

## RESERVOIRS OF INFECTION: THE EPIDEMIOLOGICAL CHARACTERISTICS OF AN EMERGING PATHOGEN, *ESCHERICHIA ALBERTII*

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### INTRODUCTION

*Escherichia albertii* was described in 2003 based on isolates taken from children in Bangladesh suffering from diarrhoea (Huys et al., 2003). *E. albertii* has also been implicated as the causative agent of mass avian mortality events in the northern hemisphere, as well as the cause of mortality in captive birds and poultry (Oaks et al., 2010). Strains of the species encode the well-known virulence determinants intimin and cytolethal distending toxin (Oaks et al., 2010) and have been shown to disrupt intercellular tight junctions in gut epithelial cells, which can lead to diarrhoea (Donato et al., 2008).

Although implicated as a pathogen of humans, little is known about its host or geographic distribution, or any other aspect of its epidemiology. One of the reasons for our ignorance, despite its apparent medical importance, is due to the fact that isolates of this species are misidentified as *Escherichia coli*, *Hafnia alvei*, *Shigella boydii*, or *Yersinia ruckeri* using commercial identification kits exploiting the biochemical attributes of an isolate.

Our goal here is to summarise our current understanding of *E. albertii* and present the results of a recent survey of *E. albertii* in Australian birds. These results are discussed in relation to the potential impact of *E. albertii* on native Australian birds, as well as what the reservoirs of infection and transmission dynamics of the pathogen might be.

### PHYLOGENETIC RELATIONS OF *E. ALBERTII*

Walk et al. (2009) analysed the nucleotide sequence of 22 core genes in a variety of *Escherichia* species. This analysis revealed that *E. albertii* is the most divergent clade of the genus *Escherichia* (Fig. 1)

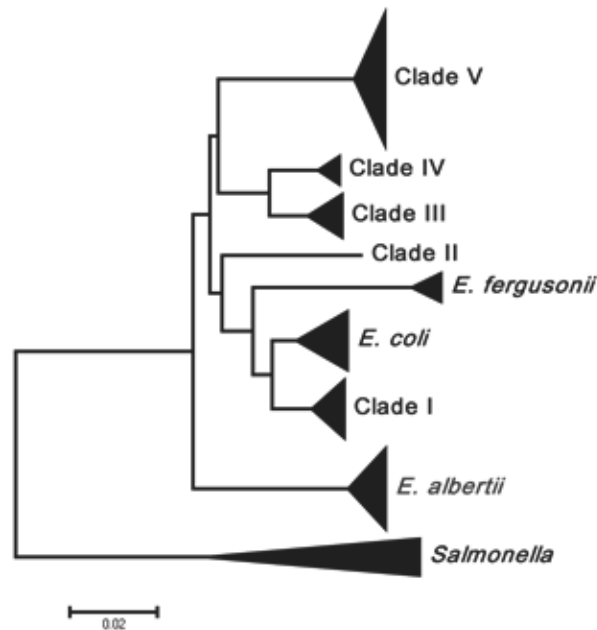


Figure 1. Phylogenetic relationships of *Escherichia* and *Salmonella*.

#### BIOCHEMICAL ATTRIBUTES AND IDENTIFICATION OF *E. ALBERTII*

Oaks et al. (2010) characterised 35 *E. albertii* isolates from birds and humans. The biochemical analysis revealed the isolates were oxidase negative, fermented glucose but not lactose, sucrose, or xylose, usually produced indole from tryptophan, and were non-motile at 35°C. The isolates also produced lysine decarboxylase, ornithine decarboxylase, and fermented D-glucose, D-mannitol, and L-arabinose. The isolates did not utilise citrate; did not produce arginine decarboxylase, hydrogen sulfide, urease, tryptophan deaminase, acetoin, or gelatinase, and did not ferment inositol, L-rhamnose, D-sucrose, D-melibiose, or amygdalin. Most isolates used  $\beta$ -galactosidase. Fermentation of D-sorbitol varied. Isolates that produce indole from tryptophan and are also sorbitol positive will generally be identified as *E. coli* with a high degree of confidence using most commercial identification kits.

There is some evidence to suggest that the biochemical profile of *E. albertii* isolates from humans may differ from the profiles observed for isolates from birds. To date, all *E. albertii* isolates taken from humans are negative for indole production, whilst the majority of isolates from birds are positive for indole production (Oaks et al., 2010; unpublished data).

