Clean bill of health? Towards an understanding of health risks posed by urban ibis

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Clean bill of health? Towards an understanding of health risks posed by urban ibis

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Abstract

Urban waterbirds are considered both serious pests and inspiring wildlife. Ibis and gulls are often vilified due to their dirty appearance and disruption of outdoor activities, while ducks are affectionately fed in parks. However, all waterbirds are potential reservoirs of zoonotic pathogens. In Sydney (Australia), we documented the relative prevalence of arbovirus exposure and Salmonella shedding in 72 Australian White Ibis (Threskiornis moluccus) at 2 urban sites in 2003 during a management cull and in 2015 as a response to increased public interest. We sampled during a period of peak human arbovirus and Salmonella infection risk in late summer and early autumn. In 2015, antibodies for the endemic West Nile virus Kunjin strain (WNVKUN) were detected in one bird. While not indicative of immediate public health risk, this highlights that an animal with a history of exposure was present or moved into a region not previously known to have endemic WNVKUN activity. However, the movement patterns of this individual and WNVKUN host competency of this species are unknown. An absence of other antibody responses suggests that ibis are not important viral reservoirs or flaviviruses are not widespread in Sydney. Assays failed to detect Salmonella in 2015, but 25% of individuals were positive in 2003. Further monitoring of the arguable health hazard represented by urban T. moluccus will facilitate informed decisions and solutions to urban bird and wetland management challenges.

Key words: Threskiornis moluccus, flavivirus, Salmonella, West Nile virus, Kunjin virus, zoonotic

Introduction

Conservation of wetlands and their associated wildlife is of critical importance for ecosystem function and human aesthetics (Lemly, Kingsford, and Thompson 2000), but as human activity encroaches on wetlands, and constructed wetlands create habitat for waterbirds in residential areas, contact between humans
and wildlife increases. With increased development and growth of adaptable wildlife populations in urban areas, it is likely that the frequency of actual, and perceived, human health risks may increase. However, there is a paucity of information on the complex ecology and epidemiology of pathogens associated with urban wetlands and waterbirds that are assumed to directly or indirectly be transmitted to people (Shutes 2001; Murray and Hamilton 2010). Uncommon but lethal cases of Salmonella in urban birds have coincided with sickness in humans (Tizard 2004; Lawson et al. 2014), and urban birds have been identified as playing an important role in outbreaks of mosquito-borne West Nile virus (WNV) in North America (Blitvich 2008; Johnson et al. 2012). News of such events, and the aggressive feeding and nesting behaviour of some urban birds has led to the unsubstantiated perception that these animals are nuisance pests and a possible health risk to humans and/or livestock (Martin, French, and Major 2010). The alternative hypothesis, rarely considered, is that these birds are not drivers of the spread of these diseases, but passengers (MacDougall and Turkington 2005) representing sentinels of the presence of zoonotic diseases in the environment rather than being a part of the transmission pathway. For example, gull species are commonly found to carry a large variety of Salmonella serotypes in their gut for short periods due to environmental contamination of food or water, while songbirds such as finches often suffer explosive lethal outbreaks of Salmonellosis from environmental sources which are very likely to represent significant sources of infection for humans (Tizard 2004). Currently, the host competency and role of most bird species in zoonotic disease transmission in Australia is unknown (Boyle, Dickerman, and Marshall 1983; Iverson et al. 2009; Bingham et al. 2010). Nevertheless, in response to the perceived risks, local authorities may implement lethal management of native bird populations (Smith and Carlile 1993; Shaw 1999; Martin, French, and Major 2007), without evidence of definite risks.

The assumption that urban wetland birds are a nuisance and hazard has led to the intensive management of the native Australian White Ibis, Threskiornis moluccus, as a pest in Australian cities (Martin, French, and Major 2007; McKiernan and Instone 2016). This species was once common in land, but has declined in its former natural wetland habitat, due to drought and the diversion of water from natural areas to agriculture (Kingsford 2000). The movement of T. moluccus to urban areas was likely aided by the abundance of novel food resources, particularly at landfills (Martin, French, and Major 2010). By 2005, fewer than 3000 birds were surveyed breeding in inland areas (Porter, Kingsford, and Hunter 2006), while over 25 000 birds are now estimated to breed near the coast and in urban centres (Martin, French, and Major 2010; Martin 2016).

