AUSTRALIAN VETERINARY JOURNAL
AUSTRALIA'S PREMIER VETERINARY SCIENCE TEXT
PRODUCTION ANIMALS

SHORT CONTRIBUTION

Identification of *Chlamydia gallinacea* in a parrot and in free-range chickens in Australia

HS Stokes,[a]* JM Martens,[a] A Chamings,[b,c] K Walder,[c] ML Berg,[a] Y Segal[d] and ATD Bennett[a]

Chlamydia gallinacea is a recently described bacterial species in a genus known to infect and cause disease in animals and humans. Our report describes the identification of *C. gallinacea* infection in free-range laying chickens (*Gallus gallus*) in Australia, and the identification of *C. gallinacea* infection in a parrot, a wild Australian galah *(Eolophus roseicapillus)*. There is currently little knowledge of the effects of *C. gallinacea* infection on avian hosts, but it has been linked to respiratory disease in humans and could potentially cause similar disease in other species. Our report highlights the need for further study and surveillance of *Chlamydia* species in both wild and domestic hosts in Australia.

Keywords *Chlamydia*; chickens; parrots; zoonosis
Aust Vet J 2019;97:398–400 doi: 10.1111/avj.12856

Introduction

The *Chlamydia* are a diverse genus of bacteria affecting many animal species, and also humans, with 11 species currently described[1]. One recently described species is *Chlamydia gallinacea*,[2,3] identified in poultry in several European countries, China and the United States.[4–6] *C. gallinacea* currently has no reported clinical signs in birds, except for reduced weight gain in chickens *(Gallus gallus)*.[5] However, *C. gallinacea* has also been found in cattle, and has been linked to respiratory symptoms in humans,[2,4] so it may pose a zoonotic threat. A *C. gallinacea*-type strain has been reported once before in Australia, in two cage-laying chickens in 2010, prior to formal species classification.[7] To our knowledge, however, *C. gallinacea* has not previously been reported in free-range chickens or wild birds in Australia.

At least two other *Chlamydia* species are known to infect birds: *Chlamydia psittaci* and *Chlamydia avium*.[3] The most well-known is *C. psittaci*, which primarily infects birds, but can also cause severe respiratory disease in humans, called 'psittacosis'.[8] *C. psittaci* commonly infects parrots, but can also infect many other bird species.[8,9] *C. psittaci* may cause no clinical signs in birds, but it can have acute and chronic effects[9] and has previously caused disease outbreaks on poultry farms.[8] In chickens, it may cause reduced egg production and weight loss.[10] *C. avium* is a more recently characterised chlamydial species, found in pigeons and parrots[3] with the majority of infections reported to be asymptomatic.

There is little known about the prevalence of chlamydial species in wild birds, despite the zoonotic risk posed by *C. psittaci.* One recent report estimated *C. psittaci* prevalence in wild Australian birds to be 0.67%.[11] Here we provide the first reports, to our knowledge, of *C. gallinacea* presence in free-range chickens in Australia, and the first evidence of *C. gallinacea* infection in a wild parrot, a galah *(Eolophus roseicapillus)*.

Methods

From February 2017 until March 2018, free-range egg-laying chickens were sampled at six farms located within 30 km of Geelong, Victoria, Australia. Each farm was visited once every three months, and a total number of 247 chickens were randomly sampled. From October 2016 until February 2018, 116 adult wild parrots were also sampled, including crimson rosellas *(Platycercus elegans,* [n = 57]*)*, galahs [n = 31], sulphur-crested cockatoos *(Cacatua galerita,* [n = 10]*)*, and blue-winged parrots *(Neophema chrysogaster,* [n = 12]*)*. Parrots were captured at four sites: Bellbrae (S38°19' E144°11'), Freshwater Creek (S38°27' E144°24'), Meredith (S37°51' E144°06') and Steiglitz (37°52' E144°18'). A cloacal swab was collected from each bird, and tested for the presence of *Chlamydia* using conventional PCR and a combination of three different sets of primers: the Chlamydiales-specific 16SIG primers[12] and *C. psittaci/C. gallinacea*-specific CTU/CTL primers[13] were used for initial detection, and the *C. gallinacea*-specific gidA primers[4] were then used to distinguish between species. A subset of *C. gallinacea* positive samples (n = 3) were confirmed using Sanger sequencing, and analysed using MEGA software and NCBI BLAST, to compare similarity with chlamydial sequences on GenBank.[14,15] All parrot serum samples (n = 116) and a subset of *C. gallinacea*-positive chicken serum samples (n = 2) were tested for *Chlamydia* antibodies using the ImmunoComb® solid-phase ELISA (Biogal, Kibbutz Galed, Israel) and compared with positive and negative control serum.