Although some *E. albertii* isolates have been serotyped as O86:K61 not all isolates react with *E. coli* O antisera.

#### VIRULENCE CHARACTERISTICS AND PATHOLOGY OF *E. ALBERTII*

All strains of *E. albertii* possess the intimin locus (*eaeA*), the cytolethal distending toxin operon (*cdtB*), and the outer membrane hemin receptor (*chuA*). The shiga-toxin genes (*stx1*, *stx2*) are absent. Many strains encode the invasion of brain epithelium operon (*ibeA*), and some, the iron uptake system aerobactin (*iutA*) (Oaks et al., 2010; unpublished data). All isolates from birds carry two types of

cytolethal distending toxin, while strains from humans apparently carry only one version (Oaks et al., 2010; unpublished data).

*In vitro* studies with polarized epithelial cells (MDCK-1 and T84) revealed that *E. albertii*, like *E. coli* O157:H7, decreased transepithelial electrical resistance in MDCK-1 cells, but not T84 cells, and increased cell permeability. Both *E. coli* O157:H7 and *E. albertii* caused a redistribution of the tight junction protein zona occludens-1, but unlike O157:H7, *E. albertii* did not redistribute the tight junction protein claudin-1 (Donato et al., 2008). *E. albertii* strains, like *E. coli*, are also able to adhere and invade Hep-2 cells *in vitro* (La Ragione et al., 2002).

*In vitro* studies would suggest that *E. albertii* initiates disease through the formation of attaching and effacing lesions in a manner similar to *E. coli* O157:H7. As well, the recovery of *E. albertii* cells from liver and spleen tissues within 24 hours of day-old chicks being exposed to *E. albertii* clearly demonstrates that the gut barrier is breached (La Ragione et al., 2002). However, in infected chicks little pathology of the gastro-intestinal mucosa is observed (La Ragione et al., 2002). In birds found dead in the wild, the primary lesions are consistent with enteritis, but the classic attaching and effacing lesions are not detected (Oaks et al., 2010).

Oaks et al. (2010) presented an analysis of nucleotide variation for six housekeeping genes (*aspC*, *clpX*, *fadD*, *icdA*, *lysP*, and *mdh*). The phylogenetic relationships inferred using these data do not provide any indication that *E. albertii* isolates from diseased birds are a distinct group of strains as compared to those isolated from asymptomatic birds (Fig. 2).

There is some data to indicate that *E. albertii* isolates from humans differ from isolates from birds. Isolates from humans are less likely to be indole positive and do not appear to encode two copies of the cytolethal distending toxin operon. However, the phylogenetic relationships observed do not provide any indication that isolates of the pathogen from birds are distinct from those isolated from humans (Fig. 2).

