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A single genotype of *Chlamydia psittaci* (ST27) found in multiple species of birds in a zoological collection in New Zealand

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ABSTRACT

Aims: To investigate the genotypes of *Chlamydia psittaci* in birds associated with two clusters of disease from a zoological collection in New Zealand.

Materials and methods: Samples were collected over two time periods from birds resident at Auckland Zoo (Auckland, NZ). In 2016, two little penguins/kororā (*Eudyptula minor*) showed respiratory disease on admission to the zoo hospital. Post-mortem samples of liver and lung were collected from the penguins and from 10 other birds from the zoo's collection that died without clinical signs. Further, 128 conjunctival, choanal and cloacal swabs were collected from 27 different bird species, all housed within the zoo and without clinical signs.

In 2019, a cluster of deaths of four diamond doves (*Geopelia cuneata*) and two superb parrots (*Polytelis swainsonii*) occurred in one mixed-species aviary. Twenty post-mortem samples were collected from these birds and other birds that died around the same time across the zoo. DNA was extracted from all samples and initially tested for *C. psittaci* using a high-resolution melting quantitative PCR (HRM qPCR) protocol. We applied multi-locus sequence typing (MLST) on 10 *C. psittaci*-positive samples from four different avian species, including one sample from 2016 (little penguin) and nine post-mortem samples from 2019.

Results: *C. psittaci* was detected in 14/140 (0.10; 95% CI = 0.061–0.161) of the samples from 2016 from seven species. A penguin sample was sequenced aligning with *ompA* genotype B and was later characterised by MLST as *C. psittaci* strain ST27. With the exception of the sample from the sick penguin, the positive results yielded very low DNA copy numbers in the HRM qPCR, potentially indicating latent infections. In the 2019 cluster, *C. psittaci* was detected in 9/20 post-mortem samples from three bird species (diamond dove, superb parrot, and zebra finch (*Taeniopygia guttata*)). All nine sample sequences aligned with *ompA* genotype B and were characterised by MLST as *C. psittaci* strain ST27.

Conclusions and clinical relevance: *C. psittaci* was present within the zoological collection in a variety of bird species associated with two disease clusters. Most of these infections were asymptomatic, but a cluster of deaths due to avian chlamydiosis in 2019 affecting three species of birds was due to a single genotype, ST27, that was also present in a wild penguin in 2016. This provides evidence of pathogenicity in birds for this genotype.

Abbreviations: C-C-C: Conjunctival, choanal slit and cloacal swabs; Cq: Cycle threshold; HRM qPCR: High resolution melting quantitative PCR; MLST: Multi-locus sequence typing; *ompA*: Outer membrane protein A; ST: Sequence type; WGS: Whole genome sequencing

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Introduction

Chlamydia psittaci is an obligate intracellular bacterial species within the family *Chlamydiaceae*. This globally widespread pathogen with proven zoonotic potential infects, and may cause disease in, a wide range of mammalian hosts, including humans, and in birds is the causal organism for the avian disease chlamydiosis (Borel *et al.* 2018; Stokes *et al.* 2021). In birds, clinical signs of *C. psittaci* infection can include upper respiratory tract infection, severe pneumonia and air-sacculitis, and septicaemia with hepatitis and splenitis (Harkinezhad *et al.* 2009). Globally, the prevalence of avian chlamydial infections (including, in addition to *C. psittaci*: *C. gallinacea, C. avium* and avian *C. abortus*) has been estimated at approximately 20% in all birds since 2012 (Sukon *et al.* 2021).

First identified in New Zealand in 1953, *C. psittaci* was believed to have been imported into the country in parrots, subsequently causing an outbreak of disease in the keepers who cared for them (Fastier and Austin 1954). Since then, the organism has been reported in New Zealand in non-native psittacine species (McCausland *et al.* 1972; Gartrell *et al.* 2013),

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feral pigeons (Motha *et al.* 1995) and as the cause of a mortality cluster in wild Malay spotted doves (*Streptopelia chinensis*; Rawdon *et al.* 2009). Asymptomatic infections have been reported in native New Zealand birds including hihi (*Notiomystis cincta*; Gartrell *et al.* 2013), and kea (*Nestor notabilis*) that were exported from New Zealand to an overseas zoo (Johnson *et al.* 1984). Most recently, a surveillance study in waterfowl showed that 24% of wild mallards (*Anas platyrhynchos*) were positive for *C. psittaci* by PCR and these birds likely represent a significant wild reservoir of the organism throughout New Zealand (Soon *et al.* 2021).

The genotypes of *C. psittaci* described, based on the analysis of the major outer membrane protein A (*ompA*) gene, are A, B, C, D, E, F, E/B, WC, M56, 1 V, 6N, Mat116, R54, YP84, and CPX0308. Genotypes A – F and E/B are linked to avian hosts (van Lent *et al.* 2012) with the following host predilection: genotypes A (psittacine), B (pigeon), C (duck and turkey), D (turkey), E (pigeons and ducks), F (psittacine), and E/B (duck) (Read *et al.* 2013; Knittler and Sachse 2015). These host predilections are not exclusive and there is variation in host-genotype interactions beyond these broad groupings. The severity of infection mainly depends on the genotype of the strain and the host, making predictions of pathogenicity difficult. Globally, genotype A is the most common genotype and is considered highly virulent to birds (Wang *et al.* 2018). A recent analysis of *C. psittaci* infections in native and non-native birds in New Zealand identified genotypes A and C (Gedye *et al.* 2018).

