Avian influenza

H5N1 highly pathogenic avian influenza outbreak dynamics and drivers in Indonesian poultry, 2008 to 2010

Background
H5N1 highly pathogenic avian influenza (HPAI) was first reported in poultry in Indonesia in 2004 and outbreaks have subsequently continued to affect domestic poultry populations and humans in many parts of the country. By early August 2011, 178 human cases had been reported, of which 146 were fatal. In early 2008, the Indonesian Government implemented a revised participatory disease surveillance response (PDSR) programme to allow rapid identification and response to H5N1 HPAI outbreaks. Through this programme, data were collected from a combination of random and targeted active surveillance, passive surveillance based on events reported to the government, and follow-up visits to villages where a potential or actual outbreak had previously occurred. During visits, villages were assigned one of five HPAI status indicators: infected, suspect(14), suspect(60), controlled, or apparently free. Infected status was assigned to villages where an HPAI-compatible event had occurred within the previous 60 days and an Anigen® rapid test at the time of the visit was positive. A village was defined as suspect(14) or suspect(60) when there were no positive rapid tests at the time of the visit although an HPAI-compatible event had occurred within the previous 14 or 60 days, respectively. A village received controlled status if follow-up investigations found no HPAI-compatible events for 14 days after implementation of control measures. Apparently free villages were those where no HPAI-compatible event was present at the time of the visit and none had been reported over the previous 60 days.

Data collected by the PDSR programme between April 2008 and September 2010 for Java, Bali and Lampung Province of Sumatra (Figure 1) provided the opportunity to examine the spatial-temporal dynamics of outbreaks in Indonesia, using ecological approaches to identify potential drivers of outbreak maintenance and spread. Although the PDSR data were gathered at the village level, the analysis was conducted at the district level for nine 90-day rolling periods, with data for the five HPAI outbreak status indicators being summarized at the district level. During each 90-day period, if an outbreak was recorded in a village anywhere within a district, it was defined as the presence of HPAI in that district for that period. For the other outbreak status indicators, the number of each reported outcome type was counted for each district and time period and the results were used as covariates (not outcome variables). These covariates included the numbers of villages reported controlled, suspect(14) and disease-free in each district for each 90-day period.

The study set out to determine: i) the probability of a district becoming infected after a period of freedom (90 days), referred to as the “colonization probability”;1

1 Colonization probability refers to the probability of HPAI infection in a district where there was no infection in the previous 90-day period. It could be interpreted as being the probability of new outbreaks in a district.
ii) the probability of an outbreak persisting in a district, referred to as the “persistence probability”;2 iii) how a district’s HPAI status in a previous period influenced the occurrence of outbreaks (colonization and persistence) in that district; and iv) the effect of risk factors such as human and poultry population densities on the probability of outbreaks (colonization and persistence) at a district.

**Spatial and temporal patterns of outbreaks**

The data available suggested that there were strong temporal and spatial differences in outbreak probabilities across the areas examined. For all districts in the study area, the average outbreak probability over time followed a distinct seasonal pattern (Figure 2). The probabilities of outbreaks increased during the early months of the year (January to March) and declined for the July to September and October to December periods, in both 2009 and 2010. This seasonality in H5N1 HPAI outbreak dynamics has been reported previously for several Asian countries. The risk factors for seasonality were shown to differ among locations.

Over the 30-month study period, the average outbreak probabilities by district (Figure 3) ranged from 0.17 to 0.60 in Bali and East Java Provinces, with a gradient of increased outbreaks towards the west. In Central Java and Yogyakarta Provinces outbreak probabilities were noticeably higher, usually ranging from 0.50 to 0.92. Western districts of Java had moderate outbreak probabilities, which appeared to be more heterogeneous than in the rest of Java, ranging from less than 0.10 to more than 0.80. All districts in Lampung Province of Sumatra showed outbreak probabilities ranging from 0.17 to 0.60.

---

2 Persistence probability refers to the probability of HPAI infection being maintained in a district from the previous to the current 90-day period. It could be interpreted as being the probability of HPAI maintenance.
ties of more than 0.70, with several exceeding 0.80, suggesting that levels of virus circulation were nearly constantly high in these areas, mainly because of the movement of poultry and products through poultry market chains.

