

SURVEILLANCE AND MONITORING FOR AVIAN INFLUENZA VIRUSES IN MIGRATORY BIRDS IN BALI

Tjokorda Sari Nindhia and I.G.N.K. Mahardika
Animal Biomedical and Molecular Biology Laboratory
Udayana University
Campus Bukit Jimbaran Bali 80364

INTRODUCTION

Highly-pathogenic avian influenza (HPAI) is currently panzootic in several countries around the world, especially in Asia. The panzootic has developed after HPAI outbreaks were first identified in rapid succession in six Asian countries in December 2003 and early 2004 - Vietnam, Thailand, China, Lao, Cambodia and Indonesia (Office International des Epizooties - <http://www.oie.int/>). These countries are characterised by high densities and close proximities of poultry and wild birds, suggesting a link between migratory birds and poultry. Incidence is also sporadic and seasonal, according to migration. Should the role of migratory birds be scientifically confirmed, it will mark a change in a long standing stable relationship between the H₅N₁ virus and its natural wild bird reservoir (Normile, 2006; Ducatez et al., 2006).

Recent outbreaks of avian influenza in both domestic poultry and the human population are a source of considerable concern (Poland et al. 2007). Given their potential impacts on both humans, and domestic and wild bird populations, it is important that the dynamics of influenza viruses are assessed on a global scale, and that similarities and differences among potential transmission pathways in different regions are considered (Melville and Shortridge, 2006; Olsen et al., 2006; Kilpatrick et al., 2006). Avian influenza viruses fall into two groups, termed low pathogenicity (LPAI) and high pathogenicity (HPAI). LPAI viruses constitute the majority of viruses in wild birds (Olsen et al., 2006). The possibility that wild birds are involved in the spread of H₅N₁ geographically between poultry flocks has been investigated and the findings are contradictory. Kilpatrick et al. (2006) concluded that there was little empirical evidence that wild birds were responsible for the movement of H₅N₁. Another study, however, compared viruses from different outbreaks in birds and found that viruses from countries that are along migratory routes are almost identical to viruses recovered from dead migratory birds at Qinghai Lake, CHina (Chen et al., 2006).

The research of Mahardika (2005) on avian influenza in Bali, shows that the whole regency is an avian influenza infected area. The H₅N₁ subtype of avian influenza A virus can be detected in domestic poultry, pure breed chickens, ducks, geese and pigeons. H₅N₁ is considered to have been spread in Bali by a number of different known vectors, including the movement of live poultry and its products, legal and illegal trade in birds, and imported chicken faeces for agricultural organic manure from outside Bali.

Large concentrations of endemic and migratory wild birds breed in Bali in close proximity to humans and their poultry providing an ideal circumstance for avian influenza transmission between domestic and wild birds. Despite this, there are no studies on the prevalence and serotypes of avian influenza viruses found in wild birds that live near or visit poultry houses in Bali.

The aim of this study was to investigate the potential role of wild birds in the dissemination of avian influenza in Bali.

MATERIALS AND METHODS

Sample collection

Samples were collected in November 2006 at two sites of wild and migratory bird aggregation in Bali, Petulu heron sanctuary of Gianyar Regency (n=117) and Serangan island of Denpasar Regency (n=105).

The Petulu heron sanctuary is located in Petulu village in Ubud, Bali. Petulu village is a countryside which consists of Banjar Petulu Gunung and Banjar Petulu Desa with a population of up to 315 families. There are 3 kinds of herons living in Petulu. the little egret, cattle egret, and Javan pond heron. Herons (Kokokan birds in Balinese) will normally emerge at the start of sasih (Balinese month) *kelima* until *kesanga* (fifth until ninth month) or in October to March. During this period thousands of kokokan birds will be nesting and hatching. Colonies densely packed with birds and may be as much as 3 km long. Trees and the surrounding environment are heavily contaminated with faeces providing ample opportunity for disease transmission. In March fledgling kokokan are able to fly. During the period of April to September, kokokan normally migrate to the other areas to hunt for food. During the nesting period, birds will perch and make their home on trees along the village road and residents' homes, and even on the rooftops of houses.

Serangan Island is a transit zone for the migratory birds. Birds that visit Serangan Island during the migration period in September include the striated heron (*Butorides striatu*), cattle egret (*Bubulcus ibis*), great egret (*Casmerodius albus*), little egret (*Egretta garzetta*), Sunda teal (*Anas gibberifrons*), common sandpiper (*Tringa hypoleucos*), curlew sandpiper (*Callidris ferruginea*), gull-billed tern (*Sterna nilotica*), great crested-tern (*Sterna bergii*), small blue kingfisher (*Alcedo caeruleascens*). Other species in the genera *Charadrius*, *Pluvialis*, *Numenius*, *Calidris*, and *Tringa*, including many migratory species are also found there.