In 2000, surveys in sub-tropical Queensland suggested that T. moluccus was a potential reservoir of Salmonella, non-zoonotic strains of Avian Influenza Virus (AlV) and, at times, flaviviruses (Epstein et al. 2007). The prevalence of arbovirus (insect-borne) infections in humans has increased in southeast Australia, raising questions as to which urban wildlife are the likely reservoirs of viral diseases (Mackenzie and Williams 2009; Faddy et al. 2015). While the reservoir hosts of alphaviruses responsible for the most commonly reported mosquito-borne diseases, such as Ross River Virus (RRV) and Barmah Forest Virus, are generally considered to be marsupial macropods, the reservoir hosts for flaviviruses such as Kunjin (WNVkun) and Murray Valley Encephalitis Virus are thought to be waterbirds (Russell and Dwyer 2000; Van Den Hurk et al. 2014; Claflin and Webb 2015; Stephenson et al. 2018). Due to their abundance in habitats commonly shared with mosquitoes that are competent vectors of arboviruses, it is possible that waterbirds are hosts and/or reservoirs for mosquito-borne viruses other than MVE and WNVkun alone. When assessing the public health risks associated with mosquito and waterbird populations, consideration must be given to specific bird species present, as not all waterbirds will be locally important viral reservoirs (Pérez-Ramírez, Llorente, and Jiménez-Clavero 2014). Factors other than wildlife abundance, such as wetland management, mosquito control, and the promotion of personal protection from mosquito bites are likely to be more effective control actions (Claflin and Webb 2015; Hongoh et al. 2016; Webb and Hess 2016; Hardy and Barrington 2017).

The aim of this study was to document the levels of the common viral (flavivirus) and bacterial (Salmonella) pathogens in ibis that currently inhabit urban parks and begin to identify what risks exist in urban wetland environments. We chose to focus our surveys on documenting ibis exposure to (i) Salmonella, because this bacteria is commonly quoted as a pathogen of human health concern carried by ibis (Ecosure 2018), and (ii) flavivirus, given that infections in humans and horses have been a topic of recent public interest in eastern Australia (Van Den Hurk et al. 2014; Toi et al. 2017). Without information about the potential frequency of zoonotic pathogens in key waterbird species, calls for ibis culling and similar resolutions of wildlife-human conflicts in urban wetlands are likely to increase in response to growing urban ibis populations (Martin et al. 2007; Camden Council 2013; Ecological Pty Ltd 2013; Ecosure 2018). Conversely, if ibis are important reservoirs of pathogens of human health significance, failure to manage increasing populations could have negative outcomes for human health.

Methods

Study sites

The Sydney metropolitan region in New South Wales, Australia has a temperate climate with hot summers and unpredictable rainfall (mean maximum temperature 25.6°C; mean minimum temperature 18.3°C; mean annual rainfall 297.2 mm; Australian Bureau of Meteorology 2016). The population of Sydney is close to 5 million people, covers an area of ~1800 km² and has a relatively high proportion of green-space, with an average of 3 ha of parkland to every 1000 residents (Searle 2011; Australian Bureau of Statistics 2015). Sydney’s parkland provides most of the feeding and breeding habitat for urban wetland birds. We sampled T. moluccus adults from two regions within the Sydney metropolitan area. In the central business district (CBD), we sampled ibis from the Royal Botanic Gardens (33°51’51.07"S, 151°12’59.65"E) and the nearby Centennial Park (33°53’58.11"S, 151°14’12.06"E). Both CBD parks were comprised of freshwater ponds, extensive lawns, picnic facilities and gardens planted with trees and shrubs. The second study region was located at the west at Sydney Olympic Park (33°51’2.67"S, 151°4’40.44"E), which is adjacent to extensive estuarine and freshwater wetlands on the Parramatta River and includes educational gardens and picnic grounds (13 km apart from the CBD). This site was chosen due to the high abundance of mosquitoes in the area and a history of regular monitoring for arbovirus infection (Webb and Russell 1999; Webb et al. 2001; Claflin and Webb 2017).
Bird sampling

