

# Identifying and managing pathways of AI transmission by wild birds on meat chicken farms in eastern Australia

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## Research article

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# Abstract

Conjecture surrounds the reservoir and bridge species potentially involved in introducing, maintaining and transmitting avian influenza (AI) on Australian meat chicken farms. This is mainly due to a lack of AI prevalence data and in-depth understanding of wild bird ecology on farms and across regions. For risk assessment purposes, we identified candidate species likely to be involved in AI maintenance and transmission during 68 bird surveys conducted across ten commercial meat chicken farms from Winter 2016 to Summer 2018 in southeastern Queensland. Using an AI-risk classification processes described in this paper, we speculate that 57 of the 139 species recorded in and around production facilities and nearby water bodies pose a medium to high risk, particularly on free range farms. On the farms with permanent waterbodies, resident and semi-resident dabbling ducks (Genus *Anas*) could maintain AI indefinitely on-site, creating opportunities for these species and several bridge species to potentially infect poultry by being a vector between the dam/water habitat and poultry facilities. We suggest that other types of wild birds that may be involved in AI transmission including nomadic waterfowl, grazing ducks (Australian Wood Ducks and Plumed Whistling-duck), Waterhens, Lapwings, resident scavengers (corvids, ibis), birds of prey and mud nests builders (e.g. Fairy Martins). Disrupting AI maintenance cycles on farm dams would reduce the chances of transmission of environmental AI by potential bridge species. This may be achievable by proactively preventing higher risk waterfowl becoming resident and habituated on farms and deterring potential bridge species from accessing poultry houses. Targeted AI surveillance of suspected bridge species is required to determine the real risk. Ideally, this should occur on non-commercial poultry farms where AI transmission to poultry can be objectively investigated.

## Introduction

Of all commercial poultry diseases, avian influenza (AI) is the most feared in poultry-producing countries. The 2014–2015 highly pathogenic outbreak in the United States resulted in the loss of nearly 50 million birds—the largest animal health disaster in the country’s history—and is a timely reminder how vulnerable poultry producers are, even in the most developed countries.

AI ecology and epidemiology is complex. Wild birds, particularly waterfowl and shorebirds, maintain and spread low pathogenicity avian influenza (LPAI) viruses naturally in the environment. Transmission of LPAI to naïve domestic birds can be detrimental. Domestic poultry are particularly vulnerable to subtype H5 and H7 LPAI strains. These strains readily mutate and cause high pathogenicity avian influenza (HPAI) outbreaks in commercial poultry flocks, leading to high mortalities and socioeconomic impacts [1].

The unforeseen and unprecedented spread of HPAI subtypes by migratory birds through Asia, Eastern Europe, Africa and North America over the past two decades has resulted in an increased frequency of HPAI outbreaks worldwide. The magnitude of some outbreaks has significantly impacted poultry production, trade and prices in several countries [2]. Furthermore, AI has become a public health concern following human fatalities from H5N1 and H7N9 strains [3, 4].

Australia is a relatively isolated island continent and not a destination for migratory Northern Hemisphere waterfowl. Australia's native waterfowl are non-migratory and their nomadic movements are generally restricted to the Australia–Papua region. All seven Australian HPAI outbreaks have been traced to endemic H7 subtypes. Therefore, Australia's experiences with AI and HPAI outbreaks in commercial poultry are atypical in the overall global picture. Australia also has a rich, diverse avifauna of around 780 species, excluding vagrants [5]. Around a third are potentially found in poultry production regions, including most of the waterfowl (Anseriformes), of which dabbling ducks (genus *Anas*) are considered the main reservoir of AI [6, 7].

Australia is also the driest inhabited continent. Unpredictable, erratic rainfall is normal over much of Australia's interior and prolonged, widespread droughts can result. Consequently, many native species are nomadic, especially waterbirds. Several species only breed when inland conditions are favourable following floods [8, 9].

The importance of dabbling ducks (genus *Anas*) in maintenance and transmission of AI on poultry farms is debatable. Due to the low incidence of HPAI in Australia, relatively few monitoring studies have been conducted to understand the ecology and epidemiology of Australian avian influenza viruses (AIVs) [10]. Research indicates migratory and endemic Australian species are both likely to be involved, and that the low prevalence in the usual reservoir species (e.g. dabbling ducks) may not be indicative of the prevalence and role other species play in Australia [11, 7].