Findings

C. gallinacea was identified in cloacal samples from 36 chickens, from two different farms, with all chickens (n = 27) testing positive during one farm visit (21st November 2017). The prevalence of *C. gallinacea* in chickens was 14.6% (95% confidence interval [CI] = 10.4, 19.6) however there was considerable variability between farms: all positive chickens were identified on two farms, whilst the

*Corresponding author.
[a]Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Victoria, Australia; hsstokes@deakin.edu.au
[b]Geelong Centre for Emerging Infectious Diseases, Geelong, Victoria, Australia
[c]Centre for Molecular and Medical Research, School of Medicine, Deakin University, Waurn Ponds, Victoria, Australia
[d]Department of Jobs, Precincts and Regions, Attwood, Victoria, Australia

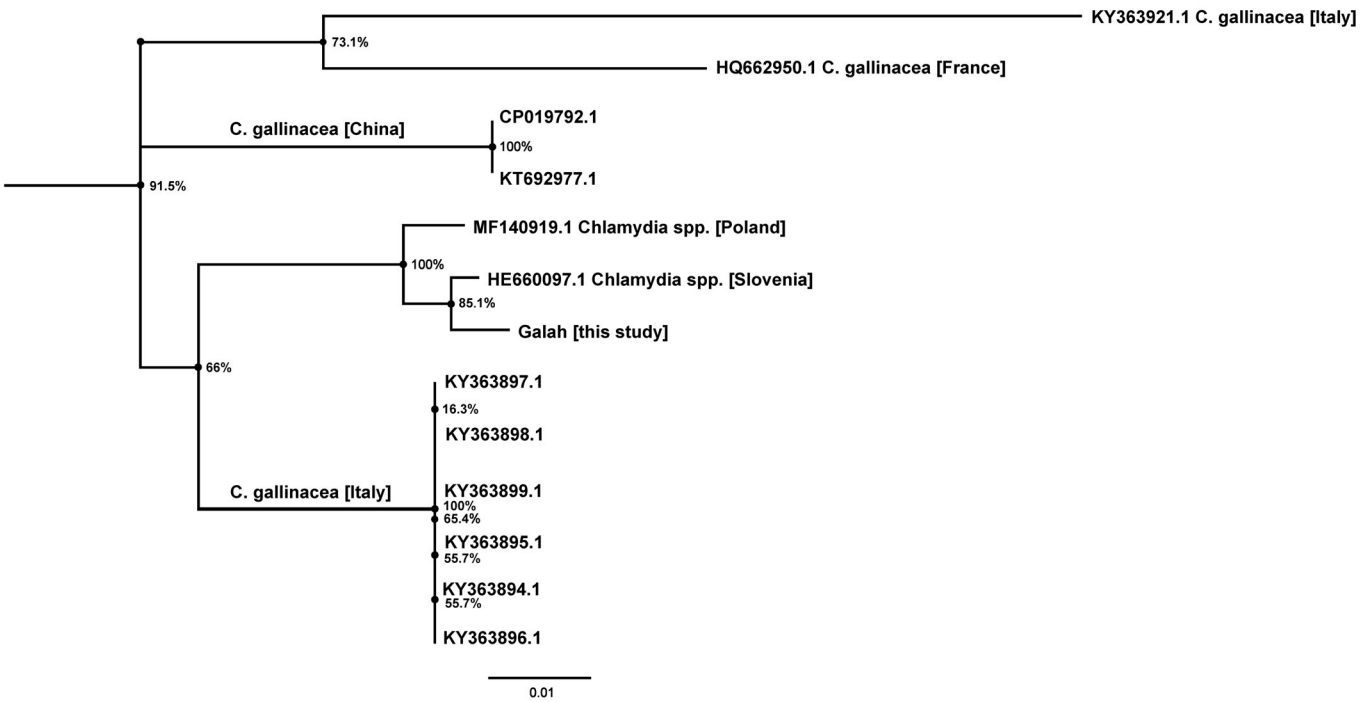

Figure 1. **Neighbour-join dendrogram based on analysis of the (463-490 nt) OmpA sequence obtained using the forward CTU/CTL primer. Sequences were aligned in Geneious Prime 2019.0.4 (https://geneious.com) using the Translation Align method. The dendrogram was created in Mega-X using the neighbour-joining method with 1000 bootstrap repetitions. It should be noted that although both are listed as *Chlamydia* spp. on GenBank, MF140919.1 grouped with *C. gallinacea* in publication, and HE660097.1 was published before *C. gallinacea* was listed as a recognised species.**