Ibis were surveyed for pathogen exposure in 2002–3 and 2015 at the CBD parks, and in 2015 at Sydney Olympic Park. Birds were sampled in summer during both years, which corresponds with historical peaks in mosquito surveillance, human cases of arbovirus infection and Salmonellosis (Russell and Dwyer 2000; D’souza et al. 2004; Crocker et al. 2017). Birds were culled from the Royal Botanic Gardens Sydney between September 2002 and June 2003 (as part of population management), and blood and faecal material were collected within 2 h of the birds being shot. Culls were conducted by trained shooters (Royal Botanic Gardens and Domain Trust licence MWL000100601) and occurred on 11 September 2002 (n = 10), 15 October 2002 (n = 1), 20 November 2002 (n = 9), 11 February 2003 (n = 1) and 24 June 2003 (n = 8). Blood was collected directly from the heart by sterile syringe, and two samples were stored: one sample in a blood clot tube on ice, prior to extraction and freezing of serum; and the other expressed onto filter paper, dried and frozen. A swab of faecal material was taken from the dissected caecum and placed in sterile Amies transport medium (Interpath Services COPAN) before samples were moved to the laboratory for Salmonella culture.

Foot-snares were used to capture adult ibis from the CBD parks and Sydney Olympic Park between March and July 2015. Snares consisted of a slip-knot tied loop of fishing line kept on the ground using small wooden pegs. Birds were fed bread to encourage them to approach the snare and step within the loop, after which the observer pulled the snare closed and fitted it with a uniquely numbered stainless-steel leg band (Australian Bird and Bat Banding Scheme) to prevent resampling of the same individual.

Arbovirus isolation

Serum from the 2015 sample of birds was taken from −80°C storage, thawed rapidly at 37°C and placed on ice. A 50-μl of each serum was inoculated in duplicate onto monolayers of C6/36 mosquito cells and incubated for 2–3 h, before removing, washing twice with sterile PBS and adding 150 μl per well RPMI supplemented with 2% foetal bovine serum (FBS). Following 7 days incubation at 28°C, two blind passages were performed (1/5 dilution), firstly on C6/36 cells and then on Vero cells. Replication of positive ssRNA and dsRNA viruses in the C6/36 cells was assessed by fixing the cell monolayers and detection of dsRNA using anti-dsRNA monoclonal antibody (mAb) 3G1 in ELISA using published protocols (O’Brien et al. 2015). The inoculated Vero cells were examined for cytopathic effect as evidence of replicating viruses.

Viral antibody response

Nineteen of the 28 samples dried on filter paper in 2003 were tested for antibodies to generic flaviviruses using an ELISA (Corner and Bagust 1993) at the Arbovirus Laboratory at Westmead Hospital, Westmead NSW. Serum was used to test for antibodies to AIV using an ELISA test (Corner and Bagust 1993), both performed at the Elizabeth Macarthur Agricultural Institute (Menangle, NSW).

All samples collected in 2015 were initially assessed for flavivirus antibodies in a blocking ELISA using the flavivirus group-reactive mAb6B6C-1 (Roehrig, Mathews and Trend 1983; Prow et al. 2013). Based on the flavivirus group ELISA test, a subset of four sera were selected and assessed for antibodies to WNV using the WNV-specific mAb1112G in a blocking ELISA (Hall et al. 1995; Blitvich et al. 2003b). In both assays, a lysate of WNVinfected C6/36 cells in lysis buffer (0.1% SDS, 1% Triton X-100 in B59 buffer (120 mM NaCl, 50 mM H3BO3, pH9.0)) was used as the coating antigen. The ability of the test sera to block the binding of mAb6B6C-1 or 3.1112 G to the WNV antigen was compared with the blocking ability of control chicken serum without antibody to WNV. Data were expressed as relative percentages and inhibition values, where >30% indicated the presence of viral antibodies (Blitvich et al. 2003a).

Microneutralisation

To determine the relative quantity of WNV antibodies in sera positive for the blocking ELISA, we performed a microneutralisation analysis. Heat-inactivated test sera were titrated in doubling dilutions from 1:10 to 1:80 in modified Eagle’s medium (DMEM) containing 2% FBS in a 96-well microtitre plate. Approximately 100 infectious units of WNV (MMR16strain) diluted in DMEM/2% FBS were added to each well (50 μl) containing 50 μl of the diluted test sera and incubated at 37°C for 1 h. Vero cells (50 μl at a density of 2× 105 cells/ml) were added and the plates incubated at 37°C/5% CO2 for 5 days. The cells were examined for CPE, fixed in situ and subjected to an ELISA using the anti-flavivirus Eprotein antibody, mAb4G2, to ascertain viral replication. As part of the neutralisation assay, a back titration was performed to determine the actual virus input and was calculated to be 372 infectious units.