To improve poultry farm biosecurity, awareness and a better understanding is required of the AI transmission pathways that wild birds initiate and create on poultry farms. This involves identifying the species, behaviours and conditions that create potential pathways. In the context of a farm, this can be assessed firstly by the risk posed by the presence of natural AI reservoirs (ducks, geese, swans, gulls, terns and waders); secondly, by the risk posed by the presence of other aquatic bird species; and thirdly, by the risk posed by bridge species (non-aquatic bird species that utilise both waterbird habitats and poultry production facilities). This knowledge will assist in improving the risk assessment and modelling of patterns of influenza virus in wild birds [12–14].

The challenges in assessing and managing the AI risk posed by the presence of wild birds on commercial poultry farms are two-fold. Firstly, no AI testing of wild birds is conducted on commercial poultry farms primarily because of the ramifications should notifiable (H5 and H7) LPAI or HPAI strains be found. Secondly, the National Wild Bird AI Surveillance Program has targeted the main natural reservoirs (Anseriformes and Charadriiformes) to stay abreast of the strains circulating in the wild [15]. The inherent assumptions are that any strains in non-reservoir species will be detected in reservoir species, and will be phylogenetically the same.

Consequently, prevalence data for those species considered to be non-reservoir species is non-existent or too small a sample to be statistically meaningful. Therefore, the potential role of other species as reservoirs, maintenance hosts (in the absence of dabbling ducks), intermediate hosts (by association with reservoir species), bridge species (these being the more likely vector to commercial poultry flocks) or

combinations thereof needs to be investigated. This should be done judiciously, by first narrowing down the field based on their ecology, on-farm behaviours and perceived risk.

Apart from the Anatids, the species involved in AI ecology and epidemiology in poultry production areas in Australia remains largely unknown. Wild bird studies on Australian poultry farms have only occurred in recent years [16], and that particular study only used camera traps for a short period without the benefit of manual surveys to augment the interpretation of the data and bird behaviour. Experts have estimated the probability of LPAI introduction to free range and conventional meat chicken and layer flocks by contact with wild birds directly and indirectly [17] without the benefit of objective data.

This study aimed to identify species of wild birds that could be instrumental in introducing, maintaining and transmitting AI to meat chicken flocks, in three biogeographically different poultry production regions in eastern Australia. We also aimed to identify how AI pathways are enabled. The implications, and ways of improving biosecurity, are discussed.

## Materials And Methods

### Sites and wild bird surveys

Bird surveys were conducted by the senior author (40 years birding experience) on 10 meat chicken farms (Table 1) in southeastern Queensland, from the Australian Winter of 2016 through to the Summer of 2018. The farms were representative free range and conventional (fully housed) farms. Seven of the farms were surveyed seasonally in parallel with camera trap surveillance undertaken by Fielder et al. [18]. The other three were surveyed opportunistically. Five sites had farm dams within 200 m of poultry houses. The single dam at the Somerset region site was small, ephemeral, and dry throughout the study. The other dams were larger and always contained water.

Table 1  
General details of meat chicken farms used for survey sites

| Type         | Region                   | n= | Farm locations  |
|--------------|--------------------------|----|---|
| Conventional | Coastal SEQ <sup>a</sup> | 4  | Southern Moreton Bay  |
|              | Non-coastal SEQ          | 3  | Lockyer Valley and Scenic Rim Region (east of Great Dividing Range) |
| Free range   | Coastal SEQ              | 1  | Southern Moreton Bay  |
|              | Non-coastal SEQ          | 2  | Logan and Somerset regions  |
|              | Southern Downs           | 1  | Southern Darling Downs (west of Great Dividing Range)               |

<sup>a</sup>SEQ stands for southeastern Queensland

A database (Excel spreadsheet) of species was used to record sightings. Surveys were conducted seasonally on all farms (usually on the same days camera traps were checked), and consisted of a walk around the farm noting the species and the situation they were seen in (e.g. on range areas, on/in poultry houses, hawking over production areas, flying over, around silo, on access roads, on dam, around dam, hawking over dam, in vegetation on the range, in adjacent ecotone, at temporary surface water on the range). If unusual behaviour was observed, photographs were taken for the record wherever possible. Bird abundance was also recorded, but not analysed because of the differences in time of visit, type of farm and size of farm, and in the case of the free range farms, the inability to observe inside the poultry houses on most farms during a grow-out.