**Determinants of disease outbreaks**

The analysis showed that the occurrence of outbreaks in each study district was affected by poultry and human densities and the number of villages in the district assigned to controlled status during a previous period. The occurrence of new outbreaks

---

**Figure 2:** Outbreak probabilities for 90-day periods, averaged across all districts in the study area, July 2008 to September 2010

**Figure 3:** Spatial distribution of outbreak probabilities in districts across the study area, averaged across 90-day periods

(colonization probability) was higher in districts with relatively low poultry densities, while outbreak persistence was favoured by high poultry densities. In addition, the occurrence of new outbreaks dropped as poultry density increased, while persistence probability remained relatively high at all but the highest densities. The observed relationship between poultry density and new outbreaks (colonization probability) suggests that districts with relatively low poultry densities that had not reported outbreaks in a 90-day period were susceptible to new outbreak incursions. The drop in colonization probability at higher poultry densities possibly indicates that as poultry density increases, the virus is more likely to persist year-round, rather than to colonize seasonally.

The relationship between outbreak persistence and poultry density was non-linear; however the poultry density at which disease persistence was highest was almost double the density that maximized new outbreak probability. This pattern suggests that high probability of incursion of H5N1 HPAI into a previously unaffected district can occur at relatively low poultry densities within a district; however, a much higher poultry density is required to sustain a high persistence probability across a 90-day period. This suggests that at lower densities there are too few susceptible hosts to maintain the transmission chain between 90-day periods, and that control efforts are more likely to be successful where poultry densities are lower. The study also shows that virus survival in the environment appears to be an important factor in the epidemiology of the disease in the study area. Taken together, the patterns of colonization and persistence of H5N1 HPAI in relation to poultry density support the view that H5N1 is a seasonal disease in Indonesia, with year-round persistence more likely in areas with sufficient poultry density to support continuously a basic reproductive number greater than one, regardless of the time of year.

The occurrence of outbreaks was affected by poultry and human densities and the number of villages in the district assigned to controlled status during a previous period.

The effect of human density on outbreak colonization probability was linear, meaning that as density increased the chance of new outbreaks within any 90-day period increased. This may reflect greater movement of live poultry between market networks and local markets within a given area, through facilities such as live-bird markets and collector yards. New outbreaks were maximized at moderate human density (6 250/km²) and relatively low poultry density (fewer than 2 000/km²). Although this is a relatively low human density compared with districts with large urban populations, such as Jakarta (14 493/km²), it is higher than the density in most of the rural districts considered.

In a district, the more villages assigned to controlled status in a previous period the greater the probability of outbreak persistence (i.e., the continuous presence of outbreaks over time). This indicated that districts that experience large numbers of outbreaks, and hence have large numbers of villages with controlled status reported in the surveillance database, continue to experience relatively large numbers of outbreaks into the future. Such districts are likely foci of the endemicity of HPAI in Indonesia, and so should be targeted for enhanced disease control activities. PDSR activities do not include surveillance in the commercial sector, so it is not possible to give an indication of the impact of disease on this part of the poultry population; only village poultry are under surveillance.
Conclusions

In general, H5N1 HPAI outbreaks reported in poultry in Java, Bali and Lampung Province of Sumatra between 2008 and 2010 demonstrated marked seasonality, with increased outbreaks during the early months of the year (January to March). The spatial distribution of outbreaks varied across the study area, with highest probabilities in Lampung, Central Java and Yogyakarta Provinces, implying endemic disease in these areas. Districts with relatively low poultry densities were likely to have new outbreak incursions after a period of freedom, while those with high poultry densities were likely to have continuous outbreaks over time. An increase in the number of villages reported with controlled status in a previous period increased the possibility of repeated outbreaks.

Acknowledgements

Much of this report was extracted from the original publication by Farnsworth et al. (2011), with some important differences, including an attempt to simplify the technical language, to highlight the practical implications of analysis presented by Farnsworth et al. This report demonstrates the value of the disease data collection and analysis that long-term surveillance of HPAI in Indonesian village poultry makes possible. The findings will support the development of technical approaches to refining surveillance and disease control, and provide policy-makers with insights into the mechanisms of HPAI persistence in poultry in Indonesia.

Bibliography


Contributors: Caryl Lockhart (FAO), Julio Pinto (FAO)

Eastern Africa selects a regional laboratory for highly pathogenic avian influenza and Newcastle disease

The Eastern Africa Regional Laboratory Network (EARLN) for Highly Pathogenic Avian Influenza and other Transboundary Animal Diseases was launched in June 2008 at the FAO regional workshop in Debre Zeit, Ethiopia. Its members are national veterinary laboratories of 12 eastern African countries: Burundi, the Democratic Republic of the Congo, Djibouti, Eritrea, Ethiopia, Kenya, South Sudan, Rwanda, Somalia, the Sudan, the United Republic of Tanzania and Uganda. The overall technical and operational capacity of each national laboratory member of EARLN was reviewed during the regional workshop, along with their specific abilities and capacities to carry out highly pathogenic avian influenza (HPAI) diagnosis and differential diagnosis. Workshop participants also determined the need to designate a regional laboratory for avian influenza (AI) and Newcastle disease (ND), and the minimum requirements for such a laboratory. Subsequent network meetings held in Kigali, Rwanda (July 2009) and Dar es Salam, United Republic of Tanzania (July 2010) provided additional opportunities to
discuss and define the specific roles and modalities for selecting the regional laboratory, and it was concluded that a clear picture of the technical and operational level of each laboratory member of EARLN was required, through detailed assessments of each national laboratory facility.