Salmonella culture

For the 2002–3 samples, faecal swabs were incubated for 24 h at 37°C in Rappaports medium (10 ml, Oxoid). The medium was then streaked onto Xylose-Lysine Desoxycholate (Oxoid) and incubated for a further 24 h at 37°C. Black colonies were further characterised using an EnterotubeB (BBL, Becton Dickinson). Salmonella sp. isolates were sent to the Salmonella Reference Laboratory at the Institute of Medical and Veterinary Science, Adelaide, SA for serotyping. Faecal samples collected in 2015 were incubated for 16 h at 38°C in 10 ml of tetrathionate broth (Becton Dickinson, Wayville, SA). Tetrathionate broth was streaked onto brilliant green agar (Becton Dickinson) and incubated for a further 16 h at 38°C. Nonlactose fermenting colonies were considered to be Salmonella sp. if they agglutinated with Salmonella poly A-1 and IVserum (Diffco Laboratories, Mount Pritchard, NSW).

Statistical analysis

Variation in the prevalence of Salmonella infection between adult and juvenile birds was determined using Chi Square analysis. We tested for differences in sexes within each age group in 2015, but sample sizes were insufficient in the 2002–3 sample. Associations between viral infection rates and
population demographics/condition were not analysed statistically because only a single bird had viral antibodies.

Results
Seventy-two birds were sampled: 28 (17 adults and 11 juveniles) from the CBD botanic gardens in 2002–2003, and 44 adults in 2015 (27 from the CBD and 17 from Sydney Olympic Park).

Virus detection
Flavivirus antibodies were not detected in blood samples (n=19) of *T. moluccus* collected in 2002–3. Two *T. moluccus* samples tested positive for NDV (HI titres of 32, 8), and ten birds had a positive ELISA for antibodies reactive with AIV. Of the 44 serum samples collected in 2015, we detected flavivirus antibodies from only a single female bird from Sydney Olympic Park (Table 1). Additional testing in a WNV-specific competitive ELISA (Hall et al. 1995) indicated that this bird had seroconverted to the Australian native WNV<sub>KUN</sub> and these data were further supported by the detection of neutralising antibodies to WNV<sub>KUN</sub> (Table 1). No arboviruses could be cultured from any of the 2015 sera.

The WNV blocking ELISA has previously been shown to be highly specific for the detection of anti-WNV antibodies (Blitvich et al. 2003a). To confirm the specificity of the blocking ELISA to WNV<sub>KUN</sub>, the assay was further challenged via the assessment of equine sera known to have high levels of neutralizing antibodies to Stratford (STRV) and/or Kokobera virus (KOKV). While each of the control equine sera were positive in the generic flavivirus blocking ELISA, they were negative in the WNV blocking ELISA. This additional testing supports the determination that serum sampled from one *T. moluccus* in 2015 was positive for WNV<sub>KUN</sub> antibodies, and not positive for other closely related flaviviruses.

Salmonella detection
Seven of the 28 *T. moluccus* sampled in 2002–3 were positive for *Salmonella*. Isolates of *Salmonella enterica* serotype Typhimurium (n=2), S. Bovismorbificans (n=2), S. Virchow (n=1), S. Muenchen (n=1) and S. Sofia (n=1) were identified. None of the 44 faecal samples collected in 2015 were found to contain *Salmonella*. The positive results from 2002 to 2003 represent 25% of the total sample of birds from that year, 36% (4/11) of the juveniles and 18% (3/17) of the adults. Despite this trend towards higher *Salmonella* rates in juvenile birds, no statistically significant differences in prevalence between the ages was detected (χ² = 0.23, df = 1, P = 0.27), although statistical power was low.

Discussion
In addressing concerns that *T. moluccus* poses a significant health hazard by measuring the actual levels of common pathogens in urban populations, our surveys suggest that there was a low prevalence of viral pathogen exposure and no *Salmonella* shedding in the birds sampled during 2015. It is possible that our small sample sizes could underestimate the prevalence of antibody responses, but they are less likely to underestimate

