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Epidemiology of avian influenza in wild aquatic birds in a biosecurity hotspot, North Queensland, Australia

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ABSTRACT

Migratory birds may introduce highly pathogenic H5N1 avian influenza from Southeast Asia into Australia via North Queensland, a key stopover along the East Asian-Australasian Flyway, with severe consequences for trade and human health. A 3-year repeated cross sectional study on the epidemiology of avian influenza in Australian nomadic wild aquatic birds was conducted in this potential biosecurity hotspot using molecular and serological techniques. Avian influenza virus subtypes H6 and H9 were commonly present in the studied population. It is likely that one of the H6 viruses was newly introduced through migratory birds confirming the perceived biosecurity risk. The matrix gene of another H6 virus was similar to the Australian H7 subtypes, which suggests the reassortment of a previously introduced H6 and local viruses. Similarly, a H9 subtype had a matrix gene similar to that found in Asian H9 viruses suggesting reassortment of viruses originated from Australia and Asia. Whilst H5N1 was not found, the serological study demonstrated a constant circulation of the H5 subtype in the sampled birds. The odds of being reactive for avian influenza viral antibodies were 13.1(95% CI: 5.9–28.9) for Pacific Black Ducks over Plumed Whistling Ducks, highlighting that some species of waterfowl pose a greater biosecurity risk. Antibody titres were slightly higher during warm wet compared with warm dry weather. Routine surveillance programmes should be established to monitor the introduction of avian influenza viruses from Asia and the interactions of the introduced viruses with resident viruses in order to better detect emerging pathogens in aquatic birds of North Queensland. Surveillance should be targeted towards highly susceptible species such as the Pacific Black Duck and carried out during favourable environmental conditions for viral transmission such as the wet season in northern Australia.

1. Introduction

Wild aquatic birds are considered a natural reservoir of avian influenza viruses (AIV) and harbour all known subtypes of the influenza A viruses including the highly pathogenic avian influenza (HPAI) strains (Brown et al., 2007; Munster et al., 2007). Australia has recorded five outbreaks of HPAI caused by H7 subtypes in commercial

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chickens (Selleck et al., 2003: Westbury, 2003). In at least two of the five Australian HPAI outbreaks, surface drinking water contaminated with Australian nomadic wild aquatic bird faeces was suspected to be the source of the spill-over infection. Therefore, understanding the epidemiology of endemic viruses in reservoir hosts such as the Australian nomadic wild aquatic birds is important in order to better evaluate and mitigate the risk of spill over.

Unlike all other human-inhabited continents, Australia has remained free of HPAI H5N1, but there are increased concerns that the virus could be introduced via migratory birds which travel between Australia and Southeast Asia where HPAI H5N1 is endemic (East et al., 2008). An H5N1 outbreak in wild migratory birds at Lake Qinghai, China in May 2005 (Lei et al., 2007; Wang et al., 2008) posed serious concerns because the lake is a major breeding site for migratory birds whose flyways extend to Southeast Asia, India, Siberia, Australia and New Zealand. Moreover, the HPAI H5N1 strain has been confirmed in Australia's close neighbour Indonesia (Capua and Alexander, 2004) and notably in West Papua on the island of New Guinea (McCallum et al., 2008). Migratory birds could introduce HPAI into Australia via North Queensland, the first point of entry into Australia for many migratory birds. Australia could provide another substantial reservoir for the virus resulting in impacts globally on trade and biosecurity. Therefore, conducting surveillance for these exotic viruses and understanding their risk of introduction into Australia is important. This can be achieved through avian influenza (AI) surveillance in high risk biosecurity areas like North Queensland and the genetic characterisation and subsequent molecular epidemiological understanding of AI subtypes in Australian nomadic wild aquatic birds.