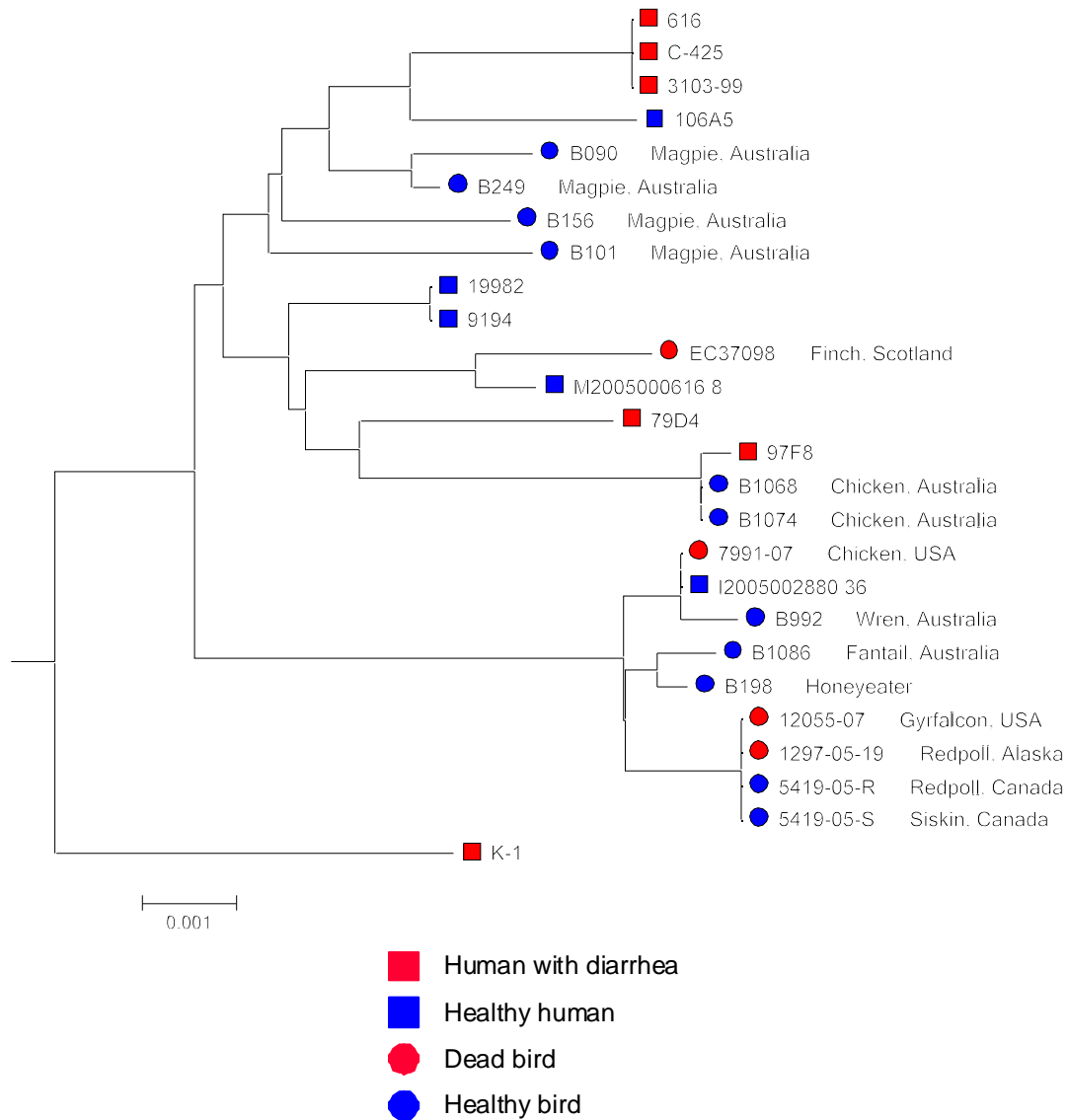


Figure 2. Among strain relationships of *E. albertii* isolates with respect to source of isolation.

### HOST AND GEOGRAPHIC DISTRIBUTION OF *E. ALBERTII*

*E. albertii* has been isolated from human stools and urinary tract, and stools of pigs and bats. *E. albertii* has been isolated from 14 species of bird representing 10 families: Accipitridae, Artamidae, Fringillidae, Maluridae, Dicruridae, Meliphagidae, Psittacidae, Cacatuidae, Rallidae and Phasianidae.

*E. albertii* has a cosmopolitan distribution as it has been isolated from hosts living in Europe, Africa, Asia, Australia, North America, and South America.

## THE IMPACT OF *E. ALBERTII* ON BIRDS

The first indication *E. albertii* caused bird mortality came from Scotland (Pennycott et al., 1998; Foster et al., 1998). In April and May 1994, dead finches were reported at feeding stations from ten sites around Inverness, Scotland. Examination of 34 carcasses resulted in 27 of the birds being diagnosed with *E. coli* O86 colibacillosis. In the following year, additional finches from the same region were diagnosed as having died from O86 colibacillosis. Dead finches found in the Strathclyde region in 1994 and 1995 were also diagnosed with avian colibacillosis. The finch species affected were siskins (*Carduelis pinus*), chaffinches (*Fringilla coelebs*), and greenfinches (*Carduelis chloris*). In all cases, the total number of birds affected was unknown, but was considered to be much larger than the number of reported deaths. O86 colibacillosis was reported as the cause of death in all of the above cases, but the available evidence indicates that the pathogen responsible was actually *E. albertii* (Oaks et al., 2010).

