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The challenge of avian influenza to the veterinary community

Ilaria Capua[1*] and Dennis J. Alexander[2]

[1]*OIE, FAO and National Reference Laboratory for Newcastle Disease and Avian Influenza, Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro, Padova, Italy, and* [2]*Unaffiliated Consultant Virologist*

Avian influenza (AI) is a listed disease of the World Organisation for Animal Health (OIE) that has become a disease of great importance both for animal and human health. The increased relevance of AI in the fields of animal and human health has highlighted the lack of scientific information on several aspects of the disease, which has hampered the adequate management of some of the recent crises. Millions of animals have died, and there is growing concern over the loss of human lives and over the management of the pandemic potential.

The present paper aims to identify areas of knowledge of veterinary competence that need to be improved in order to generate information to support the global AI crisis, and highlights the major changes in AI legislation, including regulations related to trade. It also reviews the human health implications of AI, including the mechanisms by which a human pandemic virus may be generated, and the food safety issues related to this infection. The application of control policies, ranging from stamping out to emergency and prophylactic vaccination, are discussed on the basis of data generated in recent outbreaks, and in the light of new regulations, also in view of the maintenance of good animal welfare.

Poultry veterinarians working for the industry or for the public sector represent the first line of defence against the pandemic threat and for the prevention and control of this infection in poultry and in wild birds. However, given the current situation, it is imperative that close collaboration is sought and achieved by health officials involved in the veterinary, agricultural and medical aspects of the disease. Only through the exchange of data, experiences, views and information will it be possible to combat this zoonosis, which represents a major threat to public health and animal well-being.

Introduction

Avian influenza (AI) represents one of the greatest concerns for public health that has emerged from the animal reservoir in recent times. Over the past 5 years there has been a sharp increase in the number of outbreaks of AI in poultry, compared with the previous 40 years. It has been calculated that the impact of AI on the poultry industry has increased 100-fold, with 23 million birds affected in a 40-year period between 1959 and 1998 and over 200 million from 1999 to 2004 (Capua & Alexander, 2004). In fact, from the late 1990s AI infections have assumed a completely different profile both in the veterinary and medical scientific communities. In recent times some outbreaks have maintained the characteristic of minor relevance while others, such as the Italian 1999 to 2000, the Dutch 2003, the Canadian 2004 and the ongoing Eurasian epidemics, have led to devastating consequences for the poultry industry, negative repercussions on public opinion and, in some cases, have created significant human health issues, including the risk of generating a new pandemic virus for humans via the avian–human link.

The importance of the human health implications of AI infections were revealed during the 1997 Hong-Kong outbreak, in which the H5N1 virus was shown to have infected 18 people, six of whom died. Following this episode, human infection and one death resulted from the H7N7/2003 outbreak in The Netherlands, and the H5N1 virus that is currently endemic in Asia has been shown to have caused the death of 73 individuals during 2003 to 2005 (as of 31 December 2005). The human health implications of these AI infections are, however, not limited to the sporadic occurrence of human infections already reported. Studies performed on human pandemic viruses have shown that, except for the 1977 H1N1 pandemic, they always contain an avian component. This component may be acquired by genetic reassortment between an avian virus with a human influenza virus during concurrent infection of a single host. Alternatively, as suggested recently for the "Spanish" influenza virus responsible for the pandemic of 1918 to 1919, the virus could be entirely of avian origin and develop mutations that enable the virus to acquire the characteristics that allow it to transmit easily in humans

*To whom correspondence should be addressed. Fax: +39 498084360. E-mail: icapua@izsvenezie.it

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(Taubenberger, 2005). Either of these mechanisms could represent the basis for the generation of a new human pandemic virus with inauspicious consequences.

The increased relevance of AI in the fields of animal and human health has highlighted the lack of scientific information on several aspects of the disease. This has hampered the adequate management of some of the recent crises, thus resulting in millions of dead animals and concern over loss of human lives and over management of the pandemic potential.

For this reason and for the devastating effects on the poultry industry, international organisations such as the World Health Organisation, (WHO), OIE, and the Food and Agriculture Organisation (FAO) have worked together and established a coordinated set of guidelines and action plans to combat the ongoing H5N1 epidemic. These efforts include the establishment of an OIE/FAO network of expertise on AI, the OFFLU network (www.offlu.net).

It has been recognized that due to the low profile of AI until 1999, a significant amount of information and the specific tools necessary to manage AI epidemics adequately are lacking. This refers both to the European Union (EU) situation and to the ongoing H5N1 crisis. However, in recent years some scientific data have been generated and what is available should represent the initial grounds for an international approach to combat this disease.

The first issue that needs to be addressed encompasses legislative and regulatory aspects such as the inclusion of low-pathogenicity avian influenza (LPAI) viruses of H5 and H7 subtypes in the definition of AI for which surveillance, control and trade restriction measures should be applied. This proposal logically follows the scientific evidence that LPAI viruses of H5 and H7 subtypes are the progenitors of highly-pathogenic avian influenza (HPAI) (Garcia *et al*., 1996; Perdue *et al*., 1998; Suarez *et al*., 2004). This represents a crucial aspect for prevention and control of future outbreaks and to limit the circulation of AI viruses, which is among the primary risk factors for the generation of reassortant viruses. It is therefore imperative that official veterinary services identify surveillance and early detection measures for AI in poultry as a priority, and manage LPAI outbreaks caused by viruses of the H5 and H7 subtypes in an appropriate manner.

Prior to the ongoing H5N1 epizootic, HPAI had only once affected wild birds significantly. This outbreak occurred in South Africa in 1961 and caused the death of approximately 1300 common terns (Becker, 1966). It appeared that HPAI was a disease of domesticated birds and that wild birds usually only harboured the low-pathogenic form of these viruses. The unprecedented situation occurring in Asia has resulted in the spill-over of infection to naïve populations of wild birds. Although to date all these birds were either dead or dying, the incubation period of this disease in Asian migratory birds is unknown, and probably shows considerable variability among families and species. In very simple terms, at the moment the scientific community only has an indication of the species that may be infected and succumb to the virus. Knowledge and information on all species that are susceptible to infection, including the incubation period for those birds that do develop a clinical condition, their ability to fly significant distances if infected and data on the route, duration and titre of viral shedding, are unavailable. At this stage only hypotheses can be formulated on the eco-epidemiological consequences of this spill-over.

At the moment it is unclear whether or not HPAI H5N1 is truly endemic in the Eurasian wild bird population or merely limited to spill-over events from domestic birds. If the latter is true, then provided the domestic source of infection is eliminated, and the infections are responsible for the death of the wild avian hosts, presumably the prevalence of infection will gradually be reduced to zero. In contrast, if HPAI infection does not bring about the death of the wild bird host and becomes compatible with normal behavioural patterns and migration in at least some species, this will result in the development of an endemic cycle in wild birds, mimicking the well-known LPAI ecology. The consequences of such a situation are unpredictable.

Recent HPAI outbreaks in Europe, North and South America, the Republic of South Africa and particularly the ongoing H5N1 outbreaks have necessitated the development of control and management strategies in an unprecedented eco-epidemiological situation. As an example, recent outbreaks of HPAI have affected avian species that exhibit a reduced clinical susceptibility to this virus. There is evidence that birds such as waterfowl and ostriches undergo a completely different pathogenesis in comparison with the poultry species (mainly *Galliformes*) that have traditionally been affected by this disease (Ellis *et al*., 2004a). It would therefore seem reasonable to make cautious assumptions and statements and to develop coordinated actions within well-defined research priorities.

The scientific veterinary community has a key role in planning the control and eradication of HPAI, the adequate management of the outbreaks and ultimately in the outcome of the efforts that are being made to combat this global threat. Retrospective analysis of recent outbreaks has permitted the identification of weak points in the management system that represent areas of uncertainty for which improvement is required. On the basis of the eco-epidemiological situation in each country, these areas of uncertainty should be focused and prioritized in order to maximize the outcome of the international effort.

The unprecedented eco-epidemiological situation caused by the A/H5N1 virus is in constant evolution as the virus encounters new ecosystems and new hosts. The virus has spread to the African continent, in which it is likely to become endemic. Spread to countries in which hygienic standards are not respected increases the virus's pandemic potential and raises concerns about food security for rural villages. It is imperative that the scientific community analyses in a timely manner all information that is obtained from new outbreaks in order to understand the epidemiology of this disease to develop appropriate control and prevention strategies.

Definition of Avian Influenza

The marked variation in disease caused by LPAI and HPAI viruses of the same subtype and the fact that, to date, only two subtypes H5 and H7 have been shown to be responsible for HPAI means that a careful, specific definition is required for statutory control and trade purposes.

