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COST-EFFECTIVE TOOLS AND STRATEGIES FOR THE EARLY DETECTION OF AVIAN INFLUENZA IN POULTRY

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UNIVERSIDAD DE ZARAGOZA
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Cost-effective tools and strategies for the early detection of Avian Influenza in poultry

Memoria presentada por **Daniel Beltrán Alcrudo**

Para optar al grado de Doctor

Octubre 2015

El Dr. IGNACIO DE BLAS GIRAL, Profesor Titular del Departamento de Patología Animal de la Facultad de Veterinaria de la Universidad de Zaragoza, como Director,

C E R T I F I C A:

Que D. DANIEL BELTRÁN ALCRUDO ha realizado bajo mi dirección los trabajos correspondientes a su Tesis Doctoral titulada "*Cost-effective tools and strategies for the early detection of Avian Influenza in poultry*" que se ajusta con el Proyecto de Tesis presentado y cumple las condiciones exigidas para optar al Grado de Doctor por la Universidad de Zaragoza, por lo que autoriza su presentación como compendio de publicaciones para que pueda ser juzgada por el Tribunal correspondiente.

Y para que conste, firmo el presente certificado

En Zaragoza, a 28 de octubre de 2015

Dr. Ignacio de Blas

Agradecimientos/ Acknowledgements

Esta tesis ha sido un larguísimo proceso. Más de una década trabajando en ella. De forma más bien intermitente, aunque siempre muy presente en mi lista de tareas a terminar. Han sido muchos los que me han ayudado a lo largo de mi proceso de desarrollo académico y como investigador. Mucha gente a la que agradecer su apoyo, consejo y amistad. Intentaré ser breve.

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Parte importante de mi vida academica se ha desarrollado en la Universidad de California-Davis, de donde han salido tres de las cinco publicaciones de esta tesis. Querria resaltar sobre todo la figura de Carol Cardona, supervisora de mi tesis de maestría y co-autora de mis tres artículos californianos. De ella he aprendido casi todo lo que sé de la influenza aviar, el arte de escribir un artículo de manera clara y concisa, y cómo hacer crítica constructiva. Espero que volvamos a publicar juntos algún día. También de mi etapa en Davis querría acordarme de Tim Carpenter, siempre de buen humor, que me enseñó cómo desarrollar un modelo, así como de Dave Bunn, David Hird y el resto de la comunidad docente del máster que me introdujo en el fastcinante mundo de la epidemiología.

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1 Summary / Resumen

Summary

Avian Influenza (AI), a highly contagious disease affecting the respiratory, digestive and nervous systems of domestic and wild bird species, has become a major veterinary and public health concern (due to its potential to infect humans). AI occurs worldwide. There are numerous virus strains and aquatic birds are the natural virus reservoirs of all of them.

AI can be transmitted from wild birds to poultry, but afterwards it is often perpetuated in poultry by transmission via human-driven factors, i.e. direct contact with infected poultry, or through fomites, i.e. people, vehicles, etc. Live bird markets and unregulated backyard bird populations with low biosecurity play critical roles in AI spread.

In poultry, AI can lead to a variety of clinical presentations, depending mostly on the strain and species affected. Highly pathogenic avian influenza (HPAI) mortalities can reach 100% in terrestrial poultry (e.g. chickens and turkeys), but often lead to no signs in domestic waterfowl. Instead, low pathogenic avian influenza viruses (LPAIVs) may result in inapparent infections or mild respiratory disease that often go unnoticed. HPAI have to be reported and controlled according to all national and international regulations, but the situation is not always so clear for LPAI. The current H5N1 HPAI panzootic has attracted great attention due to its historically unprecedented magnitude.

Since there is no effective treatment for AI, preventing it the entry into poultry populations, and controlling it as soon as it is detected are the best ways to minimise the impact of the disease. Both prevention and control largely rely on and effective surveillance system to provide with early detection and to inform on the disease status and the effectiveness of measures in place. Awareness raising and training of all relevant stakeholders is a cross-cutting approach with direct impact in the implementation of all three components, i.e. prevention, control and surveillance activities.

Surveillance strategies for AI vary from country to country and over time, depending on the infection and risk status of the country, and whether dealing with HPAI, reportable LPAI or LPAI. In any case, surveillance strategies should include activities to monitor wild bird populations and poultry using a combination of passive and active approaches.

Surveillance, particularly active approaches, can be very expensive. When dealing with limited, often insufficient budgets, cost-effectiveness becomes the top criterium in the design a surveillance program. The use of regular risk assessments will help to prioritize the sites, populations and species to target. Costs can also be reduced if surveillance tasks are combined with the implementation of other field activities, e.g. biosecurity assessments and improvement.

Of all surveillance types, passive surveillance is the most cost-effective if implemented effectively. However, for LPAI, where clinical signs may be inapparent or very mild, conventional passive surveillance will not be efficient, and syndromic surveillance, a very novel approach of passive surveillance, presents a promising alternative.

Resumen

La Influenza Aviar (IA) es una enfermedad altamente contagiosa que afecta a los sistemas respiratorio, digestivo y nervioso de aves domésticas y silvestres. La IA se ha convertido en un importante problema de salud veterinaria y pública (debido a su potencial zoonótico). La IA tiene una distribución mundial. Existen numerosas cepas de virus y las aves acuáticas son los reservorios naturales de todos ellos.

La IA puede transmitirse de las aves silvestres a las aves de corral, pero después se perpetúa en las aves de corral debido a factores humanos, es decir, el contacto directo con aves de corral infectadas, o por medio de fomites (personas, vehículos, etc.). Los mercados de aves vivas y las poblaciones de aves de traspatio no reguladas juegan un papel crítico en la propagación de la IA.

La presentación de la IA puede conducir a una variedad de presentaciones clínicas, dependiendo principalmente de la cepa y las especies afectadas. La influenza aviar de alta altamente patógena (IAAP) pueden alcanzar mortalidades de hasta el 100% en las aves domésticas terrestres (pollos y pavos), pero a menudo no producen ningún signo clínico en aves acuáticas domésticas. En cambio, la influenza aviar de baja patogenicidad (IABP) se presenta a menudo como infecciones inaparentes o enfermedad respiratoria leve, por lo que a menudo pasa desapercibida. La IAAP tiene que ser notificada y controlada de acuerdo con todas las normas nacionales e internacionales, pero la situación no siempre es tan clara para la IABP. La epidemia mundial de H5N1 IAAP ha atraído una gran atención por su magnitud sin precedentes históricos.

Dado que no existe un tratamiento eficaz para la IA, impedir su entrada en las poblaciones de aves de corral, y controlarla tan pronto como se detecta son las mejores formas de minimizar el impacto de la enfermedad. Tanto la prevención como el control dependen en gran medida de que el sistema de vigilancia epidemiológica sea eficaz, lo que permite la detección temprana y proporciona información sobre el estado de la enfermedad y la eficacia de las medidas vigentes. La sensibilización y la formación de todos los involucrados es un enfoque transversal con impacto directo en la ejecución de los tres componentes, es decir, la prevención, el control y las actividades de vigilancia epidemiológica.

Estrategias de vigilancia para la IA varían de país a país y con el tiempo, dependiendo del estado de la infección y el riesgo del país, y si se trata de la IAAP, IABP notificable o IABP. En cualquier caso, las estrategias de vigilancia deben incluir actividades para monitorear las poblaciones de aves silvestres y de corral utilizando una combinación de métodos pasivos y activos.

La vigilancia epidemiológica, en particular los métodos activos, puede ser muy caro. Cuando se trabaja con presupuestos limitados, a menudo insuficientes, la rentabilidad se convierte en el criterio de mayor importancia en el diseño de un

programa de vigilancia. El uso de evaluaciones de riesgo regulares ayudará a identificar en qué localidades, poblaciones y especies enfocar la vigilancia epidemiológica. Los costos también pueden reducirse si las tareas de vigilancia se combinan con la aplicación de otras actividades sobre el terreno, como las evaluaciones y mejoras de bioseguridad.

De todos los tipos de vigilancia epidemiológica, la pasiva es la más rentable si se aplica de manera efectiva. Sin embargo, para la IABP, donde los signos clínicos pueden ser inaparentes o muy leves, la vigilancia pasiva convencional no será eficiente. En estos casos, la vigilancia sindrómica, un enfoque muy novedoso de vigilancia pasiva, presenta una alternativa muy prometedora.

2 Justificación como compendio de publicaciones

La presente Tesis Doctoral se presenta como un compendio de trabajos de investigación ya publicados, de los cuales cuatro son en revistas científicas indexadas en ISI-JCR, tal y como se establece en la normativa de la Universidad de Zaragoza.

A continuación se presentan las referencias bibliográficas de los cinco trabajos publicados ordenadas cronológicamente, centrados todos ellos en el estudio de diferentes estrategias para la detección temprana de influenza aviar en poblaciones aviares domésticas:

1) Daniel Beltran-Alcrudo, David A. Bunn, Christian E. Sandrock, Carol J. Cardona. Avian flu school: A training approach to prepare for H5N1 highly pathogenic avian influenza. ***Public Health Reports*** 2008; 123(3): 323-332
2) Daniel Beltran-Alcrudo, Tim E. Carpenter, Carol Cardona. A flock-tailored early warning system for low pathogenic avian influenza (LPAI) in commercial egg laying flocks. ***Preventive Veterinary Medicine*** 2009; 92: 324–332
3) Daniel Beltrán-Alcrudo, Sergei Khomenko, Sherrilyn Wainwright, Jan Slingenbergh. Main animal disease threats in 2010: pathogen types, drivers and challenges. ***EMPRES Transboundary Animal Diseases Bulletin*** 2011; 37: 2-13
4) David Bunn, Daniel Beltran-Alcrudo, Carol Cardona. Integrating surveillance and biosecurity activities to achieve efficiencies in national avian influenza programs. ***Preventive Veterinary Medicine*** 2011; 98: 292–294
5) Anke K. Wiethoelter, Daniel Beltrán-Alcrudo, Richard Kock, Siobhan M. Mora. Global trends in infectious diseases at the wildlife-livestock interface. ***Proceedings of the National Academy of Sciences of the United States of America (PNAS)*** 2015; 112(31): 9662-9667

3 General introduction

Avian influenza (AI) spread is one the biggest threats to animal health, not just because of the important damages to poultry production, local livelihoods and trade, but also because of the public health implications due to its zoonotic potential. Conventional veterinary approaches have failed to stop its spread and bring it under control. Beltran-Alcrudo et al. (2011) lists the major threats to animal health by geographical region, AI being one of the most critical. The paper also stresses the importance to use a one health multidisciplinary approach that confronts the root causes of disease emergence at the animal-human-environment interface, as opposed to the traditional veterinary approach, which has been so far unsuccessful in preventing avian influenza spread around the world.

Avian influenza viruses (AIV) originate in wild bird species. Wild birds have shown to be able to spread the virus over large distances along migratory routes, which has translated into wild bird outbreaks and poultry outbreaks in some instances, when there are low biosecurity measures. Wiethoelter et al. (2015) proved that the wild bird-poultry interface is quite well researched, in fact the most researched of all wildlife-livestock interfaces. However, although the link between wild birds and poultry cannot be denied, the role of wild birds in AI outbreaks in poultry is largely outweighted by human driven factors, i.e. movements of poultry, poultry products and fomites.

For the early detection of highly pathogenic avian influenza (HPAI), an effective surveillance system is crucial to minimize the costs of control before the disease has spread any further. For surveillance to be cost-effective and sustainable, it needs to be mostly based on passive surveillance, which relies on reporting, and some limited targeted active surveillance. Multidisciplinary training programs such as Avian Flu School (Beltran-Alcrudo et al., 2008) are critical to increase awareness and reporting from all stakeholders in the field, which is indispensable for passive surveillance, as well as to ensure that veterinary services are knowledgeable on how to design and implement targeted surveillance.

Implementing HPAI prevention, surveillance and response national programs is a very expensive endeavour, which is often not affordable by the developing countries mostly affected by AI. As shown by Bunn et al. (2011), costs can be significantly reduced if some of the tasks are combined, particularly the implementation of field activities like surveillance and biosecurity improvement.

Low pathogenic avian influenza (LPAI) infections often go undetected by traditional passive surveillance due to the very mild clinical signs. However the early detection of LPAIV infections is still very important because it may cause significant losses for commercial poultry producers if allowed to persist. In addition, LAIVs may become zoonotic and contribute genetic material to HPAIVs. Moreover, H5 and H7 LPAIV strains can mutate to HPAIVs. Since active surveillance for LPAI would be economically unsustainable, it is necessary to develop cost-effective approaches like syndromic surveillance based on monitoring production parameters (e.g. mortality or egg production) like the one proposed by Beltran-Alcrudo et al. (2009). This can be particularly powerful for the commercial poultry production, where these parameters are recorded on a daily basis.

4 Background

4.1. World avian production

Poultry farming is the raising of domesticated birds for the purpose of producing meat or eggs for food, but also for their feathers, hunting or restocking purposes. This includes chickens, ducks, turkeys and geese, but also some game birds (e.g. quails, pheasants, etc.) and other minor species (e.g. ostriches, guinea fowls, pigeons, etc.). Poultry are farmed in great numbers (almost 24 billion in 2013). In terms of species, production is widely dominated by chickens (91%), followed by ducks (6%) (Table 1). Birds can also be farmed for ornamental purposes.

Table 1. Poultry stocks at year 2013 (Source: FAOSTAT, 2015)

Type of bird	x 1,000 head	%
Chickens	21,744,361	90,7%
Ducks	1,335,312	5,6%
Geese and guinea fowls	389,457	1,6%
Pigeons, other birds	32,355	0,1%
Turkeys	459,419	1,9%
TOTAL	**23,962,917**	**100%**

Poultry meat represents over 30% of the meat produced and consumed worldwide, i.e. 88 million tons, with chickens and turkeys representing 87% and 7% of poultry production, respectively. Similarly, hen eggs represent 92% of the egg production worldwide (FAO/EBRD 2010). Despite these impressive figures, the sector continues growing (Figure 1), getting more and more industrialized in many parts of the world.

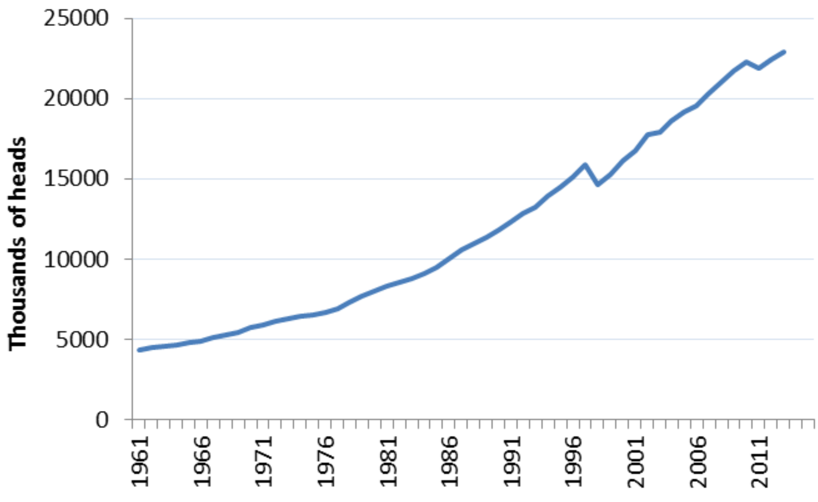

Figure 1. Worldwide evolution of poultry heads in thousands of heads (1960-2013)

This has been largely driven by an increasing human population, greater purchasing power and urbanization. Poultry production is characterized by a large reproductive ratio, turnover rate and excellent feed conversion. In addition, poultry meat presents some advantages when compared to other meats, namely value/price, good nutritional profile/low in fat, convenience/ease of preparation, and versatility (FAO/EBRD, 2010). This explains why the average per capita consumption of poultry meat has almost quadrupled since the 1960s (FAO/EBRD, 2010).

The poultry sector is widely distributed worldwide, concentrating primarily in Asia (mostly South, Southeast and East Asia) and the Americas (mainly North America, Mexico and Brazil), as depicted in Figure 2. The top four producing countries are China (with almost 6 billion heads), the USA, Indonesia and Brazil (Figure 3).

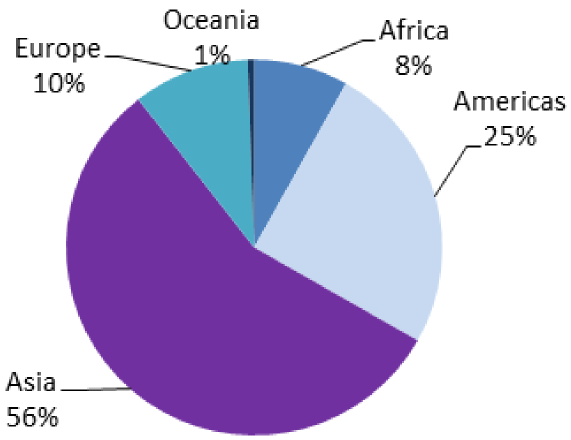

Figure 2. Poultry heads by region (%) (Average 2010-13) (Source: FAOSTAT, 2015)

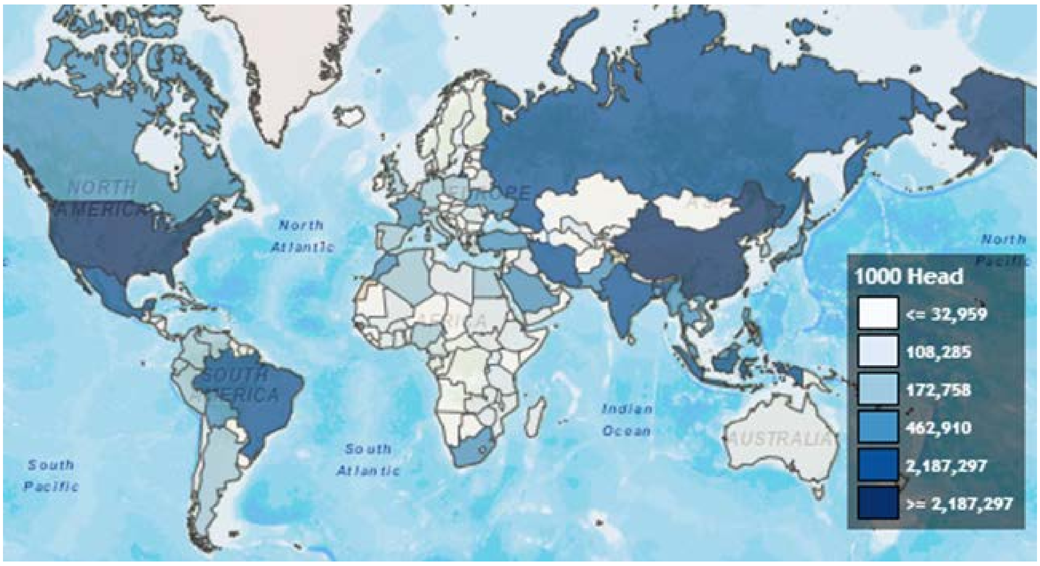

Figure 3. Number of poultry heads by country (Average 2010-2013) (Source: FAOSTAT, 2015)

There is a clear division between large-size, industrialized production systems, and extensive, usually small-scale, production systems. Industrialized production systems are largely organized and integrated into value chains (i.e. about 74% of the world's poultry meat and 68% of eggs) and use highly selected poultry breeds specialized for meat or egg production. The continuous advances in feed, slaughter, processing technologies and health management have led to increased productivity, vertical integration and large-scale units associated to the feed industry, which concentrate themselves around input sources and final markets. On the other hand, extensive systems are traditional small-scale, rural, family-based poultry systems that use indigenous breeds and mostly support rural and periurban livelihoods and supply local markets in developing countries. This small-scale poultry, although progressively reducing its relative market share, still help generating (often secondary) income (particularly to women) and providing a high quality cheap source of protein (FAO, 2014).

Diseases such as avian influenza (AI), Newcastle disease (ND), etc. depress production and cause economic losses, particularly in developing countries. Many of these diseases also affect wild birds, thus complicating the epidemiology, prevention and control. The fact that some can also jump the species barrier into humans (i.e. zoonoses), raises public health concerns.

Avian pathogens have often a transboundary nature. The capacity to rapidly detect, diagnose and control diseases is crucial to minimize losses and spread. While commercial poultry sites can exclude pathogens through biosecurity programmes, in developing countries, the often weak or absent biosecurity at farm level, predisposes for emerging pathogens to become endemic, as recently illustrated by the highly pathogenic avian influenza (HPAI) H5N1 pandemic.

4.2. Avian influenza

Avian influenza (AI) is a highly contagious disease affecting the respiratory, digestive and nervous systems of a variety of both domestic and wild bird species (Alexander, 2000). AI occurs worldwide. There are a large number of virus strains and aquatic birds are the natural virus reservoirs, representing a continuous source of infection. In poultry, AI can lead to a variety of clinical presentations, which often cannot be differentiated from endemic poultry diseases (Swayne and Suarez, 2000). The disease can spread to new geographic regions both through the trade (legal and illegal) of poultry, poultry products, and wild birds, and the movements of migratory wild birds.

AI has become a major concern for veterinary and public health due to its ability to infect humans, amongst a variety of mammals. The H5N1 HPAI panzootic since 2003 has attracted much attention over the past two decades, due to its historically unprecedented magnitude in terms of the number of infected flocks, geographic spread, and economic consequences for agriculture, trade and livelihoods, together with its human health implications (Sims et al., 2005).

Disease emergence in general is triggered by multiple, interrelated factors: human and animal demographics, climate change, increased mobility and globalization, urbanization, land degradation, and mass animal rearing. The resulting changes to host environments, and therefore to pathogen dynamics, can lead to adjustments such as expanding geographic range, jumping host species and/or shifts in virulence (Beltrán-Alcrudo et al., 2011).

From all major animal threats in recent years, H5N1 HPAI perhaps best illustrates the complexity of the factors involved in the local, national, regional and even global spread of a newly emerged animal pathogen. H5N1 HPAI has demonstrated what happens when a new virus enters a new host population (chickens) from where it can jump to further species (human infections, illustrating how the virulence of an agent can vary), and what happens when a new virus can spread across very large distances to new susceptible populations (Beltrán-Alcrudo et al., 2011).

During the current fight against HPAI, it became clear that animal disease management has to be viewed in the broader context of sustainable agriculture and rural development, natural resource management and socio-economic development. Eastern Asia provides the setting for the mixing of poultry, pig and human influenza A viruses, which together constitute an expanding gene pool of diverse virus subtypes, clades and lineages circulating in the avian, swine and human host reservoirs, and thus representing a serious risk of emergence of new highly pathogenic transmissible viruses through recombination (Beltrán-Alcrudo et al., 2011).

This rapidly evolving situation highlights the urgent need for a new approach for disease prevention and control. Current approaches to animal disease prevention and control are based on the disruption of disease transmission (through stamping out, vaccination, quarantine and other veterinary sanitary measures). While these have proved effective in both short- and long-term disease control programmes, such as national responses to foot-and-mouth disease outbreaks and global rinderpest eradication, they have been less successful in some instances, as shown by the current persistence of H5N1 HPAI, despite significant national and international efforts. This is because most current approaches apply strong veterinary science and medicine disciplines in isolation from other relevant disciplines, such as economics, sociology, anthropology, communication, and ecology and land management. Such straightforward veterinary approaches do not confront the root causes of disease emergence at the animal-human-environment interface. Beyond core laboratory and epidemiological surveillance functions, veterinary services now need to expand into an agro-ecological approach to control diseases better. This means focusing on identification of the drivers of disease flare-ups, depicting disease behaviour in the context of host availability and farming landscape dynamics, and investigating the role of ecosystem dynamics and wildlife as the source of pathogens infecting domestic animals and humans. The international community is increasingly converging on such a multi-sectorial, multidisciplinary approach to addressing the increasing disease threats. This approach, termed “One Health”, outlines a collaborative, international, cross-sectorial, multidisciplinary mode of addressing threats and reducing risks of infectious diseases at the animal-human-ecosystem interface, including the wildlife component. Again, H5N1 HPAI has been perhaps the first time that the One Health concept has been applied for an international threat (Beltrán-Alcrudo et al., 2011).

4.2.1. Aetiology and strain classification

Avian influenza is caused by viruses belonging to the Orthomyxoviridae family, genus Influenzavirus A. Of the three influenza genera (A, B and C), only influenza A viruses infect birds. Influenza A is an enveloped virus containing eight segments of single stranded RNA, which encode ten structural viral proteins. Among these, haemagglutinin (HA) and neuraminidase (NA) are used to classify the different AIV into subtypes. To date, 16 HA subtypes (H1-H16) and 9 NA subtypes (N1-N9) are currently recognised in birds, which can occur in any combination, plus two additional HA and NA types been identified, to date, just in bats (Tong et al., 2013). There is usually little or no cross-protection between different HA or NA types.

4.2.2. Official classification, i.e. notifiable AI; HPAI vs. LPAI

Avian influenza viruses (AIVs) can be pathotyped into two groups depending on the severity of the disease they cause in naıve chickens. The HA is considered to be the major determinant of virulence (Senne et al., 1996). The virulent types, termed highly pathogenic avian influenza viruses (HPAIVs) are associated with mortality approaching 100% and severe decreases in egg production. To date, HPAI have been associated only with H5 and H7 subtypes. However, the majority of AIV isolates, including H5 and H7, are of low virulence, i.e. low pathogenicity avian influenza (LPAI) viruses.

There is the risk of a H5 or H7 LPAI becoming virulent by mutation. Although it does not always occur, the transmission of AIVs from their natural reservoir to other species is related to the acquisition of virulence, i.e. HPAIVs arise after a mutation of H5 or H7 LPAIVs that have been introduced to poultry from wild birds (Capua and Marangon, 2006). The HA of LPAIV is cleaved by enzymes present in epithelial cells and respiratory secretions (Swayne, 2007). This explains why LPAI viruses, when entering the host by inhalation or ingestion, remain in the respiratory and gastrointestinal tracts. Instead, the HA of HPAIV is cleaved by enzymes found throughout the body, which translates in HPAI infections being systemic and more severe (Swayne, 2007).

Although, the timing of a mutation is unpredictable, it can be assumed that the wider and longer the circulation of LPAIVs in poultry, the more opportunities for the virus to mutate into an HPAIV (Alexander, 2007). Examples of changes in virulence include the outbreaks due to H7N3 in Canada (Bowes et al., 2004), H7N3 in Chile (Bean et al., 1985; Rojas et al., 2002), H5N2 in USA (Bean et al., 1985), H5N2 in Mexico (Swayne et al., 1997), H5N2 in Italy (Capua and Marangon, 2000) and H7N7 in the Netherlands (Elbers et al., 2004). Because of the risk of a H5 or H7 LPAI becoming virulent by mutation, all H5 and H7 viruses have to be reported to the World Organization for Animal Health (OIE) (OIE, 2015a).

According to the OIE definition, all infections of poultry caused by HPAIV or H5 or H7 viruses, regardless of their pathogenicity for chickens, have to be reported to the OIE (OIE, 2015a). These reportable avian influenzas can be HPAI and LPAI. HPAI include all HPAIV plus those H5 or H7 isolates with a HA0 cleavage site amino acid sequence similar to those of HPAI viruses. Reportable LPAIVs are all H5 and H7 viruses that are not HPAIVs, i.e. H5 and H7 isolates that are not pathogenic for chickens and do not have an HA0 cleavage site amino acid sequence similar to any of those observed in HPAIVs (OIE, 2015b).

4.2.3. Host range

All AIV subtypes and most HA/NA combinations have been found in birds (Olsen et al., 2006). Although many wild bird species may harbour influenza viruses, aquatic birds are believed to be the source of most influenza A viruses (Webster et al., 1992). Anseriformes (especially ducks, geese, and swans) and two families within the Charadriiformes (the Laridae, i.e, gulls and terns, and Scolopacidae, i.e. shorebirds) harbour the widest variety of antigenic subtypes, constituting the major natural LPAIV reservoir (Webster et al., 1992; Olsen et al., 2006). Before the spread of Asian H5N1 HPAI, very few HPAI outbreaks had been described in wild birds. Ever since, Asian H5N1 HPAIV has been isolated in over 150 different species of from 15 orders have been reported and are compiled by the US Geological Survey (USGS) National Wildlife Health Centre at http://www.nwhc.usgs.gov/disease_information/avian_influenza/affected_species_chart.jsp.

A wide array of domestic birds may also be affected by avian influenza viruses, including domestic poultry (commercial Muscovy and mallard ducks, geese, quails, turkeys, guinea fowl, ostriches, pheasants, chukars, partridges and psittacines) and caged pet birds (Alexander, 2000).

Although mammals are considered atypical hosts for AI, respiratory infections have been sporadically reported in minks, seals and whales, as well as some self-limiting sporadic infections in swine and humans (Swayne and Swayne, 2008). The host range is continuously expanding, and only the Asian H5N1 HPAI has been reported in 16 mammal species already, as reported by the USGS, including humans. At the time of writing (4 September 2015), the WHO reports 844 cases of H5N1 HPAI infection, including 449 fatalities (53% case fatality rate) in 16 countries (WHO, 2015a). Also H7N9 has recently caused 662 human infections in humans in China with a 40% case fatality rate (WHO, 2015b). In contrast with the severity of H5N1 HPAI, other avian influenza A viruses - LPAI (H7N2, H7N3, H9N2, or H10N7) and HPAI (H7N3 or H7N7 HPAI) - have caused sporadic human infections, usually after exposure to poultry, causing a wide spectrum of clinical presentations, from conjunctivitis and upper respiratory tract disease to pneumonia and multiorgan failure (Fouchier et al., 2004; Hirst et al., 2004; Nguyen-Van-Tam et al., 2006; Arzey et al., 2012; WHO, 2015b). Fortunately, persistent human-to-human transmission of these AIVs has not occurred. The most notable examples of the zoonotic potential of AI are the human influenza pandemics of 1957 (H2N2) and 1968 (H3N2), in which the HA genes probably originated from a reassortment of avian and human viruses (Scholtissek et al., 1978; Kawaoka and Webster, 1985; Kawaoka et al., 1989). A zoonotic virus can also be generated by mutation of an AIV.

4.2.4. Clinical presentation

Avian influenza viruses circulate naturally in wild bird populations, the natural reservoir, where virus and host have reached an evolutionary equilibrium over time without usually causing any clinical disease. However, infection may be followed by a deteriorated body mass (Latorre-Margalef et al., 2009), and foraging and migratory performance can be hampered due to LPAI (van Gils et al., 2007). In addition, HPAIVs such as the H5N1 can cause massive mortalities.

Low pathogenic avian influenza viruses may result in inapparent infections, particularly when the virus has recently been introduced from the wild to the domestic host (Swayne and Suarez, 2000) or in some poultry species, e.g. domestic waterfowl (Shortridge, 1982; Alexander, 2003). Other LPAIV infections may result in mild respiratory disease, depression, moderate egg production decline in laying birds, and low mortality (Capua and Alexander, 2004), which are easily mistaken for other disease syndromes and can cause substantial losses if allowed to persist in poultry populations. Some LPAI outbreaks may under certain circumstances lead to more severe symptoms similar to that of HPAI, especially in the presence of secondary infections, stressors or environmental conditions (Capua et al., 2003; OIE, 2015b).

HPAI clinical presentation in fully susceptible birds (i.e. non-immunized) can vary depending on the species, age and type of bird, the AIV strain involved, and environmental factor (OIE, 2015a). According to the OIE Terrestrial Manual (2012), clinical signs "*may vary from one of sudden death with no overt clinical signs, to a more characteristic disease with variable clinical presentations including respiratory signs, such as ocular and nasal discharges, coughing, snicking and dyspnoea, swelling of the sinuses and/or head, apathy, reduced vocalisation, marked reduction in feed and water intake, cyanosis of the unfeathered skin, wattles and comb, incoordination and nervous signs and diarrhoea. In laying birds, additional clinical features include a marked drop in egg production, usually accompanied by an increase in numbers of poor quality eggs. Typically, high morbidity is accompanied by high and rapidly escalating unexplained mortality. However, none of these signs can be considered pathognomonic*". The clinical signs are usually more pronounced in chickens and turkeys, while in waterfowl (both domestic and wild), birds usually do not show severe disease or may no show disease at all (Koch and Elbers, 2006).

The incubation period in poultry can range from a few hours to a few days. Also in mammals is short, e.g. as little as 1-2 days.

4.2.5. AIV geographic distribution

Avian influenza's natural reservoirs, mostly Anseriformes and Charadriiformes, have a global distribution, except for the most arid regions, thus translating to an almost global coverage of AIV (Webster et al., 1992; Olsen et al., 2006).

In terms of the distribution of AI in poultry (Figure 4), much more information is available on reportable outbreaks (particularly HPAI) than on LPAI outbreaks. The first reliable scientific report on HPAI outbreak in poultry took place around Torino, Italy, in 1877-1878 (Perroncito, 1878), but the first outbreak caused for sure by a HPAIV was reported in Scotland, UK, in 1959. Since then, detailed compilations of HPAI outbreaks show their presence in most of the world, but mostly in North America, Europe and, more recently, Asia (Capua and Alexander, 2004; Alexander, 2007; OIE, 2015b). The most severe AI outbreak ever is the ongoing Asian H5N1 HPAIV.

