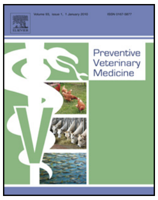
Preventive Veterinary Medicine 120 (2015) 96–105

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed



Surveillance at the molecular level: Developing an integrated network for detecting variation in avian influenza viruses in Indonesia☆

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ARTICLE INFO

Article history:
Received 4 July 2014
Received in revised form 13 February 2015
Accepted 16 February 2015

Keywords:
Antigenic cartography
Avian influenza
Bioinformatics
H5N1-HPAI
Molecular surveillance system
OFFLU
Surveillance networks

ABSTRACT

Since 2006, Indonesia has used vaccination as the principal means of control of H5N1-HPAI. During this time, the virus has undergone gradual antigenic drift, which has necessitated changes in seed strains for vaccine production and associated modifications to diagnostic antigens. In order to improve the system of monitoring such viral evolution, the Government of Indonesia, with the assistance of FAO/OFFLU, has developed an innovative network whereby H5N1 isolates are antigenically and genetically characterised. This molecular surveillance network ("Influenza Virus Monitoring" or "IVM") is based on the regional network of veterinary diagnostic laboratories, and is supported by a web-based data management system ("*IVM Online*"). The example of the Indonesian IVM network has relevance for other countries seeking to establish laboratory networks for the molecular surveillance of avian influenza and other pathogens.

1. Introduction

The H5N1 subtype responsible for the ongoing epizootic of highly pathogenic avian influenza (HPAI) was first recognised in 1997 in Hong Kong (Shortridge et al., 1998). During 2003–04, it underwent an unexpected re-emergence, wherein it spread throughout the poultry flocks of the countries in east and south-east Asia (Sims et al., 2005). Initially, this transboundary spread was thought to be via migratory wild-bird reservoirs of the virus

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Abbreviations: DIC, Disease Investigation Center; DGLAHS, Directorate General of Livestock and Animal Health Services; GOI, Government of Indonesia; HI, haemagglutination inhibition (test); HPAI, highly pathogenic avian influenza; IVM, Influenza Virus Monitoring (network).

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http://dx.doi.org/10.1016/j.prevetmed.2015.02.015
0167-5877/

(Li et al., 2004); however retrospective analyses have indicated an equal (or greater) role for the legal and illegal movement of poultry and poultry products (Kilpatrick et al., 2006; van den Berg, 2009).

H5N1-HPAI was first detected in Indonesian poultry farms in central Java in August 2003 (Wiyono et al., 2004). Subsequently, the disease spread rapidly, and by 2005 had been detected in poultry flocks in 30 out of Indonesia's 33 provinces (Sawitri Siregar et al., 2007). From June 2005, it has also caused sporadic zoonotic transmissions from infected poultry to humans (Samaan, 2007; Adisasmito, 2010), and as at late-2014, 165 fatalities have been recorded by the World Health Organisation (WHO), making Indonesia by far the country with the highest reported number of cases (WHO, 2014).

Initially the veterinary authorities in Indonesia attempted a stamping out policy, but when the extent of the spread of the disease became clear, this strategy was changed to one of vaccination (Naipospos, 2005; Sawitri Siregar et al., 2007). By 2009, about 20 vaccines, all inactivated, were registered by the veterinary regulatory authority (Domenech et al., 2009). As such, Indonesia is only one of four countries, along with China, Egypt and Vietnam, that have consistently used vaccination as a control strategy for H5N1-HPAI within the past 10 years (Swayne et al., 2011).