The Food and Agriculture Organization of the United Nations (FAO) requested the World Organisation for Animal Health (OIE)/FAO Reference Laboratory for Avian Influenza and Newcastle Disease at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Padova, Italy to carry out a series of independent assessments of central veterinary laboratories (CVLs) in the region. These assessments took place between June 2008 and August 2010, and their findings were presented and discussed during a meeting of East African chief veterinary officers (CVOs) and heads of CVLs, organized in Zanzibar, United Republic of Tanzania from 24 to 26 August 2010 by the Emergency Centre for Transboundary Animal Disease Operations (ECTAD) Unit for Eastern Africa. The meeting was attended by the CVOs of ten countries and representatives of OIE, the African Union Inter-Union Bureau for Animal Resources (AU-IBAR), IZSVe, the Southern African Centre for Infectious Disease Surveillance (SACIDS), the United States Agency for International Development’s Emergency Pandemic Threats (USAID/EPT) RESPOND programme and the FAO regional office for Africa (Accra, Ghana).

Based on the assessment findings, criteria for selecting the regional laboratory for eastern Africa were agreed. These are outlined in the box on the next page. Based on these criteria, the CVOs at the meeting short-listed the CVLs of Ethiopia, Kenya, the Sudan and the United Republic of Tanzania as candidates. The CVOs of these countries were then asked to confirm their commitment to hosting the regional laboratory by formally submitting applications to ECTAD in Nairobi (Kenya). These applications had to provide evidence that the CVL could serve as the regional laboratory. An EARLN interim secretariat (IS) consisting of FAO-ECTAD, AU-IBAR, OIE, two representatives from EARLN Member States (one for livestock and the other for wildlife), and the regional economic communities – the East African Community (EAC) and the Intergovernmental Authority on Development (IGAD) – was mandated to deliberate on the applications and review the dossiers submitted. The CVOs agreed on the following terms of reference or roles for the regional laboratory:

- assist in building the capacity of other laboratories in the region by providing training on AI/ND diagnostic techniques and organizing technical and coordination meetings;
- assist in the procurement (or production) and/or maintenance of stocks of AV/ND reagents, such as reference antigens and antisera, for emergency release to the region;
- contribute to the preparation, harmonization or review of technical reference documents, such as manuals and standard operating procedures, for use within the region;
- receive samples for AI diagnosis, perform required tests and report the results in a timely manner;
1. A statement of the government’s commitment to supporting the role and responsibilities of the regional laboratory, issued by a high-level government member.

2. A strategic location within the region, making the laboratory easily accessible to eastern African countries (for the sending of samples, communications, scientific visits, training, etc.).

3. An organizational set-up that includes:
   - institutional and technical management arrangements;
   - commitment to and effective implementation of a quality management system, following the technical and management requirements set out in International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025: 2005 standard guidelines with the ultimate objective of eventual accreditation for AI diagnostics;
   - willingness to provide AI confirmatory services to other countries;
   - willingness, experience and means for submitting infectious agents to OIE/FAO reference laboratories;
   - evidence of activities and training capabilities for AI/ND and other transboundary animal disease (TAD) diagnostic procedures;
   - experienced and qualified personnel capable of undertaking AI virology and molecular diagnostics;
   - adequate functional equipment;
   - reliable electricity supply and water services, with back-up;
   - functional incinerators;
   - a proper waste disposal system;
   - laboratory conditions of at least biosafety level (BSL) 2, with plans to improve to at least BSL2+;
   - animal housing facilities;
   - sufficient laboratory rooms dedicated to AI/ND diagnosis;
   - good local and international networking with laboratories, research institutes and universities;
   - good level of funding – from government, own funds or potential funding agencies;
   - participation in inter-laboratory proficiency testing for AI/ND;
   - experience of international collaboration for receiving and submitting samples and providing training in AI/ND diagnosis;
   - experience of hosting trainees from other countries or receiving and processing samples from other countries;
   - capability or potential ability to produce diagnostic reagents for AI/ND (e.g., facilities for research and development, and demonstrated experience in this field);
   - experience of handling AI virus (e.g., numbers of samples handled and tests conducted over the past three years);
   - capability of maintaining a repository of animal pathogens.