A multi-locus sequence typing (MLST) approach for initial molecular characterisation of strains was developed for *Chlamydiales* and is now the preferred methodology for molecular characterisation of strains, barcoding them as sequence types (ST) (Pannekoek *et al.* 2008, 2010). In addition to *ompA* genotyping and whole genome sequencing (WGS), *Chlamydiales* MLST is a useful tool for molecular characterisation of strains due to its high congruence with WGS phylogeny (Mattmann *et al.* 2019; Anstey *et al.* 2021; White *et al.* 2022). Using the MLST database, *C. psittaci* strains can be classified and tracked in outbreaks, subsequently inferring host associations. In Australia, cases of *C. psittaci* causing equine placentitis with further zoonotic spillover were attributed to the clonal and dominant ST24 strains, originating from parrots (Jelocnik *et al.* 2017; Anstey *et al.* 2021) and an ST24 strain was found to be infecting fulmars (*Fulmarus glacialis*) in the Faroe Islands where an epidemic of psittacosis connected to hunting and consuming fulmars occurred in the 1930s (Wang *et al.* 2020).

The zoonotic nature of *C. psittaci* infections poses special challenges where there is close contact between birds and humans, such as for pet owners, bird breeders, and in zoological collections for zoo staff and visitors. This is of relevance in zoos that house collections of birds in walk-through aviaries, where visitors may potentially be directly exposed to infected birds (Chaber *et al.* 2021; De Meyst *et al.* 2022). In most countries, psittacosis in birds is a notifiable disease and zoos thus have protocols in place to manage this risk using frequent surveillance testing of birds and maintaining high levels of biosecurity (Balsamo *et al.* 2017).

Recently, advances in the specificity and sensitivity of PCR diagnostics have increased detection rates in captive and wild birds, which possibly reflects the identification of latently infected birds that are intermittently shedding organisms at low levels but do not display any clinical signs (Stokes *et al.* 2021). The risk of *C. psittaci* transmission to other birds, and zoonotic spill over to humans from latently infected birds, is currently unknown, as the risk of birds shedding low levels of organisms is unclear. At present, the advice is to isolate and treat all *C. psittaci*-positive birds regardless of whether they are asymptomatic or diseased (Balsamo *et al.* 2017). Similarly, the zoonotic and pathogenic potential of different *C. psittaci* genotypes is only generally understood, and therefore treatment and isolation has still been advised regardless of the genotype identified. A more nuanced understanding of *C. psittaci* infections in birds will aid in managing this organism in a more targeted way to prevent outbreaks and zoonotic transmission in captive collections. At the same time, treatment and quarantine protocols can be tailored so that unnecessary long and often stressful antibiotic treatments can possibly be avoided. The aim of this study was to investigate the genotypes of *Chlamydia psittaci* in birds associated with two clusters of disease from a curated zoological collection.

Materials and methods

Samples used in this study

The study site for this project was the New Zealand Centre for Conservation Medicine at Auckland Zoo (Auckland, NZ). Birds cared for at the zoo and wild species brought in for veterinary care were considered the target population, which included both non-native and native species.

In 2016, two wild little penguins/kororā *(Eudyptula minor)* that had resided for an extended time at a bird rescue centre were admitted for assessment of suspected respiratory disease and shortly after were euthanised due to the severity of disease. These penguins were diagnosed with avian chlamydiosis using ante-mortem swabs and later post-mortem tissues. Post-mortem samples of lung and liver were collected from the little penguins and from 10 other birds from the collection at Auckland Zoo: budgerigars

Table 1. Bird species at Auckland Zoo (Auckland, NZ) from which samples were collected from May to December 2016 and tested for *Chlamydia psittaci* by high resolution melting quantitative PCR (HRM qPCR) showing the number positive and genome copy number for *C. psittaci*.

Species sampled[a]	Number sampled	Number *C. psittaci*-positive	Copy number (*ompA* gene/µl DNA)[b]
Antipodes parakeet (*Cyanoramphus unicolor*)	1		
Bellbird/korimako (*Anthornis melanura*)	2		
Brown kiwi (*Apteryx mantelli*)	2		
Brown teal/pāteke (*Anas chlorotis*)	17		
Budgerigar (*Melopsittacus undulatus*)	8	2	< 4–97
Cockatiel (*Nymphicus hollandicus*)	10	3	< 4–9
Crested pigeon (*Ocyphaps lophotes*)	5	2	5–14
Diamond dove (*Geopelia cuneata*)	7		
Eclectus parrot (*Eclectus roratus*)	1		
Grey teal/tētē-moroiti (*Anas gracilis*)	6		
King parrot (*Alisterus scapularis*)	2	1	<4
Kookaburra (*Dacelo novaeguineae*)	1		
Little penguin/kororā (*Eudyptula minor*)	2	2[c]	1767
Masked lovebird (*Agapornis personata*)	22	3	< 4
Musk lorikeet (*Glossopsitta concinna*)	4		
Orange fronted parakeet/kākāriki (*Cyanorhamphus malherbi)*	5		
Paradise duck/pūtangitangi (*Tadorna variegata*)	2		
Rainbow lorikeet (*Trichoglossus moluccanus*)	1		
Red-tailed black cockatoo (*Calyptorhynchus banksii*)	2		
Saddleback/tīeke (*Philesturnus carunculatus*)	4		
Scaup/pāpango (*Aythya novaeseelandiae*)	2		
Shoveler (*Anas clypeata*)	4		
Stilt (*Himantopus* spp.)	3		
Sun conure (*Aratinga solstitialis*)	2		
Takahē (*Porphyrio hochstetteri*)	6		
Turquoise parrot (*Neophema pulchella*)	19	1	< 4
Blue duck/whio (*Hymenolaimus malacorhynchos*)	2		
Sample type			
Ante-mortem samples	128	9	
Post-mortem samples	12	4	
Total	140	13	