### AI risk classification

Each observed species was classified as high, medium or low AI risk. These rankings are based on the criteria used for H5N1 risk assessments in Europe [19], and expanded to accommodate additional criteria for Australian studies. Scores for water habitat usage / feeding style (0–3), gregariousness (0–3), inter-species mixing (0–3), range usage (0–3), silo usage (0–1), poultry house usage (0–1), poultry house nesting (0–1), and mud-nesting (0–1) were summed. Species with a total score of 7 and above were considered high risk, those with scores of 5 or 6 were considered medium risk, and the remainder low risk.

## Statistical analysis

At each survey (farm/date), each species was recorded as present (observed) or not. These binary observations were subjected to a generalised linear model [20] under the Binomial distribution and logit link, using Genstat [21]. Adjusted mean proportions, and their standard errors, were estimated. The factors analysed were –

Region, as listed in Table 1

Year/Season combined. Initial analyses showed interactions between years and seasons, i.e. the seasonal effect was inconsistent over the years. Hence a pooled ‘continuous-time’ year/season factor was adopted.

Farm type – conventional (fully housed) or free range.

A significance level of 5% ( $P < 0.05$ ) has been adopted throughout, as indicated by a \* in the tables.

## Results

A total of 139 avian species were recorded across all sites during the survey period (See Additional files 1 and 2). Assuming that any species could be infectious then, based on observed behaviours, around 40% of these species could be deemed medium to high risk reservoir species (introduction risk), bridge species (transmission risk to poultry) or both (See Additional file 3).

The higher risk species were generally dabbling ducks and those species that feed in the shallows and around the margins of the dam. They included terrestrial species that also visit poultry production facilities routinely. The medium risk species were other waterbird species and terrestrial species that would only occasionally come into contact with reservoir species or waterbird habitat. Where there was doubt, it was assumed a species was a medium or high risk if there was prevalence data for related species. For example, overseas AI prevalence data was used as a basis for increasing the ranking of pipits and bushlarks. All other observed species were considered low-risk.

Significant interactions between the design factors occurred only intermittently and at about the rate expected from random chance. Hence all interactions were omitted from the final models, and the results focus on the main-effects.

Table 2 shows the mean number of high, medium and low risk species observed during each survey by region, year–season and type of farm. The Southern Downs site was the most frequented by high and medium risk species, followed by the Coastal SEQ farms and Non-Coastal SEQ farms. There was an increase in the higher and medium risk species in Spring 2017 and Summer 2018. We suggest that this was possibly due to the drought over much of inland Australia during that period.

Table 2  
Mean numbers of species observed during each survey, by AI-risk.

|                 | High-risk species |      | Medium-risk species |      | Low-risk species |      |
|-----------------|-------------------|------|---------------------|------|------------------|------|
|                 | Mean              | s.e. | Mean                | s.e. | Mean             | s.e. |
| Region          |                   |      |                     |      |                  |      |
| Coastal SEQ     | 4.46              | 0.44 | 5.00                | 0.48 | 5.17             | 0.48 |
| Non-coastal SEQ | 1.96              | 0.25 | 3.84                | 0.37 | 7.28             | 0.52 |
| Southern Downs  | 7.89              | 0.91 | 8.54                | 0.94 | 8.50             | 0.93 |
| Year-season     |                   |      |                     |      |                  |      |
| 2016 Winter     | 4.25              | 1.03 | 3.50                | 0.94 | 2.75             | 0.83 |
| 2016 Spring     | 2.50              | 0.48 | 3.75                | 0.57 | 5.54             | 0.63 |
| 2016-17 Summer  | 3.07              | 0.48 | 4.16                | 0.58 | 5.64             | 0.74 |
| 2017 Autumn     | 3.64              | 0.51 | 4.76                | 0.60 | 5.88             | 0.64 |
| 2017 Winter     | 3.87              | 0.59 | 5.43                | 0.75 | 8.67             | 0.96 |
| 2017 Spring     | 5.87              | 0.85 | 6.14                | 0.87 | 7.16             | 0.92 |
| 2017-18 Summer  | 4.33              | 0.77 | 6.30                | 0.98 | 7.61             | 1.13 |
| Farm type       |                   |      |                     |      |                  |      |
| Fully housed    | 3.79              | 0.33 | 4.06                | 0.34 | 6.09             | 0.41 |
| Free range      | 3.36              | 0.36 | 5.45                | 0.48 | 6.73             | 0.53 |