Following the Zanzibar meeting, all four short-listed countries submitted their application dossiers to the ECTAD unit. These were reviewed at a meeting of the IS on 9 and 10 May 2011, in Nairobi, which was attended by representatives of FAO-ECTAD, OIE, AU-
IBAR, EAC, the Veterinary Service of Ethiopia, the Wildlife Service of the United Republic of Tanzania and a representative of Kenya’s CVO. The IS decided to select one regional laboratory, in the understanding that network members may decide to designate a second regional laboratory at a future date, should the need arise. The IS reached consensus on the methodology to be used for reviewing the dossiers and ranking the four laboratories. At the end of the process, the National Animal Health Diagnostic and Investigation Centre (NAHDIC) of Ethiopia was ranked first. Accordingly, the IS meeting recommended the designation of NAHDIC, Sebeta, Ethiopia as the eastern Africa regional laboratory for AI and ND.

The newly designated regional laboratory needs additional support to be able to fulfil its new responsibilities. Such support should include the provision of reagents, laboratory materials, equipment and capacity building. This laboratory is considered a priority for twinning arrangements with an OIE/FAO reference laboratory for AI and ND.

Key information about NAHDIC

The National Animal Health Diagnostic and Investigation Centre (NAHDIC) is the national referral veterinary laboratory of Ethiopia. It was established in 1995 in Sebeta and was initially named the National Animal Health Research Centre, changing its name in October 2007. This name change brought a broadening of responsibilities and duties from the centre’s primary focus on research. NAHDIC now:

1. generates internationally acceptable laboratory diagnostic results to support the export trade of livestock and livestock products;
2. coordinates and performs national surveillance and diagnosis of livestock diseases of economic and public health importance, whose occurrence can lead to lengthy export bans for livestock and livestock products;
3. builds capacity in all Ethiopia’s regional veterinary laboratories, to help improve the national veterinary service so it can address the problems facing poor farming and pastoral communities;
4. undertakes a regulatory role for the control and eradication of animal trypanosomosis and tsetse fly;
5. coordinates the control and eradication of hide and skin diseases in Ethiopia, contributing to increased income generation from the animal hides sector;
6. contributes to the expansion of high-quality veterinary education at all the veterinary faculties in Ethiopia, by hosting 12 to 15 graduate students (M.Sc., DVM, B.Sc., and Ph.D.) a year for their dissertation research;
7. runs and coordinates national and international research projects such as vaccine trials for peste des petits ruminants and capripoxes, and modelling of disease dissemination.

NAHDIC has 121 staff members excluding its satellite laboratory for trypanosomosis control. Most of its activities support the generation of foreign income from exports of livestock and livestock products. The South African National Accreditation Service (SANAS) has recommended the centre for accreditation under ISO 17025 for five TADs: brucellosis (Rose bengal plate test), Rift Valley fever (enzyme linked immunosorbent assay [ELISA]), peste des petits ruminants (ELISA), foot-and-mouth disease (ELISA), and ND virus and AI (molecular diagnosis). In November 2009, it established a national laboratory network with 15 regional laboratories, and it participates in proficiency tests for Rift Valley fever, foot-and-mouth disease, peste des petits ruminants, brucellosis, HPAI and ND virus. NAHDIC has applied for OIE laboratory twinning projects, as a means of improving its diagnostic capacity and compliance with OIE standards.
Four-way linking of epidemiological and virological information on human and animal influenza

Preface

In recent decades there has been an unprecedented increase in the numbers of new and highly threatening viral diseases of humans and animals. One example has been the rapid spread of highly pathogenic avian influenza (HPAI) H5N1 viruses among poultry populations, and the subsequent threat to humans, especially in Asia, Europe and Africa. In the scientific community, discussions continue about the best control strategies for such pandemics, but it is clear that successful management and containment of HPAI depend on the ability of the animal and human health sectors to work together before, during and after epidemics. Efficient epidemiological and virological information management systems are necessary for effective collaboration and timely response by the public health (PH) and animal health (AH) sectors. Such systems need to manage the variety of data required to assess the public health risk of influenza at the human-animal interface, at the national, regional and global levels, so that actions by the different actors can be harmonized. Unfortunately, coordination between PH and AH sectors has so far fallen short of the basic requirements for efficient control of HPAI.