[a]All samples were ante-mortem combined swabs of the conjunctiva, choana slit and cloaca except for two little penguins (wild birds that died in quarantine with clinical signs of avian chlamydiosis) and one turquoise parrot and one budgerigar (aviary birds that were found dead without clinical signs) for which post-mortem samples of liver and lung were taken.
[b]As calculated from the HRM qPCR standard curve.
[c]Only sample from 2016 with sufficient DNA for multi-locus sequence typing.

(*Melopsittacus undulatus*) and turquoise parrots (*Neophema pulchella*) that died without clinical signs (Table 1).

Further samples were collected ante-mortem from 128 non-native and native zoo birds (Table 1). The samples were taken from asymptomatic birds housed either in the zoo's aviaries or in its quarantine area. Sample collection was carried out from May to December 2016 by trained bird keepers and veterinary staff during handling for scheduled health checks or relocation within the zoo. A combined sample from the conjunctiva, choanal slit and cloaca C-C-C) of each bird was taken using sterile plain dry cotton tipped swabs. This live bird sampling was conducted under Massey University animal ethics permit 19_26.

In 2019, a cluster of avian chlamydiosis occurred in one aviary housing multiple bird species at Auckland Zoo. In this aviary, a total of six birds died (four diamond doves (*Geopelia cuneata*) and two superb parrots (*Polytelis swainsonii*)), and another six asymptomatic birds from four species (crested pigeons (*Ocyphaps lophotes*), Gouldian finches (*Erythrura gouldiae*), turquoise parrots, cockatiels (*Nymphicus hollandicus*)) were confirmed with *C. psittaci* infection by PCR testing and are not further included in the molecular analysis presented in this paper. All in-contact birds with no clinical signs were moved and treated for avian chlamydiosis with 100 mg/kg doxycycline for 45 days without mortality. The three formulations of doxycycline that were used included a compounded formulation (Optimus, Auckland, NZ) given SC as an initial treatment in some but not all birds; oral doxycycline (Doxyvet; Vetafarm, Wagga Wagga, Australia) given in water or nectar, and for some parrots, oral doxycycline (Vibravet, Zoetis, Auckland NZ) was given in peanut butter. In a separate aviary, five zebra finches (*Taeniopygia guttata*) died without pathological signs of avian chlamydiosis but tested positive by PCR.

From the birds that died as part of the disease cluster (four diamond doves, two superb parrots, five zebra finches), positive tissue samples (lungs and/or livers) were later sent to Massey for MLST testing (Table 2). Tissue samples were also collected during post-mortem and stored at −20°C, as per usual zoo protocol, from nine birds (two little penguins, two kākā (*Nestor meridionalis*), four orange-fronted parakeets/kākāriki (*Cyanoramphus malherbi*), and a North Island saddleback/tīeke (*Philesturnus rufusater*)) that died in the months preceding or in the 1 month following the April 2019 outbreak (Table 2). Samples were selected to represent a wide range of species, housed over different areas of the zoo, and for which gross post-

Table 2. Species of birds sampled[a] post-mortem from Auckland Zoo (Auckland, NZ) in 2019, and tested for the presence of *Chlamydia psittaci* by high resolution melting quantitative PCR.

	C. psittaci-positive	
Species	Yes	No
Diamond dove (*Geopelia cuneata*)[b]	4	0
Superb parrot (*Polytelis swainsonii*)[b]	2	0
Zebra finch (*Taeniopygia guttata*)	3	2
Little penguin/ kororā (*Eudyptula minor*)	0	2
North Island kākā (*Nestor meridionalis*)	0	2
Orange-fronted parakeet/ kākāriki (*Cyanoramphus malherbi*)	0	4
Saddleback/tīeke (*Philesturnus rufusater*)	0	1

[a]Liver and/or lung
[b]Birds present in the mixed species aviary affected by the avian chlamydiosis mortality cluster. All other birds were from different aviaries within the zoo and had died in the months preceding or in the 1 month following the April outbreak.

mortem and histology had been inconclusive or respiratory disease was seen.

DNA extraction and C. psittaci *qPCR testing*

Genomic DNA was extracted from the 140 C-C-C swabs using a Chelex 100 (Bio-Rad, Auckland, NZ) extraction protocol, with minor modification (Martin-Galvez *et al.* 2011). The extracted DNA was stored at −20°C until used for PCR analysis. DNA was extracted from the lung and liver tissue using the DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol and was quantified using NanoDrop 2000 (ThermoFisher Scientific, Waltham, MA, USA).

