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WILDLIFE RESEARCH

Chlamydia in wild Australian rodents: a cross-sectional study to inform disease risks for a conservation translocation

Fiona Knox[A,B,*], Martina Jelocnik[C,D], Nahiid Stephens[A], Colleen Sims[B], Bethany Jackson[A], Saul Cowen[B], Kelly Rayner[B], Sean Garretson[B], Lian Yeap[A], Kristin Warren[A] and Rebecca Vaughan-Higgins[A]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Fiona Knox
School of Veterinary Medicine, Murdoch University, Murdoch, WA 6150 Australia
Email: Fiona.knox@murdoch.edu.au

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ABSTRACT

Context. *Chlamydia* species impose major global burdens on both human and animal health. However, chlamydial infections of wild rodents have been understudied, posing limitations on assessments of disease risks for rodent conservation translocations. This is particularly true when there is evidence of infection in proposed source populations, as occurred for the Shark Bay mouse (*Pseudomys gouldii*) translocations to Dirk Hartog Island. **Aims.** The aim of this study was to reduce uncertainty surrounding the risks posed by *Chlamydia* for these translocations by: (1) determining the presence, prevalence and diversity of *Chlamydia* in rodent populations in the Shark Bay region of Western Australia; (2) identifying associations with health parameters; and (3) assessing for evidence of cross-species transmission. **Methods.** Swab, faecal and tissue samples from 110 wild-caught individuals (comprising five rodent and two marsupial species) were collected across four islands in Western Australia. These samples were analysed by a *Chlamydiaceae* 23s rRNA qPCR in a 14-month cross-sectional study conducted between 2020 and 2021. **Key results.** In total, 20% of all individuals (22/110; 95%CI 13.6–28.4) from five species, including 19% (19/100; 95% CI 12.5–27.8) of rodents, were positive by the *Chlamydiaceae* qPCR, although in low loads. Further attempts at species identification of the *Chlamydiaceae* were unsuccessful. Our results found no detectable adverse health associations, or significant associated pathological findings, with low molecular loads supporting an asymptomatic infection state. Additionally, there were no disease associations in Shark Bay bandicoots (*Perameles bougainville*) despite the presence of an ocular disease syndrome previously linked to chlamydial infection in this species. **Conclusions and implications.** Our findings suggest that sub-clinical chlamydial infections in wild Australian rodents may be widespread, but for the Dirk Hartog Island translocations, the risks of *Chlamydia* associated with movement of Shark Bay mice are likely low. The results highlight how current knowledge gaps pertaining to wildlife health can be addressed through collaborative approaches to translocation planning and implementation.

Keywords: bandicoot, *Chlamydiaceae*, *Leporillus*, mouse, *Pseudomys*, reintroduction, Shark Bay, stick-nest rat, wildlife health.

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Introduction

With growing threats to global biodiversity, wildlife translocations are an increasingly employed conservation tool utilised for either ecological restoration purposes or to enhance the conservation status of threatened species. This includes population augmentation and genetic rescue efforts, or establishment of new populations through assisted colonisation or reintroductions (Seddon *et al.* 2012). However, wildlife translocations are associated with a high biosecurity risk, with the potential for release of pathogens into naïve ecosystems, exposure of translocated species to novel pathogens and disruption of ecological processes and subsequently, infectious disease dynamics. Although definitively linking disease to translocation failures can be complex, and disease may be overlooked in the absence of targeted monitoring, there are several examples across multiple taxa where the

impacts of disease have triggered significant adverse consequences for translocation programs (Stockwell *et al.* 2008; Kock *et al.* 2010; Hlokwe *et al.* 2016; Beckmann *et al.* 2022). To identify and mitigate associated disease risks, guidelines for wildlife disease risk analyses (WDRAs) have been developed and the utilisation of WDRAs in translocation planning is considered gold-standard practice (International Union for Conservation of Nature 2013). With long-distance movements underpinning many Australian wildlife translocations, WDRAs should be considered an essential element of Australian conservation translocation planning, particularly given the increased biosecurity risk posed by movements across geographical barriers (Short 2009; Sainsbury and Vaughan-Higgins 2012; Wildlife Health Australia 2018).