Former EU legislation for statutory control purposes (EC, 1992) defined AI as:

> "an infection of poultry caused by any influenza A virus that has an intravenous pathogenicity index in 6-week-old chickens greater than 1.2 or any infection with influenza A viruses of H5 or H7 subtype for which nucleotide sequencing has demonstrated the presence of multiple basic amino acids at the cleavage site of the haemagglutinin."

However, on the basis of the evidence that HPAI viruses emerge in domestic poultry from LPAI progenitors of the H5 and H7 subtypes, there is a case that not only HPAI viruses but also their LPAI progenitors should be controlled in domestic poultry (Capua & Marangon, 2000; Alexander, 2003, 2005). As a result, the EU Scientific Committee on Animal Health and Animal Welfare put forward a proposal for a new definition (SCAHAW, 2000), which is:

> "an infection of poultry caused by either any influenza A virus that has an intravenous pathogenicity index in 6-week-old chickens greater than 1.2 or any influenza A virus of H5 or H7 subtype."

A very similar definition has recently been adopted by the World Organisation for Animal Health (OIE) during its 73rd General Session (OIE, 2005a):

> "For the purposes of this Terrestrial Code, avian influenza in its notifiable form (NAI) is defined as an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described below. NAI viruses can be divided into highly pathogenic notifiable avian influenza (HPNAI) and low pathogenicity notifiable avian influenza (LPNAI):HPNAI viruses have an IVPI in 6-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in 4-to 8-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% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the precursor haemagglutinin molecule (HA0); 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."

The term LPAI is then used to define all infections caused by AI viruses that are not NAI viruses. Following the application of this revised definition there will be significant changes in the obligations to notify of AI and in trading regulations with reference to AI. The main difference compared with the past is that in order to trade, countries/zones/compartments must demonstrate freedom from NAI infection. In the past when only HPNAI was notifiable, freedom from infection relied primarily on the absence of clinical cases. With LPNAI being included in the definition, it follows that it is not possible to rely on clinical evidence only, but that freedom must be demonstrated through appropriate surveillance programmes.

The revision of the definition of AI has resulted in modified trade requirements, as these now also apply for LPAI of H5 and H7 subtypes (OIE, 2005a).

It would appear logical that if a unique definition of AI for regulatory purposes is adopted worldwide this would simplify communication and understanding of problems whether they refer to control purposes or for trade regulations. There are, however, several differences in the definition and application of terms and concepts between those recommended by the EU and those recommended by the OIE.

In the EU Directive for the control of avian influenza finalized recently (Directive 2005/94/EC of 20 December 2005) (CEC, 2006), although control measures are extended to include LPAI viruses of H5 and H7 subtype a different nomenclature and terminology to that used by the OIE has been adopted. It is inevitable that this will result in confusion and misunderstanding, not least because the same terms as used by the veterinary and

Table 1. *Comparison of OIE and proposed EU avian influenza definitions and terminology*

Term	Meaning OIE Code and Manual	Meaning proposed EU Directive
Avian influenza (AI)	Infection of birds by any virus of the influenza A genus*	infection of birds by virulent or H5 or H7 influenza A viruses
Notifiable avian influenza (NAI) viruses	Virulent AI viruses and all H5 and H7 viruses present in poultry	Not defined
Highly-pathogenic avian influenza (HPAI) viruses	Not defined, but by earlier definitions and common usage means AI viruses that fulfil a virulence criterion	H5 and H7 subtype viruses with molecular virulence qualification and other viruses that are virulent in IVPI tests
Highly-pathogenic notifiable avian influenza (HPNAI) viruses	NAI viruses that have been shown to fulfil a virulence criterion	Not defined
Low-pathogenicity notifiable avian influenza (LPNAI) viruses	H5 and H7 viruses that do not fulfil a virulence criterion	Not defined
Low-pathogenicity avian influenza (LPAI) viruses	All AI viruses that are not NAI	Viruses of H5 and H7 subtypes that do not fulfil a virulence criterion

**Note*: the recently finalized EU Directive has no term defining these viruses; it uses the term "avian influenza viruses", despite the fact this term is used to define virulent H5 or H7 influenza A viruses.

scientific community are given a different meaning (see Table 1).

The Concept of Freedom from Infection for Trade Purposes

To comply with the new OIE Code for AI, before trade in birds or their products may occur, the exporting country must be able to certify the specific health conditions of the birds or products being traded. Basically, the exporting country needs to demonstrate that its commodities are safe, and meet the recommendations of the OIE Code. In most cases, the import regulations developed will rely in part on judgements made about the effectiveness of animal health procedures undertaken by the exporting country, both at its boundaries and within its territory. Clearly the quality and credibility of the veterinary service is becoming critically important

The veterinary services of an exporting country that is establishing a zone or compartment within its territory for international trade purposes should define clearly the animal subpopulation in accordance with the measures stipulated in the relevant chapters in the Terrestrial (OIE, 2005a) or Aquatic Code (OIE, 2005b), and should be able to explain to an importing country the basis for its claim of a distinct animal health status for the zone or compartment in such terms.

The procedures used to establish and maintain the distinct health status of a zone or compartment should be appropriate to the particular circumstances, and will depend on:

- the epidemiology of the disease (including methods of disease spread and species affected);
- environmental factors (including the presence of natural barriers);
- appropriate and applicable biosecurity measures (including movement controls, use of natural and artificial boundaries, commercial management and husbandry practices); and
- disease surveillance.

The exporting country should be able to demonstrate, through detailed documentation published via official channels, that it has implemented the measures stipulated in the Terrestrial or Aquatic Code for establishing and maintaining such a zone or compartment, based on the claimed health status of the animal subpopulation. In such a case, an importing country should recognize the existence of this zone or compartment and accept the application of the appropriate measures recommended in the *Terrestrial Code* corresponding to the animal health status of the zone or compartment with regard to the importation of commodities from the zone or compartment.

Surveillance guidelines to demonstrate freedom from infection have been developed by OIE and are available (OIE, 2005a). However, given the current situation with AI, for certain countries or enterprises it may be very difficult to demonstrate freedom from NAI in the whole country. For this reason, and particularly with the poultry industry in mind, the OIE has extended the concept of zoning to a functional approach—compartmentalization in order to facilitate trade.

Zoning and compartmentalization are procedures implemented by a country under the provisions of the *Terrestrial Code*, with a view to defining animal subpopulations of different health status within its territory for the purpose of disease control and/or international trade. Compartmentalization applies to a subpopulation when commercial management systems related to biosecurity are applied in order to separate it from other subpopulations of different health status. Zoning applies when a subpopulation is defined on a geographical basis.

The following definitions have been adopted for the *Terrestrial Code*:

- *Zone/Region* means a clearly defined part of a country 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.
- *Compartment* means one or more establishments (premises in which animals are kept) under a common biosecurity management system containing an animal subpopulation 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.

The fundamental requirement for application of either concept is that the subpopulation maintains a functional separation through geographic/legal boundaries or biosecurity management, which allows a clear epidemiological differentiation from populations of different health status. The measures taken to ensure the identification of the subpopulation, and the recognition and maintenance of its health status, need to be documented in detail.

The concept of compartmentalization is particularly useful and applicable for the poultry industry. In general terms it implies that if a given country cannot provide sufficient evidence of freedom from NAI, an enterprise, in collaboration with the official veterinary service, within that country may generate a compartmentalization programme demonstrating that it is free from infection. In addition, an enterprise that has one exporting production line (e.g. layers) and other production lines that are not destined for export may certify only the layer compartment as free, provided the management practices comply with those required for a separate compartment within the enterprise.

Principles for Defining a Zone or Compartment

In conjunction with the above considerations, defining a zone or compartment should be based on the following principles:

- The extent of a zone and its limits should be established by the Veterinary Administration on the basis of natural, artificial or legal boundaries, and made public through official channels.
- The requirements regarding a compartment should be established by the Veterinary Administration on the basis of relevant criteria such as biosecurity management and husbandry practices, and made public through official channels.

- Flocks belonging to subpopulations should be clearly recognizable as such. The Veterinary Administration should document in detail the measures taken to ensure the identification of the subpopulation.
- The measures necessary to establish and maintain the distinct health status of a zone or compartment should be appropriate to the particular disease.

Thus defined, the zones and compartments constitute the relevant subpopulations for the application of the disease specific recommendations in the *Terrestrial Code* and *Aquatic Code*.