To determine if the samples were positive for *C. psittaci*, a high-resolution melting (HRM) qPCR assay targeting a 418-bp fragment of the variable domain 1 of the *C. psittaci ompA* gene was performed as described previously (Soon *et al.* 2021). *C. psittaci*-positive samples (run in duplicate) were defined as those for which the amplification produced a cycle threshold (Cq) value < 35 but also generated an HRM melt curve within the expected range of dissociation temperatures (*ompA* genotype A: 86.86 ± 0.5°C; and *ompA* genotype C: 85.31 ± 0.5°C) (Soon *et al.* 2021). A standard curve was generated by testing serial 10-fold dilutions (2.3×10^8–2.3×10^1 copies/µL) of the previously cloned 418-bp fragments of the *ompA* gene (genotypes A and C) in triplicate. This was used to determine the *C. psittaci* genome copy number per µL DNA in the samples.

C. psittaci *genotyping*

ompA *genotyping*

To confirm the *C. psittaci* genotype, the 418-bp fragment of the variable domain 1 region of the *ompA* gene was sequenced from 12 samples (from 2016 or 2019) that had Cq values < 28 or a copy number > 100 (Table 2). Endpoint PCR for *ompA* (Jelocnik *et al.* 2019) using the same primers as used for the HRM qPCR analysis was performed as follows for all reactions: 1 x HOT FIREPol Blend Master Mix (10 mM $MgCl_2$; Solis BioDyne, Tartu, Estonia), 200 nM of each primer and 50 ng of a template made to a total volume of 20 µL with nuclease-free water. Thermal cycling was performed in a Master Cycler Nexus X2 (Eppendorf; Hamburg, Germany) with the following times and temperatures: 15 minutes at 95°C, followed by 40 cycles of 95°C for 30 seconds, 50°C, 55°C or 60°C (dependent on primer) for 30 seconds, 72°C for 60 seconds, followed by a final extension at 72°C for 7 minutes. PCR products were separated by gel electrophoresis. PCR amplicons were excised from the gel and eluted overnight at 4°C in elution buffer (10 mM Tris, pH 8.0) and sent for bi-directional Sanger sequencing to Massey Genome Service (Massey University, Palmerston North, NZ). Upon sequencing, *ompA* chromatograms were aligned using Geneious (v10.1.3; Kearse *et al.* 2012), and the newly generated *ompA* sequences were subjected to BLASTn analysis to determine sequence similarity to known *C. psittaci ompA* genotypes.

Multilocus sequence typing

Chlamydia psittaci MLST was applied to 10 samples from four bird species (2016: little penguin; 2019: diamond dove, superb parrot, zebra finch). Endpoint PCR for *C. psittaci* MLST genes was performed using primers described in Jelocnik *et al.* (2019). Briefly, PCR reactions were performed as above for *ompA* fragments, also followed by sequencing at Massey Genome Service. All chromatograms for each allele were manually trimmed and aligned to the corresponding reference allele using pairwise alignment with Geneious v10.1.3. The MLST alleles were then concatenated, and ST was determined against the *Chlamydiales* database using PubMLST/Chlamydiales.[1] The 3,098 bp alignment of the 44 concatenated *C. psittaci* MLST sequences were generated using Clustal Omega 1.2.2 as implemented in Geneious Prime 2021.1.1 (Biomatters, Auckland, NZ). The mid-point rooted Bayesian tree was constructed using the concatenated MLST 3,098 bp alignment with the MrBayes 3.2.6 program (Ronquist *et al.* 2012) as implemented in Geneious. The tree parameters included: GTR + I +G nucleotide model, with four Markov chain Monte Carlo chains with a million generations, sampled every 1,000 generations and with the first 100,000 trees discarded as burn-in. The MLST sequences generated in this study are deposited in PubMLST/Chlamydiales.[1] The *ompA* and MLST sequences from a captive little penguin, diamond dove, superb parrot, and a zebra finch