From late December through to late February 2004, the deaths of Redpoll finches (*Carduelis flammea*) were reported from around Fairbanks, Alaska (Oaks et al., 2010). The local at risk population was estimated to be around 8,000 redpolls, and although more than 100 deaths were documented, the actual number was undoubtedly higher. Not all birds exhibited obvious pathology, but when present histological lesions were consistent with necrotizing proventriculitis, multifocal enteritis, or small-crypt abscessation. Bacteriological examination suggested that the pathology was a consequence of *E. albertii* infection.

In the Scottish and Alaskan mortality events, the birds were usually found dead with no obvious signs of disease. Further, the bird's body condition was good, suggesting acute death. Similarly, *E. albertii* has also been diagnosed as the cause of death in a captive gyrfalcon that appeared healthy just prior to its death. By contrast, *E. albertii* was implicated in the death of a chicken that was obviously ill for about a week before its death (Oaks et al., 2010).

We have little understanding of the virulence of *E. albertii* in different bird species. The data suggests that many species can be infected without developing obvious symptoms. *E. albertii* might act in birds in a manner similar to that of salmonella, which is maintained by sub clinical carriers and causes disease outbreaks when environmental conditions increase stress (Pennycott et al., 1998). The body mass of the host is an important determinant of the extent to which a pathogen elevates host mortality (Cable et al., 2007). Post infection, symptoms appear earlier and death occurs more rapidly in smaller-bodied hosts (Cable et al., 2007). For similar reasons large animals are often less likely to be affected by stressful conditions, and can tolerate them for longer, than small hosts.

## SURVEYS OF *E. ALBERTII* IN AUSTRALIA

Two extensive surveys of enteric bacteria in Australian vertebrates have been undertaken. The first spanned the decade 1994-2004 and included a wide range of Australian vertebrates (Gordon and Cowling, 2003). The second survey took place from October 2010 to July 2011 and was restricted to birds.

The first survey revealed that *E. albertii* could not be detected in any Australian fish, frog, snake, lizard, crocodile or mammal host (Table 1). It was, however, detected in birds. Although the overall prevalence of *E. albertii* was low, it was found to be quite common in some species. *E. albertii* was detected in 4/22 (18%) magpies (*Gymnorhina tibicen*), 1/10 (10%) honeyeaters (*Melithreptus brevirostris*), 1/38 (3%) wrens (*Malurus cyaneus*), 1/15 (7%) fantails (*Rhipidura fuliginosa*), 1/13 (8%)

rainbow lorikeets (*Trichoglossus haematodus*) and 1/26 (4 %) galahs (*Cacatua roseicapilla*).

Table 1. Number of Australian vertebrate host species and host individuals sampled for enteric bacteria between 1994 and 2004.

Host Group	Number of Host Species	Number of Hosts Sampled	Prevalence of <i>E. albertii</i>
Fish	6	138	0 %
Frogs	20	106	0 %
Crocodiles	2	33	0 %
Lizards	87	314	0 %
Snakes	18	44	0 %
Turtles	5	56	0 %
Birds	113	634	1.3%
Mammals	92	1063	0 %

*E. albertii* was also detected in a third of nine chickens sampled at a local Canberra agricultural fair in 2002 (unpublished data).

The Wildlife Exotic Disease Preparedness program of the Department of Agriculture, Fisheries and Forestry, and the Australian Wildlife Health Network funded the second survey of *E. albertii*. This survey was restricted to birds and largely targeted native birds being treated in veterinary clinics and wildlife rehabilitation centres. In addition, a large number of poultry were sampled at a regional poultry show held in Canberra during June 2011. The methods used to isolate *E. albertii* and to confirm its identity are described in Appendix 1.

Samples representing mostly native birds came from 5 main areas: Perth WA (304 birds sampled), Healesville VIC (17 birds), Canberra ACT (76 birds), Hobart TAS (125 birds) and Brisbane, QLD (154 birds). Overall, 110 species of bird were sampled (Appendix 2).

Across all birds sampled, there was a significant difference in the frequency with which *E. albertii* was detected in birds from the 5 main sampling regions (Likelihood Ratio  $X^2 = 20.17$ ,  $p = 0.0005$ ). *E. albertii* was not detected in any bird sampled from Perth, Healesville, or Hobart. *E. albertii* was detected in 3.25 % of the birds from Brisbane and in 5.26 % of birds from Canberra.