On the other hand, there is not such a wealth of information regarding LPAI outbreaks; despite they occur rather frequently in poultry, particularly in some regions such as North America. In fact, only between 2002 and 2006, Alexander (2007) lists sixty LPAIV strains isolated from poultry and other captive birds. The real number will likely be higher, since many outbreaks remain undetected or unreported (Alexander, 2007). Sometimes, when LPAIVs circulate in poultry populations for long periods, this may lead to the formation of stable virus lineages in poultry that can then spread considerably. Notorious examples include the H7N1 LPAIV in Italy (1999), which is still regularly reported in Italy and other European countries, H5N2 in Mexico (1993-present), which is circulating in Central America, or H7N3 in Pakistan (1995) and H9N2 (1998) (Marangon et al., 2003; Lee et al., 2004; Naeem and Siddique, 2006; Cecchinato et al., 2010). The US has experienced multiple LPAI outbreaks, e.g. Pennsylvania (H5N2 1985-1986; H7N2 1996-1998 and H7N2 2001-2002) and California, US (H6N2 2000-2004) (Dunn et al., 2003; Henzler et al., 2003; Kinde et al., 2003).

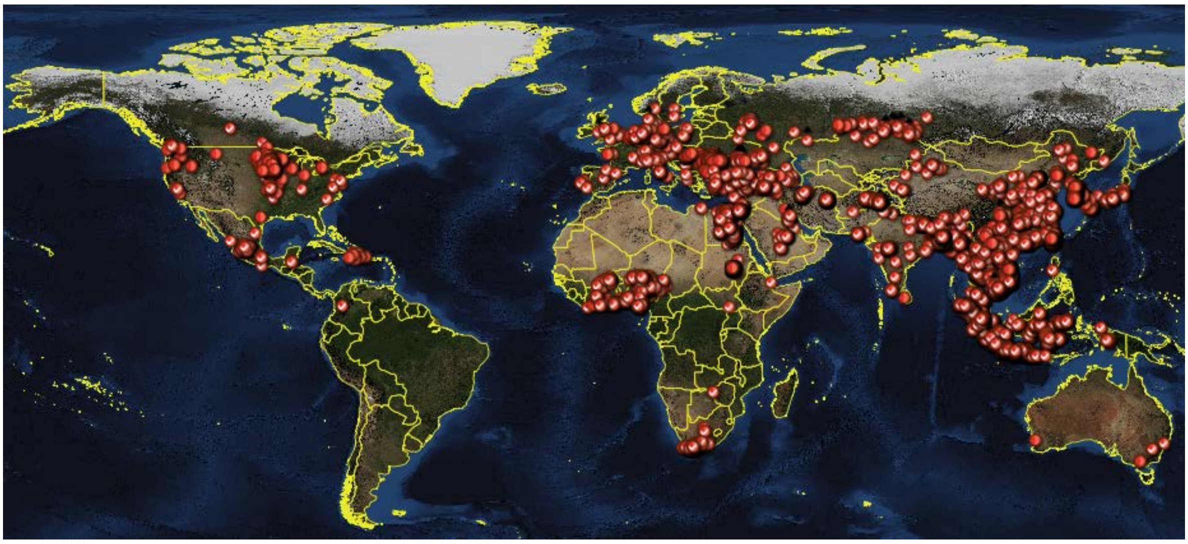

Figure 4. Avian influenza outbreaks in domestic poultry in 2005-October 2015 (Source: EMPRES-i)

4.2.6. The impact of AI

Avian influenza outbreaks, like most other transboundary animal diseases, pose a serious threat not only to the poultry industry, but also to food security and livelihoods. In addition, with AI there is a public health component not to be forgotten. This section applies mostly to HPAI, since the losses caused by non-reportable AI are not usually calculated.

The effects of HPAI on animal production can be divided into three main types: 1) direct losses to producers and other actors of the poultry market chain due to morbidity and mortality, risk mitigation (e.g. investment in animal housing), replacement birds, etc.; 2) cost of government intervention, e.g. public investment in animal health infrastructure and epidemic preparedness; and 3) market reactions, which can be particularly severe when there are public health implications (Otte et al., 2008).

The quantification of the above costs is complicated by numerous factors, e.g. control measures affect even producers not infected by HPAI, and similarly, market reactions can affect HPAI-free countries. Also, the direct impacts on farmers will spread through the supply and distribution networks. However, losses to the poultry sector will be to some extent compensated by gains in other livestock subsectors. Because of these 'systemic' reactions, the structure and flexibility of the poultry industry, its links with other sectors and its integration with global markets have to be taken into account (Otte et al., 2008).

In terms of the costs related to the death and destruction of birds, the outbreaks of 2003 and 2004 in Asian countries are good examples of the magnitude of H5N1 HPAI. Direct losses were highest in Vietnam (44 million birds, i.e. 17.5% of the poultry population, and a cost estimated at 0.3%-1.8% of GDP), Thailand (29 million; 14.5%; 1.5% of GDP) and Indonesia (16.2million) (McLeod et al., 2005). Thailand, at the time the world's 5th exporter of poultry meat, loss big part of the market for fresh poultry meat, also because of the international competition and high dynamism of the global poultry market allowed for other countries (mostly Latin America) to quickly fill in the market lost by Thailand (McLeod et al., 2005). Overall, export shortages due to HPAI and higher prices led to an unprecedented 8% drop in global poultry trade, with Asian exports (particularly Thailand and China) declining from 1.8 million to less than 1 million tonnes. Based on some of these figures, it was estimated that a single large outbreak could lead to a reduction of up to 1.5% of GDP growth considering the effects on the poultry sector alone. The reality is that other associated losses would arise, e.g. to tourism, as actually reported by both Thailand and Malaysia (McLeod et al., 2005).

The impact of HPAI vary by the type of production system: industrial chains suffer mostly from export loss, while large commercial producers serving domestic markets will be penalized by the loss of consumer confidence. Small producers will lose most relative to their assets and income, particularly backyard poultry farmers with no alternative livestock production to which to switch to (McLeod et al., 2005).

Obayelu (2007) pointed out a number of socio-economic impacts in his analysis of the H5N1 HPAI impact in Nigeria, such as job losses, or the temporary loss of consumer confidence, i.e. about 80% of regular poultry consumers shifted to other protein types, which lead to a drop in prices of poultry products (and an increase in other livestock products). About 75% of the poultry suppliers contemplated to change business. The cost of prevention and control was estimated at 0.1-0.2% reduction in the GDP (Obayelu, 2007).

Moreover, avian influenza viruses pose a real zoonotic threat, which adds a whole public health dimension to be considered. The H5N1 HPAI strain, despite its high case fatality rates, has not fully adapted to humans, and human-to-human transmission is still anecdotal. However, the risk that an AIV will adapt and cause a pandemic is a continuous threat, as shown in previous human influenza pandemics of 1957 (H2N2) and 1968 (H3N2), probably the result of reassortments of avian and human viruses.

4.2.7. Persistence of AIV in the environment

The successful transmission of AIVs to susceptible hosts is largely determined by the persistence of the virus in the environment. This knowledge will allow the development of prevention (e.g. effective biosecurity measures and cleaning and disinfection protocols), surveillance and other interventions against AI.

Avian influenza viruses do not replicate outside the body of susceptible animals, but can persist in the environment for substantial periods of time (i.e. up to several weeks under the right conditions). Persistence is strongly influenced by the pH, temperature, and salinity, with most stability observed at a mildly basic pH (7.4-8.2), low temperatures (<17°C), and fresh to brackish salinities (0-20,000 parts per million (ppm)). On the other hand, the viruses' persistence was lower in acidic conditions (pH<6.6), warmer temperatures (>32°C), and high salinity (>25,000 ppm) (Brown et al., 2007; Brown et al., 2009). As a result, AIV may persist in freshwater bodies for 2-3 months at 10°C and for over 6 months at a 0°C (Nazir et al., 2011) and will also survive well in faeces and lake sediments (Chumpolbanchorn et al., 2006; Lang et al., 2008; Nazir et al., 2011). This ability of AIVs to remain infective in water bodies is key for the disease transmission within aquatic birds.

The transmission of AIVs may also occur through contaminated fomites. A study on twelve different porous and non-porous materials and objects routinely found on poultry farms showed that AIV survived on some of the surfaces for up to 6 days (e.g. in latex and feathers) (Tiwarï et al., 2006). Nasal secretions and faecal material protect AIVs, increasing their resistance to chemical and physical deactivation, which is critical in farm conditions. Survival of AIV in faeces is influenced by many variables, e.g. the viral strain, the host or the temperature (De Benedictis et al., 2007). For example, the H5N2 HPAIV from the 1983-1985 Pennsylvania outbreak was shown experimentally to survive in wet faeces at 4°C for 35 days, but only 2 days at 25°C (Beard et al., 1984). The same virus under natural field conditions was still detectable in wet manure after 44 and 105 days, as reported by Utterback (1984) and Fichtner (2003), respectively, although the range of temperature was not reported. On the other hand, H5N1 HPAIV was completely inactivated within just 30 min of direct sunlight at 32-35°C, although the virus was still infective after 4 days in the shade at 25-32°C (Songserm et al., 2006). This information will be critical to determine how long to keep premises vacant after an outbreak.

When looking at cleaning and disinfection protocols, it can be concluded that AIVs are readily deactivated at temperatures of 56°C at 60 min (Muhammad et al., 2001; Lu et al., 2003), ionizing radiation, extreme pH (pH 1-3 or pH 10-14), and by a wide range of disinfectants, particularly in the absence of organic matter. The sensitivity of AIVs to chemicals is explained by the lipid viral envelope, which makes the virus highly susceptible to disinfectants (Benedictis et al., 2007). A comprehensive list of chemical product available for disinfecting procedures and their main recommendations and limitations of use can be found at De Benedictis et al. (2007).

4.2.8. Between-host transmission

The AIV strain, replication site and species involved will determine the presence, concentration and duration of viral particles in each secretion and tissue, and thus the role of each in disease transmission. In poultry, LPAI viruses replicate primarily in the epithelial tissue of the respiratory and gastrointestinal tracts, with some viruses preferring one versus the other (Perkins and Swayne, 2001; Swayne and Beck, 2005), which explains why LPAIV mostly concentrate in faeces and/or secretions from the respiratory tract (Spickler et al., 2008). Shedding can start as early as day 1 in respiratory secretions and day 2 in faeces (Spickler et al., 2008). In its natural reservoir, i.e. waterfowl, LPAI results in large amounts of virus excreted in faeces for 3 to 4 weeks, often without clinical signs.

Instead, HPAIVs, after initial replication in the same organs, follow a systemic spread, meaning that the virus can be detected in numerous tissues including the muscle (meat), blood, bone marrow, upper and lower respiratory tract, kidney, spleen, liver, thymus, pancreas, bursa, adrenal gland, gastrointestinal tract, ovary, testis, comb, wattles, feather follicles and brain (Spickler et al., 2008). Shedding typically occurs within a day or two, both in faeces and respiratory secretions of chickens, but sometimes in just a few hours (Spickler et al., 2008).

Transmission of LPAIVs occurs primarily via the faecal-oral route, as a result of waterfowl suffering from asymptomatic enteric infections shedding the virus via faeces into the water (Webster et al., 1992; Fouchier and Munster, 2009). Respiratory secretions and the faecal-cloacal transmission are also potential routes (Ellström et al., 2008). Since LPAIVs can remain infectious in water for a long time (Brown et al., 2007), the virus can be transmitted to other aquatic birds, including domestic waterfowl.

There is a seasonality attached to AIV transmission in their wild reservoirs, which depends on the geographical location and species involved. Prevalence rates and shedding patterns vary throughout the year. Aquatic birds tend to shed large amounts of virus when they are immunologically naïve juveniles (Webster et al., 1992). For example, AIV prevalence in North American waterfowl ranges from <1% (during spring migration) to 30% and even 60% just before and during fall migration, due to the large number juvenile birds (Hinshaw et al., 1980; Krauss et al., 2004). In terms of the species, although geese and swans are less frequently infected than ducks (Olsen et al., 2006), their tendency to congregate in large groups on agricultural fields makes it more likely for them to infect domestic waterfowl.

The wild bird-poultry interface is quite well researched, in fact the most researched of all wildlife-livestock interfaces ranking first in Asia, Europe, and North America and second in Oceania, Africa, and South America. Of all publications citing a bird-poultry interface, 22% were associated with avian influenza (Wiethoelter et al., 2015). Eventually, all AIVs circulating in poultry were initially introduced from the wild reservoir. In areas where AIVs are frequently isolated in chickens or turkeys, e.g. Missessotta, USA, the variation in virus subtype and the seasonality observed suggest multiple primary introductions rather than an endemic situation in poultry (Alexander, 2000). Between 1978 and 2000, Minnesota turkey farms experienced 108 LPAIV introductions from migratory ducks (Halvorson, 2002). However, although the link between wild birds and poultry cannot be denied, the role of wild birds in AI outbreaks in the long time spread in poultry is largely outweighted by human driven factors, i.e. movements of poultry, poultry products and fomites (Wiethoelter et al., 2015).

Transmission of AIVs from the wild bird reservoir to poultry may occur as a result of direct or indirect contact. By sharing the same water body, wild birds may transmit AIVs to domestic waterfowl. Indeed, the presence of scavenging ducks and ducks raised in rice fields has been shown to be an important risk factor for H5N1 HPAI in South East Asia (Tiensin et al., 2005; Gilbert et al., 2006). A study using sentinel ducks placed on ponds in turkey-rearing areas in Minnesota showed a direct correlation between the infection in the sentinel ducks and the wild ducks at the monitoring sites. Moreover, AIVs were also isolated in the water (Halvorson et al., 1983). Turkey flocks were also monitored in the study. Surveillance results of a 4-year period showed that: 1) AI followed seasonal patterns in both sentinel ducks and turkeys, but usually with a 6-8 week delay in turkeys; and 2) most of the AIVs involved in the turkey outbreaks were also detected in the ducks and other avian species. These results suggest the transmission chain from wild waterfowl to domestic waterfowl via water bodies, and then to turkeys (Halvorson et al., 1983; Halvorson et al., 1985). Domestic waterfowl can also transmit the disease to chickens and other terrestrial poultry through commercial transportation and particularly at live bird markets (Senne et al., 2003; Sims et al, 2003; Yee et al., 2008).

Direct transmission from wild birds to poultry can also occur, particularly in the case of outdoor free-ranging poultry (e.g. turkeys, chickens or ostriches) (Koch and Elbers, 2006), and especially when these are situated on migratory waterfowl routes, near open storage of drinking water or artificial ponds, or in the absence of bird-proof feed stores (Lang, 1981; Alexander, 2000; Koch and Elbers, 2006). Also, because of the mixed backyard flocks that often include domesticated geese and swans, which may attract wild related species (Alexander, 2000). Feral birds are believed to play the role of a bridge species between wild waterfowl and poultry, although of course, the opposite also occurs, with wild birds, particularly feral birds, being affected following HPAI outbreaks in poultry (Alexander, 2000). Wild birds may also become infected from poultry by feeding on infected poultry carcasses (Kwon et al., 2005) or, potentially, through the fertilization of fish ponds with poultry manure, which is widespread in Asia (Melville and Shortridge, 2006).

Poultry and other land-based birds do not share water bodies, as oppose to aquatic wild and domestic birds. Therefore, AIV transmission among them occurs via different routes, i.e. direct contact with other infected poultry, or through fomites, i.e. people (e.g. farmers themselves or service providers), vehicles, and other inanimate objects such as cages moving from one farm to farm can vector the spread of AIV. Also contaminated feed or water may be involved. Live bird markets (Senne et al., 2003; Sims et al., 2003; Yee et al., 2008), and unregulated backyard bird populations with low biosecurity (Meleigy, 2007; Chantong and Kaneene, 2011) play critical roles in AI spread.

4.2.9. Between country spread

A study by Kilpatrick et al. (2006) looking at the H5N1 HPAI introductions to countries worldwide found that, in Asia, 9 out of 21 introductions were most likely through poultry and 3 out of 21 through migratory birds. On the other hand, most introductions (20/23) in Europe were attributed to migratory birds, while it was more balanced for Africa (2/8 by poultry and 3/8 by migratory birds).

Outbreaks in poultry via infections from migratory birds, as already discussed in the previous section, will be mostly related to farming in migratory routes, the farming of free-ranging of domestic waterfowl, the presence of attractants in farms, and the existence of live bird markets.

For the introduction via infected/contaminated poultry, poultry products or fomites, we have to consider that, a priori, the whole world is at risk due to globalized trade. Movements of infected poultry and their products can take place through both formal (van den Berg, 2009) and informal trade (mostly between neighbouring countries) (Beato et al., 2009).

There are few documented cases of legal shipments infected with HPAI, since it is uncommon to import from high-risk countries. Still, an outbreak of H5N1 HPAI in Tibet was traced to a legal shipment of live chickens from Lanzhou (1,500 km away) (Normile, 2005). This may happen particularly if birds are moved during the pre-clinical phase of the infection. The transboundary spread of HPAI through the movement of live birds was reported in the Netherlands, Belgium and Germany in 2003 (Beato and Capua, 2011). H5N1 HPAI was also found during routine surveillance in duck meat legally imported from China to Japan (Mase et al., 2005). In 2005, imported birds destined for the UK pet market tested positive for H5 HPAI at a quarantine station (DEFRA, 2005). In 2007, H5N1 HPAIV was also reported in Houbara bustards after importation into Saudi Arabia (Monne et al., 2008).

The highest risk occurs through the informal trade of live birds, usually across the border of neighbouring countries. Because of their nature, these informal movements are difficult to trace. Sequencing of AIV has proven very useful in establishing links and tracing back possible sources of introduction (van den Berg, 2009). Examples of informal trade of wild birds include the finding of two mountain hawk eagles infected with H5N1 HPAI illegally imported to Belgium from Thailand (van Borm et al., 2005). Similarly, the Taiwanese authorities discovered a container from China with 1,037 exotic birds infected with H5N1 HPAIV (ProMED, 2005).

The trade of products represents a low risk. Although AIVs have been isolated following field or experimental studies in almost any poultry product, the fact that they are generally heat-processed before consumption decreases considerably the risk for poultry outbreaks or zoonotic infections. Field studies have found H9N2 LPAIV in imported chicken carcasses from China to Japan (Kishida et al., 2004), H5N1 HPAIV in duck meat from China to South Korea (Tumpey et al., 2002) and from China to Japan (Mase et al., 2005), H5N2 HPAI in eggs collected at an outbreak in Pennsylvania and Virginia, USA (Cappucci et al., 1985), and H5N1 HPAIV in shell washes of duck and goose eggs from Viet Nam to China (Li et al., 2006). In addition, Yamamoto et al. (2007) showed experimentally the presence of H5N1 HPAIV in duck feathers.

4.3. Prevention and control

There is no effective treatment for AI. Therefore, preventing the entry of AIVs into poultry populations, and controlling and eradicating it as soon as it is detected are the best ways to minimise the impact of the disease. The prevention and control activities/measures involved can be implemented through either private or public initiatives, but reaching an optimal level generally requires a combination of both (Beach et al., 2006). Farmers play a key role, but they may need technical and financial support. Probably the most cost-effective measure, common to both prevention and control, but also surveillance, is the creation of awareness and the provision of information and technical assistance. This will help poultry producers to make efficient decisions in the adoption of prevention and control measures. Awareness raising and training of all relevant stakeholders is a cross-cutting approach that will have a direct impact in the implementation on all disease prevention, control and surveillance activities. Everyone in contact with birds should be made aware, not just those taking care of the birds, but also further along the poultry market chain, i.e. those involved in the transport, marketing and butchering of birds, as well as service providers (e.g. private veterinarians, feed distributors, etc.).

Producers, particularly backyard farmers, need to be aware of the potential severity of AI, as well as the clinical presentation and the need to report suspected outbreaks to the authorities (i.e. passive surveillance), particularly since farmers may accept significant poultry losses as "normal" in many developing countries (Rushton et al., 2005). Information on measures to reduce the likelihood of infection (i.e. biosecurity), the importance of acting quickly to contain outbreaks and the importance to protect themselves (i.e. the zoonotic risk), should also be provided. Even information on the control policy, e.g. stamping out, vaccination, compensation and restocking will help farmers to understand their role in the whole process and be more willing to cooperate.

The development and dissemination of this kind of information may be provided through extension and outreach services, mostly by public authorities (sometimes also NGOs), rather than by the private sector. This information will lower the private cost of prevention and control measures for producers by reducing the time and human capital needed to identify and adopt them (Beach et al., 2006).

A number of different approaches can be used for the delivery of this information, e.g. leaflets, booklets, posters, TV and radio messages, meetings by religious leaders or village chiefs, etc. In some cases, however, a more thorough training is needed. As for awareness materials, there are multiple formats available, from distance learning on-line type of courses, to face-to-face traditional capacity building. Based on the assessment that there is a need to deliver information to large numbers of people, a train-the-trainer (TOT) model might be the best approach in some cases. These are programs designed for training people, who will in turn train others. Also known as "cascade training," the TOT approach is commonly used in the fields of animal health, public health, and agricultural extension.

The TOT approach has been used, in both developed and developing countries, for a whole range of issues such as Newcastle Disease vaccination in chickens, acquired immunodeficiency syndrome (AIDS), asthma, care for disaster survivors, health promotion for childbearing, promotion and risk reduction in pregnancy, neonatal intensive care, and the use of pesticides by farmers (Williams, 1978; Lowe, 1988; Anonymous, 1991; Anonymous, 1994; Armstrong, 1999; Oswalt and Boyce, 2000; Burgess et al., 2001; Gennaro et al., 2001; Slutsky and Bryant-Stephens, 2001; Alders et al., 2002; Gennaro et al., 2002; Tetteh et al., 2005; Normile, 2007; Koffel and Reidt, 2015). There have been TOT programs focusing on H5N1 HPAI, like Avian Flu School, which covered all aspects related to the prevention and control of the disease and was implemented in several countries (Beltran-Alcrudo et al., 2008), and a participatory surveillance TOT in Egypt (Rushton and Rushton, 2009).

4.3.1. Prevention

The risk of introducing AIV (or any other avian pathogen) to poultry (or other birds) is reduced by the adoption of good biosecurity practices, not just at farm, but at each and every step of the poultry market chain. Special attention should be paid at small commercial and backyard premises, which are characterized by low biosecurity standards, and at live bird markets, which bring together domestic (and sometimes also wild birds) of different species and multiple sources. Although the same biosecurity concepts apply to them, specific sets of measures/manuals have been specifically developed for these settings (Nyaga, 2007).

The purpose of biosecurity is two-fold. On the one hand, it is aimed at preventing any contact with potentially infected domestic or wild birds, mechanical vectors and fomites including feed and water sources (as described in the earlier section on between-host transmission). On the other hand, biosecurity will slow down or stop the spread of the disease within the farm and to other premises or wild birds.

Avoiding the contact with wild birds can be achieved by keeping poultry in closed housing and ensuring that wild birds cannot access poultry feed and water supplies. New poultry introduced into the farm should be first isolated/quarantined, and the access of people, vehicles or equipment should be limited and only allowed prior cleaning and disinfection or changes of clothing. One critical activity for AI spread is the transportation and marketing of poultry, especially at live bird markets. As a general rules, birds should not be returned to the farm from live bird markets. Other recommended biosecurity measures include the reduction of contamination by cleaning and disinfection, or the practice of all-in/all-out production systems.

Biosecurity is also a concept that can be applied at the national level. Measures may include trade regulations and quarantines restricting the importation of live birds and bird products, and the implementation of government policies restricting outdoor rearing of birds, or closure of live bird markets during high risk periods. These measures should be dynamic according to the risk situation, as assessed via the risk analysis of all potential routes of entry and spread (Zepeda and Salman, 2003; Murray, 2004). The OIE Terrestrial Animal Health Code also provides detailed guidelines (OIE, 2015a). Regulatory and quarantine services should be equipped to effectively intercept foodstuffs and other risk materials at international airports, seaports and border crosses. Confiscated risk materials should be destroyed or disposed, and never dumped where they can be accessed by scavengers (wild birds or humans).

4.3.2. Control

While guidelines for HPAI control have been clearly described and established, the situation with LPAI is often not that clear. However, there are four important reasons why LPAI outbreaks should be controlled: 1) LPAIV infections may cause significant losses for commercial poultry producers; 2) H5 and H7 LPAIV strains can mutate to HPAIV; 3) AIVs may expand their host ranges to new species, including humans; and 4) LPAIV strains can contribute genetic material to HPAIV (Chin, et al., 2002). Although there is no disagreement that LPAI should be swiftly controlled, there are often no government policies (Halvorson, 2002). Therefore, control measures such as stamping out or vaccination are only sometimes applied to non-H5/H7 LPAIVs outbreaks (Senne, 2007).

In any case, the control measures against AI can be classified as follows:

4.3.2.1. Depopulation and disposal

Stamping out consist on the culling of infected animals, plus usually also in contact animals, and even neighbouring premises or dangerous contacts. The slaughter of animals must be conducted in a humane way, i.e. respecting animal welfare. After stamping out is completed (if possible on-site), carcasses must be disposed of also on-site in a safe manner, i.e. burnt, buried or composted to prevent carcasses utilized for consumption, and to avoid scavenging animals accessing them (Martin et al., 2006). The disposal of very large numbers of birds in a short time presents major logistic, but also environmental problems. The destruction of carcasses should be followed by the thorough cleaning and disinfection of all premises, vehicles and equipment. The manure, feathers and feed should be removed and if the floor is earthen, the top of the soil should be removed (OIE, 2009). Following cleaning and disinfection, depopulated premises should not be restocked for 21 days at least (OIE, 2009), and it is advisable to start with a small number of sentinel poultry first to be monitored daily before full repopulation (Martin et al., 2006).

The single most important challenge arising from stamping out is that farmers will reject to have their animals killed in the absence of timely and adequate forms of compensation in place. The absence of compensation may lead to 1) outbreaks not being reported; 2) emergency slaughter by farmers either for their own consumption or sale; 3) hiding of animals or their movement to other premises; or 4) inappropriate carcass disposal in areas accessible to scavengers. Therefore, no stamping out should be applied in the absence of a sound compensation program. For further information on how to establish a good practice for compensation as part of HPAI stamping-out strategies, there are guidelines available (Delgado et al., 2006).

While HPAI-infected poultry flocks are usually depopulated and disposed, the measures taken with LPAI-infected flocks may vary with the virus and the country's legislation. H5 and H7 strains are sometimes dealt with as if they were HPAI, regardless of their pathogenicity. For LPAI, rather than stamping out, controlled marketing of infected and vaccinated flocks is applied as a means to reduce bird density in an area to limit disease transmission (Halvorson, 2002).

4.3.2.2. Movement controls

Following an outbreak or suspected case, strict quarantines should be imposed on the premises (both those infected and those under suspicion) as soon as possible, i.e. no movement of birds, meat and potentially infected materials allowed off the property. No one should leave the farm without changing (or disinfecting) clothes and footwear and pets should be confined. The idea behind movement control is to prevent disease spread. Its success depends on the early identification of the index flock and the tracing of all movements off infected farms.

When applied to a whole area or territory, effective quarantine and movement control requires continuous monitoring, patrolling, etc. by the police or military forces to ensure that only authorised personnel are allowed to enter and supervise the movements of residents. A type of movement control is the temporary closure of live bird markets, which has been often used in the control of H5N1 HPAI in Asia, as well as any other bird concentrations in the outbreak area, e.g. cockfighting, pigeon racing. etc. (Martin et al., 2006).

4.3.2.3. Vaccination

Vaccination can be useful to prevent disease and death (by reducing the susceptibility of the population), increase resistance to infection, and reduce (although not eliminate) virus replication, shedding and transmission (Marangon et al., 2008). Emergency vaccination can be an alternative to culling and to protect valuable species such as zoo birds. Vaccination is a costly and logistically demanding endeavour. For a successful vaccination program, numerous factors should be taken into account: the bird density, predominant production systems, virus strain, and the availability of vaccine, equipment and personnel.

The decision to vaccinate has to be carefully analysed, because of the associated implications in terms of 1) disease freedom declarations and re-establishment of trade (since many countries will not import poultry products from countries that vaccinate); 2) the phenomenon known as silent spread related to the fact that even the best vaccines do not provide sterilizing immunity and vaccinated birds continue to shed, despite being protected from infection; 3) vaccine-resistant isolates can emerge; and 4) the difficulty to differentiate vaccinated birds from naturally infected birds. Regarding the latter, it can be overcome by the use of DIVA strategies (differentiating infected from vaccinated animals) (Marangon et al., 2008), although they are difficult to implement. Vaccination has been used as part of control efforts in a number of LPAI and HPAI outbreaks all over the world (Marangon et al., 2008).

4.3.2.4. Zoning and compartmentalization

Both concepts apply to the establishment of animal subpopulations defined on a geographical basis (using natural, artificial or legal boundaries) in the case of zoning, or by management and husbandry practices related to biosecurity in the case of compartmentalization, e.g. to separate the high biosecurity commercial sector from the low biosecurity backyard (OIE, 2015a). Where the disease is already present, but only in part of a country, zoning becomes an important strategy towards progressive elimination or eradication efforts. For zoning to be applied, it is key for the national authorities to be able to establish infected and disease-free zones and enforce tight controls on the movement of poultry and products between zones. Zoning and compartmentalisation are aimed to facilitate the implementation of control measures while maintaining trade to some level.

4.4. Surveillance

The Terrestrial Animal Health Code of the OIE defines surveillance as the systematic ongoing collection, collation, and analysis of information related to animal health and the timely dissemination of information so that action can be taken (OIE, 2015a). The objective is to detect and monitor changes in health-related events in a defined animal population with specific predetermined goal/s, the most important being: 1) to detect disease incursions; 2) to describe the spatio-temporal distribution of the disease to inform prevention and control efforts; 3) to assess the progress of control or eradication efforts, e.g. vaccination campaigns; 4) to demonstrate disease freedom (for trade purposes); or 5) to monitor antigenic drift.

Surveillance strategies for AI vary from country to country and over time, depending on the infection and risk status of the country concerned, and whether dealing with HPAI, reportable LPAI or LPAI. Because of their economic impact, all HPAIVs in poultry should be reported to the OIE. The same goes for all H5 and H7 LPAIVs, due to the possibility they may become HPAIVs through mutation. Surveillance in these cases must be closely linked with a clear response, e.g. stamping out, movement restrictions, etc. This is not the case for non-reportable LPAI, for which there is no mandatory international regulation.

In any case, surveillance strategies should include activities to monitor wild bird populations and poultry using a combination of passive and active approaches. For example, for an HPAI surveillance strategy, it may be appropriate to rely on passive (i.e. clinical) surveillance for chickens or turkeys, which usually exhibit clear clinical signs, and use active (virological and serological) surveillance to target species that may not show clinical signs (e.g. ducks or geese) (OIE, 2015a). Regardless of the surveillance approach, the characteristics of the diagnostic tests to be employed (e.g. sensitivity and specificity) should be carefully considered in the design, sample size determination and interpretation of results (OIE, 2012).

FAO has listed the minimum requirements for effective surveillance (FAO, 2004):

- HPAI must be notifiable;
- The official veterinary services must have a formal system for detecting, investigating and reporting internationally, in accordance with OIE guidelines;
- To have the technical capability to diagnose;
- To have a system for recording, managing and analysing surveillance data;
- To participate in the regional surveillance and diagnostic network;
- A minimum frequency of surveillance of six months.

When considering HPAI, the main goal of the surveillance strategies of free countries will be early detection that allows an early response. Passive surveillance will be paramount, relying on the reporting of unusual mortalities in domestic poultry and wild birds. Countries will need access to detailed and updated information on the risks of introduction through different routes, in order to focus their surveillance efforts at the highest risk points (Martin et al., 2006). Sites and populations were some active surveillance could be implemented at times of high risk, depending on the most likely route of introduction, include borders and international entry points (particularly next to infected countries), domestic waterfowl, and live bird markets (FAO, 2004).

Infected countries will instead have some or all of the following surveillance goals: 1) description of the spatio-temporal distribution of the disease to inform prevention and control efforts; 2) assessment of the efficacy of vaccination campaigns and other control programmes; 3) monitoring of antigenic drift; and, eventually, 4) seeking free status. *I*nfected countries, while still mostly relying on passive surveillance, will need to step up their active surveillance efforts, particularly following outbreaks (tracing back and forward as part of outbreak investigation protocols). In addition to the high risk areas and populations already mentioned for free countries (borders and international entry points, domestic waterfowl and live bird markets), the monitoring of sentinel villages may also be considered (FAO, 2004). Infected countries should perform the molecular characterization of all isolates and molecular epidemiological studies, send isolates to international reference laboratories, and upload molecular data to international gene sequence databases (e.g. Genbank) (FAO, 2004). The Terrestrial Animal Health Code provides information on the specific surveillance requirements when there are ongoing AI vaccination campaigns. The Code also provides specific guidelines related to possible strategies to seek recognition of disease free status for a country, zone or compartment, both for historically free countries and after an outbreak (OIE, 2015a).

