EcoHealth 3, 290–298, 2007
DOI: 10.1007/s10393-006-0064-2

EcoHealth

The Australian White Ibis *(Threskiornis molucca)* as a Reservoir of Zoonotic and Livestock Pathogens

Jonathan H. Epstein,[1,5] Jeffrey McKee,[2] Phil Shaw,[3] Vicki Hicks,[4] Gino Micalizzi,[4] Peter Daszak,[1] A. Marm Kilpatrick,[1] and Gretchen Kaufman[5]

[1]*The Consortium for Conservation Medicine, 460 West 34th Street, 17th Floor, New York, NY10001, USA*
[2]*Department of Veterinary Sciences, The University of Queensland, St. Lucia, QueenslandAustralia*
[3]*Eco-Sure Environmental Consultants, Tugun, QueenslandAustralia*
[4]*Queensland Health Scientific Services, Coopers Plains, QueenslandAustralia*
[5]*Department of Population and Environmental Health, Tufts University Cummings School of Veterinary Medicine, 200 Westboro Road, North Grafton, MA01536, USA*

Abstract: Over the last 20 years, Australian white ibis populations *(Threskiornis molucca)* have expanded into urban areas, leading to increased contact between ibis, domestic animals, and humans. This has led to concern that ibis may transmit pathogens that threaten public health or food production. Here we report results from a study of ibis viral serology and bacterial culture that indicate that ibis are hosts of zoonotic and livestock pathogens such as *Salmonella* spp., Newcastle disease virus, avian influenza virus, and flaviviruses in Australia. We also performed a behavioral study to measure contact rates among ibis, people, and livestock that determine the potential for disease transmission.

Key words: zoonoses, *Salmonella*, avian influenza, Newcastle disease, wild birds, public health

Introduction

In coastal southeastern Queensland and northern New South Wales, Australia, urban populations of Australian white ibis (hereafter, ibis), *Threskiornis molucca*, have increased significantly since 1983 (Shaw, 2000). A range of factors have changed ibis distribution and led to population increase: drought; escape of captive birds; and increased availability of anthropogenic food sources due to rapid urban expansion in the region (Woodall, 1985; Shaw, 2000). Ibis are generalist predators and scavengers that naturally feed on saltwater and freshwater wetland invertebrates and fish (Marchant and Higgins, 1990). They have become habituated to humans and are frequently observed in close contact with people, particularly in urban recreational areas (Shaw, 2000). They have learned to forage at landfills, public recreational areas, and on farms. These ibis–human and ibis–livestock interactions provide opportunity for disease transmission.

Many wild avian species, including some related to ibis, carry pathogens which are of particular importance to human or animal health such as influenza A virus (AIV), *Salmonella* spp., Kunjin virus (and other flaviviruses), and Newcastle disease virus (NDV) (all but NDV have zoonotic potential) (Stallknecht and Shane, 1988; Mackenzie et al., 1995; Feare et al., 1999; Alexander, 2000; Craven et al., 2000; Yu et al., 2002; Delogu et al., 2003a). *Salmonella* spp.,

Published online: December 8, 2006
Correspondence to: Jonathan H. Epstein, e-mail: epstein@conservationmedicine.org

NDV, and AIV can lead to economically significant loss in domestic poultry industries (Webster, 1998; Degefa et al., 2004). It has been suggested that some historical influenza outbreaks in humans that originated from avian viruses have used pigs as an intermediate host in which avian and human influenza viruses have re-assorted, making them transmissible to humans (Alexander and Brown, 2000). However, in 1997, highly pathogenic H5N1 avian influenza (HPAI) was directly transmitted from domestic poultry to humans in Hong Kong (Capua and Alexander, 2004). More recently, there have been global outbreaks of H5N1 in domestic poultry populations, some of which have led to human infection and mortality in Southeast Asia without the involvement of porcine hosts (Li et al., 2004; Olsen et al., 2006). Migratory waterfowl and shorebirds are considered the natural reservoir for influenza A viruses and have been shown to carry all subtypes of influenza A viruses (Webster et al., 1992). Several subtypes have been isolated from Australian species of waterfowl, although little is known about how influenza viruses are transmitted between wild birds and poultry (Mackenzie et al., 1984; Webster, 1998). Although the vast majority of cases of highly pathogenic avian influenza (HPAI H5N1) in humans have been caused by contact with infected poultry, another subtype of influenza A virus (H11N9) has been transmitted directly from wild birds to humans, suggesting that it also may be possible for transmission of other subtypes to occur in this way (Gill et al., 2006).

Salmonella and *Campylobacter* spp. are two of the most common food-borne zoonotic pathogens in developed countries including USA, UK, and Australia (Thorns, 2000). In Australia, salmonellosis continues to be one of the major causes of foodborne gastroenteritis (Hall et al., 2005), largely through consumption of infected poultry and beef. However, wild birds have been implicated as a source of livestock contamination or direct transmission to humans (Craven et al., 2000; Thorns, 2000). *S. typhimurium, S. virchow*, and *S. Birkenhead* are among the 10 most common serovars found in people in Australia (Blurner et al., 2003).

Newcastle disease, while having little clinical impact on humans, is a globally important pathogen of poultry, causing mortality rates often greater than 50% in its most pathogenic form (Alexander, 2000; Swayne and King, 2003). Newcastle disease virus has been isolated from several wild waterfowl species in Australia, which supports the idea that wild birds may act as a reservoir for this virus (Mackenzie et al., 1984).