<table>
<thead>
<tr>
<th>Table 1: Serological analysis of a subset of sera collected in 2015</th>
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<tbody>
<tr>
<td><strong>Sample ID</strong></td>
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<tr>
<td>----------------</td>
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<tr>
<td><strong>Flavi (686C-1)</strong></td>
</tr>
<tr>
<td>Ibis sera</td>
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<tr>
<td>Ibis 295</td>
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<td>Ibis 8</td>
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<td>Ibis 353</td>
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<td>Ibis 355</td>
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<td>Ibis 297</td>
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<td>Ibis 298</td>
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<td>Ibis 274</td>
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<td>Ibis 277</td>
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<td>Ibis 300</td>
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<tr>
<td>Ibis 12</td>
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<tr>
<td>Control sera</td>
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<tr>
<td>Naïve chicken</td>
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<tr>
<td>Chicken 74198</td>
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<tr>
<td>Equine 1</td>
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<tr>
<td>WNV-infected&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Equine 2</td>
</tr>
<tr>
<td>WNV-infected&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Equine T113</td>
</tr>
<tr>
<td>STRAT 80/KOKV20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Equine T124</td>
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<tr>
<td>VNT STRATV 160&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Equine T104</td>
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</table>

<sup>a</sup>Value in brackets indicates serum dilution at which partial neutralisation was observed.

<sup>b</sup>Sera from Hobson-Peters et al. (2008).

<sup>c</sup>Neutralisation values from Prow et al. (2013).

Bold values show where neutralisation was observed. West Nile Virus ELISA was not done for samples designated with ‘ND’.
the role of ibis as a flavivirus reservoir. Wildlife species that are important reservoirs have a high level of seroprevalence of flavivirus, i.e. 43% of Western Australian kangaaroos were positive for RRV, hence their status as a key reservoir (Potter et al. 2014). While key information on the host competency of T. moluccus and almost all other Australian bird species is non-existent (Boyle et al. 1983; Bingham et al. 2010), results from international research suggest that related species exhibit low to no viremia in response to WN strains (Mcintosh, Dickinson, and Mcgillivray 1969; Pérez-Ramírez et al. 2014). The current results are encouraging, suggesting that it is possible that the urban T. moluccus population in Sydney poses less of a bacterial health risk than previously assumed, and also suggests that this species may not be an important reservoir of hazardous flaviviruses at this time.

Our sampling was confined to only two parts of the Sydney metropolitan area, and although the birds at these sites might not be representative of all urban T. moluccus, the sampling sites span the range of conditions from wetland interface to inner city. While these constraints place limitations on interpretation of the results, they provide key insights and allow us to suggest future surveys to determine whether ibis have a ‘clean bill of health’.

Viral prevalence

Threskiornis moluccus showed different levels of antibody responses between years of sampling and locations within Australia. The finding that 10/100 (10%) of the captured Sydney T. moluccus tested positive for general AIV antibodies in 2003 is similar to birds sampled in Queensland in 2000 (Epstein et al. 2007). Values varied depending on the year of sampling and population measured; for instance, 0–41% of Queensland T. moluccus tested positive for AIV (n = 38–88; Epstein et al. 2007).

A single ibis in Sydney, NSW was confirmed to have an antibody response to a flavivirus in 2015 and further tests confirmed that this response was specific to WNVKUN via a specific blocking ELISA and microneutralisation test. Queensland surveys found a 1–15% generic flavivirus antibody response in T. moluccus sampled in 1997 and 2000 (n = 54–88), and a 2% antibody response to WNVKUN in 2000 (n = 88; Epstein et al. 2007). Although these results suggest that T. moluccus may be a potential reservoir of viral pathogens, more recent testing of a range of species suggests that depending on the season, year and location, it is common for rates of AIV antibody responses to vary widely between 0 and 45% (Grillo et al. 2015). In addition, the host competency of T. moluccus for any virus is unknown, as primary viremia responses have been tested in only four species of heron using experimental infection of WNVKUN in Australia (Boyle et al. 1983). This suggests that the crucial information needed to identify ibis as viral reservoirs is missing (Viana et al. 2014).

More regular wild bird surveillance and experimental virus infection would be necessary to determine the patterns and significance of viral seroprevalence in T. moluccus.

Though we did not detect WNVKUN in mosquitoes, humans or birds in Sydney in 2015, the antibody response of one T. moluccus suggests that this virus may potentially have been present in vectors and other hosts. It has been thought for many years that WNVKUN was not active along the eastern seaboard of Australia, but was endemic to north-western Australia, occasionally extending to restricted inland regions of NSW, South Australia and Victoria (Hall et al. 2002; Jansen, Ritchie, and Van Den Hurk 2013). However, there was an unusual outbreak of WNVKUN in horses in NSW and Victoria in 2011 (Prow 2013), and the virus was detected in mosquitoes in the Hawkesbury region of north west Sydney (Knope et al. 2013), signifying that though this virus is normally endemic to inland and northern Australia, outbreaks in the south east, including coastal regions, are possible (Frost 2012; Roche et al. 2013).