[1]https://pubmlst.org/chlamydiales/

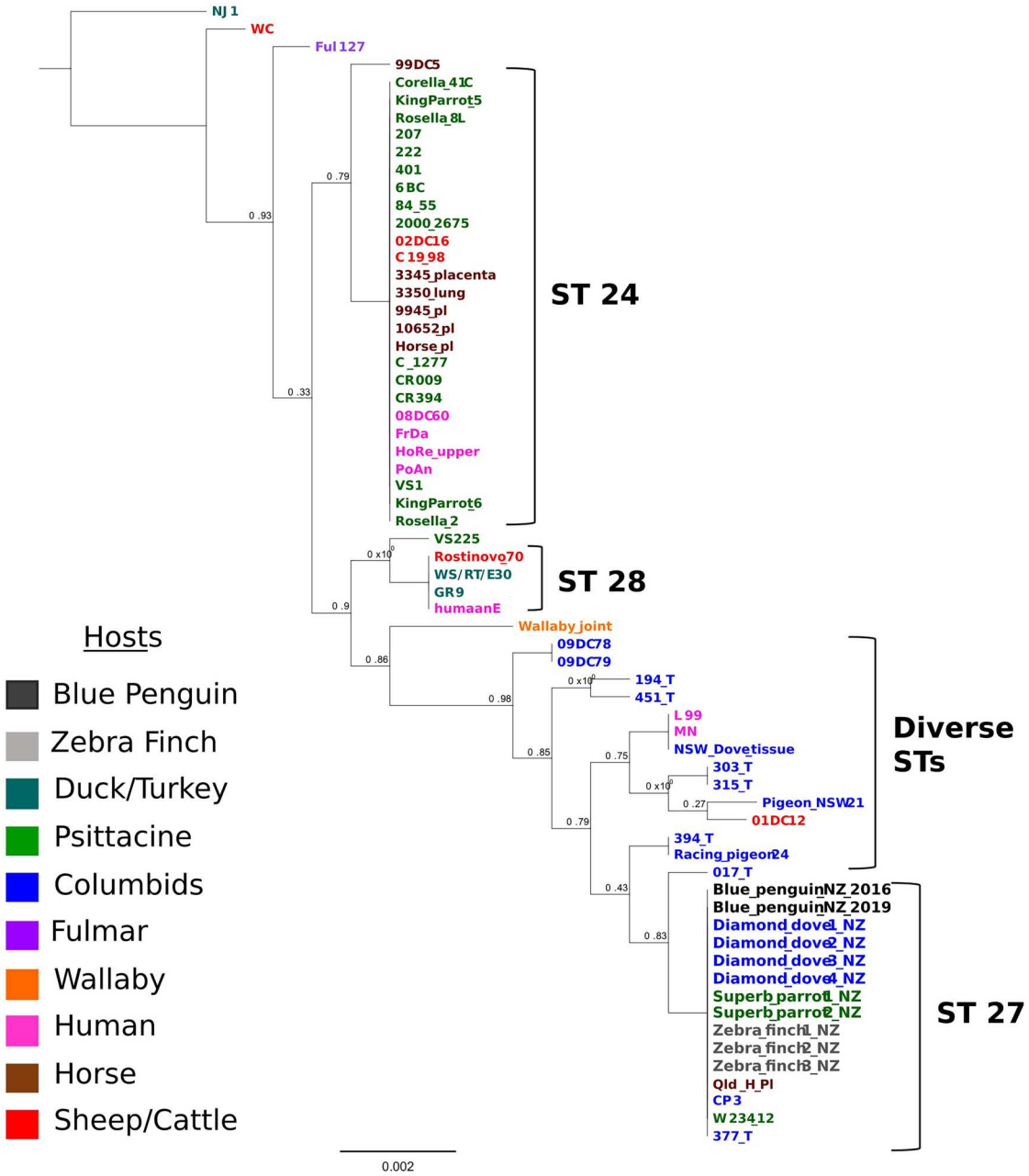

Figure 1. Bayesian phylogenetic tree of multi-locus sequence typing sequences from *Chlamydia psittaci* strains isolated from birds that died of avian chlamydiosis at Auckland Zoo (Auckland, NZ) in 2016 or 2019 (BluePenguin_NZ, Diamond_dove_NZ, Superb_-parrot_NZ, Zebra_finch_NZ) and from a range of global avian and mammalian hosts. Strains are coloured by the species of the hosts, and the strain from this study is in bold text. Sequence types (ST) in a clade are indicated by brackets. *C. psittaci* M56 was used as an outgroup as it is a strain associated with mammalian hosts.

have previously been published in a comparative genomics paper (Kasimov *et al.* 2023).

Results

HRM-qPCR C. psittaci *detection*

Chlamydia psittaci was detected at a total prevalence of 0.1 (14/140; 95% CI = 0.061–0.161) of the survey samples collected in 2016, from seven species of birds: five species of parrots, crested pigeon (*Ocyphaps lophotes*) and little penguin (Table 1). In the 2019 cluster, *C. psittaci* was detected by HRM qPCR in 9/20 post-mortem samples collected from three different bird species (diamond dove, superb parrot, and zebra finch; Table 2).

C. psittaci *genotyping using* ompA *and MLST*

From the 2016 survey, 13 of the positive samples had very low *C. psittaci* copy levels, with only the post-mortem sample from one of the sick little penguins showing high copy numbers. The sequence obtained from the little penguin aligned to *ompA* genotype B (AF269265) while the sequences from post-mortem samples from a budgerigar and a turquoise parrot

aligned to *ompA* genotype A. All nine positive samples from the 2019 collection were similarly sequenced and found to align with the *C. psittaci ompA* genotype B.

Upon examination of the genotype B sequences from the MLST analysis, the nine *C. psittaci*-positive samples from birds in the 2019 cluster and a sample from a little penguin from the 2016 cluster (10 in total) showed identical alleles in all seven loci examined. This result indicated the presence of only one strain of *C. psittaci* in the birds from the 2019 collection which was identical to the sample collected from the little penguin in 2016. This strain has been designated "BluePenguin_NZ" and was added to pubMLST along with sequences from 2019 designated "Diamond_dove_NZ" and "Superb_parrot_NZ".

The BluePenguin_NZ strain was identified as belonging to the "pigeon type" sequence type (ST) 27 clade as it clustered in a well-supported broader clade with other ST27 strains (Kasimov *et al.* 2023). The BluePenguin_NZ strain was identical to and formed a sub-clade with other ST27 strains detected from Australian sulphur-crested cockatoo (*Cacatua galerita*; W234_12), thoroughbred horses (QLd_H_Pl) and pigeons from Europe (CP3, 377_T) (Figure 1).