The concept

The four-way linking framework is a collaborative effort among the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE), the OIE/FAO network of expertise on animal influenza (OFFLU) and the Global Early Warning and Response System for Major Animal Diseases, including Zoonoses (GLEWS) to improve national, regional and global qualitative risk assessments for animal and zoonotic influenza. This framework seeks to establish a national-level mechanism for routine, integrated and qualitative assessments of virological and epidemiological influenza data from humans and animals. Decision-makers can use the information from the risk assessments to develop and implement new scientifically based measures for prioritizing and managing the risks identified and for evaluating the effects of measures already in place.

To ensure the availability of appropriate information for conducting such assessments it is necessary that systems be in place for collecting relevant epidemiological and virological information on influenza from both animals and humans, and for establishing linkages within this information according to where and when events took place, which samples and isolates belong to which human cases or animal outbreaks, and which humans were associated with which animals and when. This linked information can be examined and assessed by experts in different fields to: i) improve understanding of the overall situation, including the animal and public health risks from influenza; and ii) identify gaps in information availability or national systems.

---

3 Four-way linking focal points: Filip Claes (FAO), Gwenaelle Dauphin (FAO), Liz Mumford (WHO), Kate Glynn (OIE).
The national process for collecting relevant information and assessing risks is expected to be iterative within the four-way linking framework; gaps in the available information, and areas where national systems need strengthening would be identified during risk assessments, and used to suggest areas for improvement. Subsequently, improved information would allow better assessments, which in turn would suggest additional refinements to national systems. It is envisioned that the four-way linking framework could therefore act as a national platform for the alignment of internationally mandated capacity building and other national-level projects and activities designed to improve the systems.

The framework will be tested in three pilot countries and is expected to improve the linkage between national-level information about human influenza epidemiology and virology and about animal influenza epidemiology and virology (including sequence analysis). Regular risk assessments will also increase awareness and understanding of the process among the technical staff involved and among the stakeholders and decision-makers receiving the information. At the global level, the project aims to develop a standard mechanism for joint qualitative risk assessment of linked data for zoonotic influenza. Country-level implementation will be in partnership with human and animal health institutes and the respective ministries.

Assessment missions
WHO, FAO and OIE carried out joint assessment missions in two pilot countries – Egypt and Viet Nam – to identify key partners, national initiatives and current efforts, and existing operational tools and systems for epidemiological and virological surveillance of influenza in both the PH and AH sectors. The team assessed the existing systems for data collection, data traceability, data exchange and reporting of influenza within the PH and AH sectors, and identified major gaps in and constraints to these systems.
Observations in the pilot countries found positive trends, but also revealed opportunities and challenges for working towards more efficient information management. The following are some of the important issues to emerge:

- Increased veterinary capacity for surveillance and investigation is required.
- Human cases and positive poultry cases should be investigated by combined PH-AH teams, but cooperation between the two sectors is sometimes incomplete.
- Data sharing between sectors is incomplete, and there is no organization or output that combines intelligence from all aspects. Concerns over intellectual property and perceived lack of organizational support are the main barriers to data sharing.
- More analysis of epidemiological data for both sectors is necessary. At the moment, data are often compiled without analysis and interpretation.
- It is difficult to obtain exposure information for human cases, as late reporting generally makes it impossible to obtain real-time follow-up on the level of disease in the related poultry population. It is also difficult to obtain exposure information from poultry outbreaks where no human cases are reported.
- Translating surveillance outcomes into policy decisions is difficult.

National workshops

National-level workshops will help address and improve collaboration among the sectors in pilot countries. The first four-way linking workshop was held in Ain Sokhna, Egypt from 26 to 28 September 2011 with participation from the four main sectors involved in control of HPAI in Egypt – public health epidemiology (Ministry of Public Health, Epidemiology Unit), public health virology (Central Public Health Laboratories [CPHL]), animal health epidemiology (General Organisation for Veterinary Services [GOVS]) and animal health virology (Central Laboratory for Quality Control of Poultry Production [CLQP]) – and academia (two professors). Through didactic presentations, group work, plenary discussions and scenario-based training, the workshop addressed principles and applications of (joint) risk assessment; communication and data sharing among laboratory staff and epidemiologists; and the benefits of joint AH-PH outbreak investigations. The workshop identified gaps in daily work at the animal-human interface, and possible solutions to these gaps, leading to concrete proposals for improving mechanisms and communications among the four sectors. The outcome of the process will inform policy- and decision-makers for HPAI control. The national workshop in Viet Nam will be held in February 2012.