Surveillance activities can be a very expensive endeavour (i.e. personnel, fuel, vehicles, equipment and reagents), which can be extremely challenging for the developing countries mostly affected by AI. When dealing with limited, often insufficient budgets, cost-effectiveness has to be considered as a top criteria when designing a surveillance program. Of all surveillance types, passive surveillance is the most cost-effective if implemented effectively (i.e. with the active cooperation of all poultry-related stakeholders). In addition, costs can be significantly reduced if surveillance tasks are combined with the implementation of other field activities like biosecurity assessment and improvement (Bunn et al., 2011).

4.4.1. Surveillance in wild birds

Infection in wild birds, although not mandatory, can still be reported to the OIE on a voluntary basis when detected with no impact on trade.

Where there is a risk is of AI introduction from migratory birds, the first step is to identify the migratory patterns of different species (e.g. the origins, destinations and timing of migration) (Martin, Forman et al., 2006). Wild bird surveillance can be considered an early warning system and a way to estimate the risk of AI entry into poultry from infected wild birds. Wild bird surveillance has three components: 1) sampling of wild birds found dead or sick; 2) targeted capture and sampling of defined wild bird species during the period of migration; and 3) sampling of hunter-killed birds.

The former refers to passive surveillance, which should be strengthened by alerting and training wildlife personnel and others in contact with wildlife (e.g. hunters, hikers, etc.) in designated (high risk) surveillance areas to report unusual deaths or sickness in wild birds,. This applies specifically to some HPAIVs like the current H5N1 HPAI, since most AIVs in wild birds do not cause disease or mortality. Reports should be investigated and wild birds found dead or sick with influenza-like symptoms should be sampled, for the samples sent to the national reference laboratory for virological diagnosis.

Active surveillance of wild birds involves the sampling of (apparently) healthy animals, either captured or hunted. Oropharyngeal and cloacal swabs or fresh faecal samples are the recommended methods for live birds. By finding out the prevalence of LPAIVs (particularly those of the H5 and H7 subtypes) in wild birds, it becomes possible to estimate the likelihood of transmission from infected wild birds into poultry. Active surveillance targets those wild birds species most likely to harbour AIVs, i.e. Anseriformes and Charadriiformes. Also, sampling juvenile birds will increase the number of viruses isolated (Stanislawek et al., 2002). Virological assays only give positive results if the animal was sampled during an active AIV-infection. On the other hand, serology, although not yet optimized for wild birds, could be used to assess longer term past exposure to AIVs (Charlton et al., 2009). Although results from these active surveillance programs can be very valuable from a scientific point of view, the low rate of positive samples (rarely above 2%) and the high costs involved means that it is not a very cost-efficient activity and resources may be best reallocated elsewhere when budgets are tight. In fact, active surveillance of wild birds is no longer compulsory in the EU since 2011 onward s (AHVLA, 2013).

An interesting study evaluated a number of methods to detect H5N1 HPAI in wild waterfowl: live bird trapping, hunter-killed birds, birds caught in fishing nets, dead birds found by the public, catching live mute swans, and using sentinel flocks of mallards. Results showed that sampling dead birds found by the public and sentinel surveillance were the most sensitive approaches, while trapping live birds was least cost-effective (Knight-Jones et al., 2010).

4.4.2. Surveillance in poultry

4.4.2.1. Passive surveillance

Passive surveillance in poultry is based on the obligatory reporting of AI suspicions to the authorities, i.e. an official veterinarian or the competent authority, and is therefore based on clinical surveillance, i.e. the detection of clinical signs at the flock level. In any case, passive surveillance should be followed up by sampling for the laboratory confirmation (i.e. virological analysis) of the clinical diagnosis. Recommended samples include dead birds and tracheal/oropharyngeal and cloacal swabs focusing on sick birds. Strict movement restrictions should be imposed upon any suspected premises until avian influenza is ruled out (OIE, 2015a).

Raising awareness on avian influenza (e.g. on the risk, clinical signs, how to prevent its entry, etc.) is critical to increase the reporting of suspected outbreaks, and thus the effectiveness of passive surveillance. Everyone in contact with birds should be made aware, not just those taking care of the birds, but also further along the poultry market chain, i.e. those involved in the transport, marketing and butchering of birds, as well as service providers (e.g. private veterinarians, feed distributors, etc.). Awareness campaigns and trainings can be used to strengthen passive surveillance in areas where poultry is perceived to be at a higher risk, e.g. where migrating birds congregate.

Having in place a fair and timely compensation plan (in the event the suspicion turns out to be positive) will also be critical to encourage reporting, as discussed in the prevention and control section above.

For HPAI, passive surveillance has been shown to work well due to the obvious clinical signs (e.g. massive mortalities) that result from infection. Passive surveillance constitutes the single most important and cost-effective approach both for HPAI-free and infected countries. However, for LPAI, where clinical signs may be inapparent, very mild, or nonspecific, conventional passive surveillance will not be efficient.

4.4.2.2. Syndromic surveillance

Syndromic surveillance, a very novel approach of passive surveillance, is defined as "*the (near) real-time collection, analysis, interpretation and dissemination of health-related data to enable the early identification of the impact - or absence of impact - of potential threats*" (Triple-S, 2015). Syndromic surveillance is not based on laboratory-confirmed diagnosis, but on non-specific clinical signs and proxy measures for health. Other characteristic of syndromic surveillance is that the data are generally not collected for surveillance purposes, and are often automatically generated (Triple-S, 2015). An alert will be prompted when the recommended trigger point or threshold is reached. If combined with a rapid laboratory diagnosis, syndromic surveillance can be a very effective and fast early detection system (Beltrán-Alcrudo et al., 2009). Because of the innate characteristics of syndromic surveillance (i.e. it does not rely on laboratory testing and data are generated for a purpose other than surveillance), it can be a very cost-effective approach (Beltrán-Alcrudo et al., 2009; Rodríguez-Prieto et al., 2015; Triple-S, 2015). In addition, it can cover multiple threats at once, i.e. changes in production parameters may be indicative of multiple diseases.

Syndromic surveillance has been extensively used in human health (van den Wijngaard, 2010), and since around 2006, it is also being used for animal health purposes (Rodríguez-Prieto et al., 2015). Only within Europe, 27 veterinary projects and systems have been identified (Triple-S, 2015). Syndromic surveillance can use production data, but also data from veterinary clinics, veterinary pharmacies, diagnostic laboratories, live animal markets, or slaughterhouses (Gates et al., 2015). Production data have been proven particularly useful to detect avian influenza outbreaks in commercial/intensified poultry settings, where production parameters get systematically recorded on a very regular and frequent basis. Mortality changes in poultry were used to detect H7N7 HPAI in the Netherlands andother HPAI (Elbers et al., 2007; Malladi et al., 2011). However, it is for LPAI, where syndromic surveillance becomes particularly useful. Slight changes of production parameters, such as increased mortality, reduced feed and water consumption, clinical (respiratory) signs or a drop in egg production or quality, may be the only indication of LPAIV infection (Beltrán-Alcrudo et al., 2009; OIE, 2015b). These would not be picked up by conventional passive surveillance. However, syndromic surveillance can build on them. In fact, a syndromic surveillance system based on the monitoring of mortality and egg production changes, was shown to be effective in the (retroactive) detection of H6N2 LPAI in layers (Beltrán-Alcrudo et al., 2009).

4.4.2.3. Parameter monitoring

Parameter monitoring, although still very experimental, could also be an effective approach for AI early detection, including LPAI. Also considered a type of passive surveillance, parameter monitoring is defined as the screening of biological indicators, e.g. animal termperature, animal activity, etc. (Rodríguez-Prieto et al., 2015). Promising examples include the use of audio sensor technology to monitor the pecking sounds (i.e. feeding behavior) of broilers (Aydin et al., 2014), or subcutaneously-implanted radiotelemetry units to monitor the heart rate and body temperatura in poultry (Kettlewell et al., 1997).

A whole range of other innovative data streams measuring production parameters have been used to detect all sort of disease-related behaviours in other commercial livestock species, e.g. audio sensors to detect coughing noises in swine, cattle and horses or to monitor the feeding behavior of broilers, accelerometers to measure jaw movements as an indication of resting, eating, and ruminating periods, passive transponder tags attached to monitor feed intake in swine and cattle, electronic water flow meters to detect outbreaks of diarrhea in swine, etc. (Gates et al., 2015).

4.4.2.4. Active surveillance

Active surveillance can be a very expensive endeavour, since it involves continuous sampling and testing, with the costs these activities involve in terms of personnel, equipment and reagents. This is why early detection in HPAI-free countries is mainly based on passive surveillance, which has been shown to be very effective due to the obvious clinical signs (e.g. massive mortalities). At periods of very high risk, such as when migrating birds are arriving or when sharing borders with an infected country, it might be useful to also undertake active serological and virological surveillance, especially domestic ducks, which are most likely to be exposed and often show no clinical signs (Martin et al., 2006). Infected countries will have to add outbreak investigations and active surveillance at high risk sites, e.g. live bird markets, backyard sector, etc. and perhaps the monitoring of sentinel villages or flocks. The frequency of active surveillance should vary according to the epidemiological situation in the country and/or the risk of introduction (OIE, 2015a). Therefore, to define the most appropriate surveillance strategy, it is advisable that countries perform and update risk assessments (Martin et al., 2006).

A specific scenario when active surveillance becomes indispensable to detect AIVs is in the case of vaccinated flocks, when a DIVA surveillance strategy is required. There are several DIVA methods available, all of which involve active surveillance. These include 1) the placing and testing of marked and unvaccinated sentinel birds within vaccinated flocks; 2) using an heterologous vaccine (same HA but different NA), like in Italy, where they used a H7N3 heterologous vaccine against LPAI H7N1 (Capua et al., 2003); 3) using a subunit vaccine targeted to the HA that allows serologic surveillance to the internal proteins; and 4) measuring the serologic response to the non-structural protein 1 (NS1) (Suarez, 2005).

For LPAI, clinical signs usually go unnoticed and thus undetected by passive surveillance, so active regular surveys of poultry populations are often the only way to detect these AIVs. Evidence of this was shown by an evaluation of different surveillance approaches in a vaccination and densely populated poultry area in Italy from 2000 to 2005, which showed active surveillance to be the most effective in detecting LPAIV infection, especially when a vaccination programme is in place (Comin et al., 2011). In the EU, member states must apply active surveillance programs that allow early detection and prevention of the spread of reportable LPAIVs in poultry (European Commission, 2008), before they have the chance of becoming widespread, transform into a highly pathogenic form, or become zoonotic. Blood samples from different poultry species have to be collected for serology and if antibodies are detected, the premises are visited again for viral detection (OIE, 2012; AHVLA, 2013). Apart from detecting sub-clinical LPAI infections, this surveillance plans contribute to the demonstration of a free status according to OIE rules (European Commission, 2008).

The selection of farms may be done randomly (usually stratified), targeted (i.e. risk-based) or a combination of both (e.g. sampling higher risk premises at a higher frequency than others) (Gonzales et al., 2010). Random sampling needs to be consistent with demonstrating the absence of infection at an acceptable level of confidence

(OIE, 2015a). For targeted sampling, risk factors used include type of production (e.g. outdoor), multi-age or multi-species flocks, lifepan, live bird markets, etc. (OIE, 2015a). Targeted surveillance has shown to be more effective than random surveillance at finding LPAIVs in the EU, particularly when targeting domestic waterfowl (Gonzales et al., 2010). The same study found as well that EU countries that sampled more holdings than required, as expected, also found more positive findings. Unlike for random sampling, where only serology is recommended as a first step, when conducting targeted sampling, it is advised to use concurrently a combination of both serology and virology methods for detection (OIE, 2012).

4.4.2.5. Participatory epidemiology

Participatory epidemiology (PE) is the systematic use of participatory approaches and methods to improve understanding of diseases and options for animal disease control (Catley et al., 2012). When PE is applied to disease search, it is known as participatory surveillance (PS), which is considered a type of active surveillance (Rodríguez-Prieto et al., 2015). Early applications of PS focused on pastoral communities e.g. for the Rinderpest eradication (Jost et al., 2007), but since then the approach has became widely applied in response to the H5N1 HPAI panzootic in Asia and Africa.

In 2006, PS for HPAI was first implemented in Indonesia as a pilot programme in twelve districts (Jost et al., 2007). It was was rapidly scaled-up covering 31 provinces and 2,000 staff by 2009 (Azhar et al., 2010), showing HPAI was endenic all over the country (Mariner et al., 2014). The sensitivity and specificity of the diagnostic procedure (clinical case definition followed by a rapid test) was 84% and 100%, respectively (Robyn et al. 2012). With reducing donor funding, it became paramount to decrease costs and increase efficiency. As a result, other livestock diseases were incorporated and the system started focusing on responding to passive reports rather than actively searching for outbreaks (Mariner et al., 2014). The latter could be done thanks to the improved trust between communities and veterinary services that resulted from PS and encouraged outbreak reporting (i.e. passive surveillance), meaning that it may not be needed to carry PS as a routine (Mariner et al., 2014).

The Early Detection, Reporting and Surveillance for Avian Influenza in Africa Project (EDRSAIA) started in 2008 in 11 countries in West and East Africa (Benin, Burkina Faso, Cote d'Ivoire, Kenya, Liberia, Nigeria, Sierra Leone, Tanzania, Togo and Uganda). Although no HPAI outbreaks were detected (since the disease was not present in most countries), countries found PS to be an important tool that increased confidence on the absence of HPAI and that could be applied for other diseases (Mariner et al., 2014). Also Sudan applied PS to look both into HPAI and Newcastle disease, finding that the latter was be very common (Mariner et al., 2014).

In Egypt, the PS program started in 2008, with the first HPAI cases being reported in 2009 (57 out of 88 suspected cases in that year). The program was applied in 15 out of 28 governorates (those at high or medium HPAI risk) (Rushton and Rushton, 2009). The program is now fully integrated into the veterinary services and has been used for foot and mouth disease as well (Mariner et al., 2014).

Since PS relies mostly on clinical signs being noticeable by the poultry keeper, it may not be so useful for LPAI, given that clinical presentations are often very mild or unapparent.

4.4.2.6. Sentinel surveillance

Sentinel surveillance is defined as the repeated collection of information from the same selected sites or groups of animals to identify changes in the health status of a specified population over time (Rodríguez-Prieto et al., 2015). Sentinel flocks have been used for both LPAI and HPAI.

In Victoria, Australia, for example, 10 sentinel free-range chicken flocks are maintained in areas populated by large numbers of waterfowl (East et al., 2010). In California, backyard flocks within one mile of 22 commercial turkey flocks were tested for avian diseases, although no AIV was isolated (McBride et al., 1991). An interesting study during a 4-year period in Minnesota used sentinel turkey flocks, but also sentinel ducks (isolation-reared mallards) placed in ponds to monitor AI in wild birds sharing the same water bodies (Halvorson et al., 1983; Halvorson et al., 1985). A similar field experiment, also using domestic ducks, was performed with comparable results in an island in the Baltic Sea (Sinnecker et al., 1982). The use of sentinel flocks of mallards to detect H5N1 HPAI in wild waterfowl was more recently evaluated in Lake Constance (between Switzerland, Germany and Austria) and turned out to be the most cost-effective of all methods tried (Knight-Jones et al., 2010). General disadvantages of sentinel flocks include the expense of rearing disease-free birds, pen construction and husbandry, and the fact that sentinel flocks can be subject to predation and human disturbance (Deliberto et al., 2009).

Sentinel birds are useful when restocking, i.e. it is advisable to start with a small number of poultry first to be monitored daily before full repopulation (Martin et al., 2006). The use of sentinel birds for vaccination has already been discussed above. In addition, sentinel birds placed in commercial poultry flocks infected with LPAI could be effective in the detection of the potential mutation of the virus into a highly pathogenic form (Verdugo et al., 2005).

4.4.3. Laboratory diagnosis (extracted from OIE Terrestrial Manual, Chapter 2.3.4)

Samples taken from dead birds should include faeces or cloacal and oropharyngeal swabs. In the case of HPAI, samples from other organs may be collected and processed either separately or as a pool. Samples from live birds should include both tracheal and cloacal swabs. For small delicate birds, the collection of fresh faeces can be an alternative. The samples should be placed in isotonic phosphate buffered saline (PBS), pH 7.0-7.4, containing antibiotics. Similar swab samples can be pooled. When immediate processing is impracticable, samples may be stored at 4°C for up to 4 days, or at -80°C (without PBS) for more prolonged storage.

For agent identification, the following tests can be performed:

- *Virus isolation:* It is performed by the inoculation of specific pathogen free (SPF) embryonated chicken eggs, or specific antibody negative (SAN) eggs. Although it is considered the "gold standard", it is a laborious and time consuming technique, used mostly to diagnose the first clinical case or to obtain virus for further laboratory analysis.

- *Antigen detection:* There are several commercially available AC-ELISA kits that can detect the presence of any influenza A viruses in poultry within 15 min. However, they may lack sensitivity, may not have been validated for all bird species, they cannot identify the subtype, and the kits are expensive.
- *Direct RNA detection:* RT-PCR techniques allow rapid detection and subtype identification, including a cDNA product that can be used for sequencing. The real-time RT-PCR is a modification to the RT-PCR that reduces the testing time and has a sensitivity and specificity equivalent to virus isolation, but may lack sensitivity when using faecal swabs, faeces and tissues in some bird species, because of the presence of PCR inhibitors that result in false-negative result.

The following serological tests are used:

- *Enzyme-linked immunoassay (ELISA):* Several commercial competitive ELISA (AIV C-ELISA) and blocking ELISA (AIV B-ELISA) kits that detect antibodies against the nucleocapsid protein have been developed and validated as a more sensitive alternative to the AGID test. ELISA kits are of moderate cost and are suitable for high throughput screening, but all positive results must be followed by HI test for subtyping. Lately, some subtype-specific ELISA kits are becoming available.
- *Agar-gel immunodiffusion (AGID):* AGID tests detect the presence of antibodies to any influenza A virus. They have been widely and routinely used in chicken and turkey flocks, but they are less reliable in other avian species. The AGID is a low cost serological screening test for detection of generic influenza A infections, but must be followed by HI tests for subtyping.
- *Haemagglutination (HA) and haemagglutination inhibition (HI) tests:* Different laboratories use variations in the procedures for HA and HI tests. The HI test is primarily used to determine the subtype. While chicken sera rarely give nonspecific positive agglutination reactions, sera from other species may do so, meaning that some prior steps to prepare the sera are needed. The neuraminidase-inhibition test has been used to identify the NA type of isolates as well as to characterize the antibody in infected birds. This latter application is very valuable in DIVA strategies. Since it requires specialized expertise and reagents, this testing is usually done in an OIE Reference Laboratory.

To be kept in mind that positive AIV antibody test results can also result from vaccination against avian influenza, maternal antibodies (up to four weeks of age), and because of a lack of specificity of the test (OIE, 2012).

Apart from having the appropriate technology, a laboratory needs to ensure it has the capacity to handle a large number of samples in the event of an emergency, as well as access to international expertise to confirm any positive results and further characterization of isolates (Martin et al., 2006).

5 Objectives

5.1. General objective

The general objective of this PhD Thesis is to propose cost-effective tools and strategies for the early detection of Avian Influenza in poultry.

5.2. Specific objectives

In order to achieve this general objective, the following specific objectives have been proposed:

1. To assess the importance of avian influenza as one of the most important global threats of animal health.

2. To quantitatively characterize published literature on the infectious diseases at the wildlife-livestock interface to identify where research on this topic has been focused, analyzing more specifically the case of avian influenza.

3. To develop a comprehensive, multidisciplinary, expandible and sustainable training approach for all stakeholders involved in the surveillance, prevention and control of HPAI.

4. To define the benefits and suggest a stepwise approach for the integration of the implementation of surveillance field activities with other field activities, like biosecurity improvements.

5. To develop and evaluate an early warning system based on syndromic surveillance for LPAI in commercial egg production farms.

6 Material and methods

This PhD Thesis is a compilation of scientific papers. Therefore, the materials and methods described below are those used specific for each of the papers.

6.1. Main animal disease threats in 2010: pathogen types, drivers and challenges

EMPRES Transboundary Animal Diseases Bulletin 2011

The identification of the most important pathogens was done through the author's professional experience working at FAO, and more specifically with the Global Early Warning System (GLEWS), which is a joint system that builds on the added value of combining and coordinating the alert and disease intelligence mechanisms of OIE, FAO and WHO, through sharing of information, epidemiological analysis and joint risk assessment. In addition, the author consulted FAO colleagues in headquarters and in the field for their validation.

6.2. Global trends in infectious diseases at the wildlife-livestock interface

PNAS 2015

Standardized definitions and guidelines similar to ones available for systematic reviews are lacking for scoping reviews. To ensure an objective and comprehensive approach, this scoping study was largely based on a framework encompassing an iterative rather than linear process. It was conducted in four main steps: defining the research question, literature search, screening of search results, and analysis.

Defining the research question

The review question was structured according to a modified PICO principle (population, interest~~,~~ and context) and defined as: '*What is the current global state of knowledge based on published literature of infectious diseases at the wildlife-livestock interface?*' Livestock was broadly defined as all non-aquatic, vertebrate animals (domestic as well as non-domestic) that are farmed in agricultural systems and holdings. Depending on the degree of human influence and supervision, wildlife can comprise feral domestic, captive wild as well as wild animals. All were included. Search terms for wildlife and livestock were derived from standard nomenclature volumes for mammals and birds and comprised the Latin genus or species name and the common genus name. In addition, generic terms like 'livestock' or 'wildlife' were included to obtain publications that did not incorporate taxonomic nomenclature.

This review focused on terrestrial mammals and birds. Livestock diseases listed in the 2013 OIE Code and diseases deemed important by the OIE Working Group on Wildlife Diseases were included. Disease search terms comprised common and scientific names of pathogens including abbreviations. Terms for geographic regions were composed of United Nations' sanctioned names of countries, continents and geographical sub-regions as well as ecological regions and transboundary protected areas.

Literature search

The search strategy consisted of compiling four search strings, one for each category (wildlife, livestock, disease and geographic region) and combining these by the Boolean operator, 'AND', to obtain only the intersection. Prior to combination, all search strings were thoroughly tested and refined for each category separately to decrease the risk that publications were missed due to different spelling, notation, and nomenclature. The literature search was conducted through the platform Web of Knowledge (version 5.12). All databases were searched in English from their first entries to 2013, utilizing the topic search, which scans titles, abstracts, and keywords of each publication. Final searches were conducted between 9 and 10 January 2014.

Screening of search results

Obtained publication records were harmonized and merged into a Microsoft Access 2013 database for further data cleansing and analysis. To check for duplicates, queries targeting identical digital object identifiers, database accession numbers, titles, authors, or first 50 characters of the abstract were performed. Publications without an abstract as well as publications clearly indexed either as review, editorial, or errata were excluded.

With the aid of dynamic structured query language (SQL) statements and connecting tables between publications and search terms, all publications were automatically indexed with their corresponding search terms. In cases where no search term could be allocated, the abstract, title, and keywords of the respective record were checked manually. Publications without entries in each category (wildlife, livestock, disease and geographical regions) were excluded, as they did not meet the intersection criterion.

Analysis

Publications were analyzed by time according to year of publication as well as by diseases, interfaces, and continents to which they referred, recognizing that each publication may refer to more than one search term within each category (e.g. > 1 continents) and that percentages may therefore surmount 100%. Dynamic intersection SQL queries were used to eliminate multiple counts (e.g. publications referring to a country and its respective continent). Where possible, publications were allocated to specific livestock groups or wildlife families; otherwise these publications were summarized under the category 'generic terms' and excluded from detailed analyses. For analysis of wildlife-livestock interfaces, only publications mentioning one disease were included to avoid false attribution between species and diseases. Results were visualized as maps and plotted using the lattice and ggplot2 packages in RStudio (version 0.97.310. RStudio, Inc. Boston, MA, USA).

For each of the top-3 wildlife-livestock interfaces, piecewise models were fitted to estimate long-term trends in publication rates (1912-2013). First, a standard Poisson regression model was fitted to each series using the *glm* function in RStudio. Following this the model was re-fitted using the segmented function in the segmented package. This method takes into account potential piecewise linear relationships and provides estimates of approximate changepoints, i.e. years marked by abrupt changes in publication rates. Davies' test was used to test for a significant difference in slope before and after the estimated changepoint.

6.3. Avian flu school - a training approach to prepare for H5N1 highly pathogenic avian influenza

Public Health Reports 2008

The pilot training courses

During the initial assessment period, three pilot courses were conducted: one in Davis, USA (July 2006) with 17 participants (4 instructors, 13 trainees); one in Morogoro, Tanzania (August 2006) with 37 participants (5 instructors, 13 observers, 1 coordinator, and 18 trainees); and one in College Station, USA (September 2006) with 29 participants (4 instructors, 4 observers, 1 coordinator, and 20 trainees). Additional courses were conducted in Djibouti (2), Tanzania (1), and USA (1), in addition to courses taught by other organizations based on Avian Flu School (AFS) materials.

Course evaluation

Participants were asked to complete pre- and post-course assessments of their knowledge and to evaluate their perceived improvement in comprehension of the subject matter. An additional evaluation form was completed by trainees at the end of each module, ranking the effectiveness of each module on a scale of 1 to 5. Moreover, at the end of each workshop there was a facilitated discussion about the course's effectiveness and how to improve it. Ten months after the first pilot workshop, an anonymous online survey was emailed to participants to report on their training activities, behavioral changes, whether they would change anything in the course, and their familiarity with the learning objectives.

Training model

A train-the-trainer model might be the best approach to deliver information to large numbers of people worldwide. AFS materials are designed in tiers based on the expertise of the audiences. In Tier I, professionals and national officials from public health ministries and veterinary services are trained. Tier I trainees then conduct Tier II trainings, mainly within their districts or organizations, e.g. district veterinarians, public health workers, etc. who will, at Tier III, reach their respective communities. Tier I trainers apply for AFS and are admitted to the course based on their qualifications and expertise (e.g., poultry health, public health, epidemiology), language skills, and willingness to travel (and are classified in a dataset by these characteristics). Tier II trainers are identified by their job functions, and Tier III trainees are identified by their interest and needs.

Flexibility, lucidity and interactivity of the material

The AFS curriculum was designed in a modular format so that it can be easily and quickly adapted to an audience's needs by adding or removing modules and/or lessons.

The materials are written in simple language using short statements and formatted as bulleted lists whenever possible. Technical jargon is avoided and complementary diagrams are presented. Trainers are instructed to speak slowly and clearly, stressing the most important concepts, and monitoring trainees for comprehension. This approach facilitates the comprehension and the teaching, also since many participants do not speak English as a first language, and the translation of materials (so far into French, Kiswahili, and Spanish).

Lessons are highly interactive, mainly through the use of small group (3-5 people) review exercises, which present participants with hypothetical scenarios to practice the lessons' main concepts. The groups discuss the exercise and then report back to the full workshop group for further discussion. The exercise answers are recorded and distributed by e-mail to all participants. Discussion is highly encouraged to help understanding concepts and clarifying misunderstandings, while keeping participants involved and interested.

Relevance

AFS courses usually include local guest speakers who present their own experiences, i.e. a presentation on the poultry sector in the host country, and on the implementation of the national HPAI Plan. Instructors are encouraged to use examples from their own experience.

Training materials

Using existing documents in the public domain, an outline was developed to identify information gaps, and experts were enlisted to develop a text version of the materials to fill those gaps. The materials were then organized into short, highly interactive lessons by a commercial adult training firm (Info Pros, Sacramento, CA, USA).

Content

The complete AFS course consists of 3 days divided into four modules with short lectures and small group exercises, covering only information that is essential to understand AI. Half-day is dedicated to a practical session covering the applied skills related to the four modules. It is best taught in a location, such as a laboratory or outdoors, that allows the manipulation of live birds. Each module is supplemented with a list of the references, a feedback form, a short Microsoft PowerPoint presentation, and appendices covering specific standard operating procedures, exercises, case studies, and other complementary information.

Structure

All modules follow the same structure to aid in their ease of use. Instructors are provided the module contents, a description of the target audience, a suggested timeline, the module objectives, and the module preview, which stresses the key points of the lessons. The curriculum follows in short segments that include highly interactive review exercises.

The course manual is structured into two columns: the left containing the instructors' notes, and the right presenting the material for the participants. The instructor column includes the information to be taught, plus instructions, transitions and the course timeline. The participant column consists of highlighted key information, exercises, diagrams and space to take notes. Tier I participants are presented with both columns, while the manuals for Tiers II and III trainees show only the participant's column.

Each module has an accompanying slide show presentation with exercises, pictures, diagrams, and simple animations to help explain the most confusing concepts. Participants though focus on their manuals, so that the course can be taught in the absence of projection equipment or in the event of a power outage.

Course schedule and setup

It is recommended that full 3.5-day AFS courses be taught by a minimum of two instructors, one with a veterinary and poultry background and one other with a public health background. A third instructor with expertise in communication or national planning is helpful. The ideal number of trainees is between 10 and 15, with varied professional qualifications, which helps them bring different perspectives during the interactive exercises. The AFS laboratory requires at least one instructor per four participants.

Making the AFS materials available

To support course workshops domestically and internationally, the AFS Assessment Project developed a website, (http://www.avianfluschool.org), which provides guidance for organizing a training, for ordering AFS course materials, for locating AFS instructors, etc.

6.4. Integrating surveillance and biosecurity activities to achieve efficiencies in national avian influenza programs

Preventive Veterinary Medicine 2011

This was a letter to the editor. Therefore, no materials and methods were utilized. Rather, the authors used their own professional experience working with national authorities through Avian Flu School trainings and international work while working at the Food and Agriculture Organization (FAO).

6.5. A flock-tailored early warning system for low pathogenic avian influenza (LPAI) in commercial egg laying flocks

Preventive Veterinary Medicine 2009

Data

Daily mortality and egg production records from H6N2 LPAIV-infected flocks were obtained from 27 flocks on one commercial premises in Southern California that suffered an outbreak of H6N2 LPAI in 2002. Infection was confirmed by virus isolation from five submissions from different flocks. Subsequently, additional flock outbreaks were detected based on clinical signs (Woolcock, personal communication, 2009). The daily data available covered two months (January and February 2002), with the outbreak occurring during the second week of January. A flock was defined as hens of a single strain and age housed in a discrete building or structure. The hens were housed in cages on a multi-age egg production farm and birds were not vaccinated against AI. Information about the strain and age of the hens was also obtained. Seventeen flocks were affected during pre-molt production (9 flocks at 63 weeks of age and 8 flocks at 37 weeks of age) and the remaining 10 between the first and the second molt, at 85 weeks of age. Mean flock size was 25,040 birds (range = 8754 - 107,261).

Because data from healthy flocks from the affected farm were not available, records were obtained from another company in Southern California to estimate the baseline mortality and egg production trends for a standard flock. Data were checked for normality using the chi-square goodness of fit test. The comparison farm had no history of LPAI or any major disease event, had similar management practices, similar genetic strains, and was located in a similar environment in Southern California at around the same time. Forty-four commercial layer flocks housed between May 2002 and December 2004 in 20 poultry houses were selected. The hens were producing eggs up to 100–103 weeks of age, with one molt at around 66 weeks of age. Mean flock size was 25,726 birds (range = 16,920–75,324).

Data analysis

Baseline mortality trends (when no LPAI is present) were calculated using data from all 42 flocks for both the pre- and the post-molting periods. Mortality data over the molting period were not included in the analysis. Because molting occurs at different times in each flock, post-molt mortality data were aligned to day 1 of the post-molt production. The calculation of the egg production trend required a different approach because the slope changes depending on the stage in the production cycle, first increasing steeply to a maximum, and then decreasing gradually until the molting period. A spline regression model was used, which allows the functional form of the relationship to change at one or more points along the range of the predictor (splines). Spline models were used with five and two knots for pre- and post-molt productions, respectively. Production data over the molting period were also excluded. Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) was used to perform all calculations.

Outbreak mortality and production data

Mean daily mortality of the week prior the outbreak was used to estimate the initial baseline daily mortality. The expected mortality (if there were no outbreaks) was calculated by applying the mortality trend formulas ($y = ax + b$) shown in the results section, where b is the initial baseline daily mortality, x is time (days in production after the first week) and a is a trend coefficient. The daily mortality attributable to the outbreak was obtained by deducting the expected daily mortality from the observed daily mortality during the outbreak.

A similar process was used for egg production data. First, the initial baseline daily egg production was calculated by averaging the records of the first week. Second, depending on when in the production cycle the outbreak occurred, the corresponding egg production trend was used to estimate the expected egg production (if there were no outbreaks). Third, the expected daily egg production was deducted from the observed daily egg production during the outbreak to calculate the daily egg production loss attributable to the outbreak.