When Indonesia adopted mass vaccination as a control measure for H5N1-HPAI in poultry there was very little experience on which to base the development of an effective national vaccination strategy (Anon, 2005). Up until then, vaccination only had been used to a limited extent as a control measure for H5N1-HPAI in Hong Kong using a killed H5N2 vaccine (Ellis et al., 2004). Accordingly, at an international meeting of experts in 2005, there was little consensus except that vaccination alone was unlikely to be able to eliminate the disease from a country (Anon, 2005). There were also concerns about the wisdom of relying on long-term vaccination, as although it might effectively prevent disease, vaccination would not induce "sterilizing immunity", i.e. the virus would continue to infect and circulate within and between poultry flocks (Capua and Marangon, 2004; Savill et al., 2006).

Following on from the international meeting of experts in 2005, the World Organisation for Animal Health (OIE) and the United Nations Food and Agriculture Organization (FAO) established a joint network of expertise on avian influenza, called "OFFLU" (Edwards, 2006). With international donor support, OFFLU offered assistance to the veterinary authorities of the Government of Indonesia (GOI) to "establish sustainable mechanisms for monitoring virus circulation including viral characterization for the purpose of effective vaccine selection, and for streamlining the process to update poultry vaccines for AI" (Dauphin et al., 2010).

Here we describe the successful implementation of this OFFLU–GOI partnership project, which has led to a co-ordinated and integrated "molecular surveillance system" for HPAI, the Influenza Virus Monitoring ("IVM") network. This system, which is based on the network of veterinary laboratories within Indonesia, includes the development of standardised protocols for antigenic characterisation and a web-based data management system, called "*IVM Online*".

2. Materials and methods: development of the IVM network

2.1. Phase 1 – 2007–2010: developing the science base of the IVM network

The OFFLU–GOI partnership activities began in late 2007, with project management provided by the FAO Indonesia office working closely with the Directorate General of Livestock and Animal Health Services (DGLAHS) of the Indonesian Ministry of Agriculture. Expertise for this project was provided via three influenza reference laboratories: the Australian Animal Health Laboratory (AAHL, Geelong, Australia), the Southeast Poultry Research Laboratory (SEPRL, Athens, USA) and the Erasmus University Medical Center (Erasmus MC, Rotterdam, The Netherlands). Co-ordination of the activities of the project was provided by a dedicated OFFLU scientist (Dauphin et al., 2010).

A starting point for the work in the first phase was a vaccine efficacy challenge study undertaken by SEPRL in early 2007. For this study, undertaken under laboratory conditions, chickens were vaccinated with registered Indonesian H5N1 vaccines and challenged using a standard protocol with H5N1 isolates collected from Indonesian outbreaks during 2006. This study showed that although the antibody response to the vaccine seed strains – as measured by the haemagglutination inhibition (HI) test – was reported to be "good", indicating that the vaccines were of high quality, in some cases they were not fully protective (Kim, 2011). In particular, vaccine protection was poorest against challenge from an isolate ("A/chicken/West Java/PWT-WIJ/2006") collected from West Java (Swayne et al., 2015), a region with a high density of poultry flocks. This study demonstrated the need to determine the antigenic and genetic diversity of circulating strains within the Indonesian poultry sector, in order to be able to assess the extent of the mismatch between them and the registered vaccines.

To gain insight into the diversity of the circulating viruses, attention was directed to the collection of specimens from outbreaks in village-based poultry production systems ("sector 4"). Backyard poultry production accounts for approximately 50% of the standing population of poultry in Indonesia, and as this closely mirrors the distribution of the human population (Sawitri Siregar et al., 2007), it was considered to better reflect the H5N1 strains of direct zoonotic risk. Following isolation and confirmation of the H5N1 virus by the Indonesian veterinary laboratories, the isolates were sent to AAHL for genetic characterisation, under the Material Transfer Agreement (MTA) designed by OFFLU. Over the period 2007–10, AAHL sequenced the haemagglutinin (HA) gene of 244 H5N1 viruses isolated from 317 samples collected across 98 districts of the total of 444 districts within Indonesia (Kim, 2011). The initial genetic analysis showed that all of the Indonesian viruses grouped together as clade "2.1", which is a lineage, which apart from progenitor viruses identified in China, is unique