Understanding the potential role of T. moluccus in transmission risk of WNVKUN in coastal, particularly metropolitan, regions of Australia will require consideration of host competency, vectors and the viruses themselves. The emergence of novel strains of arboviruses needs to be considered, as recent work has shown that the 2011 strain of WNVKUN was more efficiently transmitted by the bird feeding vector mosquito, Culex annulirostris, than previous strains of the virus (Van Den Hurk et al. 2014). The prevalence of mosquito populations and habitat suitability of urban wetlands for mosquitoes and waterbirds should also be taken into account. Where this study was undertaken at Sydney Olympic Park, there are extensive wetlands that support abundant bird life and there is a long history of mosquito control and surveillance (Webb and Russell 1999; Claffin and Webb 2017). Mosquito abundance at Sydney Olympic Park has been shown to be high, and includes species of mosquitoes which are known to feed on birds and are significant hosts of several flaviviruses (Crocker et al. 2017). Also, C. annulirostris, Aedes vigilax and Ae. procax mosquitoes collected at this and nearby sites tested positive to STRV, indicating that flaviviruses were locally circulating (Crocker et al. 2017; Toi et al. 2017). Interestingly, the blood feeding preferences of mosquitoes revealed that over 71% of C. annulirostris mosquitoes fed on duck species in Sydney, compared with 0.03% which fed on T. moluccus (Jansen et al. 2009) suggesting that given a relatively similar propensity for infection and infectiveness, ducks may pose a greater potential health risk for mosquito-borne viruses than ibis. There are not yet any comparative studies of pathogen prevalence across a broad range of urban taxa at a single point in time nor is there information on the comparative viremia profiles of urban avian species in Australia; this knowledge is critical to the identification of the relative risk among waterbirds. There is also a need to consider the ‘dilution effect’, in which the relative abundance and diversity of wildlife with either a low or high capacity to infect suitable mosquito vectors may influence the potential for transmission (Swaddle and Calos 2008). It is possible that increased biodiversity in urban wetlands may reduce the relative importance of key, highly competent and reservoir hosts.

To fully understand the risk of birds transporting viruses, information on movement patterns is necessary. Wide-ranging individuals may transport pathogens to urban parks, while sedentary birds may only amplify local pathogen levels. Urban T. moluccus may not be as nomadic as in-land birds, but they do travel regionally (Martin, French, and Major 2012), with 45% travelling more than 10 km a year, and 13% of individuals recorded travelling longer distances of up to 50 km (Martin et al. 2011). To pose an elevated risk of WNVKUN introduction, infected birds will need to move from regions of endemic, or epidemic, activity into metropolitan regions while still infective, as an antibody response can develop within 4 days, after which the birds will no longer pose a risk of infecting mosquitoes that bite them (Boyle et al. 1983). While T. moluccus may be able to disperse pathogens over moderate distances of <50 km, tracking studies targeting migratory Australian, African and Asian duck, goose and swan species suggested these birds had the potential to move pathogens such as AIV more than 500 km during the time-frame of infection (Rosshier, Klomp, and Asmus 2006; Rosshier, Asmus, and Klaassen 2008; Gaidet et al. 2010; Martin...
et al. 2011). The relative risk presented by urban T. moluccus is therefore potentially smaller than the risk represented by more nomadic ducks or other urban waterbirds. Monitoring is necessary to provide a robust analysis of the viral disease epidemiology of urban T. moluccus in Sydney, but our study suggests they are not an important reservoir of flaviviruses at this time.

**Salmonella prevalence**

Salmonella cultured from Sydney’s adult bird samples was clearly less common in 2015 than in the past. In 2015, we found no evidence of Salmonella shedding in the faeces of 44 adults sampled at two locations. In contrast, T. moluccus culled in 2002–3 at one site exhibited a 25% prevalence of Salmonella shedding based on the culture of swabs from the dissected caecum. While it is possible that sampling gut contents rather than frozen faeces resulted in a higher Salmonella detection rate, it is also possible that testing a higher proportion of young birds in 2002–3 will have increased the detection rate, as the juvenile birds in our survey exhibited double the colonisation level of adults. If the later was true, this would confirm previous observations that juvenile birds are often more susceptible to bacterial infections than adults (Benskin et al. 2009). Larger numbers of samples collected from both young and adults using fresh samples from multiple sites would remove these possible confounding factors in future surveys.