Early Warning System (EWS)

The EWS was designed in a spreadsheet. Two EWS scenarios were set up: EWS1, an alert threshold, which occurs when the observed mortality exceeds the expected mortality by more than a factor *x* in a single day; and EWS2, an alert threshold, which occurs when the observed mortality exceeds the expected mortality by more than a factor *y* during each of 2 consecutive days. One reason for implementing the EWS2 is to avoid a very common inaccuracy from poultry record keeping, which may arise because daily mortality counts occur at different times during the day. This anomaly will result in a higher number of false alerts from the EWS1. In addition, a combination of both types of alerts was also evaluated, i.e. having an alert when either of the two alert levels is exceeded. The same concept of 1- and 2-day EWSs were used for the production data. The EWSs (each individually and their combination) were tested with data from both LPAIV-infected and non-infected flocks. Three outcome criteria were used to evaluate/optimize the threshold levels: detection delay (DD) of an LPAI outbreak (in outbreak flocks), the percentage of false alerts (FAs) triggered (in non-outbreak flocks), and the percentage of LPAI outbreaks missed (in outbreak flocks).

To evaluate/compare the EWS against fixed EWSs, and since no LPAI EWS was found in the literature, three EWS described by Elbers et al. (2007) to control the H7N7 HPAI epidemic in the Netherlands were adapted for LPAI by setting stricter alert levels.

7 Publicaciones

7.1. EMPRES Transboundary Animal Diseases Bulletin 2011

Daniel Beltrán-Alcrudo, Sergei Khomenko, Sherrilyn Wainwright, Jan Slingenbergh. Main animal disease threats in 2010: pathogen types, drivers and challenges. *EMPRES Transboundary Animal Diseases Bulletin* 2011; 37: 2-13

Resumen del artículo en castellano

Principales amenazas de enfermedades animales en 2010: tipos de patógenos, transmisores y desafíos.

Las enfermedades infecciosas animales pueden tener un gran impacto en la salud púlbica, la economía nacional y la cabaña ganadera. La emergencia de enfermedades está desencadenada por múltiples factores interrelacionados, que los servicios veterinarios necesitan tener en cuenta para asegurar un mejor control de las enfermedades a través de la incorporación de otras disciplinas relevantes como la economía y la sociología. Este artículo describe las principales amenazas de enfermedades animales descritas durante 2010, su transmisores y los desafíos que presentan.

EMPRES

No. 37 – 2011

Transboundary Animal Diseases **Bulletin**

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Poster in the Sofia Airport, Bulgaria

Main animal disease threats in 2010: pathogen types, drivers and challenges

Infectious animal diseases can have a major impact on public health, national economies and livelihoods. Disease emergence is triggered by multiple, interrelated factors, which veterinary services need to take into account to ensure better control of diseases through incorporating other relevant disciplines such as economics and sociology. This article describes the main animal disease threats reported during 2010, their drivers and challenges (page 2).

FAO/EUFMD mission to Turkey concerning FMD outbreaks in Bulgaria

The Food and Agriculture Organization of the United Nations (FAO) and the European Commission for the Control of Foot-and-Mouth Disease (EUFMD) sent a team to the Demirkoy District of Kirklareli Province in Turkey to evaluate what would be the most likely immediate source if spread had been through local wild boar or livestock. The team visited villages close to the recent foot-and-mouth disease (FMD) detection locations in Bulgaria to conduct interviews and provide advice on surveillance (page 14).

H5N1 HPAI worldwide in 2010

Following three years of progressive retraction, geographic expansion of the H5N1 highly pathogenic avian influenza (HPAI) virus was again observed in 2010. It is interesting to note that in late 2010 clade 2.3.2 became the most common clade in the spread of H5N1 HPAI and its invasion into new territories (page 21).

Mixing species, as in this backyard poultry farm, may lead to expansion of the host range of HPAI

World free from rinderpest:
FAO and the World Organisation for Animal Health (OIE) are about to declare that rinderpest has been eradicated from the planet (page 36).

AND...

FMD in Mongolia in 2010: FAO response (page 18)

PRRS: virulence jumps and persistent circulation in China and Southeast Asia (page 30)

WORKSHOPS:

Prevention and Control of Transboundary Animal Diseases in the Maghreb and Egypt (page 38)

One Health Workshop in Southern Africa (page 40)

MEETINGS:

African swine fever (page 42)

Information Systems and Surveillance for Major Animal Diseases and Zoonoses (page 43)

Avian influenza (page 44)

News (page 46)

Contributions from FAO Reference Centres (page 49)

Stop the press (page 51)

FAO Animal Production and Health Division

Main animal disease threats in 2010: pathogen types, drivers and challenges

Infectious animal diseases can have a major impact on public health (zoonoses), national economies (high-impact diseases), household livelihoods (enzootic diseases) and, in very serious cases, global societal stability and security (pandemics, bioterrorism). Disease emergence is triggered by multiple, interrelated factors: human and animal demographics, climate change, increased mobility and globalization, urbanization, land degradation, drug resistance, and mass animal rearing. The booming demand for animal-source protein has driven large increases in livestock production and trade during the past few decades. The resulting changes to host environments, and therefore to pathogen dynamics, can lead to adjustments such as expanding geographic range, jumping host species and/or shifts in virulence. The most common and least complicated pathogen adjustment concerns slight changes in virulence, usually following changes in susceptible host availability. Infectious diseases of animals and humans show some variability in virulence, thus accommodating local and seasonal fluctuations in susceptible host availability and consequent contact rates. For emerging disease pathogens there are three main pathways for pathogens to adjust host exploitation.

Pathogens as invaders into new territories

There are three main pathways for pathogens to adjust host exploitation

This scenario occurs when a pathogen finds access to new areas and host populations. These invasions are typically triggered by globalization, climate change and changes in land use. Invasion may take the form of a travelling wave, saltation or a mix of both. Vector-borne diseases are prominent in this group, as are pathogens that are food-borne or environmentally robust enough to be transported by fomites across wider areas. Invading pathogens may adapt to new arthropod vector species and/or different host types. More often, however, there are no dramatic changes in host range or in the infection process in the individual host but, rather, a modest change in the overall transmission pattern. The incursions of West Nile virus in North America, bluetongue in temperate climate zones of Europe, African swine fever (ASF) in the Caucasus and eastern Europe, Japanese encephalitis in southern Asia, and Rift Valley fever (RVF) in the Arabian Peninsula are among the more prominent invasions of pathogens into new locations in recent years.

Pathogens performing virulence jumps

This event typically associates with the mass rearing of host species, which first became evident during the green revolution in crop agriculture. More aggressive pest agents showed up in plant monocultures, requiring vast amounts of pesticides and triggering the development of pesticide resistance. This development was eventually contained when integrated pest management concepts were introduced. There is a parallel development in animal production; the livestock revolution translates into sharp increases in livestock numbers, particularly of poultry and pigs, with mass rearing in confined holdings. In situations where pathogens find access to rearing units of tens of thousands of animals – most of which are of the same age and sex, genetically near-clones and often immuno-compromised – there is a fitness premium for pathogens that are capable of a virulence jump and of becoming more host-aggressive and transmitting faster than competing pathogens. In situations where traditional and industrial farming systems coincide, and share distribution and marketing channels, there is opportunity for virulent pathogens to exploit the mix of production settings. Highly pathogenic avian influenza (HPAI) viruses are an example of this phenomenon in the poultry sector. Other examples in poultry are hyper-virulent Gumboro disease or infectious bursal disease virus, velogenic Newcastle disease virus and infectious bronchitis viruses; in pigs, examples include the viruses causing porcine reproductive and respiratory syndrome (PRRS) (see article on p. 30). With the livestock revolution advancing from North to South and involving emerging economies in particular, the conditions are present for other hyper-virulent pathogens to evolve and emerge as serious production problems. The appearance of antimicrobial-resistant bacteria can also be classified within the virulence jump category. Multi-drug resistance is on the increase because of the massive scale and erroneous application of antibiotics for medical treatments, and the use of drugs as growth promoters. Although animal- and human-related drug resistances usually evolve separately, drug resistance genes may traverse the bacterium species barrier, opening the door for drug resistance to pass between animal- and human-related species.

Pathogens performing species jumps

Where there are changes in the host community composition and/or the inter-host contact rate, pathogens capable of a species jump may be selected for. Many of today's human diseases passed from newly domesticated animals to humans during the early days of the agriculture revolution, starting about 10 000 years ago. A similar event started during the second half of the twentieth century, with the still ongoing livestock revolution. Increases in the animal-human contact rate and the pathogen exposure of humans also result from the bushmeat and exotic wildlife offered for sale in wet markets, while recreational activities and tourism bring humans into contact with pathogens circulating in forest and game reserves. The human immunodeficiency virus (HIV) emerged in humans following increases in the consumption of chimpanzee and other bushmeat. The severe acute respiratory syndrome (SARS) coronavirus found its way to the human host via people's encounters with civet cats on sale for consumption in wet markets. Influenza A viruses, abundant in wild water bird reservoirs, increasingly find their way into pig and poultry production systems, occasionally followed by animal-to-human spill-over or species jumps. Of the total of about 4 600 mammal species found on the planet, 43 percent are rodent and 24 percent bat species. Rodent-borne emerging zoonoses include hantaviruses, arenaviruses, plague, Lyme disease, scrub typhus and a number of rickettsial diseases. Bats harbour SARS-like viruses, Hendra, Nipah, lyssavirus (ra-

Collection of a bat trapped in a net placed in the Yoko Forest, Democratic Republic of the Congo bies), Ebola and other less well characterized ribonucleic acid (RNA) viruses. Wildlife and natural ecosystem health form part of responsible health management. The interfaces between wildlife and livestock and between animal-source food and humans are receiving increasing attention.

A new approach is needed for disease prevention and control

The One Health approach outlines a collaborative, international, cross-sectoral, multidisciplinary mode of addressing threats and reducing risks of infectious diseases

Current approaches to animal disease prevention and control are based on the disruption of disease transmission (through stamping out, vaccination, quarantine and other veterinary sanitary measures). While these have proved effective in both short- and long-term disease control programmes, such as national responses to foot-and-mouth disease (FMD) outbreaks and global rinderpest eradication, they have been less successful in some instances, as shown by the current persistence of H5N1 HPAI, despite significant national and international efforts. This is because most current approaches apply strong veterinary science and medicine disciplines in isolation from other relevant disciplines, such as economics, sociology, anthropology, communication, and ecology and land management. Such straightforward veterinary approaches do not confront the root causes of disease emergence at the animal-human-environment interface. Beyond core laboratory and epidemiological surveillance functions, veterinary services now need to expand into an agro-ecological approach to control diseases better. This means focusing on identification of the drivers of disease flare-ups, depicting disease behaviour in the context of host availability and farming landscape dynamics, and investigating the role of ecosystem dynamics and wildlife as the source of pathogens infecting domestic animals and humans. The international community is increasingly converging on such a multi-sectoral, multidisciplinary approach to addressing the increasing disease threats. This approach, termed "One Health", outlines a collaborative, international, cross-sectoral, multidisciplinary mode of addressing threats and reducing risks of infectious diseases at the animal-human-ecosystem interface, including the wildlife component.

Global animal disease situation

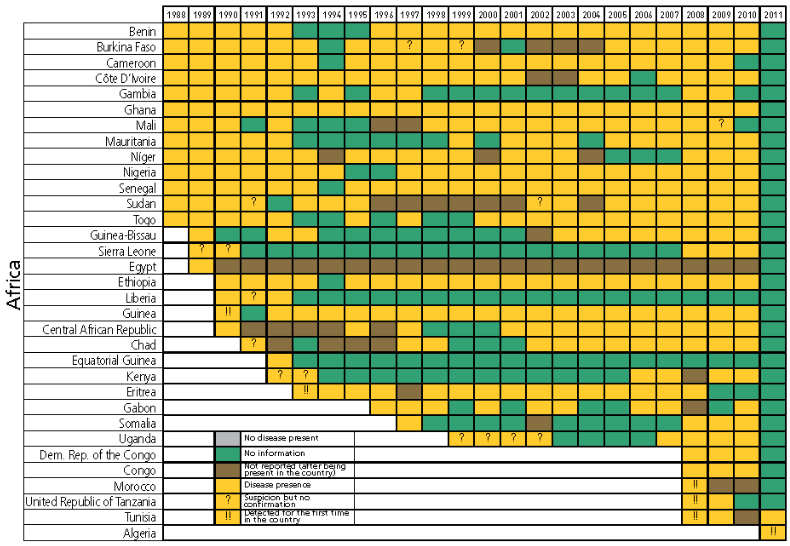
The following sections describe the main animal disease threats reported during 2010, by geographic region.

Africa

Emerging zoonotic diseases caused by viruses circulating in non-human primates, bats and rodents in sub-Saharan Africa are becoming the centre of attention in the region, particularly because of their pandemic potential. The fauna-livestock interface is also of growing importance, as pathogens that used to circulate in large game have gained a foothold in domestic ruminants, which have generally been present in greater numbers since the twentieth century. This has implications for animal and human health, and for general rural development. One example is African trypanosomosis and the impact where livestock have become infected with *Trypanosoma brucei rhodesiense*. Where the pathogen was once dependent on a tsetse fly-wildlife-human cycle there is now a tsetse-livestock-human cycle that greatly increases human exposure and the incidence of disease. Interaction of domestic livestock and wildlife is also important in the epidemiology of some animal diseases, such as FMD.

Peste des petits ruminants (PPR) distribution in Africa traditionally included the sub-Saharan countries that lie between the Atlantic Ocean and the Red Sea, where the disease has been recorded in almost every country. In recent years, PPR is rapidly expanding beyond its traditional boundaries and now poses a major threat to northern and southern Africa and Europe. On its spread south, PPR has already become endemic over recent years, first in Kenya and then in the United Republic of Tanzania, sometimes without the presence of overt disease. An incursion of PPR in 2008/2009 in Morocco was successfully contained through rigorous blanket vaccination of the entire national small ruminant flock, suggesting that PPR control presents a viable technical target. These incursions into northern Africa have been followed by the very recent finding of PPR sero-positive animals in Algeria in early 2011. Trade, transport, tourism and migration of PPR-susceptible animals may all contribute to the spread of the disease. Basic questions about the epidemiology of the disease remain unanswered, such as why the virus seems suddenly to invade and spread across PPR-free regions, sometimes in an apparently subclinical way. It is not known what modulates the virus virulence that seems to allow it to switch from virulent to non-virulent and back. The exact roles

Figure 1: Historical distribution and spread of PPR



Sources: Adapted from Sanz-Alvarez *et al.*, 2008; Food and Agriculture Organization of the United Nations (FAO) registry; World Organisation for Animal Health (OIE) animal health data, 1988 to 1995; OIE World Animal Health Database, 2005 to 2011.

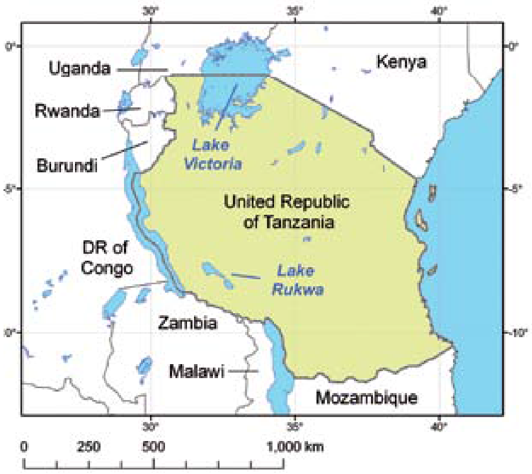
of wildlife, cattle and camels in the epidemiology of PPR also remain unknown. Some cattle herds show high rates of sero-conversion but no clinical signs, and uncertainty remains as to whether they are dead-end hosts or have ever shed the virus. PPR virus is closely related to the now extinct rinderpest. Both diseases shared similar clinical signs, and their geographic distribution was identical. For this reason, clinical surveillance often confused the two diseases, to the extent that the full global distribution of PPR has been unclear. With the last known outbreak of rinderpest reported in 2001, the world stands at the threshold of eradication of this disease. To some extent, PPR has become more apparent in the absence of rinderpest, with which it may often have been confused in sheep and goats. After the hugely successful Global Rinderpest Eradication Programme (GREP), perhaps the international community should move on to PPR as the next target disease for control/eradication. Such as initiative could build on the infrastructure, lessons learned and momentum created by GREP.

RVF is unique in that its emergence is closely linked to climate events

Rift Valley fever (RVF) is unique in that its emergence is closely linked to climate events. This allows the use of forecasting models that combine near-real-time measurements of sea surface temperatures, precipitation and vegetation cover to predict when and where an outbreak might occur. However, there are still unexpected events, such as the recent outbreak in northern Mauritania that affected camels, small ruminants and humans. In September to October 2010, unprecedented rainfall created large ponds of water in the oases of the Saharan region of Adrar, northern Mauritania. These ponds were rapidly colonized by high densities of several species of mosquito, including competent vectors for major arboviruses. A few weeks after the rains, these areas experienced severe outbreaks of malaria and RVF. For RVF alone, a total of 63 human cases, including 13 deaths, were officially reported at the end of December 2010. RVF also caused severe abortion storms in small ruminants and high rates of mortality in camels. The current hypothesis is that the virus was probably introduced rapidly through viraemic animals from RVF-endemic regions, which were transported by truck to take advantage of grazing opportunities.

As an example of changing agricultural practices influencing disease distribution, eastern and southern African ruminant populations are no longer disconnected. Important pathways for the spread of mainly ruminant diseases have opened up, facilitated by the movement of people and their stock from areas of high land pressure, such as the Lake Victoria basin, towards the agriculturally productive southern highlands, traversing the inhabitable tsetse belt that used to form a barrier for livestock and, with it, the spread of disease. The Sukuma tribe, the largest ethnic group in the United Republic of Tanzania, with an estimated population of 5.5 million people (16 percent of the country's total), traditionally lived on the southern shores of Lake Victoria in northwestern United Republic of Tanzania, but Sukuma families and their livestock have now migrated southwards into the Lake Rukwa area. This may explain the recent southwards spread of PPR to southern United Republic of Tanzania and of FMD type O to the Zambia border area. There has also been a northwards spread of contagious bovine pleuropneumonia (CBPP) and ASF, again facilitated by livestock trade or exchange along these movement pathways.

Figure 2: Migrations of the Sukuma tribe in the United Republic of Tanzania



Source: FAO.

Eastern and southeastern Asia

Eastern and southeastern Asia is the geographic setting for a relatively high number of transboundary animal diseases (TADs) in ruminants, poultry and pigs. Demographic processes, including strong economic growth and a rising demand for animal protein products, are the basis of a rapid intensification of the livestock sector, particularly poultry and pigs, in a farming landscape where traditional systems continue to play a major role in food production for the rural population. The mix of smallholder farming and intensive production results in increased vulnerability to livestock disease epidemics in eastern Asia, and poses a risk to agriculture's sustainability, environmental protection and public health. Geographic and trade corridors linking different livestock subpopulations pose increasing epidemiological challenges, and explain how highly virulent PRRS and other diseases, including FMD and classical swine fever (CSF), spread in eastern Asia.

H5N1 highly pathogenic avian influenza (HPAI) perhaps best illustrates the complexity of the factors involved in the local, national, regional and even global spread of a newly emerged animal pathogen. H5N1 HPAI has demonstrated what happens when a new virus enters a new host population (chickens) from where it can jump to further species (human infections, illustrating how the virulence of an agent can vary), and what happens when a new virus can spread across very large distances to new susceptible populations.

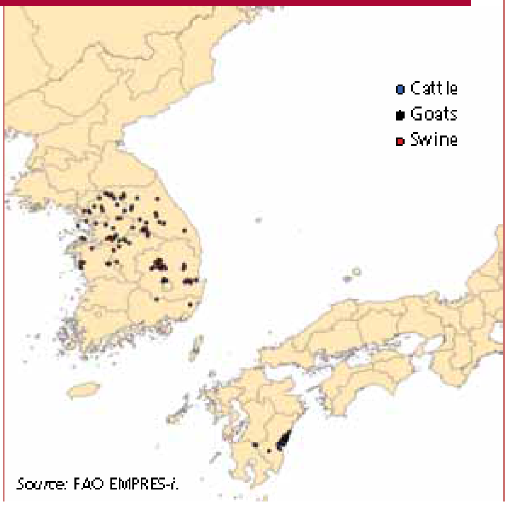
H5N1 HPAI persists in areas with rice-duck agriculture, causes outbreaks in both industrial and backyard poultry, infects humans in live-bird markets, and can spread over short and long distances through wild birds and by poultry trade. During the recent fight against HPAI, it became clear that animal disease management has to be viewed in the broader context of sustainable agriculture and rural development, natural resource

Ducks on a motobike in Hanoi market, Viet Nam

management and socio-economic development. Eastern Asia provides the setting for the mixing of poultry, pig and human influenza A viruses, which together constitute an expanding gene pool of diverse virus subtypes, clades and lineages circulating in the avian, swine and human host reservoirs, and thus representing a grave risk of emergence of new highly pathogenic transmissible viruses through recombination. The progressive control of avian influenza in eastern Asia and elsewhere continues to pose a formidable challenge. It is also recognized that H5N1 HPAI represents a threat for the rest of the world outside eastern and southeastern Asia, as demonstrated by the endemic situations in Egypt and Bangladesh, and the incursions into southern Asian and eastern European countries.

Foot-and-mouth disease (FMD) is regarded as endemic in most mainland countries in eastern and southeastern Asia, but a pattern of increased movement of viruses emerged in 2010. The drivers for this greater penetration of the FMD virus (FMDV) in the region are not clear; they may relate to the virus or they may be related more to human activity such as trade or smuggling. Since the beginning of 2010, confirmed FMD outbreaks have been reported in seven eastern Asian countries. FMD serotypes O and A were identified in these outbreaks, with serotype O predominating: Taiwan Province of China (FMDV O in February 2010), the People's Republic of China (FMDV A in January 2010, and FMDV O in February and March 2010), China, Hong Kong Special Administrative Region (SAR) (FMDV O in March 2010), Japan (FMDV O in April 2010, the first outbreak since 2000), Mongolia (FMDV O in May 2010), the Republic of Korea (FMDV A in January 2010, FMDV O in March 2010, and FMDV O in November 2010, the first outbreaks since 2002), Viet Nam and the Democratic People's Republic of Korea (FMDV O in December 2010, the first outbreak since 2002). This suggests that there is continued movement of viruses across international borders in Asia, and highlights the persistent threat posed by FMD as a TAD in the region. Livestock movements (both formal and informal) play an important role in the epidemiology of FMD worldwide and in mainland eastern and southeastern Asia. Livestock trade in this region is dynamic and can show marked seasonal variations, but it is predominantly driven by demand for meat and the price differentials this generates for livestock and livestock products. Of great concern has been the incursion of FMD into the Republic of Korea and Japan, where strict quarantine regulations govern animal imports. Both countries are continuing to investigate the route of introduction (possibly through contaminated fomites) and to review their risk management procedures. The occurrence of FMD in Mongolia, where ruminants and Mongolian gazelle populations were affected, also suggests a regional spread, most likely through livestock movements. It also shows an additional dimension of the disease: susceptible wild animals and their still unclear epidemiological role. (See article on p. 18 for more about FMD in Mongolia.) Increased coordination and sharing of data on FMD surveillance among countries in the various Asian agro-ecological zones are urgently required to identify transboundary transmission routes and ensure that suitable vaccines are available for protection against the disease (Sumption *et al.*, 2010).

Figure 3: Outbreaks of FMD type O, by species, in eastern Asia since January 2010



Source: FAO EMPRES-*i*.

Porcine reproductive and respiratory syndrome (PRRS): Starting in 2006, the pig sector of China, Viet Nam, the Philippines and Thailand has been continuously hit by atypical highly virulent strains of PRRS virus. During 2010, the disease affected additional countries in Southeast Asia, including Lao People's Democratic Republic and Cambodia, while causing a higher than expected epidemic wave in Thailand. These developments are described in greater detail in the article on p. 30.

Other pathogens: This region has also seen a number of viruses emerge and jump species, with serious consequences for animal and human health. In general, these outbreaks have been related to viruses crossing the interface between wildlife (especially bats) and livestock and/or humans. The known examples are SARS, Hendra and Nipah viruses and, more recently, the Reston Ebola virus. Given the structure of the livestock sector and the increasing incursions of humans into wildlife habitats, additional pathogens may follow.

Southern Asia

Southern Asia faces particular challenges because of the huge populations of small and large ruminants, the human population's great dependence on milk and meat as a food source, the trade that takes place across the region, and the presence of important livestock diseases. India has the largest ruminant population and the second largest dairy production in the world. Losses due to infectious ruminant diseases are of such magnitude that progressive control presents a viable economic target, provided activities are coordinated with neighbouring countries. Many additional challenges remain, however. With rapid growing urban agglomerations and poor sanitation, India faces major veterinary public health challenges, including rabies and a high prevalence of food- and water-borne diseases. HPAI is a significant threat in the region, and emerging zoonotic diseases that flared up during 2010 in southern Asia were Japanese encephalitis virus (JEV) and Chikungunya virus. India, Bhutan, Nepal, Pakistan and Bangladesh have very porous country boundaries, facilitating important exchange of trade commodities, including live animals. Livestock pathogens are shared in the process. The progressive control of TADs such as FMD and PPR across the Indian subcontinent, on an area-wide basis, is long overdue given the high density of livestock-based incomes, generated mostly by smallholder producers. India has requested FAO to contribute by facilitating a cohesive approach.

Anthrax is a somewhat neglected zoonotic disease

Anthrax is a somewhat neglected zoonotic disease. Control tools are available, but anthrax presents a challenge owing to the longevity of the spores in the soil in endemic areas. During 2010, FAO responded to a request from the Government of Bangladesh to review a flare-up of anthrax in ruminant livestock that led to human infections. Within a few weeks from mid-August 2010, Bangladesh had reported more than 500 human cases suffering from the cutaneous form of anthrax, in 11 out of 64 districts. The source of infection in most of the cases was infected cattle, which had been slaughtered, processed and sold without being inspected by a veterinary inspection authority. Anthrax is endemic in Bangladesh and outbreaks in livestock are reported every year. A large outbreak affected both livestock and humans in 2009. The Bangladesh public veterinary service is experiencing increasing difficulties in addressing a growing list of high-impact livestock and zoonotic diseases. The country is endemic for H5N1 HPAI, and confronts recurrent FMD outbreaks, PPR, brucellosis and haemorrhagic septicaemia.

Other pathogens: During 2010, in Pakistan, FAO focused mainly on the flood impact on rural livelihoods. In conjunction with this, the FAO Crisis Management Centre – Animal Health (CMC-AH) fielded a mission to draw up a strategy against *FMD*. The FMD virus strains found in Pakistan have much in common with viruses encountered in central Asia and the Near East, and are distinct from those circulating in India. At the time of the floods, Pakistan also experienced unusual episodes of *Crimean Congo haemorrhagic fever* (CCHF) and requested the World Health Organization (WHO) and FAO to investigate. A tick-borne arbovirus, CCHF virus may flare up in areas where there is unusual movement of people and animals.

Latin America

In Latin America, although the progressive control of FMD is becoming a viable target, major international animal health challenges remain, some of which are not yet adequately addressed. These include the upsurges of mainly bat-transmitted rabies in many countries, the rise of porcine cysticercosis in rural areas of central and South America, and the flare-up of mosquito-borne viruses such as bluetongue, West Nile fever, vesicular stomatitis, equine encephalitis (Venezuelan, Eastern and Western) and New World screwworm. Higher susceptibility to insect vector-borne disease incursions is a global phenomenon, triggered by the greater mobility of people, increases in trade and traffic, climate change and land utilization patterns. The control of vector-borne diseases requires contributions from experts in multiple disciplines, including meteorologists, epidemiologists, biologists and ecologists, and from local communities. Global warming trends predicted in the 2007 Intergovernmental Panel on Climatic Change (IPCC) report for South America are likely to change the temporal and geographical distribution of infectious diseases, including those that are vector-borne. Changes in distribution will be partially modulated by El Niño Southern Oscillation events, which will become more frequent and lead to more drought and flood events (Pinto *et al*., 2008).

West Nile fever is a zoonotic vector-borne disease that was detected for the first time in the Western hemisphere in New York in 1999, and subsequently spread throughout the United States of America to central and parts of South America; it continues to spread. The status of the disease in some countries of South America remains uncertain, because animal disease surveillance systems and vector surveillance are deficient in most of these countries. Wild birds are known to be reservoirs, serve as amplifying hosts and can spread the virus when migrating. The migration of birds is driven in part by seasonal climatic factors, and any change in climatic conditions may modify the direction and intensity of the spread of diseases (Pinto *et al*., 2008).

Porcine teschovirus (PTV): Since February 2009, Haiti veterinary services have been confronting PTV encephalomyelitis, a lethal pig disease in smallholder pig holdings that has spread progressively in the country. Infection with the causative PTV serotype 1 results in an acute disease that affects swine of all ages and is characterized by nervous clinical signs and mortality. Viral circulation and spread are facilitated through movement of infected pigs and contaminated fomites, such as transport vehicles, feed or people. Smallholder pig production is important in Haiti, for food and income security and the livelihood of, particularly, the lower strata of society. However, scavenging pigs are associated with waste disposal and sanitation challenges, so pig disease control and prevention forms part of a broader development equation requiring a coherent approach. The presence of highly transmissible PTV is also affecting the control efforts for other diseases, and the CSF vaccination campaign had to be postponed to avoid the spreading of PTV by vaccination teams (Pinto *et al*., 2010). Circoviruses and PRRS virus also co-circulate with PTV in Haiti pig populations today, adding to the disease burden; CSF and ASF have brought severe losses in the past.

Backyard pigs in Haiti

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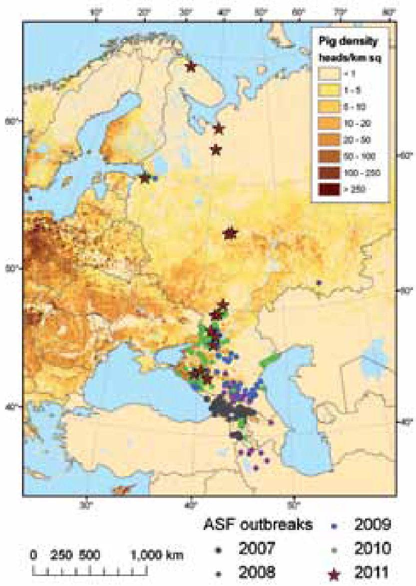
Eastern Europe

African swine fever (ASF) entered Georgia in 2007, presumably arriving in the Black Sea coastal port of Poti with a shipment from southeastern Africa. This represented a significant geographic leap for a virus that has a wildlife reservoir and for which infection of domestic pigs is a species jump. As is the case for many large shifts of animal viruses, this was probably the result of some form of illegal activity, in this case swill feeding of ship waste without any previous thermal treatment. As the veterinary services of Georgia were ill-prepared, ASF spread freely, affecting mainly backyard pigs along the main trade routes, and moved to Armenia, Azerbaijan and the Islamic Republic of Iran (wild boar). By the end of 2007, ASF had crossed to the Russian Federation and continued to spread uncontrolled, arriving just a few kilometres from the Ukraine border. The disease also circulates in areas where civil strife and military action complicate its containment. Spread is linked mainly to the movement of infected pork products, where the virus can resist for long periods.

The disease has jumped thousands of kilometres on several occasions, with repeated introductions into Leningrad Oblast (close to the Estonian and Finnish borders), Orenburg Oblast (close to northern Kazakhstan), Nizhniy Novgorod Oblast and, most recently, Murmansk Oblast (north of the Arctic Circle) and Arkhangelsk Oblast. Most of these jumps have been related to military premises, suggesting that military food supply chains play a prominent role in disease spread in the Russian Federation. The exact epidemiological role of wild boar, which have been extensively affected, and the presence of *Ornithodorus* soft ticks in the region will need to be carefully addressed in the design of ASF prevention and control plans. FAO assistance started in 2007, and has concentrated on the options available not only to halt the progressive spread of the disease, but also to address virus amplification at source. The main prospects for effective prevention and control are strengthening the first line of defence at the village level, through the engagement of pig keepers, private veterinarians, wild boar hunters, butchers, animal traders and other intermediaries. In the presence of weakened official veterinary services, empower-

Figure 4: Spread of ASF in the Caucasus and the Russian Federation, 2007 to September 2010



Source: FAO.

ing these stakeholders to take preventive and control measures against the disease may be the only viable short to medium-term solution. Again, the complexity of the situation demonstrates that disease control and prevention cannot be viewed in isolation, but need to be part of a broader sustainable agriculture and rural development agenda (Beltrán-Alcrudo, Khomenko and Dietze, 2010).