None of the farm dams surveyed supported high waterbird diversity, or significant numbers of any particular species, despite the prevailing drought conditions. Therefore, the data are likely indicative of normal conditions as well.

The chance of at least one high-risk species being present was significantly lower for Non-Coastal SEQ farms (64.3%) than Coastal SEQ farms (94.4%) and the Southern Downs (100%) whereas medium and low-risk species were almost always present ( $\approx 100\%$ ) during each survey.

These results are a reflection of the size and suitability of the water habitats. Collectively, the Coastal SEQ waterbird habitats were larger in area and more permanent so they had resident high-risk species such as Pacific Black Ducks, whereas some Non-Coastal SEQ farms did not. The Southern Downs site's dam was large and maintained at a level that favoured dabbling ducks year-round.

Major avian taxa on farms

The most-frequently occurring avian taxa (Families) represented on SEQ poultry farms were analysed by regions (Table 3), with some of these known to contain one or more species considered to potentially be AI hosts. The regional differences are largely attributable to the size and suitability of farm dams for a range of aquatic species, and the extent and proximity of native vegetation around the production areas. The inland farms happened to be located in more rural areas and were flanked by relatively undisturbed native vegetation whereas the coastal farms were smaller and mainly surrounded by paddocks and degraded remnant bushland.

Table 3  
Percent presence for the most-frequently occurring families, by regions

| Family   | Coastal SEQ | Non-coastal SEQ | Southern Downs | Avg s.e. | Sig.<br>(P < 0.05) |
|--|-------------|-----------------|----------------|----------|--------------------|
| Acanthizidae   | 4.1         | 51.9            | 49.8           | 9.7      | *                  |
| Accipitridae   | 71.2        | 56.2            | 83.1           | 9.9      |                    |
| Alcedinidae  | 36.6        | 38.4            | 37.4           | 11.0     |                    |
| Anatidae <sup>§</sup>  | 74.9        | 48.8            | 91.3           | 9.0      | *                  |
| Ardeidae   | 78.8        | 19.2            | 72.8           | 9.5      | *                  |
| Artamidae  | 52.9        | 70.6            | 100.0          | 5.7      | *                  |
| Cacatuidae   | 17.9        | 18.6            | 72.3           | 9.0      | *                  |
| Charadriidae <sup>§</sup>  | 68.0        | 7.4             | 84.9           | 7.3      | *                  |
| Columbidae   | 43.4        | 46.4            | 71.2           | 11.0     |                    |
| Corvidae <sup>§</sup>  | 37.4        | 50.9            | 100.0          | 6.5      | *                  |
| Estrildidae  | 17.7        | 25.8            | 37.8           | 10.4     |                    |
| Hirundinidae <sup>§</sup>  | 86.2        | 42.7            | 100.0          | 5.1      | *                  |
| Maluridae  | 39.1        | 44.2            | 38.1           | 10.9     |                    |
| Meliphagidae   | 44.5        | 68.2            | 38.7           | 11.2     |                    |
| Monarchidae  | 65.5        | 47.8            | 81.4           | 10.4     |                    |
| Pachycephalidae  | 25.9        | 26.1            | 27.1           | 9.9      |                    |
| Pardalotidae   | 27.3        | 51.4            | 39.0           | 9.9      |                    |
| Phalacrocoracidae  | 23.0        | 22.0            | 57.1           | 9.8      |                    |
| Psittacidae  | 32.6        | 36.9            | 29.9           | 10.4     |                    |
| Rallidae   | 64.1        | 37.5            | 44.8           | 11.4     |                    |
| Rhipiduridae   | 60.1        | 49.0            | 83.5           | 9.8      |                    |
| Sturnidae <sup>§</sup>   | 2.8         | 7.7             | 100.0          | 2.2      | *                  |
| Threskiornithidae <sup>§</sup>                                       | 54.0        | 22.3            | 66.2           | 7.2      | *                  |
| § contains one or more species considered to potentially be AI hosts |             |                 |                |          |                    |