Sequence of Steps to be Taken in Defining a Zone or Compartment

There is no single sequence of steps that must be followed in defining a zone or a compartment. The steps that the veterinary services of importing and exporting countries choose and implement will generally depend on the circumstances existing within a country and at its borders. The recommended steps are as follows:

1. For zoning:
   - The exporting country identifies a geographical area within its territory that it considers to contain an animal subpopulation with a distinct health status with respect to a specific disease/to specific diseases, based on surveillance and monitoring.
   - The exporting country identifies the procedures that are being, or could be, employed to distinguish epidemiologically the animal subpopulation in the area from those in other parts of its territory, in accordance with the measures stipulated in the *Terrestrial Code* or *Aquatic Code*.
   - The exporting country provides the aforementioned information to the importing country, and proposes that the area be treated as an epidemiologically separate zone for international trade purposes.
   - The importing country determines whether it may accept such an area as a zone for the importation of animals and animal products, taking into account:
     – an evaluation of the exporting country's Veterinary Services/competent authorities, according to the OIE Codes;
     – the result of a risk assessment based on the information provided by the exporting country and on its own research;
     – its own animal health situation with respect to the disease(s) concerned; and
     – other relevant OIE standards.
   - The importing country notifies the exporting country of its determination and the underlying reasons, within a reasonable period of time, being either:
     – recognition of the zone; in which case, the importing country and the exporting country may enter into a formal agreement defining the zone;
     – request for further information; or
     – rejection of the area as a zone for international trade purposes.
2. For compartmentalization:
   - Based on discussions with the relevant enterprise/industry, the veterinary service of the exporting country identifies within its territory one or more establishments or other premises owned by an enterprise(s) that operates under a common biosecurity management system, and which it considers contains an animal subpopulation with a distinct health status with respect to a specific disease/specific diseases.
   - The exporting country jointly examines the "biosecurity management manual" produced by the enterprise/industry for such establishment(s), and confirms through an audit that:
     – such establishment(s) is(are) epidemiologically closed throughout routine operating procedures as a result of effective implementation of its "biosecurity management manual"; and
     – the surveillance and monitoring programme in place is appropriate to verify the free status of such establishment(s) with respect to such disease(s).
   - The exporting country identifies such an enterprise to be a free compartment, in accordance with the measures stipulated in the *Terrestrial Code*.
   - The exporting country provides the aforementioned information to the importing country, and proposes that such an enterprise be treated as an epidemiologically separated compartment for international trade purposes.
   - The importing country determines whether it may accept such an enterprise as a compartment for the importation of animals and animal products, taking into account:
     – an evaluation of the exporting country's Veterinary Services/competent authorities, according to the OIE Codes;
     – the result of a risk assessment based on the information provided by the exporting country and on its own research;
     – its own animal health situation with respect to the disease(s) concerned; and
     – other relevant OIE standards.
   - The importing country notifies the exporting country of its determination and the underlying reasons, within a reasonable period of time, being either:
     – recognition of the compartment; in which case, the importing country and the exporting country may enter into a formal agreement defining the compartment;
     – request for further information; or
     – rejection of such an enterprise as a compartment for international trade purposes.

An attempt should be made to resolve any differences of opinion over the definition of the zone or compartment, either in the interim or finally, using an agreed mechanism to reach consensus. It is clear that the requirements for trade with reference to NAI have now changed significantly, and preparatory work to the fulfilment of the new requirements should be initiated. In particular, networks between the enterprises

that intend to apply the concept of compartmentalization and the Veterinary Authorities should be established.

Prevention

Outbreaks that involve significant numbers of animals are characterized by the penetration of infection into the commercial circuit. This includes industrially reared poultry but also all other poultry that is traded, including semi-intensive and backyard farms.

Concepts of disease prevention that are applied to industrially raised poultry should not, in theory, differ from management strategies that should be applied to smaller holdings. In practice, however, things differ significantly as very basic biosecurity measures (such as preventing the introduction of animals of different origin into a flock) are sufficiently well respected in the industrial system, but find very little compliance in the semi-industrial or rural environment. For this reason, in certain parts of the world, particularly where mixed species are reared together and traded through the live-bird market system, rural poultry may become a never-ending source of virus, perpetuating virus circulation and resulting in the establishment of an endemic situation.

Biosecurity (encompassing bioexclusion and biocontainment) represents the first and most important means of prevention. It follows that if biosecurity measures of a high standard are implemented and maintained, these represent a firewall against the penetration and perpetuation in the industrial circuit. However, breaches in biosecurity systems do occur. On one hand, the occurrence and extent of the breach should be evaluated and corrective measures should follow; on the other hand, they indicate the need for the establishment of early warning systems for AI. Some of these are currently being implemented in countries that have identified their densely populated poultry areas (DPPA) as areas at high risk, such as The Netherlands and Italy and some states of the USA. These early warning systems include syndrome surveillance programmes and serological monitoring systems (Akey, 2002; de Wit *et al*., 2004; Elbers *et al*., 2004a, b, 2005). However, the early warning systems will have a positive outcome only if an appropriate contingency plan has been developed conjunctly between the official veterinarians and the industry. The firm implementation of the contingency plan is crucial to the reduction of the magnitude of the outbreak.

Syndrome surveillance system for early detection of AI. In general, introductions of either LPAI or the emergence of HPAI strains in poultry are not notified immediately after they occur. In the case of HPAI this is often because the apparent duration of flock incubation time can be significant and the virus is able to spread to other farms in the meantime. The incubation period (i.e. the time between introduction into the flock and obvious clinical signs) of HPAI can vary on the basis of the strain involved, the species affected, but also the type of farming system. In caged layers for example, the incubation period of HPAI has been shown to be up to 18 days (Capua & Mutinelli, 2001). Particularly in areas with a high avian population density and lax biosecurity, infection may become widespread before the index case is identified.

LPAI infections are even more dangerous from this point of view as at early stages of infection they can spread without causing any clinical signs at all or, alternatively, causing a mild clinical condition that could be caused by virtually any bacterial or viral pathogen of poultry (Elbers *et al*., 2004a, 2005). The most difficult situation to manage is when a LPAI virus that has been circulating undetected for a period of time mutates to its highly pathogenic form. In this case the widespread low-level immunity as a result of infection with the LPAI virus confuses the situation and makes the epidemiology of the outbreaks very difficult to interpret.

For the reasons outlined, it is very difficult to ensure the identification of AI infections quickly enough to avoid secondary spread. The problem must therefore be tackled with active surveillance programmes. These programmes can be developed by encouraging farmers to submit carcases or samples from birds that exhibit a clinical condition not necessarily indicative of AI infection. Such syndrome surveillance programmes have the added value that the health condition of the farm is kept under control and, should it occur, an AI infection can be diagnosed in a timely manner.

The main difficulty encountered in applying this system is to persuade farmers and private veterinarians to submit samples on a regular basis to diagnostic laboratories. From the experience gathered within and outside the EU, it appears that farmers are reluctant to participate in such programmes. It has been demonstrated, however, that in order to avoid secondary spread from the index case, the owners/farmers and veterinarians should understand the importance of excluding AI in a very early phase. The fear of application of restriction policies should be counterbalanced by an incentive to participate in the programme.

Diagnosis and confirmation of AI in the field may be obtained within a short time (currently within 6 to 12 h) using the reverse transcriptase-polymerase chain reaction (RT-PCR) (Spackman *et al*., 2002; Cattoli *et al*., 2004). The advantage of this test is that it exhibits sensitivity equal to or higher than the standard virus isolation test and results are obtainable in less than 1 day. Although it is not yet considered an official test, molecular methods will be addressed in the AI Diagnostic Manual that will be annexed to the Directive 2005/94/EC (CEC, 2006).

Serological monitoring systems. Serological monitoring systems are based on routine serological investigations performed on selected flocks, regardless of their clinical condition. The main limit of this system is that, as it is based on a serological test, at its very best it can detect infection 7 to 10 days following its introduction in the flock. However, it does represent a means of identifying infection and can be particularly useful to detect LPAI infections.

An alternative to serological monitoring for detecting AI infections early after they are introduced would be surveillance based on the routine application of antigen or RNA detection systems on samples from birds. At the moment, these systems (rapid antigen capture kits and RT-PCR-based tests) are probably too expensive for most laboratories to be used on a routine basis for surveillance purposes. Further developments and automation of real-time RT-PCR systems may result in a more cost-effective tool for this purpose.

Ideally a monitoring programme, based on serology (or virus detection) and syndrome surveillance, would ensure a maximized outcome of monitoring efforts, and would generate data to demonstrate freedom of infection in a given country or compartment.

Improved Diagnosis and Investment in Knowledge

HPAI has had a devastating impact on the poultry industry in the countries affected by it in recent years. One of the reasons for this lies in the magnitude of the outbreaks, which may have resulted from inappropriate local management in the affected countries, at least at the beginning of the outbreak. Until recent times public and private veterinarians were not adequately trained to suspect and manage this disease. A great deal of improvement has occurred in certain countries, but in other countries this has not been the case. AI is a transboundary animal disease that has its natural reservoir in migratory waterfowl. No country can consider itself safe from introduction, and therefore specific joint training courses for public and private poultry veterinarians to face such an emergency should be considered as national priorities.