Although cross sectional studies have been conducted on the epidemiology of AI in Australian nomadic wild aquatic birds (Downie et al., 1977; Senne, 2003; Haynes et al., 2009), systematic repeated studies with the aim of estimating seroprevalence and determining risk factors for increased AIV antibody prevalence and hence the risk of spill over have not been done. The determination of the full set of hemagglutinin (H) serotypes and patterns of occurrence over time and their influence on risk of spill over have also rarely been investigated in nomadic wild aquatic birds in Australia. Systematic long-term studies with the aim of estimating AIV ribonucleic acid (RNA) prevalence and the distribution and reassortment (gene evolution) of AIV subtypes, including exotic viruses, in Australian nomadic wild aquatic birds and how these may contribute to biosecurity risks are limited. Therefore, we performed a 3-year repeated cross sectional study in North Queensland from April 2007 up to March 2010 to understand the molecular- and sero-epidemiology of AI in Australian nomadic wild aquatic birds given it is a biosecurity hotspot with perhaps the greatest risk of introduction of HPAI into Australia by migratory birds (Murray et al., 2012). Specific objectives of the study were to estimate the prevalence of AIV RNA and AIV antibodies, establish the distribution of AIV subtypes (based on molecular and serological testing), identify risk factors associated with the prevalence of AIV antibodies and determine the molecular epidemiology of AIVs in nomadic wild aquatic birds in North Queensland. This knowledge will improve our understanding of the biosecurity risks posed by AIV in Australian nomadic wild aquatic birds.

2. Materials and methods

2.1. Sites, sample size and sampling

The epidemiological study of AI was performed on nomadic wild aquatic birds in North Queensland. Birds which roosted at different wetlands located within the selected sites were considered as the sampling frame. Birds were sampled from the wetlands of four different sites in North Queensland (Fig. 1) using the most convenient sampling technique. Sites were selected based on their proximity to migratory routes, ease of access, the presence of nomadic wild aquatic birds and generally a large bird population. A repeated cross sectional study was performed on nomadic wild aquatic birds at the Billabong Sanctuary (19°22′ S and 146°54′ E) between April 2007 and March 2010 and at Green Acres Lagoon (Cromarty) (19°34′ S and 1476°90′ E) between December 2007 and 2009. Opportunistic studies were also conducted at Cape York Peninsula (15°59′ S and 141°65′ E) and the Atherton Tablelands (16°58′ S and 145°24′ E) between 2007 and 2009.

The study required 138 birds, regardless of species, to be sampled per quarter of each year. Accordingly, 1656 samples were needed for the 3-year study at Billabong Sanctuary and 1104 samples for the 2-year study at Green Acres Lagoon. The following formula was applied to calculate sample size assuming a 100% sensitivity and specificity (Noordhuizen et al., 1997): $N = Z_{\alpha}^2 \times SD^2/L^2(37)$. N = sample size; Z = 1.96 at 95% confidence level; SD^2 (variance) = 0.001 for the molecular study (MS) and 0.09 for the serological study (SS); L (absolute precision) = 0.005 (MS) and 0.05 (SS). The variance was calculated as follows: $P \times (1 - P)$. P is the expected point prevalence based on the preliminary data analysis and literature (Haynes et al., 2009) (0.01, MS and 0.10, SS). An infinite bird population was assumed.

Birds were sampled quarterly at Billabong Sanctuary and Green Acres Lagoon (Cromarty) in order to study the temporal pattern of AI. Funnel traps were mostly used to capture birds (Ethics approval no. A1175, James Cook University: JCU). Birds on Cape York were opportunistically captured for sampling using mist nets and a net launcher (licence no. WISP04524607) between 2008 and 2009. Birds on the Atherton Tableland were sporadically sampled during 2008.

2.2. Sample collection and epidemiological data recording

Cloacal and oropharyngeal swabs along with blood samples were collected from each bird. Cloacal swabs are sensitive for identifying low pathogenic AIVs, whereas oropharyngeal swabs are sensitive for identifying highly pathogenic AIVs (such as H5); serum samples were used for serological investigation of AI in cELISA and HI assays. Swabs were taken from birds by inserting swab sticks