of the OIE standards enables countries to conduct international trade safely and to avoid the imposition of unjustified sanitary restrictions.

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