The increased impact of AI in veterinary public health requires the upgrading of diagnostic capacities in laboratories worldwide. The correct application and interpretation of diagnostic tests is crucial for correct management of surveillance and eradication programmes. It is imperative that certified reagents and appropriate diagnostic strategies are used to avoid misunderstanding of test results that may impact the decision making process.

The use of appropriate serological tests and their interpretation can be at times rather complex. Appendix 1 supplies guidelines for the correct application and subsequent interpretation of serology in different avian species.

In case of an outbreak or a suspected outbreak, the identification of the agent must be performed in a timely manner. Diagnostic tests that are directed to the identification of specific antigen or of viral RNA have been developed and are available commercially. These tests can be used in conjunction with virus isolation techniques in well-defined situations but should not be used as an alternative to virus isolation. The most worrisome aspect of the use of these tests without coupling them to other systems is that for some their sensitivity may be inadequate for identifying with certainty an index case. Antigen capture assays have shown to have a sensitivity that ranges between 50 and 80%, and this limit must be kept well in mind—as must the type of sample collected. For example, cloacal swabs may not be suitable for some of these tests. PCR assays, both conventional or real-time, are known to be highly sensitive tests, provided the selection of the primers is appropriate. In all cases, virus isolation should always be pursued. The availability of a large collection of AI isolates represents an invaluable amount of information for the veterinary and medical communities, and only through genetic analysis of isolates collected in various parts of the world will it be possible to gather more information on the ecology and epidemiology of these viruses, including an improved understanding of their ability of crossing the species barrier.

Recent outbreaks occurring in areas that have been historically free from HPAI have caused extensive infection in intensively reared avian species that previously had been affected only sporadically. It should be pointed out that, for example, chickens, ducks and ostriches belong to different families within the class *Aves*, and are therefore phylogenetically quite distant and likely to respond quite differently to specific virus infections. Areas of knowledge available for gallinaceous birds are therefore not always directly applicable to birds belonging to different families. These include pathogenesis and evaluation of the carrier state, transmission dynamics and efficacy of vaccination. A coordinated effort should be made to develop joint research programmes that complement each other, thus avoiding overlapping and wasted resources.

Vaccination

Between December 1999 and April 2003, over 50 million birds died or were depopulated following HPAI infection in the EU alone (Capua & Alexander, 2004), causing significant economic losses to the private and public sectors. The pre-emptive slaughter and destruction of great numbers of animals is also questionable from an ethical point of view. This would suggest that the strategies and control measures utilized to combat the disease at the European level require improvement both from a disease control and animal welfare perspective.

Specific recommendations concerning the areas of knowledge that require improvement in the field of AI have been issued by the EU Scientific Committee of Animal Health and Welfare, in the report on "Diagnostic techniques and vaccines for Foot-and-Mouth disease, Classical Swine Fever, Avian influenza and some other important list A diseases" adopted on 24 and 25 April 2003 (SCAHAW, 2003). Guidelines on disease prevention and control with particular reference to the Asian crisis have been issued as joint OIE/FAO/WHO recommendations in the recent meetings in Rome (3 to 4 February 2004), Bangkok (26 to 28 February 2004) and Ho-Chi Min City (23 to 25 February 2005) (OIE/FAO, 2004, 2005).

These recommendations, however, need to be put into practice in a variety of different field situations, and the applicability of one system rather than another in a given situation must be evaluated bearing in mind the benefits of a successful result but also the drawbacks of a failure.

Vaccination has been shown to be a powerful tool to support eradication programmes if used in conjunction with other control methods. Previous experiences have indicated that, in order to be successful in controlling and ultimately in eradicating the infection, vaccination programmes must be part of a wider control strategy, which includes monitoring the evolution of infection and biosecurity.

In order to eradicate AI, the vaccination system must allow the detection of field exposure in the vaccinated flock. This can be achieved using both conventional inactivated vaccines and recombinant vector vaccines.

Conventional inactivated vaccines containing the same viral subtype as the field virus allow the detection of field exposure by regularly testing unvaccinated sentinels left in the flock. This system is applicable in the field but is rather impracticable, especially for the identification of sentinel birds in premises that contain

floor-raised birds. An encouraging system based on the detection of anti-NS1 antibodies has been recently developed and is applicable with all inactivated vaccines provided they have the same haemagglutinin subtype as the field virus (Tumpey *et al*., 2005). This system is based on the fact that the NS1 protein is synthesized only during active viral replication and is therefore not significantly present in inactivated vaccines. Birds that are vaccinated with such vaccines will develop antibodies to the NS1 only following field exposure. Full and field validation under different circumstances of this system is still in progress (Tumpey *et al*., 2005; Dundon *et al*., 2006) and should be made available before this system is recommended.

It is also possible that in the very near future the development of rapid and sensitive virus-detection methods, especially those that can be automated, such as real-time RT-PCR, means that these could be used for simple widespread and regular testing of vaccinated birds for the presence of field virus.

To date, the only system that enables the detection of field exposure in a vaccinated population that has been used successfully and has resulted in eradication is a Differentiating Infected from Vaccinated Animals (DIVA) system based on heterologous vaccination. This system was developed in Italy to support the eradication programmes against several introductions of LPAI viruses of the H7 subtype (Capua *et al*., 2003, 2004b). Briefly, a vaccine containing a virus possessing the same haemagglutinin but a different neuraminidase to the field virus is used. This vaccination strategy enables the detection of field exposure in a vaccinated population through the detection of antibodies to the neuraminidase antigen of the field virus. For the sake of clarity, a vaccine containing an H7N3 virus can be used against a field virus of the H7N1 subtype. Antibodies to H7 are cross-protective, thus ensuring clinical protection, increased resistance to challenge and reduction of shedding, while antibodies to the neuraminidase of the field virus (in this case N1) can be used as a natural marker of infection. Experimental data on the quantification of the effect of vaccination on transmission within a flock using this system have been generated, indicating that the reproduction ratio can be reduced to <1 by 1 week following vaccination. Such a reproduction ratio is indicative of a minor rather than a major spread of infection. In simple terms, such vaccination interventions will significantly reduce (although not prevent) secondary outbreaks (Van der Goot *et al*., 2005), although this will very much depend on the immune status of contact birds and flock.

Promising results have also been obtained with vaccines generated by reverse genetics (Tian *et al*., 2005). These vaccines are expected to have similar performances to conventional inactivated vaccines, but to date no data are available on their efficacy under field conditions.

Recombinant fowlpox vaccines expressing the haemagglutinin protein of the field virus have also been reported to be efficacious in reducing shedding levels and in providing clinical protection. They enable the detection of field exposure, as vaccinated unexposed animals do not have antibodies to any of the other viral proteins. Any test developed to detect antibodies to the nucleoprotein, matrix, NS1 or neuraminidase of the field virus can be used to identify field-exposed birds in a vaccinated population. However, there is some uncertainty on the performances of these vaccines in relation to the immune status of the host to the vector virus (Swayne *et al*., 2000). Recent encouraging studies indicate that vaccination of 1-day-old chicks with maternal antibodies against fowlpox has been successful. Data on the performances of such vaccines in a population that has been once or repeatedly field exposed to fowlpox are currently lacking. Another aspect that must be carefully considered is the host issue. These vaccines are likely to induce protective immunity only in birds that are susceptible to infection with the vector virus.

Regardless of the vaccine and companion test used, it is imperative that the occurrence of infection is mapped within the vaccinated population. This is primarily to monitor the evolution of infection and to manage field exposed flocks appropriately. The latter represent a means by which infectious virus may continue to circulate in the immune population, and, for this reason, vaccination can only be seen as part of a control strategy based on biosecurity, monitoring, controlled marketing and stamping out. A vaccination campaign that is not managed appropriately is most probably going to result in the virus becoming endemic.

Inadequate biosecurity or vaccination practices can lead to transmission between flocks and selection of variants that exhibit antigenic drift. Antigenic drift of H5N2 viruses belonging to the Mexico lineage, resulting in less homology to the vaccine strain, has been described recently (Lee *et al*., 2004). It clearly appears that the extensive prophylactic use of vaccine in Mexico has resulted in the emergence of antigenic variants that escape the immune response induced by the vaccine. Mexico has been practising vaccination since the HPAI outbreak in 1994 without applying the DIVA principle. Although, no HPAI virus has been reported following the implementation of the vaccination campaign, LPAI viruses continue to circulate. Conversely, a similar approach in Pakistan following the HPAI H7N3 outbreaks in 1995 resulted in the isolation of HPAI H7N3 virus in 2004 approximately 10 years later (Naeem & Siddique, 2005).

Emergency Vaccination

Recent outbreaks occurring in developed countries, notwithstanding an efficient veterinary infrastructure and modern diagnostic systems, have resulted in culling of millions of birds. Since the year 2000, AI epidemics in DPPA have resulted in 13 000 000 dead birds in Italy (H7N1), 5 000 000 dead birds in the United States in 2002 (H7N2), 30 000 000 dead birds in The Netherlands in 2003, and 17 000 000 depopulated in Canada in 2004. In all these episodes it appears that the biosecurity measures implemented at the farm level were insufficient to prevent massive spread of AI.