*E. albertii* was detected in four species of bird, all in care at a vet clinic or wildlife rehabilitation centre. *E. albertii* was observed in 1 of 1 (100%) spotless crakes, *Porzana tabuensis*, sampled; 1 of 7 (14.3 %) little corellas, *Cacatua sanguinea*, 6 of 42 (14.3 %) magpies, *Gymnorhina tibicen*, and 1 of 15 (6.7 %) domestic chickens.

Magpies were sampled from Perth (18 birds), Canberra (10 birds) Hobart (4 birds), and Brisbane (10 birds). There was a statistically significant difference in the frequency with

which *E. albertii* was detected among the 4 regions (Likelihood Ratio  $X^2 = 10.98$ ,  $p = 0.012$ ). *E. albertii* was not detected in any the magpies from Perth or Hobart, but was detected in 20 % of magpies sampled from Brisbane and 40 % of magpies sampled in Canberra.

A total of 241 chickens and 37 ducks were sampled at a regional poultry show held in Canberra from the 10<sup>th</sup> -12<sup>th</sup> of June 2011. The birds originated from 64 localities (flocks) in mainland eastern Australia (Fig. 3). *E. albertii* was not detected in any of the birds sampled.



Fig 3. Localities from which the chickens sampled for *E. albertii* originated.

## DISCUSSION

Over 300 birds were sampled from the Perth region in 2010/2011 and none were found to harbour *E. albertii*. Of the birds sampled approximately 100 were rainbow lorikeets and another 100 were long-billed corellas. Both of these species are shot as part of pest control programs. The balance of the birds was from a wildlife rehabilitation centre. Included among the birds sampled from the centre were 18 magpies. Rainbow lorikeets, magpies, and a species (*Cacatua sanguinea*) closely related to long-billed corellas are known hosts for *E. albertii*. Given that *E. albertii* has been isolated 10 families of bird, as well as humans, pigs and bats, there seems to be little evidence that *E. albertii* exhibits much host specificity. Consequently it appears that *E. albertii* is absent or significantly less common in Western Australia as compared to the rest of Australia.

Although, *E. albertii* does not appear to exhibit any host specificity, it is undoubtedly more common in some species than in others; most notably magpies. In the most recent survey, *E. albertii* was detected in 20 % of magpies sampled in Brisbane and 40 % of magpies sampled from Canberra. Among 8 magpies sampled from Canberra in 2000 and 2001, *E. albertii* was detected in 50% of the

birds.

The original hypothesis concerning *E. albertii* reservoirs of infection and routes of transmission is illustrated in Fig. 4. Chickens were assumed to be a reservoir host for *E. albertii*, as in the original survey of enteric bacteria from birds. *E. albertii* was isolated from 22 % of chickens sampled at a local Canberra fair. Further, because of their large size, chickens may be less susceptible to the potentially adverse effects of *E. albertii* carriage. It is not unusual, especially in Canberra, for people to maintain small 'backyard' flocks of chickens. Given that magpies are very common suburban-dwelling birds that often feed in backyards, it was further hypothesised that the high prevalence of *E. albertii* in magpies was due to their close association with backyard chickens. *E. albertii* could then spread from magpies to other native bird species. Chickens could be a potential source of infection for humans, as *E. albertii* is rarely isolated from the faeces of asymptomatic people.



Figure 4. Hypothesised reservoirs of infection and routes of transmission for *E. albertii*

However, the absence of *E. albertii* in almost 300 chickens and ducks representing birds from over 60 'flocks' makes it unlikely that poultry are a significant reservoir for *E. albertii*. In the most recent survey, the poultry were sampled during the winter while the previous poultry samples were collected in summer. However, there is no evidence to suggest that there are seasonal changes in the prevalence of *E. albertii* in poultry. First, no such phenomena has been reported for *E. coli* and; second, we know that *E. albertii* can be isolated from birds during the spring in Scotland and mid-winter in Alaska.

Consequently, it may be that magpies are the primary reservoir host and are disseminating *E. albertii* to poultry and other native species. The reason that magpies are frequent hosts for *E. albertii* is unknown, but may have something to do with their feeding habits. Whilst magpies are very opportunistic feeders, they consume a lot of invertebrate prey from the ground surface and from the soil. The only other Australian species with vaguely similar feeding habits are magpie larks and white-winged choughs. We have not detected *E. albertii* in any of the 15 magpie larks sampled and have never sampled choughs.