Table 4 lists the most-frequently occurring species represented on poultry farms by regions. Apart from Cattle Egret, which was closely associated with cattle present at the SEQ farms, the significant waterbirds reflect the differences in dam suitability. The smaller the dam, the less resources, and the more chance for an observer missing those species whose daily routines and movements extend beyond the farm. Likewise for the production areas. The Southern Downs site had the largest production area and took the longest time to survey, which increased the chances of recording more species during each visit. Being a free range farm, it also suited more species and larger populations each day, including mynas and starlings (Sturnidae).

Table 4

Percent presence for the most-frequently occurring species (observed on 19 or more of the 68 surveys), by regions

| <b>Species</b>  | <b>Coastal SEQ</b> | <b>Non-coastal SEQ</b> | <b>Southern Downs</b> | <b>Avg s.e.</b> | <b>Sig. (P &lt; 0.05)</b> |
|---|--------------------|------------------------|-----------------------|-----------------|---------------------------|
| Australasian Grebe  | 34.8               | 30.7                   | 75.5                  | 9.1             | *                         |
| Australian Magpie   | 35.1               | 64.4                   | 100.0                 | 5.7             | *                         |
| Australian Wood Duck  | 22.3               | 38.8                   | 52.1                  | 10.9            |                           |
| Cattle Egret  | 72.0               | 14.9                   | 0.0                   | 4.9             | *                         |
| Dusky Moorhen   | 64.7               | 13.5                   | 32.3                  | 10.1            | *                         |
| Magpie-lark   | 61.7               | 42.4                   | 82.6                  | 10.2            |                           |
| Masked Lapwing  | 69.2               | 7.4                    | 63.3                  | 8.0             | *                         |
| Noisy Miner   | 35.4               | 37.4                   | 8.4                   | 9.2             |                           |
| Pacific Black Duck  | 51.1               | 31.4                   | 86.9                  | 9.6             | *                         |
| Peaceful Dove   | 25.4               | 25.7                   | 58.0                  | 10.9            |                           |
| Straw-necked Ibis   | 36.8               | 21.4                   | 25.5                  | 9.0             |                           |
| Striated Pardalote  | 24.1               | 51.5                   | 38.5                  | 10.0            |                           |
| Welcome Swallow   | 81.8               | 15.6                   | 94.4                  | 6.1             | *                         |
| Whistling Kite  | 41.9               | 28.6                   | 77.6                  | 10.1            | *                         |
| Willie Wagtail  | 61.3               | 37.4                   | 83.1                  | 10.0            | *                         |
| Additional Files  |                    |                        |                       |                 |                           |
| Additional File 1:  |                    |                        |                       |                 |                           |
| Appendix A. Percent presence by regions, for species observed between five and 18 times overall |                    |                        |                       |                 |                           |
| - Survey data of the most frequently observed wild birds  |                    |                        |                       |                 |                           |
| Additional File 2:  |                    |                        |                       |                 |                           |
| Appendix B. Counts of species recorded less than 5 times  |                    |                        |                       |                 |                           |
| - Survey data of infrequently observed wild birds   |                    |                        |                       |                 |                           |
| Additional File 3:  |                    |                        |                       |                 |                           |
| Appendix C. Potential medium to high risk species for AI transmission on SEQ poultry farms      |                    |                        |                       |                 |                           |

| Species  | Coastal<br>SEQ | Non-coastal<br>SEQ | Southern<br>Downs | Avg s.e. | Sig.<br>(P < 0.05) |
|--|----------------|--------------------|-------------------|----------|--------------------|
| - Wild bird species deemed to be medium to high risk of AI transmission based on their observed behaviours |                |                    |                   |          |                    |