Discussion

To date, there is limited genotyping information available on avian *C. psittaci* strains from New Zealand. This study provides molecular descriptions of the *C. psittaci* strains from routine surveillance at a zoo in New Zealand in 2016, and an avian chlamydiosis cluster affecting multiple species of birds in 2019 at the same zoo. In the majority of *C. psittaci*-positive samples taken from the zoo species, we identified *C. psittaci* with *ompA* genotype B. In addition to the previous *C. psittaci* strains with *ompA* genotypes A and C found in New Zealand, this study indicates that at least three genetically diverse *C. psittaci* strains may be present in New Zealand (Gedye *et al.* 2018). The strains with *ompA* B genotype found at the zoo infected a range of species, of which two shared a single aviary (superb parrots and diamond doves), while the zebra finches that died were in a separate aviary. Three additional species tested positive from HRM qPCR screening, while other bird species in close contact with these birds were not affected, suggesting a variation in host susceptibility. When we further applied *C. psittaci* MLST to the avian chlamydiosis cluster samples, it was observed that all recovered infecting strains were ST27. These ST (with *ompA* genotype B) belong to a closely related yet diverse *C. psittaci* "pigeon clade", found mostly in pigeon hosts globally (Anstey *et al.* 2021; White *et al.* 2022). These results demonstrate the pathogenicity of genotype B, ST27 strain, in multiple bird species, including pigeons, parrots and penguins.

The initial survey of asymptomatic birds in 2016 was significant in identifying a range of bird species that were positive on HRM qPCR but only produced very low copy numbers in contrast to high copy numbers retrieved from birds with avian chlamydiosis. Whether these birds were also infected with the ST27 strain could not be determined as not enough DNA could be recovered for further sequencing. The potential zoonotic risk of latently infected birds is unknown and worthy of further study, given the prevalence of asymptomatic birds identified in this study. Regular testing of these birds is often cost-prohibitive, but in the event of illness or mortality, testing for *C. psittaci* and identifying any strains involved in the disease by MLST would be useful in understanding the risks associated with this pathogen.

This report describes two clusters of disease due to avian chlamydiosis in a zoological collection, but for context, it should be noted that the zoo has conducted broad surveillance of the entire bird collection for the 3 years between the two clusters, and these are the only cases of disease or detection of *C. psittaci* that have been seen in this time. Further, over a period of 5 years (2016–2020), Auckland Zoo carried out 1,450 PCR tests for *C. psittaci* (on a captive bird population of about 315) which includes several tests during quarantine for incoming birds or when transferring birds out of the facility, and for admission of wild birds. The finding of the same ST27 strain in both 2016 and 2019 raises the question as to whether the strain had become latent or endemic in the zoo collection. However, the affected penguin from 2016 was only present for 2 days before it was euthanised, and was in isolation at the veterinary department, which is geographically removed from the collection aviaries. There was no shared airspace, no keeper overlap, and strict biosecurity precautions were in place.

It is also of concern that the sick little penguin identified with *C. psittaci* ST27 from 2016 had been cared for at a private wildlife rehabilitator for several months before being transferred to the zoo. There is no information available on what other wild species the penguin was in direct or indirect contact with prior to being transferred to the zoo. We therefore cannot determine if the little penguin acquired its infection in the wild, or during its time at the wildlife rehabilitation facility. It may also have exposed a number of wild birds undergoing rehabilitation to *C. psittaci*, potentially resulting in the transmission of this strain to wild populations. Zoonotic transfer of *C. psittaci* has been previously documented between birds and people in an avian refuge centre in Belgium (Kalmar *et al.* 2014). The prevalence of *C. psittaci* in wild mallards undergoing rehabilitation has also been confirmed in New Zealand (Soon *et al.* 2021). We

recommend that the prevalence of *C. psittaci* be studied in wildlife undergoing rehabilitation to assess the risk to wildlife and also to the people involved in wildlife rehabilitation efforts.

While this research was not a study of transmission between wild birds and zoo birds, a potential source of the *C. psittaci* could be free-ranging synanthropic birds. It is of interest that the strain of *C. psittaci* that was involved in the avian chlamydiosis cluster in 2019 was identified in a sick wild penguin brought into the zoo in 2016. Since this penguin was kept in isolation at the veterinary department, with no direct or indirect contact with the zoo collection birds, it is likely that this strain is present but has not yet been described, in New Zealand. *C. psittaci* has been found in numerous free-ranging pigeons in various locations around the world (Mattmann *et al.* 2019; Stokes *et al.* 2021). The aviaries where the positive cases were found at Auckland Zoo are constructed with mesh that does not allow any wild birds to enter, although, in theory, wild bird faeces could drop into the enclosure.

These results raise numerous biosecurity questions regarding *C. psittaci* in New Zealand. Previous investigations identified *C. psittaci ompA* genotypes A and C in 13 species (Gedye *et al.* 2018; Soon *et al.* 2021). Our results have shown that *C. psittaci* can be present in New Zealand zoo birds either as an endemic asymptomatic infection or as a pathogen causing mortality. Global studies consistently show that *C. psittaci* has a broad host range, including a variety of birds, livestock and other mammals, including humans (Anstey *et al.* 2021; Kasimov *et al.* 2022). Our results also show host diversity, with a range of native and non-native bird species being affected by *C. psittaci*. While none of the zoo workers reported having symptoms of *C. psittaci* infection and wore appropriate personal protective equipment during sample collections, proximity of carers to an infected animal could see cross-species zoonotic transmission from the bird to the human, either at the zoo or in wildlife rehabilitation facilities. Globally, *C. psittaci* is recognised as a One Health pathogen (Liu *et al.* 2023). While still in its infancy, future studies on this topic in NZ should include broader surveillance of free-living birds, and genomic characterisation of avian chlamydia affecting both captive and free-ranging avian (but also mammalian) populations to assess the host range and identify opportunities for spillover.