other four farms tested negative. Serum samples from two of the *C. gallinacea*-positive chickens were seropositive according to the ImmunoComb® kit. We observed no clinical signs of disease in infected chickens, and farmers reported chickens to be healthy. Preliminary sequencing analysis of a subset (n = 2) of positive samples confirmed that they were *C. gallinacea*, with one of the samples grouping most closely with sequences identified in chickens in studies in Slovenia and France (data not shown).

C. gallinacea was identified in one sample from a female galah with healthy appearance (0.9%, 95% CI = 0, 4.7), caught on 16th October 2017 (GenBank acc. no. MN114672). Preliminary sequencing analysis suggests that this *C. gallinacea* strain groups most closely with sequences identified from chicken studies in Slovenia and Poland (Figure 1). The OmpA sequence from the galah and one of the chicken samples overlapped by ~120 nt but did not appear to be identical. The same parrot was recaptured on 16th February 2018 at the same site (Bellbrae, Victoria, S38°19' E144°11'), and tested positive by the Chlamydiales-specific 16SIG PCR, but negative with the gidA PCR; therefore on this occasion the *Chlamydia* species could not be confirmed. On both occasions, the galah tested seropositive using the ImmunoComb® kit.

Discussion

Based on the OmpA sequence, we have detected *C. gallinacea* in two free-range chicken farms and in a wild parrot, in southern central Victoria, Australia. The *C. gallinacea* prevalence on chicken farms was consistent with findings in Europe, China and elsewhere that *C. gallinacea* is likely to be a common chlamydial species circulating in poultry.[5] To our knowledge, this is only the second record of *C. gallinacea* in Australian poultry, and the first report of *C. gallinacea,* or a closely related species, in a psittacine bird or wildlife host anywhere in the world. This latter finding could be due to a combination of factors, including the possibility that *C. gallinacea* has only recently emerged in Psittaciformes, or that the actual prevalence of infection is low, and/or specific testing for this organism has rarely been performed in wild parrots. Indeed, some research suggests that several different *Chlamydia* species may be circulating in wild birds.[16] Finally, it may also be that *C. gallinacea* exposure in wild birds has previously been miscategorised as *C. psittaci* or another species: this may be particularly likely in serological studies, as there are no species-specific serological tests available for *Chlamydia.*

Our results suggest that the ImmunoComb® antibody testing kit, developed to test for *C. psittaci,* may also detect antibodies to *C. gallinacea.* It may therefore be a useful tool for screening birds for exposure to both *C. psittaci* and *C. gallinacea*, in addition to PCR. Future studies on *C. gallinacea* should consider additional serological tests to further explore this possibility. Another useful future research direction would be to investigate the effects of *C. gallinacea* on bird body weight and condition, which we were unable to investigate here.

It has been suggested that persistent endemic infection of chickens with *C. gallinacea* may represent a major disease reservoir.[5] As galah home ranges have been reported between 1,550 and 4,450 hectares[17]

it is likely that the home range of the *C. gallinacea*-positive galah included the positive chicken farms tested, as the galah was caught approximately 7-8 km from the farm which had 100% *C. gallinacea* prevalence on one sampling date (21st November 2017). It is therefore possible that *C. gallinacea* could have spread from chickens to wild birds, or vice versa. Wild birds are often suggested as a potential source of chlamydial infection for poultry or humans[16] and disease transmission from poultry to wild birds can also occur. During sample collection, galahs were observed at both farms on multiple occasions, and both had feeding stations easily accessible by wild birds (H. Stokes; personal observation). Our preliminary sequencing data suggests there may be at least two different strains of *C. gallinacea* circulating in this region, with the strain identified in the galah appearing to be slightly different from that identified in one of the chicken samples. However, this is only a preliminary analysis of one section of the *C. gallinacea* genome. Further genetic analysis *of C. gallinacea* strains is needed to elucidate possible transmission pathways between species, but it is likely unprotected feeders could attract wild birds and therefore encourage close contact, providing an opportunity for disease transmission.