The differences in Salmonella prevalence between the two sampling periods may also be influenced by a sampling bias, as birds were randomly culled from a single park in 2002–3 and trapped using bait at two park sites in 2015. Up to 68% of birds in the population of the Sydney region have been observed to forage at landfills where the risk of Salmonella infection was assumed to be higher (Martin et al. 2011). Birds caught at baits in Sydney Olympic Park and Centennial Park in 2015 may represent birds which are less likely to forage at landfills (~40% of ibis monitored at Centennial Park were only observed foraging at parks; Martin et al. 2011). The random sample of Sydney Botanic Gardens Park birds culled in 2002–3 may have been more likely to sample both birds that visit landfills as well as birds which do not, and therefore represents a less biased sampling method for the detection of Salmonella colonisation. This hypothesis is supported by a recent survey of Silver Gull (Chroicocephalus novaehollandiae) colonies in southeastern Australia which found a very low proportion (0.6–6%) of chicks shedding Salmonella in Sydney and the South Coast where there are no nearby landfills, and a 28% shedding rate in chicks near Wollongong, where parents were feeding at a waste landfill (Dolejska et al. 2015). However, in the USA, White Ibis (Eudocimus albus), were found to have higher levels of Salmonella colonisation in urban habitats than natural wetlands, most likely due to a range of exposure routes other than simple food contamination (Hernandez et al. 2016). Although birds that feed on waste from landfills, water treatment facilities and agricultural production sites are more likely to be exposed to bacterial pathogens (Benskin et al. 2009; Murray and Hamilton 2010), all urban waterbirds are likely to be exposed to bacterial pathogens in environmental food or water. Land managers should not, therefore, target specific taxa for risk management unless empirical evidence suggests they are the most likely reservoir of a particular pathogen.

Both diet and bird age may be important in determining whether urban T. moluccus are reservoirs for zoonotic bacterial disease (Tizard 2004). The spatial and temporal differences in the pathogen prevalence in urban wetland birds highlights the need for more extensive sampling to better determine if Salmonella colonisation in T. moluccus has truly declined over time due to changes in wetland or waste management in Sydney. Our analyses, although non-significant, had low power for detecting age-related differences in infection and thus, increasing the sample size of juvenile birds should be a high priority for future research.

**Conclusions**

Our study does not support the perception that ibis pose a health risk to humans in Sydney (Australia). With regard to Salmonellosis, the risk is relatively low as ibis carry only low levels of Salmonella compared with more charismatic urban ducks and gulls which often have a higher prevalence of Salmonella infection (Mitchell and Ridgwell 1971; Benskin et al. 2009). For mosquito-borne diseases, our results support the previous findings of low levels of virus exposure in surveys of T. moluccus (Epstein et al. 2007), which suggest that this species is unlikely to represent a significant reservoir of zoonotic viral diseases compared with other urban wildlife species. Given the nuisance and perceived health threats associated with urban T. moluccus, populations are increasingly subjected to lethal management (culling through egg and nest destruction; Ecosure 2018), and it is possible that improper management of urban populations could be deleterious to the national status of this native wetland species (Martin, French, and Major 2007). While urban populations of T. moluccus have increased, those of non-urban regions have fallen to the point where these populations are now small (Bino, Steinfeld, and Kingsford 2014; Martin 2016). No urban nesting sites are fully protected from lethal management, and up to 50% of nests can be destroyed or eggs oiled, even within designated ‘refuge’ colonies (Camden Council 2013; McKiernan and Instone 2016; Ecosure 2018). Continued public demands for T. moluccus control stem from the perception that these birds are a danger to aircraft, noisy, filthy, malodorous, destructive and pose a high health hazard to humans and water quality when roosting and nesting in cities (Ecosure 2018). This study demonstrates that previous arguments concerning the disease risk of this species are not well founded and should not be used as a basis for management.

**Data availability**

The data used in this manuscript can be accessed within the Advanced Ecological Knowledge and Observation System (eEKOS) Data Portal using SHaRED digital object identifier doi:10.25901/5ca9e7e5a8b9.

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