This study has failed to shed any light on the potential impact of *E. albertii* on Australian native birds, except to show that the prevalence of *E. albertii* in most bird species is low. However, this may be because it is simply uncommon, a conclusion not supported by its prevalence in magpies, or because it is highly virulent to many bird species. Highly virulent pathogens typically have a low prevalence in their host populations (Anderson and May, 1992).

Further, the circumstances under which bird mortality events occurred in North America and Europe are not common in Australia, such as snow cover against which dead birds are easily seen, and cold temperatures which reduce decomposition rates and limit insect activity. In Australia, bird feeders tend to attract larger-bodied birds such as parrots that may be less susceptible to *E. albertii* infection.



In principal, a comparison of the prevalence of carriage of *E. albertii* in sick birds turned into veterinarian clinics and wildlife rehabilitation centers with the same species in the 'wild' should provide data concerning the potential impact of *E. albertii* on bird health. The expectation would be that, if *E. albertii* is having an impact, then it should be more often observed in sick than healthy birds. Unfortunately, while it is relatively easy to sample small species readily caught in mist nets, these species seldom carry *E. albertii* and are less likely to be found and brought to care facilities. By contrast, larger species, such as magpies, that often harbour *E. albertii* and are regularly brought to care facilities, are much more difficult to capture in the wild.

### **WHERE TO NEXT?**

Additional questions to be addressed would concern three aspects of *E. albertii* epidemiology. 1. Are there bird versus human specific strains of *E. albertii*? This question could be addressed using comparative genomics/phenomics approaches. 2. What are the temporal dynamics of *E. albertii* within an individual? This question could be addressed by exposing day-old chicks to *E. albertii* and monitoring *E. albertii* cell densities through time. 3. What are the temporal dynamics and spatial distribution of *E. albertii* in Canberran magpies? This study would make use of birds brought into care facilities in the Canberra region and would attempt to collect samples from wild birds.

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## Appendix 1. Bacterial isolation, selection and identification

People agreeing to collect samples were provided with a commercial swab/transport system containing Aimes media. Aimes media is a buffered nutrient free agar-based media designed to prolong the life of bacterial cells. The swab provided was used to collect a small volume of faecal material that had been voided by the bird to be sampled. In the case of dead birds, the swab was used to collect a sample from the cloaca. Providers were asked to store the swabs at 5 - 10 °C, and to collect swabs over no more than a week prior to shipping the samples via courier to ANU.

On arrival at the ANU, the samples were immediately plated onto MacConkey agar plates. MacConkey agar plates contain crystal violet, which inhibits the growth of Gram-positive bacteria, and bile salts, which inhibits the growth of bacteria not normally found in the vertebrate GI tract. MacConkey media also contains the milk sugar lactose and a pH indicator. Bacteria that can use lactose as a growth substrate appear as red colonies while those that cannot are colourless. *E. albertii* is incapable of using lactose. However, a great many of species of enteric bacteria are also lactose negative, for example, *Salmonella enterica* and *Enterobacter cloacae*. Therefore to maximize the likelihood of detecting *E. albertii*, whilst minimizing costs, we made use of the fact that *E. albertii* cannot use the sugars L-rhamnose, D-sucrose, or D-melibiose. Although there are other lactose negative members of the Enterobacteriaceae that cannot use these sugars, they are much less common than species that are simply lactose negative. From each of the MacConkey plates up to 10 lactose negative colonies were selected. These colonies were chosen to represent the diversity of lactose negative colony morphologies present on the plate. Using a sterile toothpick, each colony was transferred onto an LB agar plate and onto a minimal media agar plate containing the sugars, L-rhamnose, D-sucrose, and D-melibiose. The order of the different colonies was the same on each of the two plates. LB agar is a general growth media and this media was used to store the bacteria until they could be processed further. The only growth substrates available in the minimal agar plates were the three sugars. Consequently, whilst *E. albertii* cells would be able to grow on the LB agar plates they would be unable to grow on the minimal plates.