Migratory waterfowl are also known to be reservoirs of several important insect-borne zoonotic encephalitis viruses (Hubalek, 2004). Murray Valley encephalitis virus (MVE), Japanese encephalitis virus (JE), and Kunjin virus are all part of the JE complex of encephalitis viruses, believed to be one of the most important flavivirus groups on a global scale (Mackenzie, 2005). This particular group of flaviviruses is enzootic to Australia, and flaviviruses in general can be found in wild birds on every other continent except Antarctica (Mackenzie et al., 1984, 1995; Kay et al., 1987; Broom et al., 2003; Johansen et al., 2004; Russell and Kay, 2004). Murray Valley encephalitis, Kunjin, and Japanese encephalitis virus have all been identified in mosquito vectors in Australia (Broom et al., 1998; Russell, 1998). Murray Valley encephalitis causes severe, sometimes fatal encephalitis. Kunjin virus, which causes less severe disease than MVE, is a genetic variant of West Nile virus and shares the same transmission cycle involving bird reservoirs and mosquito vectors (Hall et al., 2001). Japanese encephalitis virus has, so far, been limited to northern Queensland, and migratory water birds have been suggested as a likely means of entry into Australia from Papua New Guinea and the Torres Strait (Johansen et al., 2004).

In this article, we report on a study set up by The Ibis Management Coordination Group to assess the potential for ibis to transmit zoonotic and livestock pathogens in Queensland.

Materials and Methods

Survey Sites and Bird Capture

In 1995, 60 Australian white ibis were captured by cage-trapping at Coolangatta and Currumbin in southeastern Queensland, Australia (28° 08″S, 153° 29″E) between September 1994 and June 1995. There is a wildlife sanctuary in Currumbin where ibis are known to nest and breed [McKee, unpublished observations]. In 1997, 54 ibis were captured by cannon-netting at Suntown Landfill in Arundel (27° 57″S, 153° 21″E) during the same period. In 2000, 100 ibis were captured at the suburban Brown Plains Landfill, Queensland (27° 38″S, 153° 01″E) between June and August 2000, using a cannon net. This site was chosen because of its proximity to the other field sites in this study and its large population of ibis. Each captured ibis was banded to prevent repeated sampling. The age class and sex of each bird were determined by size and plumage characteristics (Marchant and Higgins, 1990).

Behavioral surveys were conducted at two locations where ibis forage and interact with either people or

domestic animals: Cascade Gardens, Gold Coast, Queensland (28° 01″S, 153° 25″E), a public recreational park with a resident population of 50 ibis; and a commercial mixed poultry and cattle farm in southeast Queensland (name and location withheld to preserve anonymity). The farm contained a laying shed with battery cages, two covered enclosures with free-ranging chickens inside, and a field in which chickens, cows, and ibis could intermingle. Ibis also roosted in a tree in the middle of this field.

Disease Survey

Seven milliliters of blood was drawn from 100 ibis using either the ulnar or tibiotarsal vein and divided between 1.0 ml EDTA tubes and 6.0 ml serum separator tubes (Vacutainer; Becton-Dickinson, Franklin Lanes, NJ). In 1995, 38/60 sera were used for serologic tests and in 2000, 88/100 were used due to hemolysis in some samples which precluded accurate ELISA interpretation. Blood smears were made on-site. Cloacal and choanal swabs were collected and stored in bacterial culture and viral transport medium, respectively.; Serum tubes were centrifuged and stored at 4°C. Choanal swabs were stored at –70°C and saved for future testing for viral pathogens. Cloacal swabs were stored at room temperature in plastic zip bags. One hundred cloacal swabs for salmonellae were cultured in Gram Negative Broth (Oxoid Australia, Adelaide, Australia) and Rapports Broth (Oxoid Australia) for 24 hours at 37°C. Both enrichments were then subcultured onto Xylose-lysine-desoxycholate (XLD; Oxoid Australia) and Bismuth sulphite agar (BSA; Oxoid Australia) and incubated at 37°C for 24 hours and 48 hours, respectively. Typical colonies, i.e., nonlactose fermenting, nonspreading, H2S colonies on XLD and H2S producing colonies with characteristic metallic sheen on BSA were picked off for further identification, initially by conventional biochemical tests and progressively by the use of Salmonella Serobact Latex kit (Medvet Australia, Adelaide, Australia) and confirmation using Microbact 24E system (Medvet Australia).

All confirmed isolates were serotyped according to the Kauffmann–White Scheme, based on their ''O'' Somatic antigens and ''H'' Flagella antigens using specific antisera (Medvet Australia), and are presented using the LeMinor and Popoff nomenclature (Leminor et al., 1990).

Parasite ova and coccidia were identified by microscopic examination of wet mounts of fecal smears following fecal flotation on a Nikon Epson 2000 microscope. Samples were collected for bacteriology and mycology using alginate swabs and immediately plated onto horse blood agar, chocolate agar, MacConkey agar, thiosulphate-citrate-bile-sucrose agar, and Sabauraud's agar. Bacterial isolates were identified by Gram stain, Microbact 24 NE (Medvet Science Pty Ltd, Adelaide, Australia) and API 20E (bioMerieux, Marcy-l'Etoile, France) systems.

Diagnostic assays for ibis pathogens and antibodies to specific pathogens are summarized in Table 1 for each study. A hemagglutination inhibition test was used to detect antibodies to Newcastle disease virus; an agar gel immunodiffusion test and cELISA for influenza A (OIE, 2004) [P. Selleck, personal communication]; cELISA for Japanese encephalitis and related flaviviruses (Hall et al., 1995); and a serum neutralization test for Hendra virus (Daniels et al., 2001) were conducted using 88 sera according to standard Office International des Epizooties (OIE) and internal protocols at The Australian Animal Health Laboratory, Geelong, Australia. Test methods were consistent across all three studies (1995, 1997, and 2000).