In conclusion, there is a definite need to build mechanisms for enabling and supporting data sharing, improving joint work on case investigations, and improving and harmonizing surveillance systems. The four-way linking platform can be instru-
ment in bringing together stakeholders from the animal and human health sectors, to facilitate virological and epidemiological information sharing. To improve the cooperation and data sharing between PH and AH, formal agreements that allow direct contacts between the two sectors are necessary.

Contributors: Filip Claes (FAO), Gwenaelle Dauphin (FAO)

**OFFLU Avian Influenza Vaccine Efficacy project in Egypt**

Highly pathogenic avian influenza (HPAI) subtype H5N1 is currently endemic in Egypt, with the first outbreaks reported in February 2006. In efforts to control the disease, vaccination of commercial flocks was introduced at the end of March 2006, and mass vaccination of household poultry was conducted from May 2007 until 2009. As in other countries applying vaccination against HPAI, these vaccination efforts met with variable success, and to increase understanding of how to improve the use of vaccination as part of an overall control strategy, the Food and Agriculture Organization of the United Nations (FAO) – in close collaboration with national agencies – implemented a three-year (2008 to 2011) World Organisation for Animal Health (OIE)/FAO network of expertise on animal influenza (OFFLU) technical project entitled Vaccine Efficacy for the Control of Avian Influenza (AI) in Egypt (OSRO/EGY/801/USA). The project aimed to promote the appropriate use of efficacious poultry vaccines as part of a comprehensive strategy to combat HPAI through understanding the characteristics and epidemiology of circulating A/H5N1 viruses; determining the efficacy of available AI poultry vaccines; and supporting the development of sustainable national systems to monitor viral evolution and ensure vaccine efficacy.

National partners included laboratories with national diagnostic responsibilities (Central Laboratory for Quality of Poultry Production – CLQP) and those responsible for veterinary vaccine quality (Central Laboratory for Evaluation of Veterinary Biologics – CLEVB). International partners were recognized leaders in influenza research: the Southeast Poultry Research Laboratory (SEPL) of the United States Department of Agriculture (USDA) (which is the OIE Collaborating Centre in Research on Emerging Avian Diseases) was responsible for laboratory capacity building activities for CLQP, conducting of and training in laboratory challenge trials, and evaluation of procedures for registration and licensing of poultry vaccines for AI; and the Erasmus Medical Centre undertook virus relationship analysis using antigenic cartography.

The project also benefited from concurrent work conducted by the *Istituto Zooprofilattico Sperimentale delle Venezie* (IZSVe, Italy) and the *Friedrich-Loeffler-Institut* (FLI, Germany), which are OIE/FAO reference centres for avian/animal influenza and Newcastle disease. Technical collaboration between IZSVe and the public and private sectors in Egypt (particularly universities and vaccine manufacturers), and the OIE twinning project between FLI and CLQP, entitled Promotion of Rapid Molecular Diagnosis and Characterization of Avian influenza and Newcastle Disease Viruses, con-
tributed data and shared information that enabled the OFFLU project to develop an even broader perspective on the Egyptian H5N1 situation. The project was funded through the United States Agency for International Development (USAID), which had funded a similar project implemented by FAO in Indonesia. Lessons learned from these projects and other FAO national programmes in affected countries contribute to the understanding and control of HPAI.

Characteristics and epidemiology of circulating A/H5N1 viruses

To improve understanding of the evolution of the H5N1 HPAI virus in Egypt and assess the impact of AI vaccines used in poultry, biologic and genetic characterization and analysis of H5N1 HPAI viruses were conducted at both national and international reference laboratories. Between 2006 and 2008, a total of 1,592 cases of H5 AI were detected using real-time reverse transcription polymerase chain reaction (RT-PCR) (confirmation of N1 was conducted on a subset of samples), and 586 cases were confirmed during 2009/2010. A total of 540 virus isolates were obtained, predominantly from household poultry: 157 from 2006 to 2008; 160 from 2009; and 223 from 2010. Genetic analysis was conducted on isolates from 2008 to 2010; SEPRL conducted sequencing of 27 isolates and supported the laboratory staff at CLQP in completing 170 H5 genes and 71 N1 genes. The data from the majority of the genes sequenced by CLQP and SEPRL have been submitted to public databases such as Genbank. Phylogenetic analysis of the Egyptian viruses indicated two major groupings – “classical” and “variant”. The classical viruses prevailed in household poultry, most of which is unvaccinated; these viruses belong to clade 2.2.1, according to the updated World Health Organization (WHO) unified nomenclature for H5N1, and demonstrated few mutations compared with the initial introduced virus from 2006. The variant viruses were predominantly detected in the commercial sector, where vaccination is routinely applied, and appeared in late 2007 (Arafa et al., submitted). These variants belong to a newly designated 2.2.1.1 clade and demonstrated a higher mutation rate (Cattoli et al., 2011) than classical viruses. It is to be noted that these variant viruses have not been associated with human infections, apart from one human case in 2009 (109 human cases were reported by WHO between 2008 and 2 November 2011). While these findings have improved the epidemiological understanding of the HPAI situation in Egypt (Arafa et al., 2010; submitted), they also highlight the need to include representative sampling from all poultry production sectors to ensure a more complete understanding of the situation, especially in efforts to monitor vaccine efficacy.