Other pathogens: The upsurge of ASF in the Caucasus and eastern Europe is only one of the many disease challenges in this region. Countries of the former Soviet Union feature the collapse, or at least weakening, of their veterinary services, together with national shifts from vertical command systems to less efficient sub-national disease management structures. At the same time, there has been an exodus of young people, particularly into the cities. Land abandonment triggers the regrowth of undergrowth, favouring rodents and other forms of wildlife, which in turn attract wild carnivores and influence the pathogens associated with rodent-wild carnivore cycles. Disease dynamics respond to changes in land use, agricultural policies and access to public services. Progressive rabies flare-up is now seen in many former Soviet Union countries, with red foxes playing a key role. Disease emergences at the animal-human-environment interface also include tick-borne encephalitis, hanta viruses, CCHF, Lyme disease and alveolar echinoccocosis, in addition to the existing TADs: brucellosis, anthrax, CSF, FMD, avian influenza and sheep and goat pox. Progressive control pathways in the form of regional roadmaps are to be pursued for FMD and brucellosis.

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Anke K. Wiethoelter, Daniel Beltrán-Alcrudo, Richard Kock, Siobhan M. Mora. Global trends in infectious diseases at the wildlife-livestock interface. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)* 2015; 112(31): 9662-9667

Resumen del artículo en castellano

Tendencias globales en enfermedades infecciosas en la interacción entre poblaciones silvestres y producciones ganaderas

El papel y la significación de la ecología de la interacción entre poblaciones silvestres y las producciones ganaderas han sido enormemente descuidados, a pesar del interés reciente en los animales como origen de enfermedades emergentes en humanos. La determinación del alcance de los métodos de revisión para evaluar objetivamente el interés relativo de la comunidad científica en las enfermedades infecciosas a nivel de la interacción entre animales silvestres y ganadería, para caracterizar las especies animales y las regiones implicadas, así como para identificar tendencias a lo largo del tiempo. Una extensa búsqueda bibliográfica combinando los términos fauna silvestre, ganadería, enfermedad y geografía permitieron localizar 78.861 publicaciones de las cuales 15.998 han sido incluidas en el análisis. Las publicaciones van de 1912 a 2013 y muestran una tendencia creciente y continua, incluyendo un cambio desde las enfermedades parasitarias a las víricas a lo largo del tiempo. En particular hay un incremento significativo en las publicaciones sobre la interacción entre artiodáctilos-bovinos y pájaros-aves de corral desde 2002 y 2003 respectivamente. Estas tendencias pueden ser atribuidas a eventos clave de enfermedades que han estimulado el interés público y la financiación para la investigación. Entre las 10 enfermedades más importantes identificadas en esta revisión la mayoría son zoonosis. La relación entre fauna silvestre y ganadería conlleva importantes interacciones entre grupos filogenéticamente próximos y/o simpátricos. La relación entre pájaros y aves de corral fue la citada con más frecuencia a nivel mundial con particularidades regionales. Esta revisión proporciona la visión más amplia de la investigación de las enfermedades infecciosas en el contexto de la relación entre fauna silvestre y ganadería realizada hasta la fecha.

Global trends in infectious diseases at the wildlife–livestock interface

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The role and significance of wildlife–livestock interfaces in disease ecology has largely been neglected, despite recent interest in animals as origins of emerging diseases in humans. Scoping review methods were applied to objectively assess the relative interest by the scientific community in infectious diseases at interfaces between wildlife and livestock, to characterize animal species and regions involved, as well as to identify trends over time. An extensive literature search combining wildlife, livestock, disease, and geographical search terms yielded 78,861 publications, of which 15,998 were included in the analysis. Publications dated from 1912 to 2013 and showed a continuous increasing trend, including a shift from parasitic to viral diseases over time. In particular there was a significant increase in publications on the artiodactyls–cattle and bird–poultry interface after 2002 and 2003, respectively. These trends could be traced to key disease events that stimulated public interest and research funding. Among the top 10 diseases identified by this review, the majority were zoonoses. Prominent wildlife–livestock interfaces resulted largely from interaction between phylogenetically closely related and/or sympatric species. The bird–poultry interface was the most frequently cited wildlife–livestock interface worldwide with other interfaces reflecting regional circumstances. This review provides the most comprehensive overview of research on infectious diseases at the wildlife–livestock interface to date.

wildlife–livestock interface | infectious diseases | zoonoses | scoping review

Pathogen maintenance within wildlife populations and spillover to livestock has been reported as a precursor to disease emergence in humans (1–3). As such, there has been growing interest in applying knowledge synthesis methods to trace and quantify the zoonotic origins of human diseases (2–6). Though zoonoses can and do impact directly on human health (7), comparatively less research has been directed toward understanding the origins of animal diseases, particularly at the wildlife–livestock interface, as well as the associated impacts on each sector (8–10).

Globally, livestock constitutes on average 37% of the agricultural gross domestic product (11) and is one of the most important and rapidly expanding commercial agricultural sectors worldwide (12). Infectious diseases cause direct losses to this sector through increased mortality and reduced livestock productivity, as well as indirect losses associated with cost of control, loss of trade, decreased market values, and food insecurity (13). Diseases that are shared between species also represent a potential burden to the whole ecosystem, affecting biodiversity, changing behavior or composition of animal populations, and even relegating species to the fringe of extinction (14, 15).

Wildlife–livestock interfaces have traditionally been characterized according to the epidemiological role of wildlife—namely, as spillover/spillback, maintenance, or dead-end hosts (16, 17). This focus and categorization reflects to some extent the human bias placed on the importance of livestock, overemphasizing the role of wildlife in transmission while neglecting the manifold values of wildlife (18). More accurate in a biological sense, wildlife–livestock interfaces are dynamic and bidirectional with pathogens transmitted freely within and between wildlife and livestock species (16) as they come into mostly indirect contact in a communal environment, through use of shared resources (e.g., pasture, water) and via vectors. Viewed this way, it can be seen that human-induced shifts in farming practices and land use changes—agricultural intensification, deforestation, and encroachment into pristine habitats, for instance—also influence observed epidemiological patterns (6, 19, 20).

Previous research on diseases at the wildlife–livestock interface has provided some insights. An inventory of known livestock pathogens revealed that 77% are capable of infecting multiple host species, including wildlife (5). Studies on certain wildlife–livestock interfaces have also identified several important diseases (16, 17, 21–25). However, no studies have characterized diseases and animal species involved on a global level.

The integration of findings from individual research studies on a given topic or question into the global knowledge base is referred to as "knowledge synthesis" (26). Of the different methodologies, scoping studies are most appropriate for mapping existing knowledge in research areas where comprehensive reviews are lacking (27). In this study, knowledge synthesis methodologies were refined to provide an overview of published research on infectious diseases at the wildlife–livestock interface. Specifically, the aim was to quantitatively characterize published literature with respect to the types of diseases, animal species involved, and temporal and regional patterns to identify where research on this topic has been focused.

Results

Overall, 15,998 publications were included in the analysis (Fig. 1), covering 113 of 118 diseases of interest. Approximately 17% of publications referred to more than one disease. Publications

Significance

Infectious diseases at the wildlife–livestock interface threaten the health and well-being of wildlife, livestock, and human populations, and contribute to significant economic losses to each sector. No studies have sought to characterize the diseases and animals involved on a global level. Using a scoping review framework we show that 10 diseases—mostly zoonoses—have accounted for half of the published research in this area over the past century. We show that relatively few interfaces can be considered important from a disease ecology perspective. These findings suggest that surveillance and research strategies that target specific wildlife–livestock interfaces may yield the greatest return in investment.

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The authors declare no conflict of interest.

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Data deposition: The data reported in this paper have been deposited in the University of Sydney Research Data Store and are available via Research Data Australia https://researchdata.ands.org.au/dwli-database/616661.

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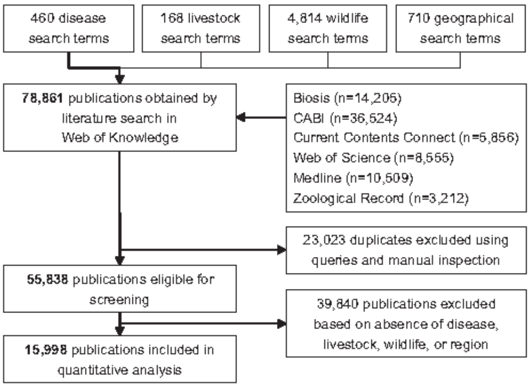

Fig. 1. Scoping review flowchart. All search categories (wildlife, livestock, disease, geographic region) were joined by the Boolean expression "AND," resulting in the intersection used for analysis.

dated from 1912 to 2013 and showed a continuous increase over time (Fig. 2). Diseases cited were caused by viruses (60%), bacteria (40%), parasites (29%), and prions (2%). Fungal diseases were not represented. Whereas early publications predominantly described parasitic diseases, the majority from 1977 onward referred to viral diseases. The Top 10 diseases are shown in Table 1 (for full list, see Table S1). Together, these diseases constituted almost 50% of published research. This trend became apparent around 1960 and has remained relatively stable since (Fig. S1).

Fig. 3 shows the prominent wildlife–livestock interfaces reported in the scientific literature. Among wildlife, birds and members of the orders Carnivora (carnivorans), Artiodactyla (artiodactyls), Rodentia (rodents), and Chiroptera (bats) were the most frequently mentioned. Poultry, cattle, small ruminants, pigs, and equines formed the most cited livestock groups. Together the matrix combination of these five wildlife and five livestock groups accounted for 74% of all publications. In addition, only a few diseases were highlighted at each interface (Table 2). For example, 22% of all publications citing a bird–poultry interface were associated with only one disease [avian influenza (AI)], whereas 16% and 24% of all publications citing an artiodactyls–cattle or carnivorans–cattle interface, respectively, referred to bovine tuberculosis (bTB).

Table 3 shows the long-term publication trends on diseases at the top three wildlife–livestock interfaces. Between 2003 and 2013, publications referring to diseases at the bird–poultry interface increased at a rate of 10.8% per year (95% CI: 8.5, 13.1), compared with only 3.9% per year between 1912 and 2002 (95% CI: 3.6, 4.2; Davies' test for slope change: $P < 0.001$). Similarly, publications on diseases at the artiodactyls–cattle interface increased significantly after 2002 (4.3% vs. 9.2% before and after 2002, respectively; $P < 0.001$). Time-series analysis of publications on AI and bTB revealed that the number of publications was highly positively correlated with media coverage and research funding for these specific diseases (Fig. 4).

Fig. 5 shows the geographic trends in diseases at the wildlife–livestock interface. The majority of publications were spatialized to Europe (38%), followed by Asia (30%), North America, including Caribbean and Central America (24%), Africa (18%), South America (8%), and Oceania (6%). The distribution of disease agents was similar across all continents, with viral diseases representing the largest fraction all over the world (Fig. 5*A*). The bird–poultry interface was the most frequently cited wildlife–livestock interface worldwide, ranking first in Asia, Europe, and North America and second in Oceania, Africa, and South America (Fig. 5*B*). Other interfaces reflected regional circumstances, as illustrated by the marsupial–cattle interface in Oceania (mostly attributable to publications on transmission of bTB between brushtail possums (*Trichosurus vulpecula*) and cattle in New Zealand) as well as the artiodactyls–cattle interface in Africa (associated with theileriosis, foot and mouth disease, and malignant catarrhal fever).

Discussion

This study is, to our knowledge, the first to apply a scoping review framework to identify infectious diseases at the wildlife–livestock interface. Results suggest a growing interest by the scientific community in this area. In some cases (such as AI and bTB) these trends could be traced to key disease events that stimulated public interest and research funding. The findings indicate that animal disease dynamics at this interface are driven by interactions between only a few wildlife and livestock groups, differing to some extent based on geographic region; they also show that relatively few diseases are transmitted at these interfaces. Scientific interest appears to have been driven largely by the zoonotic aspects of some of these diseases, with comparatively less research directed to exclusive animal diseases that impact on livestock and/or wildlife health.

Ten diseases accounted for almost 50% of the published research on diseases at the wildlife–livestock interface. The fact that the majority of these were zoonoses reflects the importance of these diseases in human health (7) and/or how funding for infectious disease research is driven by human health. It is perhaps notable that rinderpest, the only animal disease to have been globally eradicated and which affected cattle and wild artiodactyls, only ranked 29 (Table S1) despite significant scientific and political investment in this disease within the agricultural sector. Although veterinary communities have long recognized that wildlife and livestock share diseases, the importance of wildlife health only came to prominence following work by Jones et al. (3) and others (5, 8) that implicated wildlife as the origins of more than half of the diseases that emerged in humans. Recent analysis showed, however, that disease emergence in wildlife is largely driven by exposure to domestic animals and/or human-induced activities (28).

The overall increase in publications referring to diseases at the wildlife–livestock interface since 1912 is congruent with findings of bibliometric studies on other infectious diseases (29–31). These studies attributed the growth to an increased production of research data and rising demand for publication over time—in particular, during the last decades driven by China, India, and Brazil—as well as to the introduction of new journals. Nevertheless, we note that rates of publication on diseases at the bird–poultry and artiodactyls–cattle interfaces have more than doubled in the past decade, and far exceed the average annual increase for all publications in Web of Knowledge (4.5% from 1991 to 2013). In particular, we observed a significant surge in publications on diseases at the bird–poultry interface from 2003 onward, consistent with widespread transmission of highly pathogenic AI (H5N1) in Southeast Asia during 2003/2004

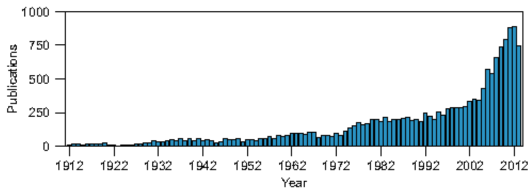

Fig. 2. Temporal trend of publications on diseases at the wildlife–livestock interface (n = 15,998).

Table 1. Top 10 diseases at the wildlife–livestock interface based on number of publications obtained

Publications (%)	Disease
1,590 (9.9%)	Avian influenza (low and highly pathogenic)
1,502 (9.4%)	Rabies
993 (6.2%)	Salmonellosis (*Salmonella enterica* excluding *Salmonella abortusovis*)*
913 (5.7%)	Bovine tuberculosis
795 (5%)	Trichinellosis
767 (4.8%)	Newcastle disease
666 (4.2%)	Brucellosis
651 (4.1%)	Leptospirosis*
609 (3.8%)	Echinococcosis
549 (3.4%)	Toxoplasmosis*

Overall, 118 diseases and 15,998 publications were included; for full presentation of all diseases, see Table S1.
*Only listed by the OIE Working Group on Wildlife Diseases.

and resulting public interest and research investment in this disease. The decline in overall publications in 2013 probably relates to the fact that the final literature research was performed in early January 2014, when not all published literature of 2013 had been added to literature databases. However, other factors such as declines in research funding cannot be ruled out.

In our review, temporal trends in publication on AI and bTB correlated strongly with media interest in and research funding for these diseases, highlighting the influence of specific disease events and sociopolitical–economic drivers of research in this area. Although pandemic human influenza cannot be denied as a serious threat, many would argue that the international response to H5N1 (including wild bird surveillance) was not commensurate with the threat or scale of the problem (32) and has failed to be effective in some regions of the world (33). In fact, international interest and funding for H5N1 has now fallen despite ongoing outbreaks in poultry and sporadic spillover to humans (34), underscoring the transient influence of public interest on research on this interface. In the case of bTB, decades of funding has been largely ineffective in reducing the disease burden in the United Kingdom, which may explain to some extent the research focus on badgers (*Meles meles*) as the problem rather than any inherent changes in the livestock systems (35). These examples show that investments have largely been proportionate to the perceptions of disease at the wildlife–livestock interface, rather than actual costs associated with, e.g., animal and human morbidity, livestock production losses, and conservation impacts.

Interfaces between phylogenetically closely related and/or potentially cohabitant species (e.g., bird–poultry, artiodactyls–cattle) were most frequently identified in this review, consistent with the view that disease dynamics are determined by interaction between sympatric species (25). However, just because a certain wildlife–livestock interface is prominently reported in the scientific literature does not necessarily mean that actual transmission is frequently occurring at this interface. For most diseases, research into true interaction, contact networks, habitat overlap, and impacts of infection (e.g., clinical vs. subclinical) in animals is limited (9, 36). Avian influenza is an example where transmission at the wildlife–livestock interface is often implied, but a functional interface is seldom documented and proven (37, 38). In fact, global spread of H5N1 was facilitated by poultry movement and trade without any proximal role of wild birds in some countries (39).

Prominent livestock groups identified by this review represent the most frequent types of livestock worldwide (40). In biologic terms, the sheer abundance of these species may contribute to contact and therefore disease transmission. The finding that cattle appear in two of the top three interfaces may reflect the historical and present day importance of the beef and dairy industries, with more substantial research and development funding in this sector (41). In recent decades, monogastric animals have risen to prominence, particularly in China (12, 41). Only 18% of the publications in this review addressed diseases at the wildlife–pig interface (vs. 33% and 25% for wildlife–cattle and wildlife–poultry interfaces, respectively), which may be an important knowledge gap considering the current trend in pig production.

Given the perceived importance of the order Chiroptera in emerging infectious diseases (42–44), we hypothesized that there would be an increase in publications exploring the bat–livestock interface. However, a relatively small number of publications referred to diseases at this interface. Emergence of viruses of proven/suspected bat origin, including Nipah (45) and Ebola Reston virus (46) in pigs and Middle East respiratory syndrome (MERS) coronavirus in camels (47), do however illustrate the potential importance of bat–livestock interfaces in emerging zoonotic diseases. The trend in agricultural expansion and intensification of the wildlife interface is particularly strong in recent decades in tropical systems, associated with external economic and development pressures and may drive spillover/emergence (48). These new diseases did not manifest strongly in findings presented here, most likely due to a lag in research and subsequent publication. Filovirus infections (e.g., Ebola) were included, but ranked 58 in this review (Table S1); coronaviruses were not considered because they are not listed diseases according to the World Organization for Animal Health (OIE), which represents an important gap in animal health surveillance, and likely reflects the limited understanding of the role of coronaviruses in wildlife and livestock disease.

There are several limitations to this study. Because this review is based on scientific publications, it is prone to publication bias influenced by country, language, institution, author career stage, study outcome, research topic, research sponsor, and timeline (49). Spatial bias also plays an important role as research and publication are linked to economic indices and therefore concentrate in developed countries, particularly in Europe and Northern America (31). As experience with H5N1 has shown (34), efficacy of disease surveillance and control measures are also largely dependent on resources available, which could be another reason for the spatial

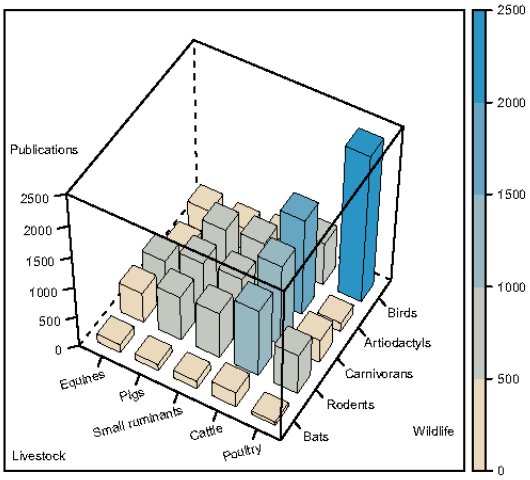

Fig. 3. Prominent wildlife–livestock interfaces reported in scientific literature. Shown are the five most frequently cited wildlife and livestock groups (Dataset S1); only publications with one disease (n = 13,293) were included.

Table 2. Top three wildlife–livestock interfaces including the five predominant diseases

Publications (%)	Wildlife	Livestock	Diseases
2,378 (17.9%)	Birds	Poultry	Avian influenza, Newcastle disease, salmonellosis, avian chlamydiosis, poxvirus infections
1,570 (11.8%)	Artiodactyls	Cattle	Bovine tuberculosis, brucellosis, malignant catarrhal fever, foot and mouth disease, theileriosis
1,324 (10%)	Carnivorans	Cattle	Rabies, bovine tuberculosis, echinococcosis, leptospirosis, salmonellosis

Only publications with one disease (n = 13,293) were included in analysis; diseases are listed in descending order. For full presentation of all interfaces depicted in Fig. 3, see Table S2.

pattern observed here. Geographical biases stemming from underrepresentation of research on emerging diseases of wildlife, particularly in Africa and South America, have been noted previously (28); this may reflect limited regional capacity for wildlife surveillance in these areas. The fact that early literature primarily addressed parasitic diseases, whereas more contemporary publications focused on viral pathogens, is presumably a result of the availability of methods to study viral systems.

To keep the scope feasible, constraints related to diseases included in this review were inevitable. No attempt was made by the authors to quantify the importance of these diseases, in terms of health, economic, or conservation impacts; rather, we deferred to lists of livestock and wildlife diseases deemed important by the OIE and OIE Working Group on Wildlife Diseases, respectively. The latter includes all OIE-listed (notifiable) diseases that affect wild animals, as well as some nonlisted diseases that have particular relevance to wildlife health and conservation (e.g., filoviruses). Because admission to the OIE disease list is subject to several criteria, such as international spread and zoonotic potential, use of this list may have biased the findings toward diseases already known to be important. However, no other comprehensive lists for livestock or wildlife diseases exist. To keep this scoping review broad, all diseases listed in the 2013 OIE Terrestrial Animal Health Code were included regardless of whether any wildlife–livestock interface was known or suspected a priori. Not surprisingly, five diseases did not yield any publications following database extraction; these diseases are not known to involve a livestock (white-nose syndrome, elephant herpes virus, feline leukemia, immunodeficiency virus infections) or wildlife host (bovine genital/venereal campylobacteriosis) in their transmission cycle. Since its establishment in 1924, the OIE list has undergone major changes in 1963/1964 (raised from 9 to 49 diseases) and 1985/1986 (from 47 to 86 diseases). These changes were not temporally associated with a surge in publications in this review (Fig. 2).

The automatic search and indexing approach used here was advantageous in processing a large number of publications. However, identified interfaces may not necessarily occur in situ. For example, experimental work like infection trials, as well as serosurveys with negative results would return the same index pattern. Likewise, cell lines or laboratory reagents incorporating species names would result in findings. Additionally, the categorization of species into livestock and wildlife is already to some extent arbitrary and not straightforward. Given the constraints of the methodology, we could not distinguish between free-ranging, captive and semicaptive wildlife. In reality, this distinction can have profound implications on the interface and transmission (25).

In the light of these limitations, it should be emphasized that findings presented here reflect perceived interest by the scientific community and should not be confused with incidence of diseases or absolute occurrence of interfaces. Likewise, high numbers should not be taken to mean high frequency of actual transmission at these interfaces as noted earlier for AI. Indeed, a good understanding of ecosystem dynamics for most multihost infectious diseases is still widely lacking (50). More basic research into these areas is needed, including specific quantitative research at the interface itself, to further elucidate the transmission pathways and specific role of wildlife and livestock species.

Our scoping review shows where research in this area has been focused over the past century. In the future, more detailed analyses using this database will focus on specific diseases, affected animal species, and geographic regions to deepen the knowledge of these interfaces and identify gaps as well as areas of knowledge saturation. These results will be useful to policymakers, donors, and other stakeholders, who require an understanding of global disease and research priorities to make informed investments in animal health programs. Combined with comprehensive field studies, more specific knowledge will help refine and adapt surveillance strategies to better monitor diseases at the wildlife–livestock interface.

Methods

Standardized definitions and guidelines—similar to ones available for systematic reviews (51, 52)—are lacking for scoping reviews (53). To ensure an objective and comprehensive approach, this scoping study was largely based on a framework encompassing an iterative rather than linear process (27); it was conducted in four main steps: defining the research question, literature search, screening of search results, and analysis.

Defining the Research Question. The review question was structured according to a modified population, interest, and context (PICO) principle and defined as "What is the current global state of knowledge based on published literature of infectious diseases at the wildlife–livestock interface?" Livestock was broadly defined as all nonaquatic, vertebrate animals (domestic as well as nondomestic) that are farmed in agricultural systems and holdings (40). Depending on the degree of human influence and supervision, wildlife can comprise feral domestic, captive wild, and wild animals (54). We did not differentiate between these groups; all feral and nondomestic animals—whether free-ranging, captive, or semicaptive—were included. Search terms for wildlife and livestock were derived from standard nomenclature volumes for mammals (55) and birds (56) and comprised the Latin genus or species name and the common genus name. In addition, generic terms such as "livestock" or "wildlife" were included to obtain publications that did not incorporate taxonomic nomenclature. This review focused on terrestrial mammals and birds; hence, fish, amphibians, reptiles, and invertebrates as well as infectious diseases thereof were excluded. Livestock diseases listed in the 2013 OIE Terrestrial Code (54) and diseases deemed important by the OIE Working Group on Wildlife Diseases (57) were included. Disease search terms comprised common and scientific names of pathogens including abbreviations. Terms for geographic regions were composed of United Nations' sanctioned names of countries (58), continents, and geographical subregions (59) as well as ecological regions and

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Table 3. Long-term trends in rates of publication on diseases at the top three wildlife–livestock interfaces

Interface	Changepoint year ± SE	Intercept (95% CI)	β1 (95% CI)	β2 (95% CI)	P value for slope change
Bird–poultry	2003 ± 1.28	−72.29 (−72.53, −67.05)	0.04 (0.04, 0.04)	0.10 (0.08, 0.12)	<0.001
Artiodactyls–cattle	2002 ± 2.22	−81.41 (−88.23, −74.59)	0.04 (0.04, 0.05)	0.09 (0.06, 0.11)	<0.001
Carnivorans–cattle	1980 ± 15.59	−74.91 (−86.53, −63.29)	0.04 (0.03, 0.05)	0.03 (0.03, 0.04)	0.121

Exponentiation of β1 and β2 yields the annual growth rate in publication before and after the changepoint, respectively. CI, confidence interval.

transboundary protected areas (60). No geographic restrictions were applied to provide a worldwide overview. Complete search strings are available upon request.

Literature Search. The search strategy consisted of compiling four search strings, one for each category (wildlife, livestock, disease, and geographic region) and combining these by the Boolean operator "AND" to obtain only the intersection. Before combination, all search strings were thoroughly tested and refined for each category separately to decrease the risk that publications were missed due to different spelling, notation, and nomenclature. The literature search was conducted through the platform Web of Knowledge (version 5.12), which provided combined access to the following six databases: BIOSIS Preview, CAB Abstracts, Current Contents Connect, MEDLINE, Web of Science, and Zoological Record, which represent the most comprehensive databases in the field of life science and biomedical research (e.g., MEDLINE accounts for more than 90% of PubMed references). All databases were searched in English from their first entries to 2013 using the topic search, which scans titles, abstracts, and keywords of each publication. Hence, non-English publications were only obtained if they included a translated title or abstract. Final searches were conducted January 9–10, 2014.

Screening of Search Results. Obtained publication records were harmonized and merged into a Microsoft Access 2013 database for further data cleansing and analysis. To check for duplicates, queries targeting identical digital object identifiers, database accession numbers, titles, authors, or first 50 characters of the abstract were performed. All query results were verified manually before excluding duplicates. In addition, publications without an abstract as well as publications clearly indexed either as review, editorial, or errata were excluded. With the aid of dynamic structured query language (SQL) statements and connecting tables between publications and search terms, all publications were automatically indexed with their corresponding search terms. In cases where no search term could be allocated, the abstract, title, and keywords of the respective record were checked manually. Publications without entries in each category (wildlife, livestock, disease, and geographical regions) were excluded, because they did not meet the intersection criterion. This deviation of query results (search in literature databases vs. SQL statements in Access) may be attributed to lemmatization causing conjunction of search terms with terms of similar meaning, varying truncation rules, different search algorithms, and other settings incorporated in the literature databases that are not under the user's control.

Analysis. Publications were analyzed by time according to year of publication as well as by diseases, interfaces, and continents to which they referred, recognizing that each publication may refer to more than one search term within each category (e.g., >1 continent) and that percentages may therefore surmount 100%. Dynamic intersection SQL queries were used to eliminate multiple counts (e.g., publications referring to a country and its respective

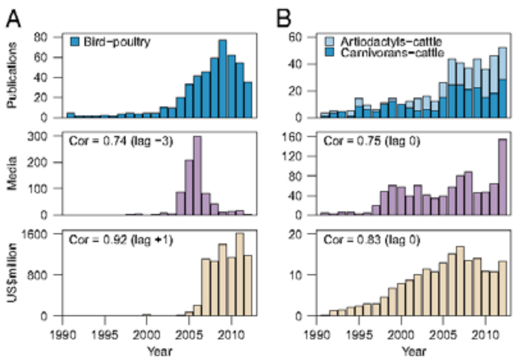

Fig. 4. Temporal trend of publications on avian influenza (*A*) and bovine tuberculosis (*B*) at the wildlife–livestock interface. The number of publications is shown in blue. For comparison, media reports and research funding directed at each disease is shown in purple and brown, respectively. Cor, maximum value of the cross-correlation (and associated time lag) between publication and media coverage/funding.

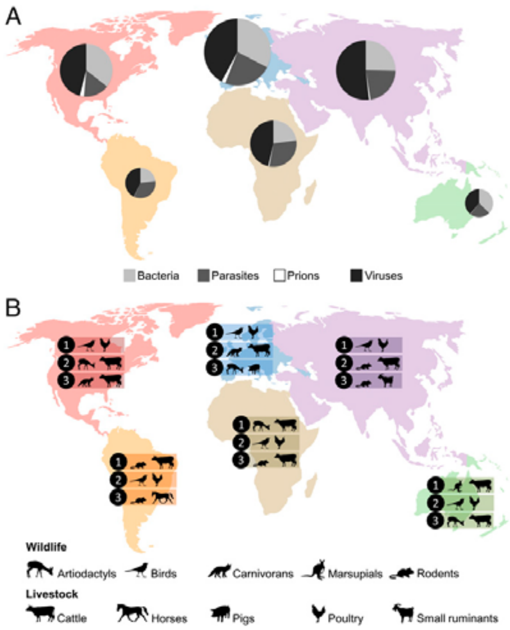

Fig. 5. Geographic distribution of disease agents (*A*) and prominent wildlife–livestock interfaces (*B*). (*A*) Size of circles is commensurate with the number of publications obtained for the corresponding continent. (*B*) Top three reported wildlife–livestock interfaces per continent (shown in pairs); only publications with one disease (n = 13,293) were included.

continent). Where possible, publications were allocated to specific livestock groups or wildlife families; otherwise, these publications were summarized under the category "generic terms" and excluded from detailed analyses. For analysis of wildlife–livestock interfaces, only publications mentioning one disease were included to avoid false attribution between species and diseases. Results were visualized as maps and plotted using the lattice (61) and ggplot2 (62) packages in RStudio (version 0.97.310; RStudio, Inc.).

For each of the top three wildlife–livestock interfaces, piecewise models were fitted to estimate long-term trends in publication rates (1912–2013). First, a standard Poisson regression model was fitted to each series using the glm function in RStudio; following this, the model was refitted using the segmented function in the segmented package (63). This method takes into account potential piecewise linear relationships and provides estimates of approximate changepoints, i.e., years marked by abrupt changes in publication rates. Davies' test was used to test for a significant difference in slope before and after the estimated changepoint. To explore potential drivers for increased publication on particular wildlife–livestock interfaces, we examined time series for two well-characterized diseases (AI and bTB) in relation to media coverage and research funding. The number of news reports on each disease by year (1991–2012) was extracted from the news service database Factiva. Search strings for indicative species of wildlife (e.g., badger, deer, elk), livestock (e.g., cattle, cow), and disease (e.g., bovine tuberculosis) were combined using the Boolean operator "AND." Major news and business circulations (as defined by Factiva) were included as the source. News reports were limited to English language; no restrictions were placed on region. Data on global research funding for AI and bTB was not available. Because the United States was by far the largest donor for global preparedness activities for AI (64), we used data from the US National Institutes for Health (65) as an indicator of timelines for research investment on AI. For bTB, we used data from the UK Department for Environment Food and Rural Affairs (66), noting that the United Kingdom and Ireland constituted 87% of the publications on bTB at the carnivorans–cattle interface. Cross-correlation analysis was applied to assess the degree to which media coverage and funding correlated with the number of publications over time.

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Supporting Information

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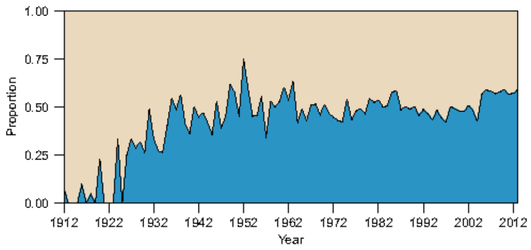

Fig. S1. Temporal trend in diseases (1912–2013). The area plot depicts the proportion of publications comprising all top 10 diseases in Table 1 (blue) and other diseases not included in top 10 (brown).