For the purpose of age and disease prevalence comparisons, data from juvenile and subadult birds were combined to form a ''young'' group which was compared to data from adults. Prevalence and proximity data were analyzed using chi-squared and Fisher's exact tests. For 3 × 2 tables, we also performed pair-wise analyses using a Bonferroni correction.

Behavioral Survey

We assessed the nature and frequency of interaction between ibis and humans, cattle, and chickens during typical ibis foraging behaviors. The One-Zero sampling technique was used for behavioral observations (Altmann, 1974). We recorded the instantaneous behavior and proximity of five randomly chosen ibis to people, food objects, free-range chickens, or laying sheds that contained caged chickens and egg racks every 2 minutes for 5 hours (1100–1600 hours) over 4 days at each location. We used binoculars (Pentax 10 × 24 ucf WR) to observe each bird. Each set of observations was termed a ''bird-observation period.'' Only one distance estimation was assigned to each bird per observation. Results of the behavioral survey are not reported here. Results of proximity observations are reported from two sites: Cascade Gardens, and a mixed poultry and cattle farm. Food objects were considered important for pathogen transmission from ibis via the fecal–oral route. A food object was defined as any item that either was food or in direct contact with food. On the poultry farm, a food object

Table 1. Summary of Prevalence of Various Pathogens Detected in AWI 1995–2000

AWI disease screening results 1995–2000				1995		1997		2000		Mean overall (±1 SE)
Organism	Disease	Potential hazard to:	Test	*N* =	Prev. (%)	*N* =	Prev. (%)	*N* =	Prev. (%)	Prev. (%)
Avian influenza virus	Avian influenza	Poultry, humans	AGID ELISA	38	0*	54	41†	88	31†	24 (±12.30)
Candida	Fungal infection	Ibis	Culture	38	26					26
Coccidia	GI tract parasitism	Ibis	Flotation	60	2					2
Flavivirus, generic	Various	Humans	PRNT			54	15*	88	1†	8 (±5.70)
Haemoproteus	Blood protozoan infection	Ibis	Blood smear	21	38					38
Helminths	Intestinal parasitism	Ibis	Fecal flotation	60	2					2
Hendra virus	Pneumonia	Humans, horses	SNT			54	0			0
Japanese encephalitis virus	Japanese encephalitis	Humans, pigs	PRNT					88	0	0
Kunjin virus	Kunjin encephalitis	Humans	ELISA, PRNT	38	0†			88	2†	1 (±0.82)
Murray Valley encephalitis virus	Murray Valley encephalitis	Humans	ELISA	38	0			88	0	0
Newcastle disease virus	Newcastle disease	Poultry	HI	38	3*	54	31†	88	32†	22 (±9.50)
Salmonella spp.	Salmonellosis	Humans and livestock	Culture	38	3*			100	7†	5 (±1.60)
S. Birkenhead								100	1	1
S. Oranienburg								100	1	1
S. Typhimurium								100	1	1
S. Virchow								100	4	4
Vibrio cholerae		Humans	Culture	38	3					3

AWI, Australian white ibis; AGID, agar-gel immunodiffusion; ELISA, enzyme-linked immunosorbent assay; PRNT, plaque reduction neutralization test; SNT, serum neutralization test; HI, hemagglutin inhibition.

*, †Items are significantly different ($P < 0.05$).

†, †Items are not significantly different ($P \geq 0.05$).

refers to chicken or cow food resources. During each observation period, if an ibis was observed within 3 meters of an object of interest, it was assigned a proximity category: 0, contact with; 1, between 0 and 1 meter of; or 3, between 1 and 3 meters of the object. Preliminary observations suggested that people or chickens tended to utilize an approximate area within a 3-meter radius of their location during eating activities, so the presence of ibis, feces, or oronasal fluids on the ground or on food objects within this area was considered as having the highest potential for contact with people or chickens. Examples of food objects included picnic tables, grills, picnic blankets, food containers, water fountains or faucets at Cascade Gardens, and feed and food and water troughs for cattle or chickens at the poultry farm. The ''frequency'' at which an ibis stood at a certain distance from an object was calculated by dividing the number of observations at that distance by the total number of observations. The ''relative frequency'' was calculated by dividing the number of observations in each distance category by the total number of observations that included an ibis within 3 meters.

Results

Pathogen Prevalence

Table 1 summarizes the results of disease surveys conducted in 1995, 1997, and 2000. The seroprevalence of AIV and NDV antibodies differed between the 3 years of the survey (AIV: $\chi^2 = 19.7$, df = 2, $P < 0.001$; and NDV: $\chi^2 = 13.8$, df = 2, $P < 0.001$). The prevalence increased between 1995 and 1997 (AIV: $\chi^2 = 20.0$, df = 1, $P < 0.001$; NDV: $\chi^2 = 11.8$, df = 1, $P < 0.001$ both significant at the 0.01 level after Bonferroni correction), but not between 1997 and 2000 (AIV: $\chi^2 = 1.5$, df = 1, $P = 0.220$; NDV: $\chi^2 = 0.03$, df = 1, $P = 0.860$). The prevalence of generic flaviviruses was

Table 2. Comparative Prevalences for AIV, NDV, and *Salmonella* spp. between Sexes and Age Classe