Emergency vaccination for AI has become an acceptable tool to combat the spread of AI, in conjunction with other measures, and therefore could represent an alternative to pre-emptive culling in reducing the susceptibility of healthy flocks at risk, by reducing the transmission rate. The effectiveness of such a policy depends on variables such as the density of poultry flocks in the area, the level of biosecurity and integration of the industry, and on the characteristics of the virus

strain involved. In addition, practical and logistical problems such as vaccine availability and adequate and speedy administration must be kept in mind. The dimension of the vaccination zone in case of a ring vaccination depends not only on the transmission rate, but also on the initial spread during the high-risk period and on the functional interconnections of the infected zone. For this reason the concept of compartmentalization may be more appropriate than zoning for AI management strategies. Based on the preliminary analyses of the Dutch outbreak, a zone up to 35 km around the index case would have had to have been vaccinated (EFSA, 2005). Even so, the risk of spread of the virus not just to adjacent farms, but by fomites over a longer distance, remains, and it is generally accepted that AI cannot be controlled when interventions strategies are based on geography only.

Another issue of relevance is that of the time interval necessary to obtain protective immunity. It is estimated that a minimum of 7 to 10 days are necessary for the initial development of the immune response, and over 2 weeks may be necessary to have protective antibody levels. This implies that the decision-making process must be fast-tracked and vaccine must be available for immediate use. In the face of an emergency, however, uncontrolled movement of vaccination crews may result in spreading of infection rather than in a means of controlling its spread. For this reason, contingency plans that include decision-making patterns under different scenarios should be formulated.

It also appears rather clear that even when vaccination is considered a valid option it is not possible to lay down general conditions for vaccination programmes that can be applied worldwide. Although the industrial system often has overlapping points, there are major differences in animal densities, species reared, husbandry systems and genetic profiles. Analysing transmission dynamics and identifying critical points for virus spread from past and future outbreaks should provide data that are required to design appropriate vaccination programmes in the different situations.

Pivotal work on emergency vaccination has been carried out in Italy, and the application of the DIVA vaccination strategy has resulted in the approval of use of vaccination as an additional tool for the eradication of two epidemics of LPAI (H7N1 and H7N3) without massive pre-emptive killing of animals. Vaccination was used to complement restriction measures already in place and was integrated with an intensive monitoring programme, targeted at identifying viral circulation in the area (Capua *et al*., 2004a) and culling of infected birds. In 2000, for the first time, heterologous vaccination was used in the field against an H7 virus as a "natural marker vaccine", and subsequently in Hong Kong vaccination using a DIVA strategy was successful in preventing further spreading of HPAI to neighbouring farms in face of an outbreak of HPAI H5N1 (Ellis *et al*., 2004b).

Although the use of a DIVA system enabled the continuation of international trade of poultry products (Capua *et al*., 2004a; Marangon & Capua, 2005), vaccination for AI is a new concept and several countries are reluctant to even consider it as a possibility.

Governmental authorities ultimately make the decision of whether vaccination should be used in a given country or not, and their reluctance is probably driven both by legislative and scientific uncertainties, coupled with doubts about how this practice will be used in the field and other considerations such as defining an exit strategy. In countries where there are no DPPA, control by rapid diagnosis and stamping out may well be the most appropriate route to the eradication of NAI. Given the current situation, however, it would be wise both for public and private entities to take into account all the possible control options, including the possibility of vaccinating poultry at high risk of exposure. This should result in the preparation of contingency manuals in times of peace that should include sourcing vaccine beforehand. The decision on whether to use of vaccination or not in the face of an emergency should then be made on the basis of the characteristics of the outbreak and of the poultry industry in the area.

With reference to trade implications, the new OIE *Terrestrial Code* Chapter on AI does enable the continuation of trade in the presence of vaccination providing the exporting country is able to produce surveillance and other data that confirm NAI is not present in the compartment from which the exports come. This is the result of extensive work done by OIE experts and by the OIE Central Bureau on the issue of reducing the impact of animal diseases through the use of vaccination, and is supported by a recommendation document issued a result of an International Conference held in Buenos Aires (14 to 17 April 2004) that strongly supports the use of vaccines for list A diseases (Capua *et al*., 2004a; OIE, 2004).

Vaccination versus Pre-emptive Culling

The financial losses due to AI epidemics can be huge for the commercial and the public sectors, especially once AI viruses are introduced in areas that have high bird densities. In these areas, the high density of poultry farms, the organization of the poultry production sector and the difficulties in applying rigorous biosecurity measures increase the risk of major AI epidemics. Epidemics in such areas have proved difficult to control despite the enforcement of draconian eradication measures based on the depopulation of farms that are infected, suspected of being infected, suspected of being contaminated or located in areas at risk of infection.

Unpublished field evidence from the current situation in Asia indicates that despite the enforcement of massive stamping out and depopulation measures, both LPAI and HPAI viruses may persist undetected in domestic reservoirs or potentially in the wild, re-emerge and rapidly spread after repopulation of poultry farms in previously affected areas. This means that frequent incursions or the re-emergence of AI viruses in densely populated areas can contribute to make these areas unsustainable in the long term.

Management of outbreaks by a stamping out and pre-emptive culling policy alone can lead to very high costs and economical losses for the public sector, the industry and, ultimately, for the consumers, and this needs to be carefully balanced against the trading advantages of rapid eradication and potentially lower costs of alternative measures.

There is no doubt enforcement of heightened biosecurity and stamping out measures on AI-affected farms can be effectively applied in areas with a low poultry density, especially if the sites first infected are promptly

detected and adequately managed. If this is the case, the depopulation of infected premises can allow the rapid eradication of the disease, at acceptable costs for both the producers and the public.

Taking into account the high risk of major AI epidemics once AI viruses are introduced in areas with high poultry densities and the reluctance of national governments or international bodies to actively discourage the formation of DPPA, alternatives to the application of stamping out alone, which will inevitably lead to pre-emptive culling in cases of LPAI or HPAI outbreaks in DPPA, should be pursued.

Prophylactic Vaccination

Prophylactic vaccination for viruses of the H5 and H7 subtypes is a completely innovative concept. This is primarily due to the fact that it is only recently that situations have been pinpointed and identified that may find in this policy a cost-effective solution.

The rationale behind the use of prophylactic vaccination is that it should be able to generate a level of protective immunity in the target population. The immune response may be boosted if there is evidence of the introduction of a field virus to avoid a situation where low-level immunity confuses and interferes with diagnosis.

Prophylactic vaccination should increase the resistance of birds and, in case of virus introduction, reduce levels of viral shedding—at the same levels of biosecurity. It should be perceived as a tool to maximize biosecurity measures when a high risk of exposure exists. Ultimately, it should result in preventing the index case, or alternatively in reducing the number of secondary outbreaks and thus minimizing the negative aspects of animal welfare and potential economic losses in areas where the density of the poultry population will otherwise result in uncontrollable spread without pre-emptive culling.

Prophylactic vaccination should only be considered when there is circumstantial evidence showing that country/area/compartment is at risk of infection. Risk of infection may be subdivided into two categories:

1. High risk of infection with either the H5 or H7 subtype (e.g. from migratory birds).
2. Risk of infection with a known subtype (e.g. live bird markets in the USA, Asian countries with H5N1).

In the first case, a bivalent (H5 and H7) vaccination programme could be implemented. Italy has recently implemented such a programme in the DPPA at risk of infection (EC 2004a). In the second case, a monovalent (either H5 or H7) programme would be sufficient.

The choice of the vaccine is crucial to the outcome of prophylactic vaccination campaigns. Ideally vaccines that enable field exposure with any AI virus should be used. Ideal candidates would be vaccines that enable the identification of field exposure flocks through the detection of antibodies to an antigen that is common to all type A influenza viruses such as NP, M or possibly NS1. Such a strategy would be able to detect the introduction of any subtype of AI.

The DIVA system using heterologous neuramindase has some limitations in its application for prophylaxis or in epidemiological situations where there is the risk of introduction of multiple AI subtypes. The main limitation is that as there is no active viral circulation in category 1 above, or in case of risk of multiple introductions it is impossible to identify a vaccine strain that has a different neuraminidase. An approach to resolving this difficulty is to use seed vaccine strains of the H5 and H7 subtypes exhibiting rare neuraminidase subtypes such as N5 or N8. This selection criterion of vaccine strains will greatly reduce the chance that an AI virus of a similar N subtype is introduced. However, for surveillance purposes unvaccinated sentinels should be present in the flock.