Main species associating with poultry and poultry production facilities

The following 12 species were regularly seen around and, in the case of free range farms, inside the poultry houses in one or more regions: Magpie-lark, Willie Wagtail, Australian Magpie, Australian Raven, White-winged Chough, Apostlebird, Australasian Pipit, House Sparrow, Welcome Swallow, Fairy Martin, Common Starling, Common Myna. Except for Fairy Martins and Welcome Swallows, all these species were observed feeding among free range poultry on the range, and entering poultry houses to feed, drink or seek shelter. Four of these species were observed at farm dams. It is possible others do likewise (to drink) and may become infectious. Australian Magpie was observed feeding on Darkling Beetle larvae between grow-outs on a free range farm. It is possible this also happened during the grow-out. Purple Swamphens were also seen in a poultry house between grow-outs and on the range at this farm. Willie Wagtail was a common sight on free range farms and considered a risk because of its penchant for visiting water habitats.

Year/season affects

The trends, or lack of, reflect Australian waterbird dynamics under extreme weather conditions. We experienced drought punctuated with a cyclone. The main goal was to establish the overall species assemblages in different regions, and use ecological knowledge of each species to determine their risk throughout the year.

## Discussion

Many of the species observed are widespread, so the results are applicable to other poultry production regions, particularly in eastern Australia. Farm type, water habitat suitability and biogeographic location largely govern the avian composition and likely reservoir and bridge species involved.

Risk classification for most species is subjective and will remain so until evidenced by AI prevalence data. There is no annual migration of waterfowl to Australia, and there is little interaction between other migratory species and poultry based on our observations. The risk is more likely to involve nomadic waterfowl and resident bridge species. Therefore, transmission pathways to poultry are of more interest than the risk from individual species in isolation.

AI maintenance on farm

Waterfowl

Dabbling ducks, specifically, Pacific Black Duck, were observed every survey at some farms. This species is adaptable and is closely related to the high AI-risk Mallard. It is therefore likely to be a key reservoir in eastern Australia. Preventing this species taking up permanent residency on farms could be a key strategy to routinely disrupting AI maintenance and therefore persistence in the farm environment throughout the year. Reducing dabbling duck numbers prior to predicted influxes is also strategic. Otherwise, the AI risk is exacerbated when nomadic species such as Grey Teal take up temporary residence, potentially introducing new AIVs, and boosting the reserves for AI maintenance.

Dabbling ducks were only observed amongst poultry once. It followed heavy rainfall on the southern Downs. Grey Teal were seen alighting amongst poultry in temporary surface water. The perceived danger is excretion of virus on the range. Pacific Black Duck were seen standing on access roads and would be attracted to spilt feed like grazing ducks are.

Unlike the Northern Hemisphere where the AI season is tied to migratory waterfowl movements, there is no clear season in Australia where waterfowl are largely nomadic. The availability of suitable waterbird habitats dictates the distribution of waterbirds and therefore concentrations of AIVs in the Australian landscape and AI prevalence in each species. The AI cycle is naturally broken far more often inland than on the coast because of the drier conditions and periodic droughts. It follows that maintenance occurs at permanent water bodies.

In Australia, prevalence data for rallids and jacanids is limited or non-existent yet species such as Dusky Moorhen, Eurasian Coot and Comb-crested Jacana are often on wetlands and dams in poultry production areas. These families may play a role in maintaining AIV in southern Africa, where they occur year-round in high abundances in many wetlands and were frequently observed foraging near dabbling and diving ducks.

Cormorants and darters (Phalacrocoridae) were often present at our study sites. They are mobile and were frequently seen roosting with ducks. Straw-necked and Australian White Ibis (Threskiornithidae) also share foraging habitats with grazing and dabbling ducks. Australian White Ibis may feed on carcasses, making them potentially vulnerable during AIV epizootics in locations (e.g. unmonitored lakes) where carcass removal is not rapid.