	AIV	NDV	*Salmonella* spp.
Males	25% (40)	28% (40)	15% (44)
Females	42% (31)	42% (31)	3% (32)
Comparison[a]	$\chi^2 = 2.5$, df = 1, $P = 0.11$	$\chi^2 = 2.00$, df = 1, $P = 0.16$	$\chi^2 = 2.99$, df = 1, $P = 0.08$
Adults	37% (54)	48% (54)	7% (57)
Juveniles + subadults	21% (33)	9%(33)	8%(36)
Comparison[a]	$\chi^2 = 13.1$, df = 1, $P < 0.001$	$\chi^2 = 11.4$, df =1, $P < 0.001$	$P = 0.702$, FET

AIV, avian influenza virus; NDV, Newcastle disease virus; FET, Fisher's exact test.
[a]Either a chi-square analysis or Fisher's exact test (FET) was used to measure differences. Significant differences have a P-value < 0.05.

significantly higher in 1997 than 2000 (and Fisher's exact test, $P = 0.002$). The prevalences of *Salmonella* and Kunjin virus infection did not vary significantly between 1995 and 2000 (Fisher's exact tests, $P = 0.44$ and $P = 1.00$, respectively).

Age and Sex

Of 100 birds sampled in 2000, 45 were male, 36 were female, and 19 were of unknown sex. There were 29 juveniles, 8 subadults, 61 adults, and 2 birds whose age was not determined. There was no significant difference in prevalence between males and females for any of the pathogens examined; however, adults had a significantly higher prevalence of AIV and NDV antibodies than younger birds (Table 2).

Behavioral Study

At Cascade Gardens, ibis were observed either in contact with or within 3 meters of a food object in 20% (610/3025) of the total observations, and people were present at the food object during 72% (432) of those observation periods (Fig. 1a). Ibis were observed making contact with a food object 5% (151) of the time (Fig. 1b). Figure 2 shows an example of ibis bathing in and drinking from a food object, in this case a water tap, which was subsequently used by two young boys who drank from it. Ibis made contact with unattended food objects more than twice as often as when people were present (3.8% vs. 1.5% (610); $\chi^2 = 6.29$, df = 1, $P = 0.012$). There were 20 bird observation periods in which people were seen either actively feeding ibis or passively feeding ibis by leaving food scraps behind after eating, and 70% of those bird observation periods included one or more ibis eating human-provided food.

At the poultry farm, 19% (402/2135) of the observations included an ibis within 3 meters of a food object, chickens, or laying shed (Fig. 1c). Ibis were only present at the poultry farm for 3 of the 4 days during the observation period. Ibis were regularly observed in close proximity to free-range hens, as well as caged hens in the laying sheds, to which they had unrestricted access. Ibis were observed between 0 and 3 meters of free-ranging chickens in 11% (43/402) of proximity measurements with 28% (12/43) of those including ibis in direct contact with chickens (Fig. 1d). Ibis were frequently observed inside the laying shed (32%, 132/402), foraging under the elevated chicken cages, poking their bills into piles of excrement under the cages and occasionally taking an egg from the rack. Ibis were also observed scavenging meat from chicken carcasses, which were laid out in an outdoor wire cage into which ibis could reach with their bills. Forty-one percent (164/402) of proximity observations included one or more ibis within 3 meters of an animal food object, and of those, 74% included an ibis making contact with the food object—most frequently the cows' water trough at the base of a large tree in which the ibis perched daily. There was no direct contact between ibis and cows observed in this study.

Discussion

This is the first published report of *Salmonella* infection, or the presence of antibodies to NDV, AIV, and flaviviruses in Australian white ibis. AIV, *Salmonella*, and flaviviruses are significant to public health, while NDV, AIV, and *Salmonella* are important livestock diseases (Alexander, 2000; Capua and Alexander, 2004). These pathogens have been previously described in other free-ranging birds (Stallkn-