In addition, prophylactic vaccination should not mean vaccination forever. Prophylactic vaccination should be carried out as long as the risk of infection exists, and can be used in a targeted manner for limited periods of time. This means a detailed exit strategy should be formulated before preventative vaccination is undertaken.

What appears to be lacking in some situations are guidelines that define an appropriate territorial approach. These guidelines may be derived from general guidelines on surveillance for epizootic diseases, but must be adapted to the local situations and must be targeted towards a well-defined and pursuable objective. In addition, due to recent exposure of a vast variety of avian species to HPAI, it is imperative that specific research programmes are developed to evaluate the efficacy of vaccination in these species and to develop and validate novel vaccination concepts that enable the DIVA system.

Human Health Implications

Due to the recent cases of human infection caused by AI viruses, and to the concern about the generation of a new pandemic virus originating from the H5N1 virus, AI infections are now considered a significant threat for public health.

Although it has been known for sometime that the human pandemic viruses of 1957 and 1968 appeared to arise by reassortment between viruses present in the human population and AI viruses (Scholtissek *et al*., 1978; Gething *et al*., 1980; Kawaoka *et al*., 1989), because of the apparent "barriers" to human influenza viruses infecting birds, and AI viruses infecting humans, it was suggested that pigs, which both human and avian viruses are known to infect readily, acted as "mixing vessels". Reassortment between human and AI viruses could therefore take place in pigs, with the emergence of viruses with the necessary gene(s) from the virus of human origin to allow replication and spread in the human population, but with a different haemagglutinin surface glycoprotein, so that the human population could be regarded as immunologically naive.

However, there has been a significant change in our understanding of infections of humans with AI viruses following recent events. A summary of reported cases is presented in Table 2. As indicated, until 1996 there had been only three reported infections and these had been the result of unknown contact, in 1959, and two laboratory accidents in 1977 and 1981 (the latter with an AI isolate from a seal). This was in keeping with the findings of Beare and Webster (1991) that in experiments human volunteers produced at best only transitory infections when challenged with AI viruses.

Table 2. *Reported cases of AI infection in humans 1959 to 2005*

Year	Country	Subtype	Number infected	Number of deaths	Symptoms	Reference
1959	USA	H7N7	1	0	Hepatitis?	Campbell *et al.* (1970)
1977	Australia	H7N7	1	0	Conjunctivitis	Taylor and Turner (1977)
1981	USA	H7N7	1	0	Conjunctivitis	Webster *et al.* (1981)
1996	England	H7N7	1	0	Conjunctivitis	Kurtz *et al.* (1996)
1997	China	H5N1	18	6	Influenza-like illness	Chan (2002)
1999	China	H9N2	2	0	Influenza-like illness	Peiris *et al.* (1999)
2002	USA	H7N2	1	0	Serologic evidence	CDC website
2003	China	H5N1	2	1	Influenza-like illness	CDC website
		H9N2	1	0	Influenza-like illness	Butt *et al.* (2005)
	The Netherlands	H7N7	89	1	Conjunctivitis Influenza-like illness	CDC website
	USA	H7N2	1	0	Influenza-like illness	CDC website
	Viet Nam	H5N1	3	3	Influenza-like illness	WHO website
2004	Canada	H7N3	2	0	Influenza-like illness	CDC website
	Thailand	H5N1	17	12	Influenza-like illness	WHO website
	Viet Nam	H5N1	29	20	Influenza-like illness	WHO website
2005	Cambodia	H5N1	4	4	Influenza-like illness	WHO website
	China	H5N1	8	5	Influenza-like illness	WHO website
	Indonesia	H5N1	16	11	Influenza-like illness	WHO website
	Thailand	H5N1	5	2	Influenza-like illness	WHO website
	Viet Nam	H5N1	61	19	Influenza-like illness	WHO website
Total number of human deaths				84		
Total number of human infections			263			

Source: WHO. CDC website, http://www.cdc.gov/flu/avian/gen-info/avian-flu-humans.htm; WHO website, http://www.who.int/csr/disease/avian_influenza/country/en/index.html.

The first reported infection of a human known to have contact with birds was the isolation of an avian virus of H7N7 from a woman in England who kept ducks and presented with conjunctivitis (Kurtz *et al*., 1996; Banks *et al*., 1998). This was the vanguard of the series of isolations from people having contact with poultry presented in Table 2. The impact of these subsequent human infections on public health issues was greatly enhanced by the high death rate in those shown to be infected. Deaths usually occurred as a result of severe respiratory disease and, although there were other symptoms, there was no evidence that virus replicated outside the respiratory tract (Yuen *et al*., 1998) and they were not comparable with the systemic infections seen in poultry.

The biggest threat resulting from of the demonstration of direct natural infections of humans with AI viruses is that pandemic viruses could emerge as a result without an intermediate host. There are two mechanisms by which this could occur: by genetic reassortment, or by progressive adaptation. The first case would occur if a person was simultaneously infected with an AI virus and a "human" influenza virus. In this case, through genetic reassortment, the potential emergence of a virus fully capable of spread in the human population but with H5, H7 or H9 haemagglutinin could occur, resulting in a true influenza pandemic. However, it seems probable that during the widespread outbreaks of H9N2 virus since the mid-1990s and the H5N1 outbreaks in Asia since 1996, many more people than those presented in Table 2 could have been infected with these viruses. For example, a serological survey of poultry workers in Hong Kong after the 1997 outbreak identified 10% seroprevalence of H5 antibodies, but without any known occurrence of clinical disease (Bridges *et al*., 2002). In relation to serological investigations in humans during the Dutch 2003 H7N7 epidemic, which also caused one human fatality and 83 confirmed cases of conjunctivitis, extensive seropositivity was reported (Fouchier *et al*., 2004). Despite this, no reassortant virus has emerged and it may well be that other, unknown, factors limit the chances of a pandemic virus arising in this way.

The second mechanism by which the generation of a pandemic virus may occur is through progressive adaptation of a virus entirely of avian origin. Recent studies on the genome of the H1N1 "Spanish" influenza virus, which affected human beings at the beginning of the twentieth century, have resulted in the speculation that this virus was entirely of avian origin and not generated by reassortment (Taubenberger, 2005), thus suggesting a virus containing all eight segments of avian origin was able to establish itself in the human population and cause more deaths than World War I. Sequencing of genes of this and other viruses that have infected humans directly from the avian source has highlighted mutations that are a result or progressive adaptation to the human host.

Regardless of the mechanism by which the new human pandemic virus may be generated, it appears logical that AI virus circulation should be reduced and that, above all, contacts at risk should be avoided. This is one of the most complex problems to be addressed in developing countries. The human cases that have occurred during the ongoing H5N1 epidemic have developed following contacts at risk between villagers and rural chickens/fighting cocks. The nature and entity of these contacts are dependent on social and behavioural practices linked to food security or hobby activities. In addition, basic hygienic standards are rarely respected. The modification of these patterns appears to be inapplicable in the

short term. Efforts should be concentrated on the reduction of viral shedding from rural poultry so that the amount of virus shed is insufficient to infect a human being. It is considered by many that vaccination interventions of rural poultry currently appear to be the only means to achieve a reduction of virus load in the rural environment. Although if this is attempted without putting in place other important measures, including stamping out where infections are detected, it is unlikely that vaccination alone will have the desired effect and may make the situation worse.

In order to control infection of rural poultry, the awareness of AI and of the risk it poses should increase. This implies the education of farmers and of poultry workers to the basic concepts of biosecurity, farming hygiene, prevention and notification procedures. Farmers should self-notify outbreaks rather than attempting to escape restrictions, and be trained in outbreak management practices. These include the recognition of the disease, the culling of infected birds and their appropriate disposal. In case of the implementation of a vaccination campaign it is imperative that it is carried out using hygienic and appropriate logistic/management practices. Vaccine must be of high quality and must be administered to each group of birds with sterile syringes. The cold chain must be respected and vaccine bottles must be shaken vigorously prior to use so that the quality of the product is maintained and efficacy is guaranteed.

In these conditions, field exposure of infected flocks can be rather difficult to assess. Laboratory diagnosis is not performed in an extensive manner when it comes to rural poultry, and vaccinated birds may not display any clinical signs and actively shed virus, thus perpetuating infection. Leaving unvaccinated sentinel birds in the flock appears to be the only pursuable system of detecting field exposure. The identification of sentinels could be achieved by leaving the male birds in the flock unvaccinated.

Avian Influenza in Poultry Commodities

The zoonotic implications of AI have raised concerns about the safety of food obtained from poultry. AI viruses have been isolated from poultry meat and from commercial eggs (Cappucci *et al*., 1985; Tumpey *et al*., 2002;). The extent of this event is dependent on a series of different factors, including the avian species affected, the viral strain and on whether the product is processed or not. Data on the presence of selected AI strains have been generated recently and other studies are in progress (Swayne & Beck, 2004). These findings will represent the basis for the assessment of the risk posed by poultry products for human consumption and spread of disease to other animals through swill feeding. Swill feeding appears to occur rather frequently in some parts of the world and appropriate measures should be taken to prevent this occurrence.