Table S1. Diseases at the wildlife–livestock interface based on number of publications obtained

Rank	No. of publications	%	Disease
1	1,590	9.94	Avian influenza (low and highly pathogenic)
2	1,502	9.39	Rabies
3	993	6.21	Salmonellosis (*S. enterica* excluding *S. Abortusovis*)
4	913	5.71	Bovine tuberculosis
5	795	4.97	Trichinellosis
6	767	4.79	Newcastle disease
7	666	4.16	Brucellosis
8	651	4.07	Leptospirosis
9	609	3.81	Echinococcosis
10	549	3.43	Toxoplasmosis
11	492	3.08	Pasteurellosis
12	443	2.77	Cysticercosis
13	423	2.64	Foot and mouth disease
14	385	2.41	West Nile fever
15	356	2.23	Babesiosis
16	347	2.17	Classical swine fever
17	330	2.06	Poxvirus infections
18	325	2.03	Avian chlamydiosis
19	303	1.89	Theileriosis
20	301	1.88	Bovine anaplasmosis
21	291	1.82	Fasciolosis
22	287	1.79	Tsetse-transmitted trypanosomosis
23	278	1.74	Bluetongue
24	271	1.69	Paratuberculosis
25	270	1.69	Q fever
26	254	1.59	Surra (*Trypanosoma evansi*)
27	226	1.41	Leishmaniosis
28	223	1.39	Aujeszky's disease
29	222	1.39	Rinderpest
30	209	1.31	Anthrax
31	186	1.16	Lyme disease
32	178	1.11	Vesicular stomatitis
33	175	1.09	Malignant catarrhal fever
34	163	1.02	Yersiniosis
35	163	1.02	Infection with *Sarcoptes scabiei*
36	163	1.02	Japanese encephalitis
37	151	0.94	Myxomatosis
38	149	0.93	Western equine encephalomyelitis
39	146	0.91	Tularemia
40	142	0.89	Avian mycoplasmosis
41	142	0.89	African swine fever
42	139	0.87	Bovine viral diarrhea
43	139	0.87	Equine influenza
44	137	0.86	Scrapie
45	135	0.84	Eastern equine encephalomyelitis
46	132	0.83	Circovirus infections
47	126	0.79	Listeriosis
48	124	0.78	Bovine spongiform encephalopathy
49	124	0.78	Rift Valley fever
50	114	0.71	Rabbit hemorrhagic disease
51	113	0.71	Heartwater
52	109	0.68	Infectious bovine rhinotracheitis/Infectious pustular vulvovaginitis
53	109	0.68	Tick borne encephalitis
54	97	0.61	Chronic wasting disease
55	96	0.6	African horse sickness
56	94	0.59	Parvovirus infections
57	89	0.56	Avian infectious bronchitis
58	86	0.54	Filovirus infections
59	84	0.53	Nipah virus encephalitis
60	82	0.51	Epizootic hemorrhagic disease
61	78	0.49	Pullorum disease
62	76	0.48	Infectious bursal disease
63	76	0.48	Morbillivirus infections

Table S1. Cont.

Rank	No. of publications	%	Disease
64	75	0.47	Hendra
65	71	0.44	Porcine reproductive and respiratory syndrome
66	70	0.44	Venezuelan equine encephalomyelitis
67	69	0.43	Infection with *Psoroptes* spp.
68	62	0.39	Crimean Congo hemorrhagic fever
69	62	0.39	Louping ill
70	57	0.36	Plague
71	55	0.34	Infection with *Fascioloides magna*
72	55	0.34	Equine piroplasmosis
73	55	0.34	Avian trichomoniasis
74	53	0.33	Fowl typhoid
75	51	0.32	Histomoniasis
76	51	0.32	Encephalomyocarditis virus infections
77	49	0.31	Enzootic bovine leucosis
78	48	0.3	Enzootic abortion of ewes
79	48	0.3	Dourine
80	48	0.3	Avian infectious laryngotracheitis
81	43	0.27	Hantavirus infections
82	42	0.26	Peste des petits ruminants
83	41	0.26	Turkey rhinotracheitis
84	41	0.26	Equine infectious anemia
85	37	0.23	Glanders
86	36	0.23	Sheep pox and goat pox
87	33	0.21	Avian paramyxovirus infections
88	29	0.18	Contagious agalactia
89	29	0.18	Equine rhinopneumonitis
90	29	0.18	Swine vesicular disease
91	28	0.18	New world screwworm
92	25	0.16	Contagious bovine pleuropneumonia
93	24	0.15	Ovine epididymitis
94	24	0.15	Transmissible gastroenteritis
95	24	0.15	Yellow fever
96	22	0.14	Marine calicivirus infections
97	21	0.13	Trichomonosis
98	21	0.13	Duck virus hepatitis
99	20	0.13	Contagious equine metritis
100	17	0.11	Lumpy skin disease
101	16	0.1	Infection with *Plasmodium* spp.
102	15	0.09	Caprine arthritis–encephalitis
103	13	0.08	Equine viral arteritis
104	13	0.08	European brown hare syndrome
105	12	0.08	Maedi-visna
106	12	0.08	Nairobi sheep disease
107	10	0.06	Contagious caprine pleuropneumonia
108	9	0.06	*Baylisascaris procyonis* infections
109	9	0.06	Old world screwworm
110	7	0.04	Camelpox
111	6	0.04	Tyzzer's disease
112	4	0.03	Salmonellosis due to *S. Abortusovis*
113	3	0.02	Hemorrhagic septicemia
114	0	0	Bovine genital campylobacteriosis/Bovine venereal campylobacteriosis
115	0	0	Elephant herpes virus
116	0	0	Feline leukemia
117	0	0	Immunodeficiency virus infections
118	0	0	White nose syndrome

Overall, 118 diseases and 15,998 publications were included.

Table S2. Prominent reported wildlife–livestock interfaces, including the five most frequently associated diseases

Wildlife	Livestock group	Publications	Diseases
Birds	Poultry	2,378	Avian influenza, Newcastle disease, salmonellosis, avian chlamydiosis, poxvirus infections
	Cattle	697	Salmonellosis, leptospirosis, babesiosis, brucellosis, bovine tuberculosis
	Small ruminants	454	Salmonellosis, avian chlamydiosis, fasciolosis, avian influenza, louping ill
	Pigs	370	Salmonellosis, avian influenza, trichinellosis, cysticercosis, leptospirosis
	Equines	401	West Nile fever, eastern equine encephalomyelitis, salmonellosis, equine piroplasmosis, rabies
Artiodactyls	Poultry	140	Salmonellosis, toxoplasmosis, trichinellosis, bovine tuberculosis, pasteurellosis
	Cattle	1,570	Bovine tuberculosis, brucellosis, malignant catarrhal fever, foot and mouth disease, theileriosis
	Small ruminants	910	Malignant catarrhal fever, paratuberculosis, rabies, echinococcosis, brucellosis
	Pigs	852	Trichinellosis, classical swine fever, African swine fever, brucellosis, toxoplasmosis
	Equines	253	Rabies, trichinellosis, bovine anaplasmosis, echinococcosis, toxoplasmosis
Carnivorans	Poultry	380	Avian influenza, rabies, salmonellosis, toxoplasmosis, trichinellosis
	Cattle	1,324	Rabies, bovine tuberculosis, echinococcosis, leptospirosis, salmonellosis
	Small ruminants	817	Rabies, echinococcosis, leptospirosis, toxoplasmosis, cysticercosis
	Pigs	793	Trichinellosis, rabies, echinococcosis, leptospirosis, toxoplasmosis
	Equines	538	Rabies, trichinellosis, leptospirosis, echinococcosis, salmonellosis
Rodents	Poultry	658	Salmonellosis, avian influenza, toxoplasmosis, Newcastle disease, rabies
	Cattle	1,103	Leptospirosis, rabies, tsetse-transmitted trypanosomosis, salmonellosis, surra (*Trypanosoma evansi*),
	Small ruminants	761	Rabies, toxoplasmosis, leptospirosis, trypanosomosis, Q fever
	Pigs	732	Trichinellosis, leptospirosis, toxoplasmosis, salmonellosis, rabies
	Equines	499	Rabies, surra (*T. evansi*), leptospirosis, Venezuelan equine encephalomyelitis, leishmaniosis
Bats	Poultry	49	Rabies, avian influenza, Japanese encephalitis, Newcastle disease, toxoplasmosis
	Cattle	248	Rabies, surra (*T. evansi*), leptospirosis, bovine viral diarrhea, foot and mouth disease
	Small ruminants	119	Rabies, Q fever, surra (*T. evansi*), leptospirosis, toxoplasmosis
	Pigs	97	Rabies, Nipah virus encephalitis, Japanese encephalitis, surra (*T. evansi*), toxoplasmosis
	Equines	134	Rabies, Hendra, surra (*T. evansi*), leishmaniosis, Venezuelan equine encephalomyelitis

Interfaces between wildlife and livestock groups belonging to the same taxonomic order are shaded; diseases are listed in descending order; only publications with one disease (n = 13,293) were included.

Dataset S1. Overview of wildlife–livestock interfaces. Stated are frequencies of respective interfaces in publications; only publications with one disease (n = 13,293) were included

Dataset S1

7.3. Public Health Reports 2008

Daniel Beltran-Alcrudo, David A. Bunn, Christian E. Sandrock, Carol J. Cardona. Avian flu school: A training approach to prepare for H5N1 highly pathogenic avian influenza. *Public Health Reports* 2008; 123(3): 323-332

Resumen del artículo en castellano

Escuela de Gripe Aviar: Una aproximación formativa para prepararse frente a la influenza aviar altamente patógena H5N1

Desde la reemergencia de la influenza aviar altamente patógena (H5N1 HPAI) en 2003, se ha desarrollado una pandemia que no tiene precedentes históricos en el número de explotaciones infectadas, la dispersión geográfica y as consecuencias económicas para la agricultura. La epidemia ha afectado a un amplio rango de aves y mamíferos, incluyendo humanos. La gestión ineficaz de los brotes, principalmente debida a la falta de conocimientos de los implicados en la detección, prevención y respuesta, apunta a la necesidad de realizar una formación específica sobre H5N1 HPAI. Los principales desafíos son el requerimiento de un enfoque multidisciplinar, la falta de expertos, la necesidad de formación a todos los niveles y la diversidad de posibles escenarios. La Escuela de Gripe Aviar (Avian Flu School) afronta estos desafíos a través de un programa de tres niveles de formar al formador que intenta minimizar el impacto sanitario y económico de la H5N1 HPAI mediante la mejora de la capacidad de la comunidad para prevenir y responder, mientras se protegen a sí mismos y a los demás. El curso enseña los hechos que se necesitan conocer utilizando materiales flexibles, interactivos y relevantes.

Avian Flu School: A Training Approach to Prepare for H5N1 Highly Pathogenic Avian Influenza

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SYNOPSIS

Since the reemergence of highly pathogenic avian influenza (H5N1 HPAI) in 2003, a panzootic that is historically unprecedented in the number of infected flocks, geographic spread, and economic consequences for agriculture has developed. The epidemic has affected a wide range of birds and mammals, including humans. The ineffective management of outbreaks, mainly due to a lack of knowledge among those involved in detection, prevention, and response, points to the need for training on H5N1 HPAI. The main challenges are the multidisciplinary approach required, the lack of experts, the need to train at all levels, and the diversity of outbreak scenarios.

Avian Flu School addresses these challenges through a three-level train-the-trainer program intended to minimize the health and economic impacts of H5N1 HPAI by improving a community's ability to prevent and respond, while protecting themselves and others. The course teaches need-to-know facts using highly flexible, interactive, and relevant materials.

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Since the reemergence of a highly pathogenic avian influenza (H5N1 HPAI) strain in 2003, the world has been experiencing a global panzootic that is historically unprecedented in the number of infected flocks, geographic spread, and economic consequences for agriculture and livelihoods.[1] The disease is believed to have spread to new geographic regions both through the movements of migratory wild birds and the trade (legal and illegal) of poultry, poultry products, and wild birds. Millions of commercial birds have died or been destroyed as a result of outbreaks in dozens of countries across three continents.[2] The epidemic has also affected a wide range of wild bird species and mammals.[3]

Moreover, HPAI H5N1 has important implications for human health: 307 cases had been detected in nine countries as of May 2007, with a lethality of 61%.[4] Most human cases have been associated with direct or close contact with infected poultry or surfaces contaminated with their feces or secretions. In addition to the current panzootic, the world now fears a pandemic similar to the one in 1918.[5] This pandemic may happen if the virus changes such that it can spread easily from person to person.

The importance of H5N1 HPAI to animal and human health has drawn attention to the sometimes ineffective management of recent outbreaks, mainly due to a general lack of practical and applied knowledge among the people involved in detection, prevention, and response. For example, confirmatory diagnosis has been delayed in some cases because of a lack of expertise and experience in packing and shipping samples for international delivery.[6] Also, many human infections to date have been caused by the unsafe handling of infected birds during preparation for consumption, which could have been prevented by the application of simple hygiene and personal protection practices. There is clearly an information gap that an applied training course on H5N1 HPAI could fill.

The nature of the current panzootic challenges the design and implementation of training programs that address knowledge gaps and needed skills. One of the key difficulties is that diverse species have been impacted by H5N1 HPAI, and that increases the professional disciplines whose input is required in prevention and response discussions. The needed multidisciplinary approach is made more difficult by the lack of collaboration between professions and the failure of health professionals to see beyond the confines of their own expertise and experience.

Additionally, the current number of qualified experts in some disciplines is inadequate to respond effectively in many parts of the world, particularly in developing countries. For example, in Tanzania there are no field veterinarians who specialize in poultry medicine or public health; only at the university level are there three or four specialists in each of these fields (Personal communication, Dr. Peter L. Msoffe, Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, December 2006). Even in a developed country the size of the U.S., there are only 275 qualified poultry veterinarians, and approximately 600 veterinarians have demonstrated expertise in preventive veterinary medicine.[7] Presumably, very few of them have both qualifications. Moreover, these few experts are fully employed and committed to full-time jobs; therefore, they lack the time to travel to the countries that need their advice on the prevention and control of H5N1 HPAI.

On one level, there is a need to provide detailed training to professionals already working in a particular discipline; however, it is the people who will directly encounter avian flu who can benefit the most from training and will have the greatest impact on the outcome of a disease outbreak. In the case of H5N1 HPAI, those individuals include poultry producers, district-level veterinarians, agricultural extension staff, medical doctors, public health workers, wildlife health experts, and, most importantly, those raising poultry at the village level. All of these individuals need to know how to detect, prevent, and respond to an H5N1 HPAI outbreak, as well as how to protect themselves and others from becoming infected. The delivery of information to all levels requires numerous trainers, and materials that can be delivered both to educated professionals and the general public.

Another training challenge is that there is no one solution that will fit all disease outbreak situations. Because of its global spread and the range of species that may be infected, an HPAI H5N1 outbreak may occur in an enormous diversity of scenarios. Each scenario is characterized by a number of factors and circumstances that may be unique to a region. For example, the poultry sector is defined in terms of production systems, species raised, distribution chains, and marketing, all of which impact how the disease is spread and, therefore, controlled. Other less obvious factors may include the geography of a region, its climate, the susceptible wildlife species in the area, the culture(s), the country's trading partners, the political/economical environs, transportation networks, and the animal and human health infrastructures. All of these factors and their complex interactions will determine the best strategy to respond to an outbreak or case in a specific setting.

AVIAN FLU SCHOOL

Avian Flu School (AFS) was created to address these training challenges and needs through a train-the-trainer program that was intended to build local capacity. The AFS development project was completed between May and December 2006. AFS, a project of the Global Livestock Collaborative Research Support Program, is based on a cooperative extension model for the delivery of information and skills, and was developed by experts from the Schools of Veterinary Medicine and Medicine at the University of California, Davis (UC Davis).

MATERIALS AND METHODS

The pilot training courses

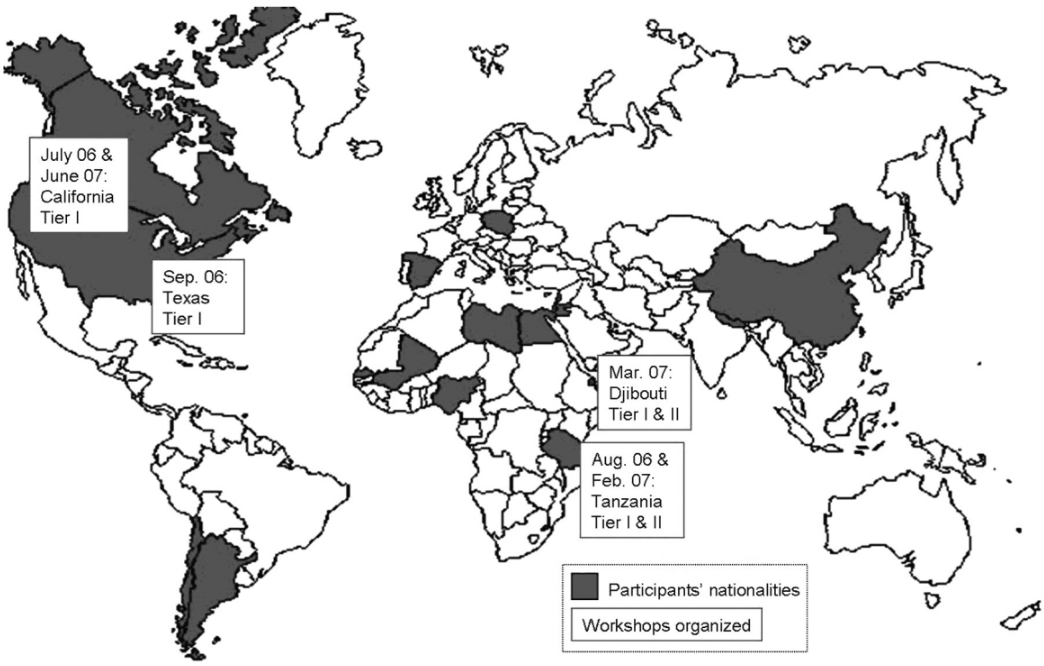
During the initial assessment period, three pilot courses were conducted—one at UC Davis (July 1–4, 2006) with 17 domestic and international participants (4 instructors, 13 trainees); one at Sokoine University of Agriculture, Morogoro, Tanzania (August 21–24, 2006) with 37 participants (5 instructors, 13 observers, 1 coordinator, and 18 trainees); and one at Texas A&M University (September 11–14, 2006) with 29 domestic and international participants (4 instructors, 4 observers, 1 coordinator, and 20 trainees). Four additional courses have been conducted in Djibouti (two), Tanzania (one), and Davis, California (one), in addition to courses taught by other organizations based on the AFS materials. The cadre of trainers is of 17 nationalities and comprises a core of 50 qualified AFS instructors. Of these, by summer 2007, 10 had conducted additional AFS courses. The location of the courses and the participants' nationalities are shown in Figure 1.

Course evaluation

Assessment of the training model's effectiveness is based on evaluations of the pilot courses only. Participants were asked to complete pre- and post-course assessments of their knowledge and to evaluate their perceived improvement in comprehension of the subject matter. An additional evaluation form was completed by trainees at the end of each module, ranking the effectiveness of each module on a scale of 1 to 5, with 5 being the highest level. Moreover, at the end of each workshop there was a facilitated discussion about the course's effectiveness and how to improve it. Most adjustments in course content were made based on feedback gathered at the pilot courses.

Ten months after the first pilot workshop, an invitation to complete an anonymous online survey was e-mailed to participants of the pilot courses. Participants

Figure 1. Locations of workshops conducted and nationalities of Avian Flu School trainers



were asked to report on their training activities, behavioral changes since the workshop, whether they would change anything in the course, and their familiarity with the main learning objectives for each module.

Training model

Based on the assessment that there is a need to deliver information to large numbers of people worldwide, it was concluded that a train-the-trainer model might be the best approach. "Train-the-trainer" is a term used to describe programs designed for training people who will in turn train others. Also known as "cascade training," the train-the-trainer approach is commonly used in the fields of animal health, public health, and agricultural extension. It has been used in the past to approach issues in both developed and developing countries, such as Newcastle Disease vaccination in chickens,[8] human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS),[9–12] asthma,[13] care for disaster survivors,[14] health promotion for childbearing,[15] promotion and risk reduction in pregnancy,[16] neonatal intensive care,[17] and the use of pesticides by farmers.[18]

AFS materials are designed in tiers based on the expertise of the intended audiences. In Tier I, professionals as well as national officials from public health ministries and veterinary service departments are trained. Tier I trainees then conduct Tier II trainings, mainly within their districts or organizations; the intended audiences are zonal and district veterinarians, agricultural extension staff, wildlife managers, and public health workers who will, at Tier III, reach their respective communities.

Tier I trainers apply for AFS and are admitted to the course based on their qualifications as health professionals with relevant expertise (e.g., poultry health, public health, epidemiology), language skills, and willingness to travel. Trainers are classified in a dataset by these characteristics so that they can be easily identified and matched to the specific requirements of requested trainings. Tier II trainers are identified by their job functions, and Tier III trainees are identified by their interest and needs.

Flexibility

The AFS curriculum is adaptable because of its interactive structure. In addition, the course was designed in a modular format so that it can be easily and quickly adapted to an audience's needs by adding or removing modules and/or lessons. Figure 2 presents a list of the modules and their content. Customizing the AFS course usually means teaching the whole or most of the Overview module and selecting only some lessons of the other four modules. While the Overview module serves as the basic background information for all audiences, the Surveillance module is not always taught because it is aimed only at individuals involved in the development and/or implementation of surveillance activities.

Figure 2. Topical outline and timeline of the Avian Flu School course

Module 1: Overview (1 day)
- Introduction
- Avian flu viruses
- History of H5N1 HPAI
- H5N1 HPAI transmission
- H5N1 HPAI risk to humans
- Impacts of H5N1 HPAI
- Virus surveillance, testing, and reporting
- Coordination and management of an H5N1 HPAI emergency
- Communications planning

Module 2: Surveillance (4 hours)
- Introduction
- Surveillance of H5N1 HPAI: steps, methods, types, and objectives
- Sample-size calculation
- Surveillance in poultry and captive populations
- Surveillance in wild birds
- Developing an H5N1 HPAI surveillance plan

Module 3: Public Health and Worker Safety (2.5 hours)
- Introduction
- General public education and protection
- Poultry farm worker protection
- Backyard/small holder poultry owner protection
- Live-bird market worker protection
- Medical worker protection and patient protocol
- Public health team protection
- First responder protection
- Health-care worker protection

Module 4: Prevention and Response (1 day)
- Introduction
- Prevention
- Response
- Recovery
- Scenarios (smallholder poultry operations, wet markets, commercial poultry facilities, zoo and aviary collections, wildlife refuges, parks)
- Developing prevention plans
- Developing response plans

Practical Session (3 hours)
- Packaging a virus sample for shipping
- Putting on and removing personal protective equipment
- Cloacal and oral cavity swabbing for samples (1 person, 2 people)
- Vaccinating a chicken
- Bleeding a chicken
- Safe slaughter and cleaning of a chicken or duck
- Use of a rapid diagnostic test

H5N1 HPAI = highly pathogenic avian influenza

As an additional option, lessons in sample-size calculation, for example, can be shortened to include only the importance of adequate sample sizes, and covered with a job aid like a laminated card with a sample-size table, which can be used as a reference.

Lucidity of the material

The materials are written in simple language using short statements and formatted as bulleted lists whenever possible. Technical jargon is avoided and complementary diagrams are presented along with the text to explain some of the more complex and confusing concepts. This approach facilitates not only comprehension, but also the teaching of the materials. Moreover, many participants do not speak English as a first language, and a more complicated writing style would compromise their understanding. The simple language structure and straightforward points also facilitate the translation of the materials into other languages. To date, the curriculum has been translated into French, Kiswahili, and Spanish. Trainers are also instructed to speak slowly and clearly, stressing the most important concepts, and monitoring trainees frequently for comprehension.

Interactivity

Lessons are highly interactive, mainly through the use of small group review exercises. The exercises generally present participants with hypothetical scenarios with which they can practice the lessons' main concepts. Most of the exercises are conducted in small groups of three to five people, who discuss the exercise and then report back to the full workshop group for further review and discussion. The main exercise answers are then recorded on a flip chart and later distributed by e-mail to all participants.

Open discussion is highly encouraged, not just during the exercises but also at any time during the lessons. Discussions help in understanding concepts and clarifying common misunderstandings, while keeping participants involved and interested in the subject. If the discussion goes off track, the trainer can note the issue or question on a chart so that it can be clarified and/or discussed at a more relevant time later on in the course. This helps to keep the modules on topic and on schedule, yet allows the curriculum to be adjusted to the audience's specific needs.

Relevance

AFS courses usually include local guest speakers who present their own experiences, especially as they relate to situations that may impact H5N1 HPAI detection, prevention, response, or recovery. Examples of such information offered during the pilot courses included presentations about the response to the first HPAI H5N1 outbreak in Nigeria, a talk on the Tanzanian National Plan, and open discussions on the diagnostic capabilities both in the U.S. and Tanzania. Instructors are also encouraged to use real-life examples and case scenarios from their own experience to illustrate the different concepts being taught. At a minimum, a presentation on the poultry sector in the host country and another one on the implementation of the national HPAI Plan are important topics to be included with the Overview (Module 1).

To encourage immediate action based on knowledge gained in the course, participants are asked after each module to identify one action that they plan to take based on what they just learned, when they plan to implement the action, what other immediate applications they may see, and with whom they will share the information.

Training materials

AFS materials were compiled and developed from existing documents in the public domain as well as from new materials. To locate appropriate background and documents, the websites of the main organizations involved in outreach, education, and messaging on avian influenza were visited to identify relevant brochures, fact sheets, slide shows, and books. These documents were collected, read, and categorized by topic. An outline was then developed to identify gaps in the publicly available information, and experts were enlisted to develop material to fill those gaps.

A text version of the materials was developed with the input of experts in relevant fields. Once the content was developed, the materials were organized into short, highly interactive lessons by a commercial adult training firm (Info Pros, Sacramento, California), which provided guidance on the course module format and structure.

Content

The complete AFS course consists of three days of workshops divided into four modules with short lectures and small group exercises, plus a half-day of practical exercises (Figure 2). The practical session covers the applied skills related to the four modules. It is best taught in a location, such as a laboratory or outdoors, that allows the manipulation of live birds. Each module is supplemented with a list of the references used, a feedback form, and a short Microsoft® PowerPoint® presentation. In addition, a series of appendices covers specific standard operating procedures, exercises, diagrams, case studies, and other complementary information. Each module covers only information essential to understanding avian influenza. For example, only general concepts in virology necessary for understanding the prevention and control of avian influenza are presented. Any extra information that the participants may want to know in an individual training is directed as questions to the instructors. Because the material is used in a variety of settings, every group of trainees will have specific questions related to their own experiences, interests, or needs.

Structure

All modules follow the same structure to aid in their ease of use. Instructors are provided the module contents, a description of the target audience, a suggested timeline, the module objectives, and the module preview, which stresses the key points of the lessons to come. The curriculum follows in short segments that include highly interactive review exercises. The module preview and the objectives are repeated at the end of the module to ensure that all basic concepts are clear.

The course manual is structured into two columns: the left column contains the instructors' notes, and the right column presents the material for the participants (Figure 3). The instructor column includes the information to be taught, plus instructions, transitions, and the course timeline. The participant column consists of highlighted key information, exercises, diagrams, and space for participants to take notes. Tier I participants are presented with both columns, while the manuals for Tiers II and III trainees show only the participant's column.

Each module has an accompanying slide show presentation with exercises, pictures, diagrams, and simple animations to help explain the most confusing concepts. It was decided not to include in the slide shows detailed text or information covered in the manual. Initially, some instructors in the pilot courses used slide show presentations that either represented the curriculum using new pictures and graphics or added illustrative material. Participants' feedback was nearly unanimous in that they wanted instructors to use the exact material in the manual to make it easier to learn in a short period of time. The current iteration of AFS keeps participants focused on their manuals, which means that the course can be taught in the absence of projection equipment, or in the event of a power outage, which is common in some developing countries.

Course schedule and setup

It is recommended that full 3.5-day AFS courses be taught by a minimum of two instructors, preferably three. Ideally, one instructor should have a veterinary and poultry background and one other should have a public health background. A third instructor with expertise in communication or national planning is helpful. The ideal number of trainees to optimize the learning process is between 10 and 15, with varied professional qualifications. The interactive exercises are most effective when trainees can bring public health and veterinary health perspectives together. Although several points of view are presented in the workshop, a more complete integration of ideas is achieved when the participants have diverse backgrounds.

The recommended room layout for the workshop is illustrated in Figure 4. The AFS laboratory requires at least one instructor per four participants, and the suggested room or facility setup is also shown in Figure 4.

Making the AFS materials available

To support course workshops domestically and internationally, the AFS Assessment Project developed a website, www.avianfluschool.org, which provides guidance for organizing a training workshop, directions for ordering AFS course materials or a custom course, guidance for locating AFS instructors, and recommended resources for instructors.

RESULTS

Pilot course evaluation

Each pilot course received higher evaluations than the preceding one, indicating that the modifications made between courses effectively improved the course's perceived quality. Figure 5 shows how the overall course evaluation score improved from 4 out of 5 during the first pilot course at UC Davis to more than 4.4 out of 5 for the last pilot course at Texas A&M; both were attended by similar audiences. Similar results were obtained for each of the modules individually. Module 2 was changed the most from the first to the third pilot course. The improvement in evaluation scores is reflective of those changes.

The online survey conducted after the pilot courses had a 66.1% response rate and showed the following about the participants:

- 80.6% were confident in their ability to use the materials to train others as a result of the course.
- 36.1% trained someone using the AFS course materials. The trainees included marine biologists, poultry producers in the U.S., national park rangers, medical officers, government leaders and

Figure 3. Avian Flu School training manual[a]

AVIAN FLU SCHOOL COURSE GUIDE

LESSON I
H5N1 HPAI SURVEILLANCE

Instructor Notes	Course Material
TIME: 30 MINUTES **START TIME:**________ **END:** ________ *TRANSITION* *As discussed in the overview, it is optimal to survey both poultry and wild birds, since H5N1 HPAI may spread into uninfected areas through their movements.*	IMPORTANT POINT To have effective H5N1 HPAI emergency management, it is critical to be ready to respond to an outbreak. Surveillance is vital for early detection. A surveillance plan should be ready to be applied **before**, **during** and after an **outbreak**.
Successful surveillance planning includes specific strategies for each the following: • **Locating** susceptible populations. • Efficient **detection**, reporting and assessment of morbidity and mortality events.	Successful surveillance planning includes specific strategies for each the following: 1. Locate: ________________________ IMPORTANT POINT Keeping an accurate and up to date database of all commercial premises, backyard poultry, wild bird congregations, zoos, and pet shops is vital to allow the quick identification and surveillance of at risk populations within an area after infection is detected. 2. Detect: ________________________

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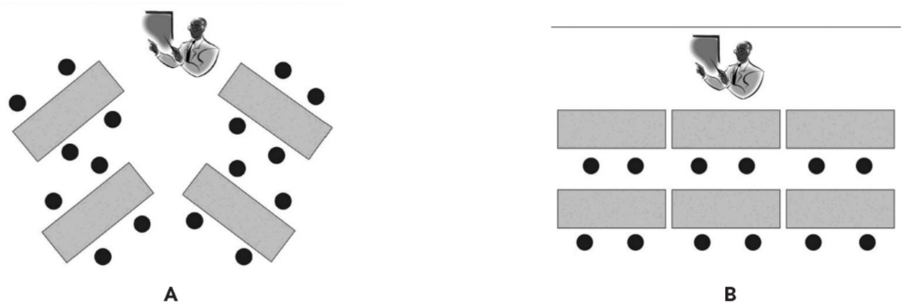
Module 2: Surveillance **7**

[a]The course manual is structured into two columns: the left column has instructor notes and the right column has material for the participants. The participant column has key information highlighted, notes, exercises, diagrams, and space for taking notes. Tier I participants are presented with both columns, while the manuals for Tier II and III trainees show only the participant's column.

H5N1 HPAI = highly pathogenic avian influenza

Figure 4. Suggested table setup for Avian Flu School[a]



[a]Setup for the workshop (A) and the laboratory (B):

A) The room should be set up with 4 to 5 tables of 4 to 5 trainees each. The tables should be angled toward the instructor so that participants can easily look forward, but also turn back to their tables for group discussions.

B) Participants should all face the instructor and have enough space to perform the bird manipulation (2 to 3 per table depending on the size of the table).

field officers in Tanzania, government veterinarians in Vietnam, and a virologist in Libya.