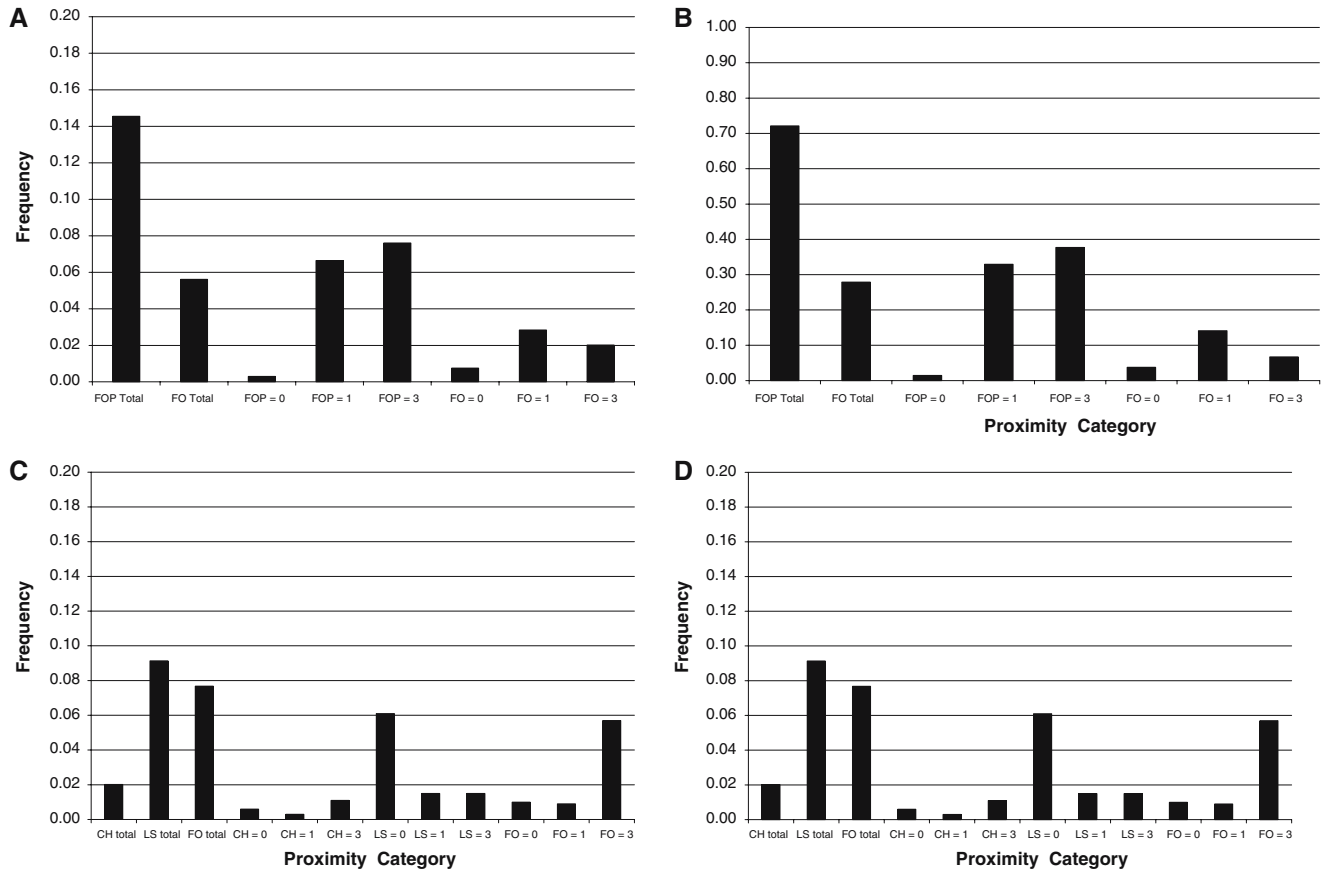

Figure. 1. a–d: Estimates of ibis' distance from objects considered important for disease transmission were obtained in three categories: 0, an ibis was either on top of, under, or otherwise in contact with an object; 1, an ibis was between 0 and 1 meter of said object; or 3, an ibis was between 1 and 3 meters from the object. The "frequency" at which an ibis stood at a certain distance from an object was calculated by dividing the number of observations at that distance by the total number of observations. The "relative frequency" was calculated by dividing the number of observations in each distance category by the total number of observations that included an ibis within 3 meters. a, b: Frequency and relative frequency of ibis within 3 meters of food objects occupied by people (FOP), and unoccupied food objects (FO), at Cascade Gardens (n = 3025 and n = 610, respectively). c, d: Frequency and relative frequency of ibis within 3 meters of free-range chickens (CH), laying sheds (LS), and FOs (n = 2135 and n = 402, respectively).

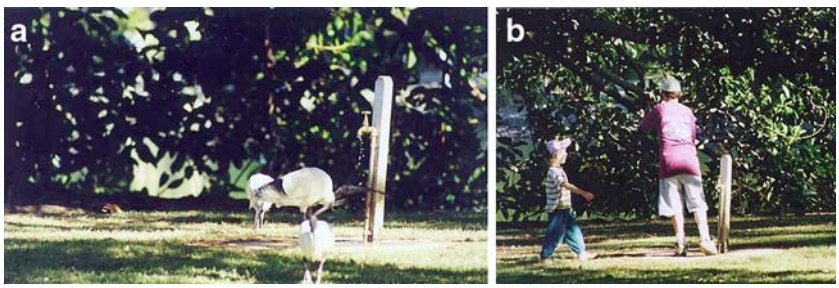

Figure. 2. a: Ibis bathing and drinking under a water tap in Cascade Gardens. Ibis were observed shaking their head and body under the tap. This could potentially contaminate the tap with excretions such as saliva, mucous, or feces—-which may contribute to transmission of zoonotic pathogens. **b:** Moments later, 2 young boys were seen drinking from the same tap.

echt and Shane, 1988; Feare et al., 1999; Pfitzer et al., 2000; Stanislawek et al., 2002; Yu et al., 2002; Delogu et al., 2003a; Mackenzie, 2005). The recent regional epizootic of highly pathogenic avian influenza (HPAI H5N1), which began in 2003 in Asia, has led to the slaughter of hundreds of millions of chickens, and 241 human cases, including 141 deaths (WHO, 2006). To date, Australia has not had any reported cases of H5N1 infection in either poultry or humans (WHO, 2006). Ducks (Family *Anseriformes*) are considered the natural reservoir for HPAI H5N1 in Southeast Asia (Webster et al., 1992; Hulse-Post et al., 2005). Ibis share aquatic environments with several duck species (Marchant and Higgins, 1990). We found 41% and 31% of ibis had antibodies to influenza A in 1997 and 2000,

respectively. It is unknown which subtypes of AIV ibis carry, or whether they maintain subclinical infections, or exhibit clinical disease and succumb to the virus. Experimental infections in several bird species have shown that influenza A viruses have variable shedding periods depending on the bird species and viral subtype. Ducks shed H5N1 between 11 and 17 days and had higher viral titers in the trachea not the cloaca, however, the opposite was true when infected with a different H5N1 strain (Hulse-Post et al., 2005). Pheasants can shed virus for up to 45 days post-infection (Humberd et al., 2006). The primary route and duration of viral shedding in ibis is unknown. Future testing should include AIV subtyping, and perhaps more importantly, fecal culture to determine the presence of specific subtypes of avian influenza viruses.