Unfortunately, epidemiological knowledge deficiencies are commonly encountered during WDRA processes. Translocations, as well as attached ecological monitoring programs, may be an opportunity to address these knowledge gaps. This may enable refinement of WDRAs as part of an iterative process for the benefit of future translocations and potentially alter understanding of how disease may influence broader species conservation priorities. A WDRA for a proposed translocation of native Australian rodents to Dirk Hartog Island (DHI), Western Australia (Knox, F., unpubl. data), identified several knowledge deficits regarding the role native Australian murids play in the epidemiology of significant disease hazards. A specific outcome of this WDRA was to recommend further investigation of the historical detection of *Chlamydia* in Shark Bay mice (SBM) (*Pseudomys gouldii*) on Bernier Island (Sims, C., unpubl. data), a proposed source population of SBM for the DHI translocations.

Chlamydia spp., members of the intracellular *Chlamydiaceae* family, impose major global burdens on both human and animal health. Some species, such as *Chlamydia psittaci*, have serious zoonotic potential (Cheong *et al.* 2019), and *Chlamydia trachomatis* is the leading cause of infectious blindness and infertility in humans (Dean *et al.* 2008; Hocking *et al.* 2023). *Chlamydia* spp. reduce livestock productivity (Reinhold *et al.* 2011) and, as in the case of *Chlamydia pecorum*, threaten the conservation of wildlife populations (Burnard and Polkinghorne 2016; Quigley and Timms 2020). The characteristics of the *Chlamydia* genus meet several of the criteria proposed by Rideout *et al.* (2017) to prioritise pathogens of concern during wildlife translocations. That is, they are possibly non-native microorganisms with broad host ranges and reservoirs, characterised by long incubation and infectious periods, and for which there is limited availability of effective treatment or vaccines applicable to free-ranging wildlife (Reinhold *et al.* 2011; Burnard and Polkinghorne 2016; Waugh *et al.* 2016; Borel *et al.* 2018).

In 2001, opportunistic sampling identified a *Chlamydiaceae* PCR-positive SBM on Bernier Island, Western Australia (Sims, C., unpubl. data). To the authors' knowledge, there have been no published studies of this genus in Australian murid rodents, and global epidemiological knowledge regarding chlamydial infections in wild rodents, particularly murids, is limited. Information regarding *Chlamydia* species diversity in wild rodents is lacking (Spalatin *et al.* 1971; Ramsey *et al.* 2016), and few identified studies have examined potential health implications of infection for wild rodent hosts (Stephan *et al.* 2014; Ramsey *et al.* 2016). Despite infertility and chronic pneumonias documented from experimental infections of laboratory murids (Rank 2006; Murthy *et al.* 2016), knowledge extrapolation is hindered by experimental manipulation to develop animal models of human infection (Murthy *et al.* 2016). However, *Chlamydia muridarum* has recently re-emerged as a natural pathogen warranting targeted exclusion in laboratory mice, due to associations with incidental pulmonary lesions and more severe disease in immunocompromised mice (Mishkin *et al.* 2022). In non-murid rodents, both *Chlamydia caviae* and a highly virulent *C. psittaci* strain (M56) have been detected in association with disease in natural infections of guinea pigs (*Cavia porcellus*) and wild muskrats (*Ondatra zibethicus*) respectively, and both have been linked to zoonotic transmission events (Murray 1964; Spalatin *et al.* 1966; Borel *et al.* 2023).