Antigenic cartography (Smith et al., 2004) quantifies the antigenic differences between the haemagglutinin (HA) proteins of tested viruses based on haemagglutination inhibition (HI) assay data. The data are displayed in a map format to enable visualization of the antigenic distances between the viruses. This method was applied

---

4 www.who.int/csr/disease/avian_influenza/guidelines/nomenclature/en/
5 www.who.int/influenza/human_animal_interface/en_gip_20111102cumulativenumberh5n1casesupdated.pdf
to aid the selection of candidate challenge and vaccine strains for further in vivo testing. Reference strains for reagent production were selected from the Egyptian strains, based on the year of isolation and biological characterization, and shared among the partners. Technical staff from CLPQ were trained in assay techniques, data management and analysis of the cartography results, and participated in reagent production with SEPRL, using a harmonized protocol.

**Challenge tests**

Trials to determine vaccine efficacy through challenge testing against both classical and variant strains were conducted at both SEPRL and CLQP, and benefited from additional information from trials conducted separately at FLI (Grund et al., 2011) and IZSVE (Terregino et al., 2010). In laboratory trials, birds were inoculated using either commercially available inactivated vaccines or experimental inactivated vaccines generated from classical or variant Egyptian strains. The level of protection and the extent of virus shedding after challenge were then evaluated.

All trials under the OFFLU project – and those carried out by FLI, IZSVE, CLQP and the Veterinary and Agrochemical Research Centre (VAR – Belgium) (Rauw et al., 2011), where a classical sub-lineage Egyptian virus was used for challenge – demonstrated 100 percent clinical protection under laboratory conditions, regardless of the vaccine strain used. For trials using challenge viruses from the variant sub lineage, protection afforded from vaccination was highly variable (ranging from nearly 80 percent to complete vaccine failure of 0 percent protection in a few cases), as was the level of virus shedding post-challenge. However, the data also suggest that vaccines with sufficient antigen content to produce high titres in the bird could be protective even against viruses with large antigenic and genetic differences (e.g., variants). This highlights the importance of the antigenic content of commercial vaccines in stimulating the appropriate immune response.

**Laboratory capacity building**

Laboratory capacity building activities and technology transfer under this project aimed to support the rapid and accurate diagnosis of H5N1 HPAI in government veterinary laboratories and to ensure the sustainability of ongoing surveillance and monitoring activities.

Laboratory staff at CLQP and CLEVB received training on-site and abroad (at SEPRL) on genetic sequencing for H5N1 HPAI and phylogenetic analysis; antigenic characterization and production of standardized reagents; safety, potency, purity testing, and challenge testing of H5 AI vaccines; and laboratory biosafety and biosecurity. Recommendations were provided, related to the CLQP and CLEVB facilities: protocols for pathogen characterization, vaccine potency and efficacy determination; and virus surveillance.

Vaccines could be protective even against viruses with large antigenic and genetic differences

---

* Antigenic cartography data should not be regarded as the only criterion for predicting vaccine efficacy.
Project partners contributed their expertise through joint data analysis and technical review of strategies and policies, developed recommendations and indicated necessary actions. These outcomes were delivered at two meetings (January 2009 in Cairo, Egypt, and June 2011 in Rome, Italy), which engaged other national stakeholders. Project activities helped strengthen national laboratory capacity, improve biosafety procedures in national partner laboratories, and support ongoing virus detection and characterization through the provision of laboratory equipment, reagents and other consumables. Support to the development of a new laboratory information management system made it possible to monitor and respond to disease outbreaks and analyse epidemiologic data in conjunction with laboratory data. The outcome of biologic, genetic and antigenic analyses of Egyptian viruses contributed to understanding of these viruses and identification of potential candidate vaccine strains for use in poultry.

CLOP should continue the surveillance and characterization of influenza viruses, to develop a complete understanding of the epidemiology of H5N1 HPAI in Egypt and ensure sound disease control planning and informed decision-making. The data generated by such surveillance and characterization are used to validate diagnostic tests and enable early detection of emerging viruses with specific mutations that have major phenotypic implications. Collection and integration of data on vaccine efficacy and effectiveness at both the laboratory and the field levels (e.g., post-vaccination monitoring, vaccination data analysis) are still needed in Egypt. Given the country’s current socio-economic situation, external funding may be required in the short term to continue this important work.