Given the variety of products originating from poultry that are intended for human consumption, specific experiments should be performed to establish whether viable virus can be detected in different commodities. These experiments should be performed bearing in mind that the pathogenesis of AI may be different in diverse avian species, and therefore any assumption may be incautious.

Little is known about the effect of processing on AI virus. Only a limited number of investigations have been performed on certain commodities, and general notions on the effect of processing commodities on AI virus persistence are extrapolated from experiments with Newcastle disease. Specific studies on the efficacy of heat treatment on the persistence of AI should be performed on a variety of commodities, including those that do not enter the food chain, such as, for example, feathers. The availability of such data would facilitate trade from countries that are major exporters of poultry commodities, in case they are unable to demonstrate freedom from NAI.

When addressing the food safety implications of AI viruses in avian products, it is necessary to consider the pathogenesis of the disease in the infected host as this will determine in which organs or products these viruses are present during the acute course of infection. Viral distribution will be highly influenced by the type of virus (LPAI or HPAI), by the strain of virus, by the animal species involved and possibly, within a species, by other factors such as age and exacerbating factors.

Infections with HPAI viruses, particularly in chickens and turkeys, are characterized by extensive viraemia, and virus may be detected not only in the respiratory and enteric tracts but also in internal organs such as the spleen, pancreas, heart, liver, kidney, nervous system as well as muscle and skin (Starick & Werner, 2003).

Theoretically, LPAI viruses are restricted to replication in the respiratory and intestinal tracts and infections should not result in infective material outside these areas. However, under exacerbating conditions, more generalized LPAI virus infections have been reported, especially in turkeys (Mutinelli *et al*., 2003). Therefore the theoretical absence of LPAI viruses in some poultry products cannot be guaranteed, particularly if the carcases are not perfectly eviscerated (Beato *et al*., 2006). In addition, the extensive replication of LPAI viruses in the intestinal tract and large amounts of virus excreted in the faeces means there is the potential that products could be contaminated with such infective faeces and therefore pose a risk to susceptible birds if adequate hygienic measures are not practised.

The presence of AI virus in avian commodities, and the food safety implications that follow, are also dependent on the characteristics of the commodity itself. Meat and eggs are among the commodities that are considered to pose a potential risk for the transmission of AI to other hosts.

How meat is prepared after slaughter may have significant effects on the survival of infectious virus. For example, all influenza viruses are considered extremely sensitive to acid pH. On the other hand, it is known that poultry meat does not always experience a significant drop in pH, which might also be species dependent (e.g. ratite meat). There appear to be no adequate studies on these aspect in the literature, but it may well be that chilled meat poses less of a risk than frozen meat and the speed at which meat is frozen or chilled after slaughter may influence the survival of infectious virus.

In most poultry species HPAI viruses cause viraemia and systemic infections with virus replication in muscle tissues, and it has long been recognized that HPAI viruses may be detected in the muscle tissues of infected birds; numerous experiments have shown this. For

example, Purchase (1931) was able to show that chickens fed on muscle tissues from HPAI-infected birds became infected. More recently there have been numerous reports of the detection of virus in meat/muscles of HPAI-infected poultry (Mo *et al*., 1998; Perkins & Swayne, 2001; Lu *et al*., 2003; Swayne & Beck, 2005). No information is currently available on whether fresh meat may contain a high enough concentration of virus to infect humans.

There seems little doubt that meat from chickens, turkeys, ducks and other poultry slaughtered during an active HPAI infection will contain infectious virus, and although titres may be low there may be sufficient virus present to infect other birds if fed to them untreated (Swayne & Beck, 2005).

Particular consideration should be given to fresh duck meat. Ducks usually remain healthy when infected with HPAI viruses, although they do become viraemic and virus may be isolated from internal organs (Wood *et al*., 1995; Kwon *et al*., 2005). Infected ducks may well pass veterinary inspection at and prior to slaughter and in recent years HPAI H5N1 virus was isolated from duck meat imported into Korea (Tumpey *et al*., 2002, 2003).

There have been very few reports in which the presence of LPAI virus in meat has been estimated in either experimental or field infections of poultry. In keeping with the assumed lack of systemic virus replication following LPAI infections, Mo *et al.* (1998), using immunohistochemical techniques, failed to detect the presence of LPAI virus in the skeletal muscles of infected birds. Swayne & Beck (2005) failed to detect any virus in the meat of chickens infected experimentally with LPAI H5N2 or H7N2 viruses. But, in contrast, Kishida *et al.* (2004) reported the isolation of LPAI H9N2 virus from imported chicken meat and were able to demonstrate virus in the muscles of chickens infected experimentally with the isolated virus.

Without the report of Kishida *et al.* (2004) the conclusion would almost certainly be that, in keeping with theory, the risk of the presence of LPAI viruses in fresh meat is likely to be very low to negligible even from birds excreting infectious virus at the time of slaughter, and that a greater risk would be the contamination of meat by infective faeces at or after slaughter. However, the presence of the H9N2 virus in meat and confirmation that it is present in muscle tissues during infections suggests that the presence of LPAI viruses in meat may be strain-specific and that the risk may need to be assessed on a case by case basis.

The potential for contamination with faeces and other potentially infective body fluids would appear to be greater for whole carcases than meat cuts.

Poultry meat may also be processed, and the assessment must therefore be whether or not that treatment is likely to reduce the potential level of viable virus contamination to an acceptable level. Most treatments for poultry products involve heat treatment. Influenza viruses are usually considered heat labile. The figures usually quoted are that influenza viruses are inactivated by heat-treating for 15 min at 56°C or for 5 min at 62°C (Easterday & Beard, 1984; King, 1991). However, there has been no proper study of the inactivation of AI viruses by heat treatment in which inactivation curves have been constructed and D_t values determined. Alexander & Manvell (2004) investigated the heat inactivation of Newcastle disease virus in artificially infected meat and calculated D_{65} as 120 sec, D_{70} as 82 sec, D_{74} as 40 sec and D_{80} as 29 sec. In the absence of any similar data on AI viruses, Newcastle disease virus could be considered sufficiently similar to AI viruses that these figures could serve as a guide for estimating the efficacy of heat treatments at reducing the risk of infective poultry meat. However, as mentioned, other factors such as the starting titre of virus, the acceptable level of probability of virus survival and in what quantity of product will need to be assessed.

The other avian commodity that raises most concerns are eggs. These products are often eaten raw, and frequently enter the animal food chain either as shells or as cracked eggs. HPAI viruses have been reported as present on the surface and in the contents of eggs laid by infected hens on most occasions this has been investigated (Moses *et al*., 1948; Narayan *et al*., 1969; Beard *et al*., 1984; Cappucci *et al*., 1985; Bean *et al*., 1985; Starick & Werner, 2003). In experiments, M. Brugh (cited by Swayne & Beck, 2004) was able to demonstrate the presence of H5N9 HPAI virus in eggs laid 3 to 4 days after infection with titres up to $10^{4.9}$ median embryo infective dose (EID_{50})/ml egg product.

Table eggs from HPAI-infected hens and egg trays and other fomites that may come in contact with such eggs therefore represent a very high risk for the potential spread of HPAI virus.

There has been no report of a natural infection of laying birds with LPAI viruses that has resulted in eggs containing virus internally. Swayne & Beck (2004) cited P. Dunn as reporting failure to isolate AI virus from the albumen of 9930 eggs tested during the monitoring of three layer flocks in Pennsylvania infected with H7N2 LPAI during 1996 to 1998. Equally, Lu *et al.* (2004) failed to demonstrate the presence of LPAI H7N2 virus in egg shell swabs, albumen or yolk of eggs laid by hens with respiratory signs and egg production problems despite the virus being present in tracheal and cloacal swabs. In contrast Ziegler *et al.* (1999) reported the isolation of virus from the oviduct in hens infected with H7N2 LPAI during the 1996 to 1998 Pennsylvania outbreak. Thus, while there may be the potential for table eggs to become infected with LPAI viruses internally, the marked absence that this has occurred suggests that this risk is very low.

However, LPAI viruses are excreted in large amounts in the faeces of infected birds and faecal material frequently contaminates the outside of eggs shells. It would seem a wise precaution that the outside of table eggs are treated in some way to reduce the likelihood of faecal and/or virus contamination, either as a routine measure or when the parent flock is known to be or suspected of being infected with LPAI virus. Egg trays and other packaging material, especially if packaging procedures take place in close proximity to the laying flock, may also be contaminated with faeces/virus or infective egg fluids from cracked or broken eggs. It would be a wise precaution that these should be disposed of after use or thoroughly washed and disinfected. Similarly, other fomites that may come in contact with eggs should be thoroughly disinfected after each use.