#### AI transmission by bridge species

AI prevalence and transmission data has not been reported for Australian poultry farms, and sampling of non-Anseriformes and non-Charadriiformes has been limited [22]. Hypothetically, AI prevalence and transmission in Australia is perhaps best gauged from countries at similar latitudes such as Africa and South America (rather than using Northern Hemisphere data).

In South Africa, overall viral prevalence was at the lower range of European values, but there was marked spatial and temporal variation, and there was no indication that prevalence was influenced by Palearctic migrants [23]. While agreeing that waterbirds appear to be the primary reservoir, they suggest passerines

may link wild birds and poultry, which would partly explain how viral transmission in South Africa can occur throughout the year.

Cumming et al [23] suggest the lack of a predictable spike in prevalence is due to the relatively stochastic nature of southern African seasonality and the nomadic behaviour of southern African ducks. This reflects the current thinking in Australia [24, 11], particularly if AI epidemiology is tied in with nomadic movements of Grey Teal and other non-seasonal dabbling ducks.

Plumed Whistling-duck is a grazing species that is a maintenance and likely bridge species. This species can congregate in huge numbers, camping on the edge of waterbodies during the day and feeding at night. Prevalence of AIVs in southern Africa appears to be twice as high in dendrocygnid (whistling) ducks (5.2%) as in anatid ducks (2.4%).

#### Mud-nesting species

Worldwide, very few species of bird build mud nests. Remarkably, Australia has five species that do, namely, Magpie-lark, White-winged Chough, Apostlebird, Fairy Martin and Welcome Swallow. All five species were recorded interacting with free range poultry on the southern Downs. Magpie-larks, Apostlebirds and White-winged choughs were observed feeding among poultry on the range and the Apostlebirds were feeding from the feeders inside a poultry house. Welcome Swallows were observed roosting in free range farm poultry houses. Fairy martins were nesting under the eaves of poultry houses in two regions.

Collecting mud from waterbird habitats during nest construction and maintenance could lead to oral intake of environmental AIVs in these species. Swallows have tested positive for LPAI overseas [25]. Until proven otherwise, it is prudent to treat mud-nesting species as potential bridge species, especially during the nesting season and during drought, when waterbirds are concentrated and the mud supply is limited to the edge of water habitats where it is more than likely contaminated.

Magpie-larks is a possible maintenance species and arguably the most likely bridge species on any farm with a waterbody because of its routines of walking and feeding around the edge of dams and visiting ranges. The mechanical transfer of AIVs shed by waterfowl is also a possibility. Magpie-larks nest opportunistically any time of year so may be infectious any time of year whereas other mud-nesting species are more predictable.

Fairy Martins and Welcome Swallows also breed when conditions suit but mainly from July through December. In the case of Fairy Martins, a colonial nester, active bottle-shaped mud nests were seen above vent openings that could potentially enable their droppings to fall through. Fairy Martins were seen building nests under the eaves of poultry houses and other structures. Old nests of welcome swallows and fairy martins were seen at other farms. Welcome Swallows were observed roosting above the entrances to poultry houses and in free range farm poultry houses.

White-winged choughs and Apostlebirds are gregarious and form family groups of 5–20 birds. Non-breeding members participate in nest-building (a large domed mud nest). Breeding season depends on prevailing conditions but ranges from July through to February. Mud may be sourced from water habitats potentially infected with AIVs, so both species are candidate bridge species, and every family member is potentially infected during the breeding season.

## Passerines

Passerines were common around free range facilities but few species were observed utilising farm dams during this study. Cumming et al [23] observed a prevalence of 4.5% in South African passerines, most of which were from resident species, suggesting a potential role by them in influenza epidemiology. Their data indicate some passeriform families (e.g. Alaudidae, which is represented in our poultry farms in Australia) may contribute to the persistence and spread of AIV. From the perspective of both humans and poultry, AIV transmission by wild birds appears to be possible at any time of the year. The opportunistic behavioural responses of waterbird populations to environmental drivers, for example rainfall, may make it possible to obtain short-term predictions of AIV risks using rainfall data or forecasts.