As free-range farming becomes more common, opportunities for pathogen transmission between poultry and wildlife may increase. Whilst there are currently few known clinical signs of *C. gallinacea* infection in birds or humans, it is plausible that *C. gallinacea* could cause significant disease: respiratory symptoms have been reported in humans exposed to infected birds,[2,4] and the closely related *C. psittaci* can be highly pathogenic in some avian and mammalian species, including humans.[9] Our findings highlight the need for further study of the prevalence and diversity of *Chlamydia* species in both domestic and wild hosts, particularly in areas with high opportunity for inter-specific transmission.

Acknowledgments

The authors thank all poultry farmers and landowners for their cooperation, and Dr. Christiane Schnee at the Friedrich-Loeffler Institut, Federal Research Institute for Animal Health (Germany) for providing control samples. This work was funded by the Australian Research Council (LP140100691 and DP180103494), Deakin University, and the Holsworth Wildlife Research Endowment. The work complied with the laws of Victoria (research permit 10007969) and Deakin University animal ethics approval (B31-2015 and B37-2016) and was carried out under ABBBS authority 2319.

References

1. Sachse K, Bavoil PM, Kaltenboeck B et al. Emendation of the family *Chlamydiaceae:* proposal of a single genus, *Chlamydia*, to include all currently recognized species. *Syst Appl Microbiol* 2015;38:99–103.
2. Laroucau K, Vorimore F, Aaziz R et al. Isolation of a new chlamydial agent from infected domestic poultry coincided with cases of atypical pneumonia among slaughterhouse workers in France. *Infect Genet Evol* 2009;9:1240–1247.
3. Sachse K, Laroucau K, Riege K et al. Evidence for the existence of two new members of the family *Chlamydiaceae* and proposal of *Chlamydia avium* sp. nov. and *Chlamydia gallinacea* sp. nov. *Syst Appl Microbiol* 2014;37:79–88.
4. Li J, Guo W, Kaltenboeck B et al. *Chlamydia pecorum* is the endemic intestinal species in cattle while *C. gallinacea, C. psittaci* and *C. pneumoniae* associate with sporadic systemic infection. *Vet Microbiol* 2016;193:93–99.
5. Guo W, Li J, Kaltenboeck B et al. *Chlamydia gallinacea*, not *C. psittaci*, is the endemic chlamydial species in chicken *(Gallus gallus)*. *Sci Rep* 2016;6:9196389.
6. Li L, Luther M, Macklin K et al. *Chlamydia gallinacea:* a widespread emerging *Chlamydia* agent with zoonotic potential in backyard poultry. *Epidemiol Infect* 2017;145:2701–2703.
7. Robertson T, Bibby S, O'Rourke D et al. Identification of Chlamydial species in crocodiles and chickens by PCR-HRM curve analysis. *Vet Microbiol* 2010;145:373–379.
8. Sachse K, Laroucau K, Vanrompay D. Avian Chlamydiosis. *Current Clinical Microbiology Reports* 2015;2:10–21.
9. Gerlach H. Chlamydia. In: Ritchie B, Harrison G, Harrison L, editors. *Avian Medicine: Principles and Application*. Wingers Publishing, Lake Worth, Florida, 1994:984–996.
10. Yin L, Kalmar ID, Boden J, Vanrompay D. Chlamydia psittaci infections in Chinese poultry: a literature review. *World's Poultry Science Journal* 2015;71:473–482.
11. Chlamydia in Australian wild birds: Fact sheet. Wildlife Health Australia, Sydney, New South Wales, 2017:1–12.
12. Everett K, Bush R, Anderson A. Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiales*, including a new genus and five new species, and standards for the identification of organisms. *International Journal of Systematic Bacteriology* 1999;49:415–440.
13. Denamur E, Sayada C, Souriau A et al. Restriction pattern of the major outer-membrane protein gene provides evidence for a homogeneous invasive group among ruminant isolates of *Chlamydia psittaci*. *Journal of General Microbiology* 1991;137:2525–2530.
14. Tamura K, Dudley J, Nei M, Kumar S. *MEGA4*: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 2007;24:1596–1599.
15. NCBI. BLAST Basic Local Alignment Search Tool. NIH, 2018.
16. Krawiec M, Piasecki T, Wieliczko A. Prevalence of *Chlamydia psittaci* and Other *Chlamydia* Species in Wild Birds in Poland. *Vector Borne Zoonotic Dis* 2015;15:652–655.
17. Noske S. Aspects of the behaviour and ecology of the White Cockatoo *(Cacatua galerita)* and Galah *(C. roseicapilla)* in croplands in north-east New South Wales. The University of New England, Armidale, 1983.

(Accepted for publication 13 June 2019)