The faecal samples were collected by placing plates of aluminium foil under trees where the birds were roosting and nesting. Faeces were collected with sterile synthetic swabs (Dacron® or polyester®) and 5 swabs were pooled in a single tube. Samples were stored in PBS glycerol medium transport (WHO 2002). The faecal samples were centrifuged, diluted 1:10 with phosphate buffered saline (PBS) containing an antifungal agent, then stored at -20°C until analysis. All samples were analysed in the Animal Biomedical and Molecular Biology Laboratory of Udayana University

Virus propagation in SPF embryonated chicken eggs

Faecal samples were inoculated into 9-day-old SPF embryonated chicken eggs. Each inoculum was prepared by mixing the faecal sample with 10 µl PBS containing of 2x10⁶ U/L penicillin and 200 mg/L streptomycin in a tube. The eggs were incubated at 37°C and observed everyday for 4 days, and allantoic fluid was harvested at the 4th day of incubation and then tested for haemagglutination activity (WHO 2002).

Haemagglutination Activity Test (HA)

Before the micro HA test was done, and initial rapid agglutination test was performed by mixing 1 drop of allantoic fluid with 5% chicken red blood cells (v/v). The presence of virus was indicated by agglutination of the red blood cells within 15 seconds. Positive allantoic fluid was then tested with the micro HA test using 2-fold dilutions of serum and an equal volume of diluted chicken red blood

cells. Negative and positive controls were included. Positive samples were defined as those that agglutinated the red blood cells after 30 minutes of shaking.

Positive allantoic fluid was further tested for the presence of AIV subtype H₅N₁ using Reverse Transcriptase-Polymerase Chain Reaction with standard primer pairs (WHO 2002).

RESULTS AND DISCUSSION

Seven (6.66 %) of the samples collected in Serangan, and 11 (9.40 %) from Petulu heron sanctuary tested positive for the influenza A but negative for H₅ and N₁ genes. After verifying that the samples were negative for H₅N₁, we did not attempt to determine which subtype of AIV had infected the bird, since the aim was to detect H₅N₁, and our funding was limited. Also during the study banded shorebirds were identified. One of these, a Gajahan Erasia (*Numenius arquata*) was originally banded in China., the country where H₅N₁ outbreak of AI originated.

Our study found no evidence of infection of wild birds and migratory birds with avian influenza subtype H₅N₁ in Bali. Given that avian influenza subtype H₅N₁ is widespread in Bali and many of these birds were breeding in close proximity to poultry, our study suggests that wild and migratory birds do not play a role in the epizootiology of this disease. Given that Bali is an important stop over stop for migratory birds, some of which migrate through countries that are infected with avian influenza subtype H₅N₁, continued monitoring of these birds is indicated.

In this study we have found avian influenza A virus in wild and migratory birds, but not H₅N₁. Wild water bird species (Anseriformes and Charadriiformes) are considered the natural reservoir of Influenza A virus subtypes (Krauss et al., 2004; Olsen et al., 2006). All known avian influenza virus subtypes have been isolated from these taxonomic orders, which usually cause only minor clinical signs after infection and are referred to as Low Pathogenic Avian Influenza (LPAI) viruses. Introduction of these viruses into poultry flocks with high population densities may result in the virus mutating into a highly Pathogenic Avian Influenza (HPAI) virus, and initiate outbreaks with mortalities up to 100% in poultry (Olsen et al., 2006). There are many subtypes of avian influenza viruses, but only some strains of four subtypes have been highly pathogenic in humans. These are types H₅N₁, H₇N₃, H₇N₇ and H₉N₂ (Leong et al., 2008). There are possibilities that the virus which we discovered in wild bird can threaten human health. Therefore this research must be continued in order to find the subtype of Influenza Virus A which are carried by these birds. An understanding of the viruses and their relationships can help us identify and modify critical control points to reduce transmission of avian influenza viruses into animal and human populations.

Very little information on shore bird migration through Indonesia is known. This study suggests that Indonesia may prove to be an important stopover site for migrating shore birds. It is critical that additional studies be done to verify these findings.

CONCLUSION

Wild and migratory birds in Serangan Island Denpasar and the Petulu heron sanctuary in Bali have been infected by avian influenza A virus but not the H₅N₁ subtype and are not involved in spreading of H₅N₁ in Bali. The surveillance network should be operated continuously after the end of this study for complete scientific data about avian influenza and the other emerging diseases in the Bali region.

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