Human infection with HPAI H5N1 from poultry is still uncommon; however, these events may lead to pandemic spread of influenza A virus (Smith et al., 2006). While there has been no reported transmission of H5N1 between wild birds and humans (WHO, 2005), transmission of H11N9 has occurred between wild ducks and hunters, raising the possibility that direct transmission of more pathogenic subtypes of influenza A viruses is possible (Gill et al., 2006). If ibis shed influenza A virus when acutely infected, like other birds, then there would be ample opportunity for them to transmit the virus to conspecifics, other bird species including domestic poultry, and possibly humans.

Kunjin and Murray Valley encephalitis viruses are endemic to Australia (Hall et al., 2001; Johansen et al., 2001; DOHA, 2006). The low antibody prevalence to flaviviruses in ibis suggests a low level of exposure to these pathogens at a population level, a poor ability to mount an immune response, or high mortality. The ELISA used in this study was unable to differentiate among antibodies to each of the three flaviviruses, although a blocking ELISA has been described that is able to differentiate between antibodies to Murray Valley encephalitis virus and Kunjin virus (Hall et al., 1995).

It was not surprising to find older birds with a significantly higher seroprevalence of NDV and AIV antibodies, assuming that horizontal transmission occurs and that antibodies persist at a detectable level after exposure, because older birds would have had more time to be exposed to these pathogens. Ibis were observed co-roosting with flying foxes (*Pteropus* spp.), a known reservoir for Hendra virus, and were tested to see whether cross-species transmission had occurred (Halpin et al., 2000). All ibis were negative for Hendra-neutralizing antibodies, which is consistent with data that show Hendra virus is primarily found in mammals (Westbury et al., 1995). The similar prevalence of *Salmonella* spp. between adult and juvenile ibis is consistent with previous studies on gulls (Monaghan, 1986). Landfills have been identified as a source of *Salmonella* for gulls and other scavenging birds, and this may be a source of infection for ibis as well (Tizard, 2004).

Our behavioral study shows that Australian white ibis interact either with humans or domestic animals directly and indirectly on a daily basis, depending on the location. To date, there have been no documented reports of salmonellosis in humans that have been linked to ibis. However, 3 of the 4 serovars isolated from ibis in this study (*S. typhimurium, S. virchow*, and *S. Birkenhead*) are among the 10 most common in people (Blurner et al., 2003). Human salmonellosis typically occurs by consumption of raw or undercooked chicken or eggs (Thorns, 2000). It is possible that these serovars that are common to people and ibis may be exchanged between ibis and poultry.

Ibis at the poultry farm had easy access to hen houses because of the open-sided design of the laying sheds. Contact was observed between ibis and free-ranging chicken, caged hens, eggs, and chicken feces deposited below battery cages. This may be a potential route of pathogen transmission *between* poultry and ibis. The dispersive nature of ibis would then provide a mechanism for the spread of these pathogens among farms.

Installing physical barriers that exclude ibis from poultry sheds and that prevent opportunities for interaction with free-range chickens may reduce the risk of NDV, AIV, or *Salmonella* transmission from Australian white ibis, or other wild birds, to poultry and other livestock. A comparative study of these pathogens between poultry flocks that are, or are not, associated with ibis would provide further insight into the occurrence of transmission between these species.

Our observations of ibis standing on picnic tables and drinking from or bathing under public water faucets also used by children for drinking indicate possible opportunities for the transfer of zoonotic agents like salmonellae or possibly influenza A virus via environmental contamination with feces or oronasal secretions (Fig. 2). Water is believed to play a central role in the interspecies transmission of influenza virus among water birds (Stallknecht et al., 1990; Delogu et al., 2003b). Influenza A viruses have been shown to survive in fresh water at 28°C for up to 60 days, depending on the strain, the temperature of the water, and the pH (Stallknecht et al., 1990). The high frequency of people feeding ibis at this park, and the higher frequency at

which ibis were close to (within 3 meters), or in contact with, a food object when people were present compared to when people were not present, suggest that ibis have not only been highly habituated to humans, but have learned to identify people as a source of food. The data suggest that people, likewise, tolerate the presence of ibis, as seen in the highest frequency of proximity observations with occupied food objects. Ibis were also seen standing on food objects when people were not present, which allows for contamination of food surfaces without a person's knowledge.

The behavioral data, coupled with the presence of *Salmonella* spp. and antibodies to AIV, NDV, and flaviviruses in ibis at different times within a 7-year period, suggests a long-term presence of these pathogens in ibis populations in southeastern Queensland. Ibis have adapted to urbanized landscapes, become overabundant, habituated to humans, and readily utilize anthropogenic food sources. The continued growth and geographic expansion of ibis populations into rural and urbanized settings provides more opportunity for ibis to interact with humans and livestock, and the nature of these interactions may represent an increased risk of pathogen transmission. Public education that leads to increased hygiene (i.e., hand-washing and cleaning surfaces of tables and grills prior to use), and reduces the interactions between ibis and people will reduce the chance of zoonotic pathogen transmission.