In conclusion, this report associates the death of birds due to avian chlamydiosis in a zoological collection with a single genotype, ST27, providing evidence of pathogenicity in birds.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

Anstey SI, Kasimov V, Jenkins C, Legione A, Devlin J, Amery-Gale J, Gilkerson J, Hair S, Perkins N, Peel AJ, *et al.* *Chlamydia psittaci* ST24: clonal strains of One Health importance dominate in Australian horse, bird and human infections. *Pathogens* 10, 1015, 2021. https://doi.org/10.3390/pathogens10081015

Balsamo G, Maxted AM, Midla JW, Murphy JM, Wohrle R, Edling TM, Fish PH, Flammer K, Hyde D, Kutty PK, *et al.* Compendium of measures to control *Chlamydia psittaci* infection among humans (psittacosis) and pet birds (avian chlamydiosis). *Journal of Avian Medicine and Surgery* 31, 262–82, 2017. https://doi.org/10.1647/217-265

Borel N, Polkinghorne A, Pospischil A. A review on chlamydial diseases in animals: still a challenge for pathologists? *Veterinary Pathology* 55, 374–90, 2018. https://doi.org/10.1177/0300985817751218

Chaber AL, Jelocnik M, Woolford L. Undiagnosed cases of human pneumonia following exposure to *Chlamydia psittaci* from an infected rosella parrot. *Pathogens* 10, 968, 2021. https://doi.org/10.3390/pathogens10080968

De Meyst A, Aaziz R, Pex J, Braeckman L, Livingstone M, Longbottom D, Laroucau K, Vanrompay D. Prevalence of new and established avian chlamydial species in humans and their psittacine pet birds in Belgium. *Microorganisms* 10, 1758, 2022. https://doi.org/10.3390/microorganisms10091758

Fastier LB, Austin FJ. Psittacosis among Australian parrots imported into New Zealand. *New Zealand Medical Journal* 53, 373–9, 1954

Gartrell BD, French NP, Howe L, Nelson NJ, Houston M, Burrows EA, Russell JC, Anderson SH. First detection of *Chlamydia psittaci* from a wild native passerine bird in New Zealand. *New Zealand Veterinary Journal* 61, 174–6, 2013. https://doi.org/10.1080/00480169.2012.740656

Gedye KR, Fremaux M, Garcia-Ramirez JC, Gartrell BD. A preliminary survey of *Chlamydia psittaci* genotypes from native and introduced birds in New Zealand. *New Zealand Veterinary Journal* 66, 162–5, 2018. https://doi.org/10.1080/00480169.2018.1439779

Harkinezhad T, Geens T, Vanrompay D. *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. *Veterinary Microbiology* 135, 68–77, 2009. https://doi.org/10.1016/j.vetmic.2008.09.046

Jelocnik M, Branley J, Heller J, Raidal S, Alderson S, Galea F, Gabor M, Polkinghorne A. Multilocus sequence typing identifies an avian-like *Chlamydia psittaci* strain involved in

equine placentitis and associated with subsequent human psittacosis. *Emerging Microbes Infection* 6, e7, 2017. https://doi.org/10.1038/emi.2016.135

Jelocnik M, Polkinghorne A, Pannekoek Y. Multilocus sequence typing (MLST) of Chlamydiales. *Methods in Molecular Biology* 2042, 69–86, 2019. https://doi.org/10.1007/978-1-4939-9694-0_7

Johnson FW, Lyon DG, Wilkinson R, Bloomfield P, Philips HL. Isolation of *Chlamydia psittaci* from newly imported keas (*Nestor notabilis*). *Veterinary Record* 114, 298–9, 1984. https://doi.org/10.1136/vr.114.12.298

Kalmar ID, Dickx V, Dossche L, Vanrompay D. Zoonotic infection with *Chlamydia psittaci* at an avian refuge centre. *Veterinary Journal* 199, 300–2, 2014. https://doi.org/10.1016/j.tvjl.2013.10.034

Kasimov V, Dong Y, Shao R, Brunton A, Anstey SI, Hall C, Chalmers G, Conroy G, Booth R, Timms P, *et al.* Emerging and well-characterized chlamydial infections detected in a wide range of wild Australian birds. *Transboundary and Emerging Diseases* 69, e3154–70, 2022. https://doi.org/10.1111/tbed.14457

Kasimov V, White RT, Foxwell J, Jenkins C, Gedye K, Pannekoek Y, Jelocnik M. Whole-genome sequencing of *Chlamydia psittaci* from Australasian avian hosts: a genomics approach to a pathogen that still ruffles feathers. *Microbial Genomics* 9, 001072, 2023. https://doi.org/10.1099/mgen.0.001072

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, *et al.* Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–9, 2012. https://doi.org/10.1093/bioinformatics/bts199