Egg products are frequently obtained from eggs downgraded from table eggs, often due to cracked shells. As a result these products may 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.

Most egg products are whole eggs or parts of the egg that have been liquefied or homogenized and subjected to some form of heat treatment, or are products that contain egg material treated in this way. Very few studies have been published that assess the survival of HPAI or LPAI viruses in egg materials subjected to heat treatments normally applied during commercial processing. Swayne & Beck (2004) conducted a series of experiments aimed at assessing the heat inactivation of a H7N2 LPAI virus and a H5N2 HPAI virus in various egg products at temperatures used commercially. They calculated D_t values (the time taken for the treatment to inactivate 1 $\log_{10}$) for the two viruses in each of the products and concluded that for homogenized whole egg, liquid egg white and 10% salted yolk, the temperature and time applied in standard industrial pasteurization was likely to reduce a level of $10^{4.9}$ EID_{50}/ml egg product to below or very close to the probability of 1:100 that 1 ml product would contain 1 EID_{50}. However, they considered that the industrial standard of 54.4°C for 7 days for dried egg white would be inadequate for acceptable heat inactivation of virus.

Assessing the risk of treated products depends on several factors; that is, the starting titre of virus, the acceptable level of probability of virus survival in a defined quantity of product, even when the D_t value of the virus in the product is known. Those suggested by Swayne & Beck (2004) do not seem unreasonable, but some recipients of the products may demand greater assurance under some circumstances.

Animal Welfare

The occurrence of major outbreaks of epizootic diseases very often results in compromised animal welfare. This is related both to the suffering caused to the animal by the disease, but also by movement restrictions imposed on animals that should be slaughtered or re-located. Moreover, the development of large-scale culling systems have not kept pace with the growth of intensive farming practices. In order to respect animal welfare, approved culling systems of proven efficacy should be made available or developed on the basis of the requirements and of the type of poultry production in the country. Staff assigned to culling operations must be trained in times of peace and organized in task forces that are able to work on a 24-h shift basis if necessary. In some situations (e.g. if culling capacity is insufficient) vaccination may be seen as a means to preserve animal welfare in the period that precedes culling. Where a control method is used, the risk of poor welfare in the birds that are the subject of the activity should be assessed and compared with the probable benefit to these and other birds as a consequence of the measures taken. An extensive review on the animal welfare aspects of AI has recently been published by the AHAW Panel of the European Food Safety Authority (EFSA, 2005). This report indicates the methods of culling that are recommended, a summary of which is reported in Appendix 2.

Conclusions

The scientific veterinary community has areas of expertise that can support AI crisis management. However, there are areas in which knowledge needs to improve and the outcome of such efforts should be made available to the international scientific community. An enormous effort should be made by national governments and funding bodies to make resources available to develop research programmes based on the priorities that have been identified globally and on the priorities that appear most relevant to the single country.

The new OIE *Terrestrial Code* Chapter on AI, enforced in January 2006, represents the first document that approaches AI in a more modern manner, taking into account the new scientific data that are available on this disease, and makes use of it for regulating trade.

It is imperative that transversal research programmes, encompassing veterinary, medical and agricultural science, are developed and sustained, in order to maximize the global effort to combat this disease.

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Appendix 1. OIE/FAO Guidelines for Correct Application and Interpretation of Diagnostic Results for the Diagnosis of AI on Serum Samples

Introduction. Serological diagnosis of AI should be performed in two steps, unless the subtype circulating in a given country is already known. The first step aims at the detection of antibodies to any AI virus. The second step, to be performed on samples that are positive to step 1, identifies the viral subtype causing infection. OIE-certified reagents should be used for diagnostic purposes, and protocols described in the *OIE Manual* should be used.

Step 1. Detection of antibodies to the group antigen (Type A). These tests are able to detect antibodies to the group antigen of influenza A viruses. The antigen–antibody reaction is against the nucleoprotein (NP) or Matrix (M) proteins of AI viruses. These antigens are present in all influenza A viruses regardless of the H or N subtype. Positivity to these tests indicates that the birds have encountered an influenza A virus but no information on the AI subtype that has caused seroconversion can be deduced. The AI subtype used for the production of the antigen contained in the test is not an indication of the virus that has caused the positive result.

Agar gel immunodiffusion test. This is a simple and reliable test in chicken and turkey sera. It is very specific but is of limited sensitivity, for this reason it must be used as a diagnostic tool on a flock basis. It can be performed in any laboratory with basic equipment. It is completely unreliable in waterfowl as these birds do not produce precipitating antibodies. It has not been fully validated in other avian species.

Enzyme linked immunosorbent assay (ELISA). This is a test that requires more advanced laboratory equipment that must include a spectrophotometer. It is a highly

sensitive test, but it lacks in specificity. In cases of indirect ELISA tests, care must be taken in ensuring that the secondary antibody in the test (anti-species) is directed against the species under examination. Competitive ELISA tests have the advantage that serum of any species can be examined. The manufacturer's instructions should be followed and assumptions on test reactivity for species other than those mentioned in the kit's specifications should be avoided. Serological positivity to type A in waterfowl (wild and domestic) is a normal finding.

Step 2. Detection of subtype-specific antibodies (H subtype). This test is to be used in birds that are known to be infected with AI—either following a positive serologic test against the group (type A antigen), or as a result of clinical history. The test is used to identify the haemagglutinin subtype of the virus causing the seropositivity.

Haemagglutination inhibition. This test is to be used for this purpose. In order to avoid wastage of diagnostic reagents it is recommended that serological positivity to notifiable avian influenza (NAI) viruses is immediately excluded or confirmed. Initial testing should be performed using H5 and H7 subtype antigens. At least two antigens of the same H subtype but with different neuraminidase subtypes (e.g. H5N1 and H5N9 and H7N1 and H7N3) should be used in the initial approach to diagnosis. A sample is considered positive if it causes inhibition of the haemagglutinating activity of 4 HA units at a titre of at least 1:16 (2^4).

Low-degree cross-reactivity with other H subtypes may be observed due to homology with the neuraminidase antigen. This cross-reactivity is generally not higher than 1:16 (2^4) and disappears with another antigen with different neuraminidase. For example: a serum sample is positive to H9N2 at a titre of 1:256 (2^8). If tested with H5N2 antigen a positive inhibition result may be observed at 1:8 (2^3). When tested with an H5N9 antigen the sample will be negative.In case of serological positivity to NAI, first detection of antibodies to viruses of the H5 or H7 subtype in a given country, this result should be confirmed by an OIE reference laboratory. Further investigations aiming at the isolation of the virus should be promptly initiated.

Appendix 2. Summary of Recommendations on Culling of Birds Recommended by EFSA

Full text available online (www.efsa.eu.int).

1. The following methods of killing poultry for AI control are recommended:
   - the killing of birds by placing them in suitable containers, including effectively restricted areas of a building, containing appropriate inert gas mixtures such as argon with not more than 2% oxygen;
   - the use of carbon monoxide provided that the birds are put into a suitable container of pure gas, that the concentration is 4 to 6% for a duration of at least 6 min and there are proper safeguards for human operators;
   - with the exception of ducks and geese for which carbon dioxide should not be used, carbon dioxide can be used provided that the birds are put into not more than 30% carbon dioxide in an inert gas such as nitrogen or argon and not more than 2% oxygen;
   - the use of a portable electrical stunner, poultry killer, or captive bolt stunner, but only if death can be confirmed in each animal;
   - injection of individual birds with barbiturates, a method that is difficult for large numbers of birds;
   - for poultry during the first week of life, dropping into a macerator that will kill the bird instantaneously.

   Other methods—such as putting birds into plastic bags and burning them; gassing with hydrogen cyanide; gassing with impure carbon monoxide; gassing with high concentrations of carbon dioxide: gassing of whole buildings without adequate restriction of the area occupied by gas; or injection with any chemical except barbiturates—should not be used.
2. When selecting a killing method, only those that can guarantee high-volume killing capacity under all weather circumstances should be used.
3. All birds that are to be killed for disease-control purposes should be handled with the same care and concern for their welfare as those that are killed for food.
4. Killing for disease control purposes and vaccination should be carried out only by properly trained persons. The training of persons to do such work should be carried out at times when there is no disease outbreak so that efficient, trained persons are available when any outbreak occurs. Resources should be made available to create a group of trained facilitators for emergency culling of large numbers of birds.
5. It is advisable to involve the local farming community in drawing up plans for each farm or type of farm during non-crisis time, so that in the event of an outbreak of a disease such as AI there will be an optimal killing process with a minimal amount of poor welfare in the animals.
6. If there is a risk that AI will spread from wild or captive birds, the welfare of these birds should be preserved.