Acknowledgments

This study was supported by an award from the V. Kann Rasmussen Foundation to the Consortium for Conservation Medicine and The Center for Conservation Medicine, Tufts University Cummings School of Veterinary Medicine, NIAID-NIH contract #NO1-AI-25490; and by funding from the Ibis Management Coordination Group, Queensland, Australia. The authors thank John Bates, Janine Barrett, Rosie Booth, Hume Field, Kell Irwin, Nicki Markus, Brad McCall, Wendy Paton, Kylie Patrick, Mark Woodin, and Craig Smith, who assisted in this work. Mobile hospital facilities and supplies were provided by Wildcare, Queensland, Australia. We are grateful for support from Logan City Council Waste Service, Queensland, Australia. Ibis trapping and sample collection for this study were conducted with permission from the Queensland Department of the Environment under permit number W4/002547/00/SAA.

References

Alexander DJ (2000) Newcastle disease and other avian paramyxoviruses. *Revue Scientifique et Technique de l'*Office International des Epizooties 19:443–462

Alexander DJ, Brown IH (2000) Recent zoonoses caused by influenza A viruses. *Revue Scientifique et Technique de l'*Office International des Epizooties 19:197–225

Altmann J (1974) Observational study of behavior-sampling methods. *Behaviour* 49:227–267

Australia's Notifiable Diseases Status, 2001: Annual Report of the National Notifiable Diseases Surveillance System (2003) Available: http://www.health.gov.au/internet/wcms/publishing.nsf/Content/cda-pubs-cdi-2003-cdi2701-htm-cdi2701a10.htm [accessed August 28, 2006]

Broom AK, Hall RA, Johansen CA, Oliveira N, Howard MA, Lindsay MD, et al. (1998) Identification of Australian arboviruses in inoculated cell cultures using monoclonal antibodies in ELISA. *Pathology* 30:286–288

Broom AK, Lindsay MDA, Wright AE, Smith DW, MacKenzie JS (2003) Epizootic activity of Murray Valley encephalitis and Kunjin viruses in an aboriginal community in the Southeast Kimberley region of Western Australia: results of mosquito fauna and virus isolation studies. *American Journal of Tropical Medicine and Hygiene* 69:277–283

Capua I, Alexander DJ (2004) Human health implications of avian influenza viruses and paramyxoviruses. *European Journal of Clinical Microbiology and Infectious Diseases* 23:1–6

Craven SE, Stern NJ, Line E, Bailey JS, Cox NA, Fedorka-Cray P (2000) Determination of the incidence of Salmonella spp., Campylobacter jejuni, and Clostridium perfringens in wild birds near broiler chicken houses by sampling intestinal droppings. *Avian Diseases* 44:715–720

Daniels P, Ksiazek T, Eaton BT (2001) Laboratory diagnosis of Nipah and Hendra virus infections. *Microbes and Infection* 3:289–295

Degefa T, Dadi L, Yami A, Mariam KG, Nassir M (2004) Technical and economic evaluation of different methods of Newcastle disease vaccine administration. *Journal of Veterinary Medicine Series A* 51:365–369

Delogu M, De Marco MA, Donatelli I, Campitelli L, Catelli E (2003a) Ecological aspects of influenza A virus circulation in wild birds of the Western Palearctic. *Veterinary Research Communications Supplement* 27:101–106

Delogu M, De Marco MA, Donatelli I, Campitelli L, Catelli E (2003b) Ecological aspects of influenza A virus circulation in wild birds of the Western Palearctic. *Veterinary Research Communications* 27:101–106

Department of Health and Aging (DOHA) (2006) *National Notifiable Diseases Surveillance System*. Available: http://www9.health.gov.au/cda/Source/Rpt_4.cfm [accessed July 7, 2006]

Feare CJ, Sanders MF, Blasco R, Bishop JD (1999) Canada goose (Branta canadensis) droppings as a potential source of pathogenic bacteria. *Journal of the Royal Society for the Promotion of Health* 119:146–155

Gill JS, Webby R, Gilchrist MJR, Gray GC (2006) Avian influenza among waterfowl hunters and wildlife professionals. *Emerging Infectious Diseases* 12:1284–1286

Hall G, Kirk MD, Becker N, Gregory JE, Unicomb L, Millard G, et al. (2005) Estimating foodborne gastroenteritis, Australia. *Emerging Infectious Diseases* 11:1257–1264

Hall RA, Broom AK, Hartnett AC, Howard MJ, Mackenzie JS (1995) Immunodominant epitopes on the Ns1 protein of Mve and Kun viruses serve as targets for a blocking ELISA to detect virus-specific antibodies in sentinel animal serum. *Journal of Virological Methods* 51:201–210

Hall RA, Scherret JH, Mackenzie JS (2001) Kunjin virus. An Australian variant of West Nile?. *Annals of the New York Academy of Sciences* 951:153–160

Halpin K, Young PL, Field HE, Mackenzie JS (2000) Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *Journal of General Virology* 81:1927–1932

Hubalek Z (2004) An annotated checklist of pathogenic microorganisms associated with migratory birds. *Journal of Wildlife Diseases* 40:639–659

Hulse-Post DJ, Sturm-Ramirez KM, Humberd J, Seiler P, Govorkova EA, Krauss S, et al. (2005) Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. *Proceedings of the National Academy of Sciences of the United States of America* 102:10682–10687

Humberd J, Guan Y, Webster RG (2006) Comparison of the replication of influenza A viruses in Chinese ring-necked pheasants and chukar partridges. *Journal of Virology* 80:2151–2161

Johansen CA, Nisbet DJ, Foley PN, van den Hurk AF, Hall RA, Mackenzie JS, et al. (2004) Flavivirus isolations from mosquitoes collected from Saibai Island in the Torres Strait, Australia, during an incursion of Japanese encephalitis virus. *Medical and Veterinary Entomology* 18:281–287