We agree with Flint et al [26] that active surveillance of live wild birds is likely the best way to determine the true distribution of AIVs. Focus should be on regions with the greatest risk for poultry losses, and where waterfowl and bridge species are in the vicinity of poultry operations. Surveillance would address the mechanism(s) of virus transfer and provide data to improve biosecurity management.

## Other bridge species

Some reservoir species may also be AI transmitters to poultry. Observations on southeastern Queensland farms suggest dabbling ducks (Genus *Anas*) are unlikely to visit production areas unless the ground is waterlogged, bringing food to the surface, or there is feed spilled around the silos. In either case, they would not remain there after feeding, retreating to the safety of the dam. They are therefore less likely to transmit AIVs than grazing ducks, such as Australian Wood Duck, which feed, roam and rest for long periods in production areas. There are several other species that were observed utilising farm dams as well as silos and ranges, leading us to concur with other researchers [26–28] that bridge species are more likely to facilitate the transfer of AIVs.

Potential bridge species may include pest birds, rodents, and/or invertebrates [29, 16]. Common Starlings (*Sturnus vulgaris*) and Rock Doves (*Columba livia*) are two potential avian bridge species for HPAI in North America. In our study, both species were more prominent on free range farms, particularly the Southern Downs site. Starlings were often inside the poultry houses and Rock Doves around the silos. However, neither species was observed at waterbird habitats during this study so they were ranked low risk for AI. Songbirds and rodents were unlikely bridge hosts in the 2015 United States outbreak [30, 31], but the role their Australian counterparts play has not been established.

## Breaking transmission pathways to enhance farm biosecurity

Identification of pathways by which LPAI and HPAI can move from waterfowl and waterfowl habitat to commercial poultry holdings can be used to enhance and actively target biosecurity. Knowing which species serve as potential bridge species at any given time of year, and understanding the factors that could align to enable AI transmission, will aid development of risk-based deterrent and control strategies. In order to minimise the risk, knowledge of abiotic factors affecting AIV persistence in the environment is also important [32, 33].

Although Anseriformes and Charadriiformes are the main natural reservoirs, higher than expected prevalence has been reported in other families, including Passeriformes. For example, in South Africa, researchers detected AI in 4.5% of passerines sampled [23]. Wild bird species that enter poultry barns may carry AIVs directly into the poultry flock, while others spending time near poultry may contaminate surfaces near barns, increasing the potential for AIVs to be carried into poultry houses and range areas by farm workers, equipment, pets, rodents or insect pests.

## Conclusions

AI ecology and epidemiology on meat chicken farms will remain a matter of conjecture until targeted surveillance of potential bridge species occurs; however, knowing which species utilise farms and range areas is necessary for identifying the likely pathways. The case for testing several potential bridge species in southeastern Queensland has been made based on field observations of wild bird behaviour. There are different bridge species for different regions and AI surveillance programs should incorporate these species. Once bridge species are identified, farmers can be given assistance to identify transmission pathways, for AI and other diseases, to their poultry. Identifying these pathways may be difficult because they can be obscure and change over time. Effective farm-specific biosecurity programs should be aimed at disrupting or dismantling the pathways or reducing the AI maintenance in the first place.

Manual surveys revealed species, behaviours and interactions on poultry farms that camera traps have missed, regardless of the length of time they are deployed for. Therefore, reliance on camera traps alone is insufficient to identify the potential AI risk pathways on farms. Conversely, short seasonal surveys are not practical for capturing certain cause-and-effect events and understanding time-budgets. Combined surveys offer a powerful tool for illuminating potential AI transmission pathways and for improving risk assessments. We suggest that machine vision might one day facilitate on-farm monitoring of wild birds and be used in conjunction with robotic deterrents to deter specific species.

## Declarations

### Ethics approval and consent to participate

This project was approved by the DAF Animal Ethics Committee under application reference number SA 2016/08/568.

## Consent for publication

Not Applicable.

## Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

## Competing Interests

"The authors declare that they have no competing interests".

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## Author's contributions

Conceptualisation of this research was by MA and DF. MA and DF collected the data, and then analysed and interpreted the data with DM. MA was the major contributor in writing the original manuscript. All authors reviewed and edited the manuscript.

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