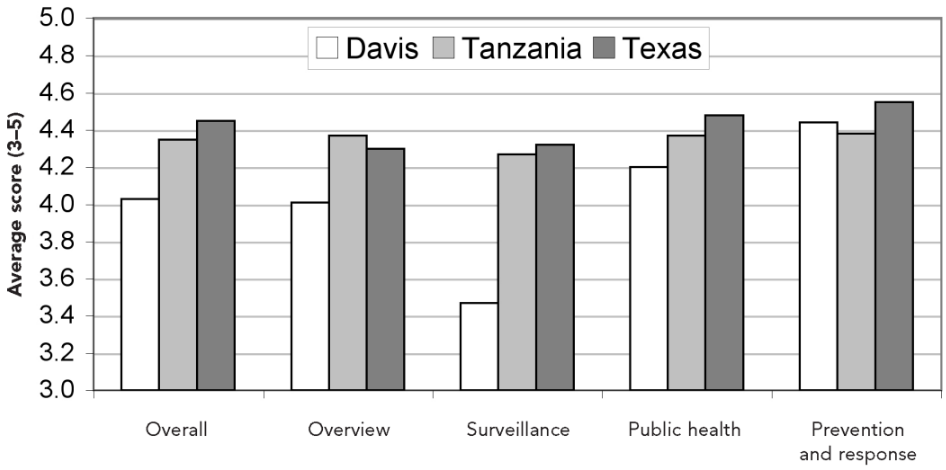
- 52.8% gave the entire manual or part of it to someone, for a total of 119 additional individuals who received the materials.
- 38.9% implemented some behavioral change based on what they learned.
- 52.9% would not modify, add, or eliminate any part of the training materials, both in terms of content and presentation.

With regard to the AFS content, trainees were asked to assess their knowledge of the course's learning objectives. The responses of the survey participants are summarized in the Table.

Project

One of the unanticipated outcomes of the AFS training courses has been the development of a village Newcastle vaccination project through collaboration by trainees from the Tanzanian pilot course. During the exercise in the Surveillance module (Module 2),

Figure 5. Overall and individual module average scores given by participants in evaluations conducted at each of the three Avian Flu School pilot courses[a]



[a]Average scores were calculated from individual scores, with 5 representing the best possible score and 1 the worst.

two groups of participants suggested a novel strategy. They proposed implementation of a Newcastle disease vaccination program in selected villages to prevent clinical disease associated with viscerotropic velogenic Newcastle disease (VVND). VVND is endemic in Tanzania and many other parts of the developing world, and is clinically indistinguishable from any HPAI, including H5N1 HPAI. The cost of the laboratory testing needed to differentiate these diseases is prohibitive, and is thus a limiting step in achieving adequate surveillance for HPAI in rural Tanzania.

The groups of trainees proposed using sentinel villages in which chickens would be vaccinated for Newcastle disease and enrolled in a reporting system for mortality events. A chicken die-off in Newcastle-vaccinated chickens is much more likely to be caused by HPAI than a mortality event in nonvaccinated chickens. If a mortality event occurs, veterinarians in the region who have been equipped with rapid flu detection kits can confirm a presumptive diagnosis. This system optimizes the use of influenza tests that are expensive and in short supply in Tanzania, as they are in many countries.

DISCUSSION

Challenges

Although the AFS was effective, it did raise some issues. There is a general need for practical training of this type. Additionally, the immediacy of those needs changes as H5N1 HPAI spreads. Although the train-the-trainer model has worked well in a short period of time, and there are more than 50 trainers with a variety of language skills, this number is equivalent to fewer than two per nation with reported H5N1 HPAI cases. Furthermore, most are fully employed and often cannot travel on short notice when an outbreak occurs.

In addition to the difficulty in developing a network of trainers, there are three main challenges for the implementation of AFS: time constraints, logistics, and funding. Generally, six weeks are required to organize an AFS workshop, although a series or regional cluster of courses may require less time to organize. Most of that time is spent organizing speakers and their visas, transportation, lodging, and meals; selecting the participants and the venue; and preparing and shipping the materials. All of these tasks are time-consuming and logistically challenging. Many laboratory materials considered common in developed countries may not be easily accessible in developing nations, so almost everything has to be packed and shipped from elsewhere. However, every effort is made to find local products that can be used effectively. For example, if a recommended disinfectant is only available in the U.S., then international trainees have no ready supply. It is then necessary to investigate locally available disinfectants and determine which ones are effective yet nontoxic to animals or people.

Workshops can also be expensive. There are substantial costs for travel, accommodations, and meals for participants and instructors, and for laboratory supplies, venue rental, and staff time. Initial workshops were more expensive than current courses. This is partly because there are trainers located in more parts of the world, with many who are located closer to workshop sites, which reduces travel expenses. Additionally, the materials have been translated into key languages, thus reducing the expense of any given workshop. Training funds are available to conduct Tier I workshops in many countries, but may be inadequate to support Tier II

Table. Results of a survey of Avian Flu School participants and their comprehension of the learning objectives for the material before and after the course

	Participant responses	
Learning objectives for AFS	*Knew before AFS (percent)*	*Learned in AFS (percent)*
Are familiar with H5N1 HPAI virus transmission pathways	54.8	96.8
Are prepared to communicate about an avian influenza emergency	12.9	87.1
Could design a surveillance plan and modify it based on status of the disease	16.1	80.6
Know how to protect themselves and others from exposure	38.7	96.8
Could give biosecurity advice for poultry flocks in various settings	22.6	96.8
Are familiar with response and recovery procedures	16.1	77.4
Can properly don and doff personal protective equipment	16.1	93.5

H5N1 HPAI = highly pathogenic avian influenza

AFS = Avian Flu School

workshops or village-level training. Within three weeks of the Tanzania course, a participating zonal veterinarian conducted a Tier II course for district veterinarians in the Arusha area. This is one indication of the need for the information and how little extra input would be needed to implement Tier II trainings.

The AFS course materials have not yet been fully adapted for education programs at the local community or village level. These Tier III trainings may be best facilitated by nongovernmental organizations and agencies that already have an existing network of field staff. There are numerous networks of people working at the village level, including agricultural extension advisors, agricultural associations, faith-based organizations, local volunteer organizations, microfinance networks, and public health field program staff (in areas such as HIV/AIDS, tuberculosis, and rabies projects). The AFS team is currently seeking to establish collaborative relationships with organizations that have the interest and necessary networks to deliver Tier III trainings.

Future of AFS

AFS is now a part of the Stop AI effort of Development Alternatives International funded by the U.S. Agency for International Development. Through the Stop AI effort, AFS will be combined with other training programs and delivered worldwide.

Avian Flu School California is another iteration of the course. The goal of this project is to educate existing networks of state cooperative extension advisors and specialists about avian influenza. California cooperative extension has lost most of its poultry specialists and advisors, although the state remains a major poultry producer. Therefore, there are few resources to address poultry disease threats like H5N1 HPAI. Through AFS, county farm advisors, youth development advisors, and nutrition, family, and consumer sciences advisors can be educated about avian influenza. After training with a shortened version of AFS that covers the basics of H5N1 HPAI and topics of special interest, they will deliver the program in their communities to audiences such as ethnic groups, health-care workers, children, hunters, backyard poultry owners, and hobby farmers.

Now that the training model, materials, and methods have been fully developed and repeatedly tested, the AFS approach can be used to develop educational programs for other diseases that threaten the sustainability of agriculture.

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7.4. Preventive Veterinary Medicine 2011

David Bunn, Daniel Beltran-Alcrudo, Carol Cardona. Integrating surveillance and biosecurity activities to achieve efficiencies in national avian influenza programs. *Preventive Veterinary Medicine* 2011; 98: 292–294

Resumen del artículo en castellano

Integrando vigilancia epidemiológica y actividades de bioseguridad para alcanzar la eficiencia en programas nacionales de influenza aviar

Basándose en la experiencia con la HPAI (Influenza Aviar Altamente Patógena), los esfuerzos para mejorar la capacidad global para prevenir y controlar enfermedades zoonóticas deberían considerar modelos nuevos y más eficientes para integrar las actividades de prevención y vigilancia.

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Letter to the Editor

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ABSTRACT

Based on the HPAI experience, efforts to improve global capacity to prevent and control zoonotic diseases should consider new and more efficient models for integrating prevention and surveillance activities.

Integrating surveillance and biosecurity activities to achieve efficiencies in national avian influenza programs

While training and consulting on highly pathogenic avian influenza (HPAI) in Africa over the past four years, we have observed opportunities for improving the efficiency of HPAI prevention, surveillance and response programs. Implementing these national programs is a very expensive endeavor, typically far exceeding a developing country's budgeted funds and thus requiring substantial donor support. Costs could be significantly reduced if some of the tasks within the various components were combined, particularly in the implementation of field activities. One of the most obvious synergies is between surveillance and biosecurity improvement activities.

Integrating biosecurity improvement training into surveillance programs would be an efficient way to improve the field implementation of both functions, because:

a. Biosecurity and surveillance professionals must engage and train the same groups such as poultry keepers, live-bird market managers, and poultry service providers. These groups must be trained on how to apply biosecurity, and on detection and notification of suspicious morbidity and mortality events.
b. Professionals implementing surveillance and biosecurity programs would benefit from cross training. Surveillance supervisors, usually epidemiologists working with laboratory scientists, would learn how to better prioritize high-risk sites, how to implement personal biosecurity, and how to assist with biosecurity training. Similarly, biosecurity personnel, usually poultry veterinarians, would learn how to better integrate passive surveillance into their biosecurity improvement field activities.
c. Biosecurity and surveillance professionals have overlapping tasks—both must gather information, evaluate risk, prioritize poultry sites and practices, and manage actions at high-risk poultry farms and live-bird markets.
d. Assessing biosecurity measures and gaps at surveillance sites provides key information for focusing surveillance.
e. Implementing biosecurity measures at surveillance sites, which includes placing limitations on the movement of poultry materials that may transmit potential pathogens, makes surveillance data more valid over a longer period of time. The pathogens are likely to have spread less since the surveillance data was collected and then confirmed.

A surveillance program (applying both passive surveillance and targeted active surveillance) for the early detection of HPAI incorporates the principle of distributing surveillance resources and efforts based on a ranking of high-risk sites and practices. These are the same sites and practices that should be targeted for biosecurity training and improvements. Thus, surveillance and biosecurity may be integrated as follows (see Fig. 1):

1. Identify poultry populations and poultry-related sites and practices and gather information about the production systems, their value, and the connection of those

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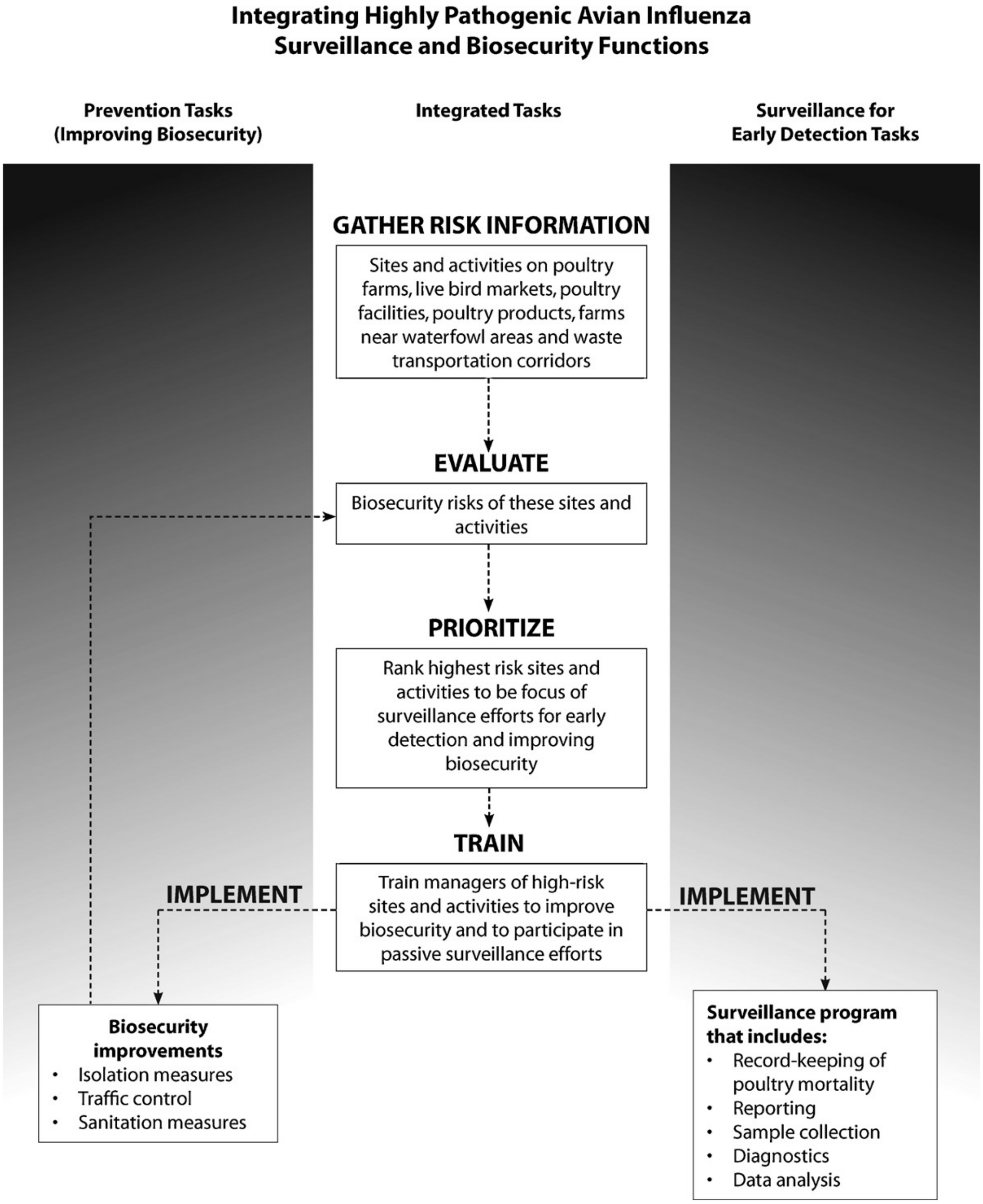

Fig. 1. Integrating highly pathogenic avian influenza surveillance and biosecurity activities.

poultry operations to poultry input and product networks.

2. Evaluate the risk of HPAI introduction and further transmission at each of these sites or poultry-related activities.
3. Rank these sites and practices based on their disease outbreak risk.
4. Train district-level officials, site managers (farm and market managers, village leaders) and service managers (transport managers, border agents, etc.) on passive surveillance procedures (detection, notification and investigation) and on how to improve biosecurity.
5. Monitor the implementation of biosecurity improvements and disease reporting, and periodically re-

evaluate and prioritize the high-risk sites and activities (see feedback loop in Fig. 1).

The H5N1 HPAI panzootic has highlighted the need to build capacity for disease prevention, detection and response preparedness. These efforts have encountered the enormous expenses of training, communication, personnel and equipment. Cross-training among professional field staff, linking activities that require similar knowledge and engage the same groups, and integrating surveillance, biosecurity and other field activities will build more efficient disease prevention and surveillance programs. We have used HPAI as an example, but of course the same principles could be applied to national prevention and control programs for other poultry and livestock diseases.

Thank you for sharing this with your readers.

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7.5. Preventive Veterinary Medicine 2009

Daniel Beltran-Alcrudo, Tim E. Carpenter, Carol Cardona. A flock-tailored early warning system for low pathogenic avian influenza (LPAI) in commercial egg laying flocks. *Preventive Veterinary Medicine* 2009; 92: 324–332

Resumen del artículo en castellano

Un sistema de alerta temprana a nivel de explotación para Influenza Aviar de Baja Patogenicidad (LPAI) en epxlotaciones comerciales de ponedoras.

El objetivo de este estudio fue desarrollar y evaluar un sistema de alerta temprana (EWS) para explotaciones comerciales de ponedoras para detectar mortalidades leves y cambios en la producción de huevos que caracterizan las infecciones por virus de influenza aviar de baja patogenicidad (LPAIV). Un EWS generará una alerta cuando un punto de alerta recomendado se alcanza o se exceda. Anteriormente los EWS utilizados se basan en niveles fijos de alerta, mientras que el que proponemos personaliza el nivel de alerta para cada explotación. A pesar de que un enfoque fijo puede ser válido para enfermedades de alta patogenicidad, conlleva una baja probabilidad de detección en enfermedades de baja patogenicidad. El EWS se basó en la recogida diaria de datos de explotaciones afectadas por la epidemia de LPAI H6N2 ocurrida en California entre 2000 y 2004. Se evaluaron tres sistemas: EWS1 que alertaba cuando la mortalidad aumentaba o la producción disminuía más de x veces los valores diarios esperados (2.75–3.50 veces la mortalidad esperada), (2) EWS2, que alertaba cuando mortalidad aumentaba o la producción disminuía más de y veces los valores diarios esperados durante 2 días consecutivos (1.75–2.15 veces la mortalidad esperada), y una combinación de ambos. Los EWS fueron evaluados de acuerdo a tres parámetros: demora en la detección de un brote de LPAI (en días), falsas alertas (%) y brotes no detectados (%). Los resultados mostraron que en un sistema basado en la producción de huevos no añade beneficios sobre uns sistema basado en la mortalidad, principalmente porque la disminución de la producción de huevos relacionada con H6N2 LPAI se produce siempre después de un incremento de la mortalidad.

(Continúa)

Combinando ambos sistemas se consiguió reducir el tiempo de detección y se detectaron todos los brotes, pero a costa de un ligero incremento del número de falsas alertas. El sistema presentado en este estudio también llevó a cabo alertas basadas en valores fijos para los tres parámetros evaluados. El sistema propuesto, si se utilizara como parte de un programa de crianza cooperativo y se combinara con un diagnóstico laboratorial rápido, podría ser una herramienta útil para detectar y controlar los brotes de LPAI y otras enfermedades que afectan a las aves de corral. Construido sobre una hoja de cálculo, el sistema podría ser barato, sencillo y rápidamente incorporado en el sistema de toma de decisiones de granjas comerciales de producción de huevos. Además, el sistema propuesto puede ajustarse rápidamente a situaciones epidémicas cambiantes, y fácilmente personalizado para cada explotación.

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A flock-tailored early warning system for low pathogenic avian influenza (LPAI) in commercial egg laying flocks

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ABSTRACT

The aim of this study was to develop and evaluate an early warning system (EWS) for commercial egg laying flocks to detect the subtle mortality and egg production changes that characterize low pathogenic avian influenza virus (LPAIV) infections. An EWS will create an alert when the recommended 'trigger point' is reached or exceeded. Previously used EWSs are based on fixed alert levels, while the proposed EWS customizes the alert level to each flock. While a fixed approach may be valid for highly pathogenic diseases, it results in a lower detection probability for low pathogenic diseases. The EWS was based on daily data collected from flocks affected by the 2000–2004 H6N2 LPAI epidemic in California. Three EWSs were evaluated: (1) EWS1, which is triggered when the observed mortality increase or production decrease exceeds more than "*x*" times the expected daily value (2.75–3.50 times the expected mortality), (2) EWS2, which is triggered when the observed mortality increase or production decrease exceeds more than "*y*" times during each of 2 consecutive days the expected daily values (1.75–2.15 times the expected mortality), and (3) a combination of the two. The EWSs were evaluated according to three parameters: detection delay (days) of a LPAI outbreak, false alerts (%) and outbreaks missed (%). Results showed that an egg production-based EWS added no benefit to a mortality-based system, mainly because H6N2 LPAI-related egg production decrease always occurred after increase in mortality. Combining the two EWSs resulted in a reduced detection delay and no missed outbreaks, but at the expense of a slight increase in the number of false alerts triggered. The system presented in this study also outperformed fixed EWSs in all three evaluated parameters. The proposed EWS, if used as part of a poultry cooperative program and combined with a rapid laboratory diagnosis, could be a useful tool in the detection and control of LPAI outbreaks and other poultry diseases. Built in a spreadsheet, the system could be inexpensively, easily and quickly incorporated into a commercial egg production farm decision support system. In addition, the proposed system could be quickly adjusted to changing epidemic situations, and easily customized to individual flocks.

1. Introduction

1.1. The virus

Avian influenza (AI) is one of the most important diseases of poultry. Avian influenza viruses (AIVs) can be pathotyped into two groups depending on the severity of

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the disease they cause in naïve chickens. The virulent types, termed highly pathogenic avian influenza viruses (HPAIVs) are associated with severe decreases in egg production and mortality approaching 100%; while low pathogenic avian influenza viruses (LPAIVs) may result in inapparent infections, particularly when the virus has recently been introduced from the wild to the domestic host (Swayne et al., 2000) or in some species, e.g. domestic waterfowl (Shortridge, 1982; Alexander, 2003). The lack of clinical signs can result in spread within and between premises, before farmers realize that the birds are infected. Other LPAIV infections may result in mild respiratory disease, depression, moderate egg production decline in laying birds, and low mortality (Capua and Alexander, 2004), which are easily mistaken for other disease syndromes.

Although LPAIVs, other than the H5 and H7 subtypes, are not notifiable to the World Organization for Animal Health (OIE), they can cause substantial losses if allowed to persist in poultry populations (Cardona, 2005). Moreover, secondary infections, stressors or environmental conditions can cause an exacerbation of LPAIV infections leading to a much more serious disease (Capua et al., 2003). In the last two decades there have been economically important LPAI outbreaks in Italy (H7N1 2000–2001), Mexico (H5N2 1993–present) and Pakistan (H7N3 1995 and H9N2 1998) (Marangon et al., 2003; Lee et al., 2004; Naeem and Siddique, 2006). In recent years, the U.S. poultry industry has been affected by LPAI outbreaks in Pennsylvania (H5N2 1985–1986; H7N2 1996–1998 and H7N2 2001–2002) and California (H6N2 2000–2004) (Kradel, 1987; Dunn et al., 2003; Henzler et al., 2003; Kinde et al., 2003).

There is strong evidence supporting the hypothesis that HPAIVs arise after a mutation of H5 or H7 LPAIVs that have been introduced to poultry from wild birds (Capua and Marangon, 2006). Although, the timing of a mutation is unpredictable, it can be assumed that the wider the circulation of LPAIVs in poultry, the more opportunities the virus has to mutate into an HPAIV (Alexander, 2007).

Avian influenza viruses have demonstrated zoonotic potential. The most notable examples are the human influenza pandemics of 1957 (H2N2) and 1968 (H3N2), in which the hemagglutinin genes probably originated from AIV generated by reassortment of avian and human viruses (Scholtissek et al., 1978; Kawaoka and Webster, 1985; Kawaoka et al., 1989). A zoonotic virus can also be generated by mutation of an AIV.

1.2. Early warning system (EWS)

There are four reasons why early detection of LPAIVs circulating in poultry flocks is important: (1) LPAIV infections may cause significant losses for commercial poultry producers (Cardona, 2005), (2) H5 and H7 LPAIV strains can mutate to HPAIVs, (3) AIVs may expand their host ranges to new species, including humans, and (4) LPAIV strains can contribute genetic material to HPAIVs (Chin et al., 2002).

Since prevention of AI is not always possible, once the virus has been introduced, its early detection becomes the key to a successful control strategy. In the case of LPAI, where clinical signs may be inapparent, very mild, or nonspecific, an EWS that detects subtle changes in the daily mortality, egg production or both, especially if combined with a rapid laboratory diagnosis, will allow the quick implementation of control actions, such as isolation and sanitation, thus preventing further spread of the virus and reducing the economic impact. An EWS will create an alert when the recommended 'trigger point' or 'threshold' is reached. In order for an EWS to be justified, it is necessary to balance the potential benefit of the early detection of an outbreak, with the cost of responding to an alert and the probability and cost of false alerts. A highly sensitive EWS will detect an outbreak very quickly, but at the cost of having an increased number of false alerts, while a less sensitive EWS that reduces the number of false alerts, will detect an outbreak later and be more likely to miss it.

Such systems have already been used to detect several animal diseases (Carpenter et al., 2007). In 2000, a temporary EWS was put in place by the Dutch veterinary authorities because of the threat of the HPAI epidemic in Italy (1999–2000). Every poultry farmer had to notify the government when daily flock mortality was >0.5% (alert level). When the first H7N7 HPAI outbreak was reported in The Netherlands in 2003, a different EWS was reinstated until June 2005, when the alert threshold was weekly mortality of 3% or a 20% reduction in either feed or water consumption. The 1-week mortality threshold was criticized, because it implicitly delayed a response for up to 1 week after the onset of disease (Elbers et al., 2007). After eradication of the disease, Elbers et al. (2007) suggested stricter notification thresholds, based on an analysis of daily within-flock mortality data. Different recommendations were made for each poultry type, e.g. a threshold of >0.25% daily mortality for 2 consecutive days for caged layers. In addition, it was recommended that a poultry farmer should consult the veterinarian in the event of a reduction of >5% for 2 consecutive days in (1) feed or water intake or (2) daily egg production in layers.

1.3. The H6N2 epidemic in California

This study uses data collected from the 2000–2004 H6N2 AIV epidemic in California. In February 2000, a LPAIV subtype H6N2 was isolated from a backyard flock in Southern California (Kinde et al., 2003). During the next 2 years, infection was detected on 16 commercial premises (Kinde et al., 2003). The flocks presented with respiratory distress, a substantial decrease in total egg production (10–40%) and a slight increase in total mortality over baseline (0.25–3%) (Kinde et al., 2003). In early 2002, the epidemic spread to the Central Valley in Northern California. The associated pathology was more severe (fibrinous yolk peritonitis, and occasionally salpingitis, oophoritis and misshapen ovules). In a few cases, decreased feed consumption was also observed. It was also noted that pullets did not develop the disease until they started laying eggs (>20 weeks of age) (Kinde et al., 2003). Genetic studies on the different isolates suggest adaptation of the virus to its chicken host (Webby et al., 2002). Not being an H5 or H7 strain, there were no control plans in place and it was only through the application of a voluntary plan developed by the California poultry industry that the epidemic was controlled (Cardona, 2005).

The purpose of this study was to develop and evaluate an EWS for LPAI for commercial egg production farms, which could be best used as part of the alert system in a cooperative control program, such as those implemented by turkey producers in Minnesota or by egg producers in California (Halvorson, 1984; Poss, 2003; Cardona, 2005). Such cooperative control programs ensure that appropriate and pre-defined preventive and control actions are taken promptly when the system is triggered, and are particularly useful for non-notifiable diseases such as (non-H5 or H7) LPAI, where governments may not take action and it is up to the producers to respond.

2. Materials and methods

2.1. Data

Daily mortality and egg production records from H6N2 LPAIV-infected flocks were obtained from 27 flocks on one commercial premises in Southern California that suffered an outbreak of H6N2 LPAI in 2002. Infection was confirmed by virus isolation from five submissions from different flocks. Subsequently, additional flock outbreaks were detected based on clinical signs (Woolcock, personal communication, 2009). The daily data available covered 2 months (January and February 2002), with the outbreak occurring during the second week of January. A flock was defined as hens of a single strain and age housed in a discrete building or structure. The hens were housed in cages on a multi-age egg production farm and birds were not vaccinated against AI. Information about the strain and age of the hens was also obtained. Seventeen flocks were affected during pre-molt production (9 flocks at 63 weeks of age and 8 flocks at 37 weeks of age) and the remaining 10 between the first and the second molt, at 85 weeks of age. Mean flock size was 25,040 birds (range = 8754–107,261).

Because data from healthy flocks from the affected farm were not available, records were obtained from another company in Southern California to estimate the baseline mortality and egg production trends for a standard flock. Data were checked for normality using the chi-square goodness of fit test. The comparison farm had no history of LPAI or any major disease event (according to farm records), had similar management practices, similar genetic strains, and was located in a similar environment in Southern California at around the same time. Forty-four commercial layer flocks housed between May 2002 and December 2004 in 20 poultry houses were selected. The hens were producing eggs up to 100–103 weeks of age, with one molt at around 66 weeks of age. Mean flock size was 25,726 birds (range = 16,920–75,324).

2.2. Data analysis

Baseline mortality trends (when no LPAI is present) were calculated using data from all 42 flocks for both the pre- and the post-molting periods. Mortality data over the molting period were not included in the analysis, because of the extremely high variability during molting between and within flocks. Because molting occurs at different times in each flock, depending on egg production and the market price for eggs, post-molt mortality data were aligned to day 1 of the post-molt production. The calculation of the egg production trend required a different approach because the slope changes depending on the stage in the production cycle, first increasing steeply to a maximum, and then decreasing gradually until the molting period. A spline regression model was used, which allows the functional form of the relationship to change at one or more points along the range of the predictor (splines). Locations of these shifts are called knot points or knots. If knot points are fixed by the analyst, like in this case, splines can be easily fitted (Dohoo et al., 2003). Egg production models for the pre- and post-molting periods were defined by the parameters shown in Table 1. Spline models were used with five and two knots for pre- and post-molt productions, respectively. Production data over the molting period were also excluded. Excel® (Microsoft Corporation, Redmond, WA) was used to perform all calculations.

2.2.1. Outbreak mortality and production data

No outbreak occurred until the second week of January, so mean daily mortality of the previous week was used to estimate the initial baseline daily mortality. This was considered a sufficient period of time, since daily mortality does not fluctuate substantially when a flock is in production (around week 20). The expected mortality (if there were no outbreaks) was calculated by applying the mortality trend formulas ($y = ax + b$) shown in the results section, where "b" is the initial baseline daily mortality, "x" is time (days in production after the first week) and "a" is a trend coefficient. The daily mortality attributable to the outbreak was obtained by deducting the expected daily mortality from the observed daily mortality during the outbreak.

Table 1
Spline model for the pre- and post-molt egg production in a commercial layer flock.

Knot no. (pre- or post-molt)	Position (time in days)	Trend after each knot (x = days)	R^2
1 (pre)	1	$y = 0x$	1.00
2 (pre)	11	$y = 0.13412x - 1.50211$	0.97
3 (pre)	18	$y = 0.19154x^2 - 7.32565x + 72.51697$	1.00
4 (pre)	35	$y = 0.00237x^3 - 0.43692x^2 + 26.81577x - 454.59275$	0.97
5 (pre)	74	$y = -0.06609x + 99.82599$	0.89
1 (post)	1	$y = 0.0203x^3 - 0.5165x^2 + 3.6533x - 5.4509$	0.96
2 (post)	22	$y = 0.0256x^3 - 2.606x^2 + 87.99x - 900.31$	0.94
3 (post)	41	$y = -0.0879x + 93.44$	0.80

A similar process was used for egg production data. First, the initial baseline daily egg production was calculated by averaging the records of the first week. Second, depending on when in the production cycle the outbreak occurred, the corresponding egg production trend (from the spline model shown in Table 1) was used to estimate the expected egg production (if there were no outbreaks). Third, the expected daily egg production was deducted from the observed daily egg production during the outbreak to calculate the daily egg production loss attributable to the outbreak.

2.3. Early warning system (EWS)

The EWS was designed in a spreadsheet. Two EWS scenarios were set up: EWS1, an alert threshold, which occurs when the observed mortality exceeds the expected mortality (as defined by the mortality trend) by more than a factor "*x*" in a single day, and EWS2, an alert threshold, which occurs when the observed mortality exceeds the expected mortality by more than a factor "*y*" during each of 2 consecutive days. One reason for implementing the EWS2 is to avoid a very common inaccuracy from poultry record keeping, which may arise because daily mortality counts occur at different times during the day, resulting in high counts recorded late in the day and lower counts the following day (and *vice versa*). This anomaly will result in a higher number of false alerts from the EWS1. In addition, a combination of both types of alerts was also evaluated, i.e. having an alert when either of the two alert levels is exceeded. The same concept of 1- and 2-day EWSs were used for the production data: EWS1 is an alert threshold, which occurs when the observed decrease in egg production is lower than the expected egg production (as defined by the egg production trend) by more than a factor "*x*" in a single day, and EWS2 is an alert threshold, which occurs when the observed decrease in egg production is lower than the expected egg production by more than a factor "*y*" during each of 2 consecutive days.

The EWSs (each individually and their combination) were tested with data from both LPAIV-infected and non-infected flocks. For the non-infected flocks, data were obtained for a total of 82 two-month periods starting at the same weeks of age as the LPAIV-infected flocks (37, 63 and 85 weeks of age). Three outcome criteria were used to evaluate/optimize the threshold levels: detection delay (DD) of an LPAI outbreak (in outbreak flocks), the percentage of false alerts (FAs) triggered (in non-outbreak flocks), and the percentage of LPAI outbreaks missed (in outbreak flocks).

To evaluate/compare the EWS against fixed EWSs, and since no LPAI EWS was found in the literature, the three EWS described in the introduction that were used/recommended to control the H7N7 HPAI epidemic in the Netherlands (Elbers et al., 2007), were adapted for LPAI by setting stricter alert levels. The same daily data from the H6N2 affected and healthy flocks were used to evaluate these adapted fixed EWSs according to the same performance indicators (detection delay, percentage of false alerts and percentage of outbreaks missed).

3. Results

3.1. No LPAI outbreak (baseline) mortality and production data

Daily mortality (%) was estimated as

$$y = 0.00010882x + 0.00971850 \quad (1)$$

and

$$y = 0.00011851x + 0.02163608 \quad (2)$$

where x = days in production, for pre- and post-molt, respectively (Eqs. (1) and (2) and Figs. 1 and 2). Data conformed to generalized linear model assumptions. Regarding the normality assumption, the error terms were not significantly different from the normal distribution, with p values of 0.41 and 0.54 for pre- and post-molt error terms, respectively.