If a colony was unable to grow on the minimal agar plate, cells from the appropriate colony on the LB agar plate were grown overnight in LB liquid media. The DNA from these cells was extracted using DNAzol<sup>®</sup> according to the manufacturer's protocol. Using a PCR-based approach, the potential *E. albertii* strains were screened using PCR primers designed to be specific to *E. albertii* (Hyma et al., 2005). All isolates confirmed to be *E. albertii* have been stored at -80 °C so they are available for further analysis.

**Appendix 2. Bird species sampled and number of individuals sampled for each of the species. Data for the 2010/2011 survey.**

Species	Common Name	# Sampled
<i>Acanthiza chrysorrhoa</i>	Yellow-Rump Thornbill	7
<i>Acanthiza lineata</i>	Striated Thornbill	3
<i>Acanthiza pusilla</i>	Brown Thornbill	2
<i>Acanthiza reguloides</i>	Buff-rumped Thornbill	7
<i>Acanthorhynchus tenuirostris</i>	Eastern Spinebill	1
<i>Accipiter cirrocephalus</i>	Collared Sparrowhawk	1
<i>Accipiter fasciatus</i>	Brown Goshawk	1
<i>Aegotheles cristatus</i>	Australian Owlet-nightjar	1
<i>Alectura lathami</i>	Brush Turkey	3
<i>Anas superciliosa</i>	Pacific Black Duck	3
<i>Anthochaera carunculata</i>	Red Wattlebird	7
<i>Anthochaera chrysoptera</i>	Little Wattlebird	1
<i>Anthochaera sp</i>	Wattlebird	5
<i>Ara ararauna</i>	Blue Macaw	1
<i>Ardea intermedia</i>	Intermediate Egret	1
<i>Ardea pacifica</i>	White-necked Heron	1
<i>Barnardius zonanius</i>	Ringneck Parrot	16
<i>Bubulcus ibis</i>	Cattle Egret	5
<i>Cacatua galerita</i>	Sulphur Crested Cockatoo	4
<i>Cacatua sanguinea</i>	Little Corella	7
<i>Cacatua tenuirostris</i>	Long-billed Corella	99
<i>Carduelis chloris</i>	European Greenfinch	1
<i>Centropus phasianinus</i>	Pheasant Coucal	2
<i>Chenonetta jubata</i>	Wood Duck	1
<i>Chroicocephalus novaehollandiae</i>	Silvergull	1
<i>Chthonicola sagittatus</i>	Speckled Warbler	5
<i>Circus approximans</i>	Swamp Harrier	2
<i>Colluricincla harmonica</i>	Grey-shrike Thrush	2
<i>Columba leucomela</i>	White Headed Pigeon	1
<i>Columba livia</i>	Domestic Pigeon	7
<i>Coracina novaehollandiae</i>	Black-faced Cuckoo-shrike	2
<i>Corvus coronoides</i>	Australian Raven	7
<i>Corvus sp</i>	Crow	3
<i>Coturnix ypsilophora</i>	Brown Quail	2
<i>Cracticus sp</i>	Butcherbird	1
<i>Cracticus torquatus</i>	Grey Butcherbird	1
<i>Cygnus atratus</i>	Black Swan	1
<i>Dacelo novaeguineae</i>	Kookaburra	32
<i>Domestic Chicken</i>	Domestic Chicken	256
<i>Domestic Duck</i>	Domestic duck	38
<i>Domestic Goose</i>	Goose	1
<i>Egretta novaehollandiae</i>	White-faced Heron	2
<i>Eolophus roseicapilla</i>	Galah	14
<i>Eudynamys orientalis</i>	Common Koel	1
<i>Falco berigora</i>	Brown falcon	2
<i>Falco cenchroides</i>	Nankeen Kestrel	2
<i>Falco longipennis</i>	Australian Westerway	1
<i>Falco peregrinus</i>	Peregrine Falcon	1
<i>Gallinula mortierii</i>	Tasmanian Native Hen	1
<i>Gallinula tenebrosa</i>	Dusky Moorhen	2
<i>Gallirallus philippensis</i>	Buff-banded Rail	2
<i>Geopelia humeralis</i>	Bar-shouldered Dove	1
<i>Glossopsitta concinna</i>	Musk Lorikeet	6
<i>Grallina cyanoleuca</i>	Magpie Lark	7
<i>Gymnorhina tibicen</i>	Magpie	42
<i>Hirundo neoxena</i>	Welcome Swallow	1
<i>Larus dominicanus</i>	Kelp Gull	3
<i>Larus novaehollandiae</i>	Silver Gull	2