Knittler MR, Sachse K. *Chlamydia psittaci*: update on an underestimated zoonotic agent. *Pathogens and Disease* 73, 1–15, 2015. https://doi.org/10.1093/femspd/ftu007

Liu S, Cui Z, Carr MJ, Meng L, Shi W, Zhang Z, Liu S, Cui Z, Carr MJ, Meng L, *et al.* *Chlamydia psittaci* should be a notifiable infectious disease everywhere. *The Lancet Microbe* 4, e62–3, 2023. https://doi.org/10.1016/S2666-5247(22)00306-8

Martin-Galvez D, Peralta-Sanchez JM, Dawson DA, Martin-Platero AM, Martinez-Bueno M, Burke T, Soler JJ. DNA sampling from eggshell swabbing is widely applicable in wild bird populations as demonstrated in 23 species. *Molecular Ecology Resources* 11, 481–93, 2011. https://doi.org/10.1111/j.1755-0998.2010.02953.x

Mattmann P, Marti H, Borel N, Jelocnik M, Albini S, Vogler BR. Chlamydiaceae in wild, feral and domestic pigeons in Switzerland and insight into population dynamics by *Chlamydia psittaci* multilocus sequence typing. *PLoS One* 14, e0226088, 2019. https://doi.org/10.1371/journal.pone.0226088

McCausland IP, Carter ME, O'Hara PJ. Clinical ornithosis in a New Zealand aviary. *New Zealand Veterinary Journal* 20, 53–4, 1972. https://doi.org/10.1080/00480169.1972.34004

***Motha MXJ, Reed C, Gibbons A.** The prevalence of *Chlamydia psittaci* in feral pigeons and native psittacines. *Surveillance* 22(4), 20–2, 1995

Pannekoek Y, Morelli G, Kusecek B, Morre SA, Ossewaarde JM, Langerak AA, Van der Ende A. Multi locus sequence typing of Chlamydiales: clonal groupings within the obligate intracellular bacteria *Chlamydia trachomatis*. *BMC Microbiology* 8, 42, 2008. https://doi.org/10.1186/1471-2180-8-42

Pannekoek Y, Dickx V, Beeckman DS, Jolley KA, Keijzers WC, Vretou E, Maiden MC, Vanrompay D, Van der Ende A. Multi locus sequence typing of *Chlamydia* reveals an association between *Chlamydia psittaci* genotypes and host species. *PLoS One* 5, e14179, 2010. https://doi.org/10.1371/journal.pone.0014179

Rawdon TG, Potter JS, Harvey CJ, Westera BF. Chlamydiosis (psittacosis) in Malay spotted doves *Streptopelia chinesis*. *Kokako* 16, 54–6, 2009

Read TD, Joseph SJ, Didelot X, Liang B, Patel L, Dean D. Comparative analysis of *Chlamydia psittaci* genomes reveals the recent emergence of a pathogenic lineage with a broad host range. *mBio* 4, e00604–12, 2013. https://doi.org/10.1128/mBio.00604-12

Ronquist F, Teslenko M, Van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. Mrbayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61, 539–42, 2012. https://doi.org/10.1093/sysbio/sys029

Soon XQ, Gartrell B, Gedye K. Presence and shedding of *Chlamydia psittaci* in waterfowl in a rehabilitation facility and in the wild in New Zealand. *New Zealand Veterinary Journal* 69, 240–6, 2021. https://doi.org/10.1080/00480169.2021.1915212

Stokes HS, Berg ML, Bennett ATD. A review of chlamydial infections in wild birds. *Pathogens* 10, 948, 2021. https://doi.org/10.3390/pathogens10080948

Sukon P, Nam NH, Kittipreeya P, Sara-In A, Wawilai P, Inchuai R, Weerakhun S. Global prevalence of chlamydial infections in birds: a systematic review and meta-analysis. *Preventative Veterinary Medicine* 192, 105370, 2021. https://doi.org/10.1016/j.prevetmed.2021.105370

Van Lent S, Piet JR, Beeckman D, van der Ende A, Van Nieuwerburgh F, Bavoil P, Myers G, Vanrompay D, Pannekoek Y. Full genome sequences of all nine *Chlamydia psittaci* genotype reference strains. *Journal of Bacteriology* 194, 6930–1, 2012. https://doi.org/10.1128/JB.01828-12

Wang C, Li L, Xie Y, Tan Y, Wen Y, Chen Q, Yu J, Song Y, Wu Y. Isolation and characterization of avian *Chlamydia psittaci* from symptomatic pet birds in Southern Hunan, China. *Avian Diseases* 63, 31–7, 2018. https://doi.org/10.1637/11932-071718-Reg.1

Wang H, Jensen JK, Olsson A, Vorimore F, Aaziz R, Guy L, Ellstrom P, Laroucau K, Herrmann B. *Chlamydia psittaci* in fulmars on the Faroe Islands: a causative link to South American psittacines eight decades after a severe epidemic. *Microbes and Infection* 22, 356–9, 2020. https://doi.org/10.1016/j.micinf.2020.02.007

White RT, Anstey SI, Kasimov V, Jenkins C, Devlin J, El-Hage C, Pannekoek Y, Legione AR, Jelocnik M. One clone to rule them all: culture-independent genomics of *Chlamydia psittaci* from equine and avian hosts in Australia. *Microbial Genomics* 8, mgen000888, 2022. https://doi.org/10.1099/mgen.0.000888

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