The comparisons between the predicted and the observed egg production curves are illustrated in Figs. 3 and 4, and the production trends are shown in Table 1.

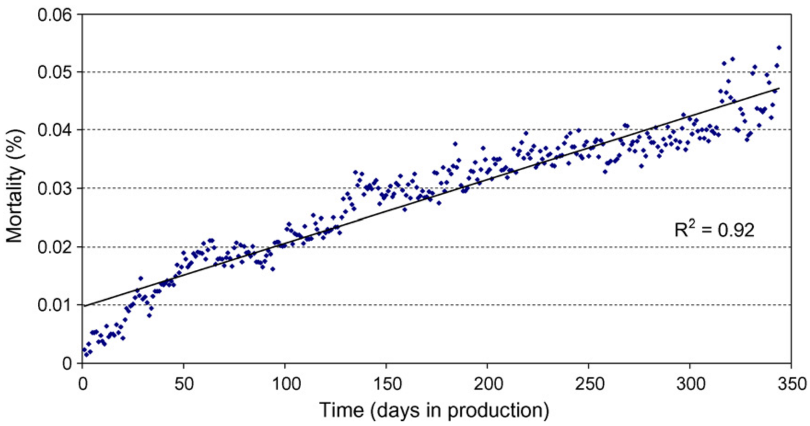

Fig. 1. Mean and predicted daily pre-molt percent mortality in a commercial layer flock (R^2 = fit of the model). The mean is based on data obtained from 44 healthy commercial layer flocks housed between May 2002 and December 2004 in California.

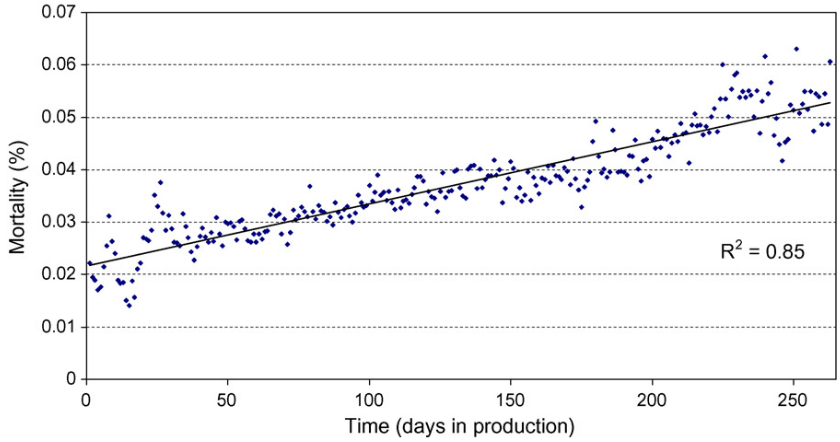

Fig. 2. Mean and predicted daily post-molt percent mortality in a commercial layer flock (R^2 = fit of the model). The mean is based on data obtained from 44 healthy commercial layer flocks housed between May 2002 and December 2004 in California.

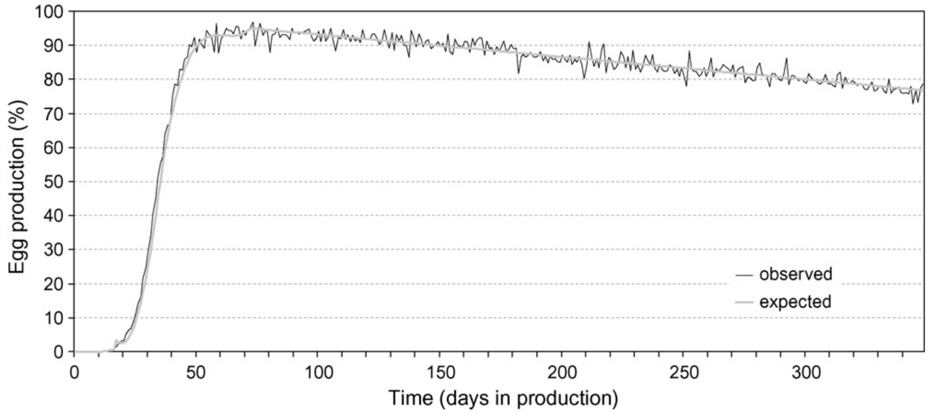

Fig. 3. Mean and predicted daily pre-molt percent egg production in a commercial layer flock. The mean is based on data obtained from 44 healthy commercial layer flocks housed between May 2002 and December 2004 in California.

3.2. LPAI outbreak mortality and production (descriptive statistics)

The mean total mortality attributable to the outbreak during the 18-day period in which LPAI-associated mortality exceeded the expected values (Fig. 5) was 1.73% (range = 0.18–5.27). Pre- and post-molt data were pooled for the calculations since no statistically significant difference was found between the two groups ($p = 0.6$). The peak daily mortality attributable to the outbreak was 0.40% (range = 0.09–2.30%). Egg production was substantially below expected for approximately 6 weeks after the onset, and remained steady at 8% below expected for the remaining period for which data were available. During the 7-week period, the LPAI-associated egg loss was 17.18% (range = 3.13–28.49%). The maximum drop in egg production (35.66%; range = 22.45–60.95%) occurred 4 days (range = 2–7 days) after the LPAI-associated mortality peak.

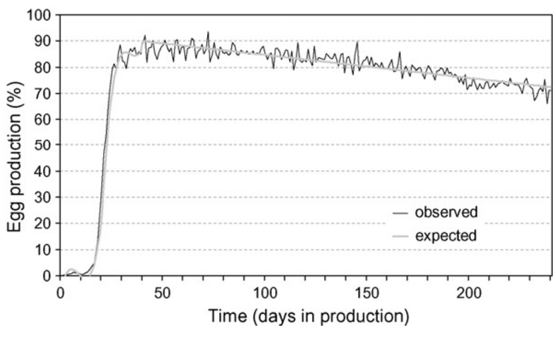

Fig. 4. Mean and predicted daily post-molt percent egg production in a commercial layer flock. The mean is based on data obtained from 44 healthy commercial layer flocks housed between May 2002 and December 2004 in California.

3.3. Early warning system (EWS)

Results assuming different alert levels for EWS1 and EWS2 are presented in Figs. 6 and 7. Only alert levels that resulted in no missed outbreaks were selected (with the exception of ESW1 mortality alert levels 3.5 and 3.25 that missed 3.7% of outbreaks). As expected, stricter alert levels resulted in more false alerts and shorter detection delays.

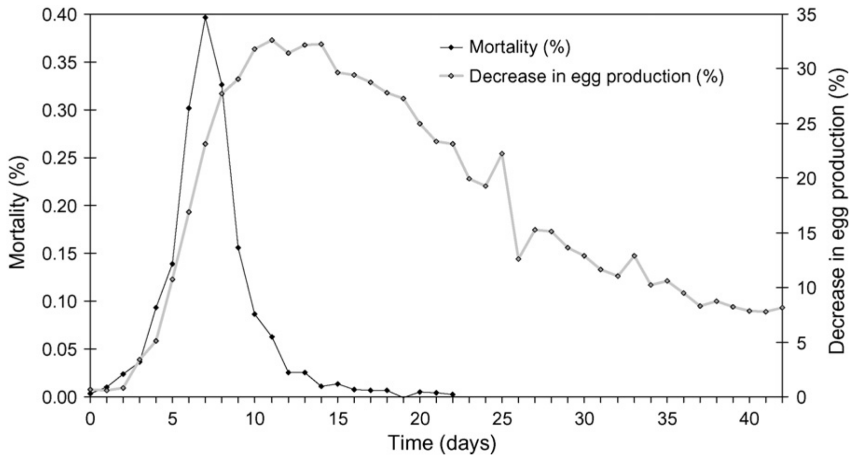

Fig. 5. Mean changes in daily percent mortality and percent egg production attributed to a H6N2 low pathogenic avian influenza (LPAI) outbreak. The mean values are based on data obtained from 27 commercial layer flocks that experienced H6N2 LPAI outbreaks in January and February 2002 in California.

The EWSs based on increased mortality were preferred over those based on decreased egg production. For EWS1, when using egg production-based alert levels, the percentage of false alerts and the detection delay were consistently at least 70% higher and 4 days longer, respectively, than for mortality-based alert levels (Fig. 6). For EWS2, when mortality and egg production alert levels that resulted in similar percentages of false alerts were compared, the detection delay was consistently approximately 4 days longer when using egg production-based alert levels (Fig. 7). Based on these findings, there was no benefit in using egg production vs. mortality as a trigger of the EWS.

For the mortality-based system, increasing the EWS1 alert level from 2.25 to 2.75 times the baseline mortality resulted in a substantial decrease in the number of false alerts from 29.3% to 2.4%, and a minor increase in detection delay from 6.0 to 7.4 days (Fig. 6). Similarly, increasing the EWS2 alert level from 1.75 to 1.85 times the baseline mortality resulted in a decrease in the number of false alerts from 8.5% to 3.7%, and an increased detection delay from 7.6 to 7.8 days (Fig. 7). Further increases in alert levels resulted in minor decreases in false alerts and minor increases in detection delays in both EWS1 and EWS2 (Figs. 6 and 7).

Combining EWS1 and EWS2, i.e. assuming an alert occurred when the trigger was exceeded in either system, as expected, resulted in reduced detection delays and increased false alerts compared with using either system individually (Table 2). Exceptions were when the alert levels for the combined EWSs were high: 2.15 and 3.50 for EWS2 and EWS1, respectively, which produced the same level of false alerts as when the EWSs were used on their own. In addition, no outbreaks were missed for any of the EWS1 and EWS2 combinations.

When comparing the performance of this study's EWSs with the three adapted (fixed) EWSs used/recommended in the Netherlands, results for the latter were worse for the three evaluated criteria as shown in Table 3. Particularly, the detection delay associated with the Dutch system

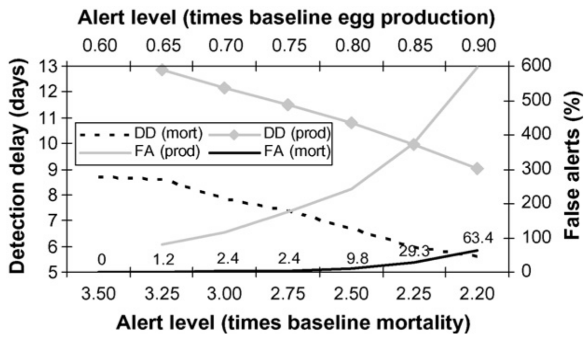

Fig. 6. Performance results of an early warning system for H6N2 low pathogenic avian influenza (LPAI) outbreaks in commercial layer flocks for an early warning system (EWS1) based on changes in egg production/mortality for 1 day. Parameters measured are detection delay (DD) (days) and false alerts (FAs) (%). Only alert levels that resulted in no missed outbreaks (with the exception of mortality alert levels 3.5 and 3.25 that missed 3.7% of outbreaks) are represented. The egg production alert level 0.6 is not represented, because it missed 40.7% of outbreaks.

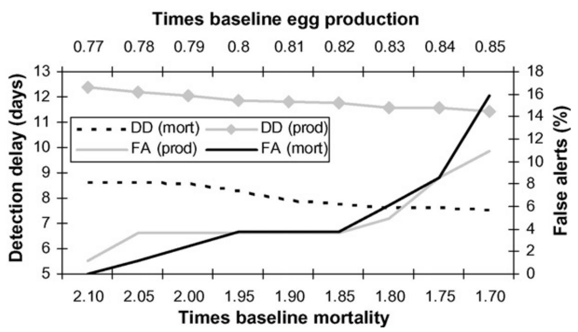

Fig. 7. Performance results of an early warning system for H6N2 low pathogenic avian influenza (LPAI) outbreaks in commercial layer flocks for an early warning system (EWS2) based on changes in egg production/mortality for each of 2 consecutive days. Parameters measured are detection delay (DD) (days) and false alerts (FAs) (%). Only alert levels that resulted in no missed outbreaks are represented.

Table 2
Performance results of an early warning system (EWS) for H6N2 low pathogenic avian influenza (LPAI) outbreaks in commercial layer flocks based on increased mortality for 1 day (EWS1), each of 2 consecutive days (EWS2), and the combination of EWS1 and EWS2. Parameters measured are detection delay (DD) (days), false alerts (FAs) (%), and outbreaks missed (%).

		EWS 1 (times baseline mortality)								EWS2 alone	
		2.75		3.00		3.25		3.50			
		DD (days)	FAs (%)	DD (days)	FAs (%)	DD (days)	FAs (%)	DD (days)	FAs (%)	DD (days)	FAs (%)
EWS 2 (times baseline mortality)	1.75	6.9	11.0	7.0	11.0	7.3	9.8	7.4	8.5	7.6	8.5
	1.80	6.9	8.5	7.0	8.5	7.3	7.3	7.4	6.1	7.6	6.1
	1.85	6.9	6.1	7.0	6.1	7.3	4.9	7.4	3.7	7.8	3.7
	1.90	6.9	6.1	7.0	6.1	7.3	4.9	7.4	3.7	7.9	3.7
	1.95	7.0	6.1	7.2	6.1	7.7	4.9	7.7	3.7	8.3	3.7
	2.00	7.2	4.9	7.4	4.9	7.9	3.7	7.9	2.4	8.6	2.4
	2.05	7.2	3.7	7.4	3.7	7.9	2.4	8.0	1.2	8.6	1.2
	2.15	7.2	2.4	7.4	2.4	7.9	1.2	8.0	0.0	8.7	0.0
EWS 1 alone		7.4	2.4	7.9	2.4	8.6[a]	1.2	8.7[a]	0.0		

[a] All 27 simulated outbreaks were detected except in the case of when EWS1 alone was used (for 3.25 and 3.50 times the baseline mortality, EWS1 failed to detect 1 outbreak = 3.7% of LPAI outbreaks missed); EWS1: an alert threshold when the observed daily mortality exceeded more than *x* times the expected mortality; EWS2: an alert threshold, which has to be exceeded during 2 consecutive days before an alert occurs. This analysis is based on data obtained from two commercial farms in California.

always exceeded 8 days, consistently higher than the EWS alternatives presented in this study.

4. Discussion

This study demonstrates that non-notifiable LPAIVs can cause considerable losses to the poultry industry: associated mortalities of nearly 2% in just over 2 weeks and egg losses close to 20%. The egg production loss may have been much higher if data after the seventh week had been available. After the sixth week, egg production stabilized at 8% below expected, indicating that depressed production may continue for the remaining of the production cycle. This may be explained by the severe reproductive disorders described by Kinde et al. (2003) in H6N2 LPAI-infected flocks, from which some hens may never recover.

Table 3
Performance results of three early warning systems (EWS) with fixed alert levels to detect H6N2 low pathogenic avian influenza (LPAI) outbreaks in commercial layer flocks. These are EWSs used/recommended in the Netherlands originally intended to detect highly pathogenic avian influenza outbreaks that have been adapted to detect LPAI outbreaks. Parameters measured are detection delay (DD) (days), false alerts (FAs) (%), and outbreaks missed (%).

Mort (%)	DD	FAs (%)	Outbreaks missed (%)
The Netherlands EWS during the outbreak in Italy (2000): >0.5% mort per day			
>0.100	8.3	26.8	0
>0.113	8.6	8.5	3.7
>0.125	8.8	3.7	3.7
>0.150	9.4	1.2	3.7
>0.200	10.3	1.2	7.4
>0.500	10.5	1.2	63
The Netherlands EWS during the outbreak (2003-05): >3% mort per week			
>0.60	10.0	23.2	3.7
>0.65	10.5	1.2	3.7
>0.70	10.7	0	3.7
>0.75	10.6	0	3.7
>1.00	11.9	0	18.5
>2.00	11.6	0	70.4
>3%	13.7	0	88.9
The Netherlands recommended EWS after the outbreak (2007): >0.25% mort for 2 days			
>0.090	9.4	12.2	0
>0.095	9.7	4.9	0
>0.100	9.9	1.2	0
>0.125	10.3	0	3.7
>0.150	10.6	0	7.4
>0.200	11.4	0	29.6
>0.250	11.7	0	40.7

Mort: mortality; FA: false alert; DD: detection delay. Actual notification values used/recommended appear in gray (Elbers et al., 2007).

The proposed EWS differs from previously used EWSs in that the latter are based on trigger values that are not adjusted to the individual flock, but fixed. While a fixed approach may be valid for very pathogenic diseases, such as HPAI, it will result in a lower detection probability for less pathogenic diseases, such LPAI. An alert level (such as the ones used/recommended in the Netherlands) that is fixed for all commercial egg production flocks in the country does not take into account that flocks with poorer standards will systematically trigger false alerts. Similarly, management factors, strain of bird used, whether the flock is pre- or post-molt or season of the year are also ignored. However, all these factors will affect both mortality and egg production. The advantage of the EWS described in this study is that it can incorporate these confounders, by using observed values for each monitored flock. The proposed EWS allows setting up tighter and, therefore, much more sensitive and realistic trigger values, resulting in consistently favorable results for the three criteria examined (detection delay, percentage of false alerts and percentage of outbreaks missed), when compared to fixed EWS.

The chosen EWS scenario and alert levels will depend on the situation: what is a satisfactory detection delay, the number of false alerts that can be economically justified, or whether it is acceptable to miss an outbreak. This is an additional advantage compared with a fixed EWS, since it allows adjusting the alert levels to a changing situation. For example, at times when the risk of infection may be higher, a farmer/poultry association may want to set stricter alert levels to increase the sensitivity of the system, e.g. during an epidemic in the region, during waterfowl migration or during breeding seasons (Halvorson, 1984). Alert levels could also be stricter for situations when an AI outbreak would imply higher losses, such as in multi-age premises, for valuable birds (breeders and layers vs. broilers), or in areas with very high poultry density, where control of the disease would be difficult. Other factors to be considered include existing biosecurity level, structure of the affected poultry industry, size of flocks and farms, existing legal/regulatory framework, past experiences with disease outbreaks, egg prices, AIV strain, whether compensation programs are present, and outbreak costs (culling, disposal or vaccination). Fewer outbreaks missed and shorter detection delays are inversely related to the number of false alerts, which cost money in the form of laboratory diagnosis expenditures. A cost-benefit analysis should be performed before setting alert levels. It is key to know (1) the cost of an outbreak and how much of that cost could be saved by detecting the outbreak earlier, thus reducing the spread within and among farms; and (2) the cost of sampling, shipping and laboratory testing, which will depend on the number of samples per flock, the pathogens tested, the types of tests used, and whether testing is paid (or subsidized) by the government or by the industry.

It was concluded that the monitoring of egg production was not appropriate for an EWS, at least for this particular scenario, because it was always triggered after mortality (see Fig. 5), while not decreasing (and sometimes actually increasing) the number of false alerts. However, it could still be used as a confirmation of a disease process in the flock. In fact, LPAI signs may differ by strain and, in some cases, decreases in egg production may precede mortality changes (Elbers et al., 2004). Since we do not always know which LPAIV strain will enter the farm next, keeping egg production as a supplemental trigger parameter may help in the early detection of other LPAIVs. The fact that signs of LPAI are non-specific and therefore similar to those of other important poultry diseases, means that this EWS will have an additional externality benefit in that it will also improve the early detection and control of other important poultry diseases such as Newcastle disease (ND), infectious bronchitis (IB), mycoplasmosis, infectious laryngotracheitis (ILT), metapneumovirus infections and infectious coryza. In the event of an alert, samples will have to be tested for other pathogens. It would be useful to test this EWS against daily data from flocks affected by other LPAIVs and other avian diseases. Moreover, this EWS could also be easily adapted to other production settings, e.g. broilers or breeders, or species, e.g. turkeys, ducks, or quails. The EWS could be further improved by adding other usual daily parameters that are usually recorded at farm level, such as egg size and egg quality, water and feed intake, or looking for clinical signs.

Applying this EWS on commercial egg production farms should present minimal additional work. Baseline mortality and production estimates required for the EWS would be based on historic data collected for the specific premises. Many of the already existing programs for data entry, record keeping and analysis of production and mortality data already utilize spreadsheets for data entry. Therefore, once expected mortality and egg production patterns were estimated, adding this EWS application would require no extra input from the farmer other than the daily data entry, which is already done at most commercial and semi-commercial farms. In addition, trigger values are easily changed in an input cell, so the system could be tailored to each farming environment. Trigger values could be dynamic, and quickly modified throughout one production cycle according to a changing situation.

In conclusion, we believe results presented here demonstrate how a custom-tailored EWS could be easily adapted by the poultry industry to detect LPAI and other diseases of low pathogenicity.

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8 General discussion

8.1. Main animal disease threats in 2010: pathogen types, drivers and challenges

EMPRES Transboundary Animal Diseases Bulletin 2011

Infectious animal diseases can have a major impact on public health (zoonoses), national economies (high-impact diseases), household livelihoods (enzootic diseases) and, in very serious cases, global societal stability and security (pandemics, bioterrorism...). Disease emergence is triggered by multiple, interrelated factors: human and animal demographics, climate change, increased mobility and globalization, urbanization, land degradation, drug resistance, and mass animal rearing. There are three main pathways for emerging disease pathogens to adjust host exploitation: pathogens as invaders into new territories; pathogens performing virulence jumps; and pathogens performing species jumps.

Because of this complexity, a new approach is needed for disease prevention and control. Current approaches to animal disease prevention and control are based on the disruption of disease transmission. While these have proved effective in both short- and long-term disease control programmes, they have been less successful in some instances, as shown by the current persistence of H5N1 HPAI, despite significant national and international efforts. This is because most current approaches apply strong veterinary science and medicine disciplines in isolation from other relevant disciplines, without confronting the root causes of disease emergence at the animal-human-environment interface. Veterinary services need to expand into an agro-ecological approach. Also the international community is increasingly converging on such a multi-sectoral, multidisciplinary approach to addressing the increasing disease threats. This approach, termed “One World, One Health”, outlines a collaborative, international, cross-sectoral, multidisciplinary mode of addressing threats and reducing risks of infectious diseases at the animal-human-ecosystem interface, including the wildlife component.

The major disease threats are then analysed by geographical region (i.e. continent). One of the most worrying and expanding threats is avian influenza, particularly in south east and East Asia. H5N1 HPAI perhaps best illustrates the complexity of the factors involved in the local, national, regional and even global spread of a newly emerged animal pathogen. H5N1 HPAI has demonstrated what happens when a new virus enters a new host population (chickens) from where it can jump to further species (human infections, illustrating how the virulence of an agent can vary), and what happens when a new virus can spread across very large distances to new susceptible populations.

8.2. Global trends in infectious diseases at the wildlife-livestock interface

PNAS 2015

The role and significance of wildlife–livestock interfaces in disease ecology has largely been neglected, despite recent interest in animals as origins of emerging diseases in humans. However, no studies have sought to characterize the diseases and animals involved on a global level. An extensive literature search combining wildlife, livestock, disease, and geographical search terms yielded 78,861 publications, of which 15,998 were included in the analysis, providing the most comprehensive overview of research on infectious diseases at the wildlife–livestock interface to date. Publications dated from 1912 to 2013 and showed a continuous increasing trend, including a shift from parasitic to viral diseases over time. Ten diseases, mostly zoonoses, have accounted for half of the published research in this area over the past century. Relatively few interfaces can be considered important from a disease ecology perspective. These findings suggest that surveillance and research strategies that target specific wildlife–livestock interfaces may yield the greatest return in investment.

The bird–poultry interface was the most frequently cited wildlife–livestock interface worldwide, ranking first in Asia, Europe, and North America and second in Oceania, Africa, and South America. Of all publications citing a bird–poultry interface, avian influenza (AI) constituted the 22%. The magnitude of this result highlights the importance of the wildlife poultry interface for AI. However, time-series analysis revealed that the number of publications on AI was highly positively correlated with media coverage and research funding, highlighting the influence of specific disease events and sociopolitical–economic drivers of research in this area. Just because a certain wildlife–livestock interface is prominently reported in the scientific literature does not necessarily mean that actual transmission is frequently occurring at this interface. Avian influenza is an example where transmission at the wildlife–livestock interface is often implied, but a functional interface is seldom documented and proven. In fact, global spread of H5N1 was facilitated by poultry movement and trade without any proximal role of wild birds in some countries.

8.3. Avian flu school: A training approach to prepare for H5N1 highly pathogenic avian influenza

Public Health Reports 2008

Since the reemergence of H5N1 HPAI in 2003, a panzootic that is historically unprecedented in the number of infected flocks, geographic spread, and economic consequences for agriculture has developed. The epidemic has affected a wide range of birds and mammals, including humans. The ineffective management of outbreaks, mainly due to a lack of knowledge among those involved in detection, prevention, and response, points to the need for training on H5N1 HPAI. The main challenges are the multidisciplinary approach required, the lack of experts, the need to train at all levels, and the diversity of outbreak scenarios.

Avian Flu School aimed to address these challenges through a three-level train-the-trainer program intended to minimize the health and economic impacts of H5N1 HPAI by improving a community's ability to prevent and respond, while protecting themselves and others. The course teaches need-to-know facts using highly flexible, interactive, and relevant materials.

8.4. Integrating surveillance and biosecurity activities to achieve efficiencies in national avian influenza programs

Preventive Veterinary Medicine 2011

Implementing HPAI prevention, surveillance and response national programs is a very expensive endeavour, typically far exceeding a developing country's budgeted funds and thus requiring substantial donor support. Costs could be significantly reduced if some of the tasks within the various components were combined, particularly in the implementation of field activities.

One of the most obvious synergies is between surveillance and biosecurity improvement activities, because: 1) Biosecurity and surveillance professionals must engage and train the same groups such as poultry keepers, live bird market managers, and poultry service providers; 2) Professionals implementing surveillance and biosecurity programs would benefit from cross training; 3) Biosecurity and surveillance professionals have overlapping tasks; and 4) Assessing biosecurity measures and gaps at surveillance sites provides key information for focusing/targeting surveillance.

The H5N1 HPAI panzootic has highlighted the need to build capacity for disease prevention, detection and response preparedness. These efforts have encountered the enormous expenses of training, communication, personnel and equipment.

Cross-training among professional field staff, linking activities that require similar knowledge and engage the same groups, and integrating surveillance, biosecurity and other field activities will build more efficient disease prevention and surveillance programs.

8.5. A flock-tailored early warning system for low pathogenic avian influenza (LPAI) in commercial egg laying flocks

Preventive Veterinary Medicine 2009

The aim of this study was to develop and evaluate an early warning system (EWS) for commercial egg laying flocks to detect the subtle mortality and egg production changes that characterize LPAI infections. An EWS will create an alert when the recommended trigger point is reached or exceeded. Previously used EWSs are based on fixed alert levels, while the proposed EWS customizes the alert level to each flock. While a fixed approach may be valid for highly pathogenic diseases, it results in a lower detection probability for low pathogenic diseases.

The EWS was based on daily data collected from flocks affected by the 2000–2004 H6N2 LPAI epidemic in California. Three EWSs were evaluated: (1) EWS1 is triggered when the observed mortality increase or production decrease exceeds more than X times the expected daily value, (2) EWS2 is triggered when the observed mortality increase or production decrease exceeds more than Y times during each of 2 consecutive days the expected daily values, and (3) a combination of the two. The EWSs were evaluated according to three parameters: detection delay (days) of a LPAI outbreak, false alerts (%) and outbreaks missed (%).

Results showed that an egg production-based EWS added no benefit to a mortality-based system, mainly because H6N2 LPAI-related egg production decrease always occurred after increase in mortality. Combining the two EWSs resulted in a reduced detection delay and no missed outbreaks, but at the expense of a slight increase in the number of false alerts triggered. The system presented in this study also outperformed fixed EWSs in all three evaluated parameters. The proposed EWS, if used as part of a poultry cooperative program and combined with a rapid laboratory diagnosis, could be a useful tool in the detection and control of LPAI outbreaks and other poultry diseases. Built in a spreadsheet, the system could be inexpensively, easily and quickly incorporated into a commercial egg production farm decision support system. In addition, the proposed system could be quickly adjusted to changing epidemic situations, and easily customized to individual flocks.

9 Conclusions / Conclusiones

Conclusions

Based on the results and the conditions of the present study the following conclusions are drawn:

FIRST: Avian influenza spread, which is mostly human-driven, is one the biggest threats to animal health, not just because of the important damages to poultry production, local livelihoods and trade, but also because of the public health implications.

SECOND: The wild bird-poultry interface is the most researched of all wildlife livestock interfaces. However, although the interface exists, the role of wild birds in Avian Influenza outbreaks in poultry has been exaggerated, since they are mostly human driven, i.e. due to movements of poultry, poultry products and fomites.

THIRD: For an effective surveillance, prevention and control of H5N1 HPAI, it is critical that all responders (i.e. farmers and others along the poultry chain, veterinary services and public health services) are effectively trained in multidisciplinary approach. In order to reach stakeholders at all levels, a train-the-trainer approach is the most sustainable and effective.

FORTH: Implementing HPAI prevention, surveillance and response national programs is a very expensive endeavour. Costs can be significantly reduced if some of the tasks are combined, particularly the implementation of field activities like surveillance and biosecurity improvement.

FIFTH: Syndromic surveillance base on monitoring production parameters (e.g. mortality or egg production) can be the most cost-effective way to detect the subtle changes that characterize LPAI infections, particularly in commercial farms.

Conclusiones

En base a las condiciones del presente estudio y a los resultados obtenidos, se ha llegado a las siguientes conclusiones:

PRIMERA: La diseminación de la Influenza Aviar, que normalmente es diseminada por humanos, es una de las mayores amenazas a la sanidad animal, no sólo por los importantes perjuicios en la producción de aves de corral, las condiciones de vida de los productores y el comercio, sino también por las implicaciones que tiene en la Salud Pública.

SEGUNDA: La interacción entre aves silvestres y aves de corral es la más investigada de todas las interacciones de la fauna silvestre y las producciones animales. Sin embargo, a pesar de que esta interacción existe, el papel de las aves silvestres en los brotes de Influenza Aviar en naves de corral ha sido excesiva, ya que en la mayoría han sido transmitidas por los humanos, fundamentalmente debido a movimientos de aves, sus productos y fomites.

TERCERA: Para una vigilancia epidemiológica, prevención y control efectivos de las cepas altamente patógenas de Influenza Aviar (HPAI) H5N1, es fundamental que todos los implicados (granjeros y otros implicados en la cadena productiva, servicios veterinarios y servicios de salud pública) estén entrenados debidamente con un enfoque multidisciplinar. Con el fin de alcanzar a los participantes de todos los niveles, un enfoque de formar-al-formador es la más sostenible y efectiva.

CUARTA: La implementación de programas nacionales de prevención, vigilancia epidemiológica y control de las cepas altamente patógenas de Influenza Aviar (HPAI) supone un importante esfuerzo económico. Los costes pueden reducirse significativamente si alguna de las tareas se combinan, particularmente la implementación de actividades de campo como la vigilancia epidemiológica y mejora de la bioseguridad.

QUINTA: La vigilancia sindrómica basada en la monitorización de parámetros productivos (mortalidad o producción de huevos) puede ser la forma más económica para detectar los cambios sútiles que caracterizan las infecciones por cepas de baja patogenicidad de Influenza Aviar (LPAI), en especial en explotaciones comerciales.

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11 Apéndices

11.1. Características de las revistas

En el presente apéndice se indican el factor de impacto (JIF) y las áreas temáticas correspondientes a las revistas donde se han publicado los trabajos incluidos en la presente Tesis Doctoral. Todos los valores se han obtenido del Journal Citation Reports® disponible en ISI Web of Knowledge.

En cada una de las áreas temáticas señaladas se indica el cuartil y entre paréntesis la posición de la revista indicada sobre el total de revistas incluidas en el área de estudio.

Revista	Public Health Reports		
JIF	1.299	**Año**	2008
Áreas temáticas	Public, Environmental & Occupational Health: Q3 (70 /105)		

Revista	Preventive Veterinary Medicine		
JIF	2.121	**Año**	2009
Áreas temáticas	Veterinary Sciences: Q1 (10 / 142)		

Revista	EMPRES Transboundary Animal Diseases Bulletin		
JIF	-	**Año**	2011
Publicada por	FAO Animal Production and Health Division		

Revista	Preventive Veterinary Medicine		
JIF	2.046	**Año**	2011
Áreas temáticas	Veterinary Sciences: Q1 (11 / 145)		

Revista	Proceedings of the National Academy of Sciences of the United States of America (PNAS)		
JIF	9.674	**Año**	2014*
Áreas temáticas	Mutidisciplinary Sciences: Q1 (4 / 56)		

11.2. Contribución del doctorando y renuncia de coautores no Doctores

El doctorando es el primer o segundo autor de todos los trabajos presentados en esta Tesis Doctoral, lo que justifica plenamente su contribución. Además, debemos indicar que todos los coautores son doctores.