There is also need for increased cooperation between government and industry, to improve the virus data and epidemiologic information regarding commercial poultry. The engagement of other stakeholders – such as private laboratories, Egyptian universities and other institutions conducting laboratory analysis in the country, including New Medical Research Unit 3 (NAMRU3) – should be encouraged to allow the sharing of data and information that can contribute to disease control efforts. The integration of epidemiological information with virus characterization data could also contribute to better early risk assessments, leading to appropriate response actions in both the animal and the human sectors and to a more effective national AI control programme. The national animal health sector should play an active and leading role in improving these linkages, in line with the four-way linking framework proposed by FAO and WHO.1

Although this study shows that most AI poultry vaccines currently used in Egypt appear to confer acceptable levels of protection when applied appropriately, the

1 A four-way linking workshop was conducted in Egypt from 26 to 28 September 2011 (see article on page 36).
proper handling and application of vaccines in the field are critical if vaccination is to be an effective part of a comprehensive control programme. Based on field data, the actual coverage of vaccination campaigns appears to be low, suggesting that application of AI vaccination in Egypt needs to be improved. Vaccination of day-old chicks (DOCs) with an effective vaccine (if and when available) may significantly decrease the virus load and subsequent circulation along the poultry value chain. The application of DOC vaccines at both traditional and modern hatcheries represents an efficient intervention that could have a significant impact on the control of H5N1 HPAI. FAO encourages the ongoing efforts of pharmaceutical companies to develop vaccines for use in DOCs.

FAO continues to emphasize that biosecurity remains one of the most important and critical tools in the fight against H5N1 HPAI; as a supportive measure to reduce virus load in the poultry population and the environment, vaccination should be applied as part of a comprehensive control programme. When a nation opts to apply AI vaccination, a clear plan for ongoing surveillance and post-vaccination monitoring must be in place (FAO, 2011). This project contributed to the development of enhanced capacity to undertake laboratory-based activities in support of field investigations and surveillance in Egypt, but inputs for implementing the recently revised HPAI surveillance programme based on the value chain and covering all sectors of poultry production are still required.

References


Contributors: Gwenaelle Dauphin (FAO), Mia Kim (FAO), Yilma Jobre (FAO), Juan Lubroth (FAO)

OFFLU contribution to the World Health Organization Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2012 (26 to 28 September 2011, Geneva, Switzerland)

Every six months, a team of specialists reviews all human influenza virus activity and virus characterization, including of the zoonotic influenzas A/H5N1 and A/H9N2. The team also describes the current status of development of new A/H5N1 and A/H9N2 candidate vaccine viruses. This process is managed by the World Health Organization (WHO) and aims to provide national authorities and vaccine companies with guidance on the selection of candidate viruses for use in vaccine development. Since 2010, the World Organisation for Animal Health/Food and Agriculture Organization on the United Nations (OIE/FAO) network of expertise on animal influenza (OFFLU) has been officially involved in this consultation process, for an initial period of three years. FAO gathers genetic and antigenic data on animal viruses from laboratory networks and publicly available sources, and epidemiological data are compiled from
animal health databases – the FAO Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases Global Animal Disease Information System (EMPRES-i), and the OIE World Animal Health Information Database (WAHID).

At the last consultancy meeting (September 2011), OFFLU provided a summary of the available epidemiological and molecular data for highly pathogenic avian influenza (HPAI) H5N1 and avian influenza H9N2 for the period 1 February to 20 September 2011. For H5, OFFLU shared new and previously unreported sequences from Bangladesh, Egypt, India, Indonesia, Israel, Lao People’s Democratic Republic, Myanmar and Viet Nam, representing clades 1, 2.1.3, 2.2, 2.2.1, 2.3.2, 2.3.4. The report included 245 H5 sequences (120 non-public and 12 public-domain sequences from 2011, and 113 non-public for 2009 to 2011). For H9, OFFLU contributed 20 pre-2011 sequences (most from 2009) and one 2011 sequence from Bangladesh. The very satisfactory and increasing level of information sharing between countries and OFFLU is to be acknowledged.

The outcomes of this consultancy process are published on the WHO Web site under Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness.7

Contributors: Gwenaelle Dauphin (FAO), Mia Kim (FAO), Flip Claes (FAO)

7 www.who.int/influenza/resources/documents/characteristics_virus_vaccines/en/