Species	Common Name	# Sampled
<i>Larus pacificus</i>	Pacific Gull	4
<i>Lathamus discolor</i>	Swift Parrot	1
<i>Lichenostomus leucotis</i>	White-eared Honeyeater	1
<i>Lichenostomus melanops cassidix</i>	Helmeted Honeyeater	2
<i>Lichenostomus penicillatus</i>	White-plumed Honeyeater	1
<i>Lichenostomus sp</i>	Honey Eater	1
<i>Lichenostomus virescens</i>	Singing Honeyeater	5
<i>Lichmera indistincta</i>	Brown Honeyeater	1
<i>Manorina flavigula</i>	Yellow Throated Miner	1
<i>Manorina melanocephala</i>	Noisy Miner	4
<i>Melithreptus brevirostris</i>	Brown-headed Honeyeater	2
<i>Melopsittacus undulatus</i>	Pet Budgie	9
<i>Neophema chrysogaster</i>	Orange Bellied Parrot	2
<i>Neophema chrysostoma</i>	Blue-winged parrot	2
<i>Ninox novaeseelandiae</i>	Boobook Owl	6
<i>Numenius sp</i>	Curlew	3
<i>Nycticorax caledonicus</i>	Nankeen Night Heron	1
<i>Nymphicus hollandicus</i>	Pet Cockatiel	3
<i>Ocyphaps lophotes</i>	Crested Pigeon	4
<i>Pachycephala pectoralis</i>	Golden Whister	2
<i>Pachyptila vittata</i>	Broad Billed Prion	1
<i>Pardalotus punctatus</i>	Spotted Pardalote	3
<i>Pardalotus striatus</i>	Striated Pardalote	4
<i>Petroica boodang</i>	Scarlet Robin	3
<i>Pezoporus wallicus</i>	Ground Parrot	1
<i>Phalacrocorax fuscescens</i>	Black-faced Cormorant	3
<i>Phalacrocorax sp</i>	Cormorant	5
<i>Phaps chalcoptera</i>	Common Bronzewing	7
<i>Phylidonyris novaehollandiae</i>	New Holland Honeyeater	1
<i>Pitta versicolor</i>	Noisy Pitta	1
<i>Platycercus adscitus</i>	Pale-headed Rosella	1
<i>Platycercus caledonicus</i>	Green Rosella	15
<i>Platycercus elegans</i>	Crimson Rosella	4
<i>Platycercus eximius</i>	Crimson Rosella	7
<i>Platycercus eximius</i>	Eastern Rosella	4
<i>Podargus strigoides</i>	Tawny Frogmouth	39
<i>Poliiocephalus poliocephalus</i>	Hoary-headed Grebe	1
<i>Porphyrio porphyrio</i>	Purple Swamphen	3
<i>Porzana tabuensis</i>	Spotless Crake	1
<i>Puffinus sp</i>	Shearwater	4
<i>Purpureicephalus spurius</i>	Red-capped Parrot	3
<i>Rhipidura albiscapa</i>	Grey Fantail	1
<i>Smicrornis brevirostris</i>	Weebill	3
<i>Sphecotheres viridis</i>	Fig Bird	1
<i>Strepera graculina</i>	Pied Currawong	3
<i>Streptopelia chinensis</i>	Spotted Turtledove	2
<i>Streptopelia risoria</i>	Ringneck Dove	2
<i>Streptopelia senegalensis</i>	Laughing Dove	6
<i>Sturnus vulgaris</i>	Starling	1
<i>Tachybaptus novaehollandiae</i>	Australasian Grebe	1
<i>Thalasseus bergii</i>	Crested Tern	1
<i>Threskiornis molucca</i>	Australian White Ibis	4
<i>Threskiornis spinicollis</i>	Straw-necked Ibis	2
<i>Todiramphus sanctus</i>	Sacred Kingfisher	1
<i>Trichoglossus chlorolepidotus</i>	Scaly-breasted Lorikeet	1
<i>Trichoglossus haematodus</i>	Rainbow Lorikeet	112
<i>Turdus merula</i>	Common Blackbird	1
<i>Vanellus miles</i>	Masked Lapwing	2
<i>Zosterops lateralis</i>	Silvereye	3