Johansen CA, van den Hurk AF, Pyke AT, Zborowski P, Phillips DA, Mackenzie JS, et al. (2001) Entomological investigations of an outbreak of Japanese encephalitis virus in the Torres Strait, Australia, in 1998. *Journal of Medical Entomology* 38:581–588

Kay BH, Saul AJ, McCullagh A (1987) A mathematical-model for the rural amplification of Murray Valley encephalitis-virus in Southern Australia. *American Journal of Epidemiology* 125:690–705

Leminor L, Popoff MY, Bockemuhl J (1990) Supplement 1989 (N-33) to the Kauffmann-White scheme. *Research in Microbiology* 141:1173–1177

Li KS, Guan Y, Wang J, Smith GJD, Xu KM, Duan L, et al. (2004) Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature* 430:209–213

Mackenzie JS (2005) Emerging zoonotic encephalitis viruses: lessons from Southeast Asia and Oceania. *Journal of Neurovirology* 11:434–440

Mackenzie JS, Edwards EC, Holmes RM, Hinshaw VS (1984) Isolation of orthoviruses and paramyxovirus from wild birds in Western-Australia, and the characterization of novel influenza-a viruses. *Australian Journal of Experimental Biology and Medical Science* 62:89–99

Mackenzie JS, Poidinger M, Lindsay MD, Hall RA, Sammels LM (1995) Molecular epidemiology and evolution of mosquito-borne flaviviruses and alphaviruses enzootic in Australia. *Virus Genes* 11:225–237

Marchant S, Higgins PJ (1990) *Handbook of Australian, New Zealand and Antarctic Birds (HANZAB)*, pp 1078–1087

Monaghan P (1986) Gulls, man and environmental contamination. *Applied Animal Behaviour Science* 15:184–185

Office International des Épizooties (OIE) (2004) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Available: http://www.oie.int/eng/normes/mmanual/A_summry.htm [accessed September 12, 2006]

Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus A, Fouchier RAM (2006) Global patterns of influenza A virus in wild birds. *Science* 312:384–388

Pfitzer S, Verwoerd DJ, Gerdes GH, Labuschagne AE, Erasmus A, Manvell RJ, et al. (2000) Newcastle disease and avian influenza A virus in wild waterfowl in South Africa. *Avian Diseases* 44:655–660

Russell RC (1998) Vectors vs humans in Australia—who is on top down under? An update on vectorborne disease and research on vectors in Australia. *Journal of Vector Ecology* 23:1–46

Russell RC, Kay BH (2004) Medical entomology: changes in the spectrum of mosquito-borne disease in Australia and other vector threats and risks, 1972–2004. *Australian Journal of Entomology* 43:271–282

Shaw P (2000) *Ibis Management Program Annual Report to the Ibis Management Coordination Group*, Gold Coast, Queensland, Australia: IMCG

Smith GJD, Naipospos TSP, Nguyen TD, de Jong MD, Vijaykrishna D, Usman TB, et al. (2006) Evolution and adaptation of H5N1 influenza virus in avian and human hosts in Indonesia and Vietnam. *Virology* 350:258–268

Stallknecht DE, Shane SM (1988) Host range of avian influenza-virus in free-living birds. *Veterinary Research Communications* 12:125–141

Stallknecht DE, Shane SM, Kearney MT, Zwank PJ (1990) Persistence of avian influenza-viruses in water. *Avian Diseases* 34:406–411

Stanislawek WL, Wilks CR, Meers J, Horner GW, Alexander DJ, Manvell RJ, et al. (2002) Avian paramyxoviruses and influenza viruses isolated from mallard ducks (Anas platyrhynchos) in New Zealand. *Archives of Virology* 147:1287–1302

Swayne DE, King DJ (2003) Avian influenza and Newcastle disease. *Journal of the American Veterinary Medical Association* 222:1534–1540

Thorns CJ (2000) Bacterial food-borne zoonoses. *Revue Scientifique et Technique de l'*Office International des Epizooties 19:226–239

Tizard I (2004) Salmonellosis in wild birds. *Seminars in Avian and Exotic Pet Medicine* 13:50–66

Webster RG (1998) Influenza: an emerging disease. *Emerging Infectious Diseases* 4:436–441

Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y (1992) Evolution and ecology of influenza-a viruses. *Microbiological Reviews* 56:152–179

Westbury HA, Hooper PT, Selleck PW, Murray PK (1995) Equine morbillivirus pneumonia: susceptibility of laboratory animals to the virus. *Australian Veterinary Journal* 72:278–279

WHO Global Influenza Program Surveillance Network (2005) *Evolution of H5N1 Avian Influenza Viruses in Asia*. Available: http://www.cdc.gov.ncidod/ED/vol11no10/05-0644.htm [accessed August 28, 2006]

World Health Organization (WHO) (2006) *Epidemiology of WHO-confirmed Human Cases of Avian Influenza A(H5N1) Infection*. Available: http://www.who.int/wer [accessed August 28, 2006]

Woodall PF (1985) Waterbird populations in the Brisbane region, 1972–83, and correlates with rainfall and water heights. *Australian Wildlife Research* 12:495–506

Yu SQ, Shinya K, Otsuki K, Ito H, Ito T (2002) Isolation of myxoviruses from migratory waterfowls in San-in District, western Japan in winters of 1997–2000. *Journal of Veterinary Medical Science* 64:1049–1052