Additionally, there is growing awareness of possible reservoir and maintenance communities in chlamydial disease ecology (Dean *et al.* 2013; Jelocnik *et al.* 2013; Polkinghorne *et al.* 2013; van Grootveld *et al.* 2018; Akter *et al.* 2021; Anstey *et al.* 2021). It has been suggested by some authors that rodents may play a role in spillover of infection in some settings, but the capacity for this to occur has received limited examination (Eddie *et al.* 1969; Cisláková *et al.* 2004; Stephan *et al.* 2014; Burnard and Polkinghorne 2016). *Chlamydia* spillover risk to sympatric species is particularly important in the context of the DHI translocations, with reported disease associations in the Shark Bay bandicoot (*Perameles bougainville*), a threatened species that has recently been reintroduced to DHI. *Chlamydia* has previously been assessed as posing a risk to translocations of Shark Bay bandicoots (Vaughan-Higgins *et al.* 2021), with *C. pecorum*, *Chlamydia pneumoniae* and other species within the *Chlamydiales* order linked to an ocular disease syndrome characterised by corneal opacities, conjunctivitis, missing or ruptured globes and purulent ocular discharge in this species (Bodetti *et al.* 2003; Warren *et al.* 2005; Kumar *et al.* 2007; Kutlin *et al.* 2007). However, the presence of shared chlamydial genotypes across wildlife populations in the region has not been previously investigated.

The aim of this study was to gather further baseline information to assess what disease risks *Chlamydia* may pose for the DHI rodent translocations. Principal objectives were to determine the infection status of rodent populations across both source and destination sites, identify chlamydial species diversity harboured, assess for evidence of cross-species transmission amongst sympatric wildlife and measure associations with physical, clinical and pathological findings. Additionally, we aimed to ascertain preferred anatomical sampling sites in these rodents to facilitate future investigations.

Materials and methods

Sampling

Samples were collected between 22 March 2020 and 25 May 2021 from 110 wild animals (representing five rodent and two marsupial species) originating from Bernier Island (n = 31), Dirk Hartog Island (n = 48), Salutation Island (n = 16) or Northwest Island (Montebello Islands Archipelago) (n = 15) (Fig. 1). Animals were caught either under existing ecological monitoring programs established across these islands, for translocation purposes, or through targeted trapping to increase sample sizes. Animals were trapped using a mix of baited Sheffield cage traps (Sheffield Wire Products, Welshpool, WA or Mascot Wire Products, Preston, Victoria), baited Elliott traps (Elliott Scientific Equipment, Upwey, Victoria) and pit-fall traps. Animals from Salutation and Northwest Island were sampled on arrival at Dirk Hartog Island during translocation.

Animals were either sampled live under general anaesthesia (n = 66) or after being found recently deceased or following euthanasia for welfare reasons (n = 14), and house mice (*Mus musculus*) were sampled immediately after euthanasia for research purposes (n = 30). All animals were subject to physical examination by a veterinarian at the time of sampling and morphometrics recorded. Age was subjectively recorded based on observer classification as either adult or subadult, guided by weight, morphometrics and reproductive condition. All deceased animals were subject to detailed post-mortem examination by a veterinarian. Gross findings were documented, and representative samples of tissue collected, both frozen and in 10% neutral buffered formalin. Blood was collected from house mice immediately after euthanasia via cardiac puncture, with packed cell volume (PCV) measured after centrifugation of heparinised micro-haematocrit tubes at 12 000 RPM (7500*g*) for a total of 5 min (ZipCombo, LW Scientific, Lawrence, GA, USA), and total plasma solids (TPS) measured via a Brix refractometer.

Live animals were removed from traps using calico bags for handling. Anaesthesia was then induced and maintained using isoflurane (Veterinary Companies of Australia, Kings Park, NSW, Australia) in 100% medical grade oxygen delivered via face mask or nose cone by a portable inhalational anaesthetic machine ('Stinger', Advanced Anaesthetic Specialists, Gladesville, NSW, Australia). Live animals with pouch young, lactating or assessed as likely pregnant were excluded from sampling (n = 2).

Samples were collected by gentle swabbing of the conjunctival, oral, rectal and urogenital spaces (or conjunctival, oral

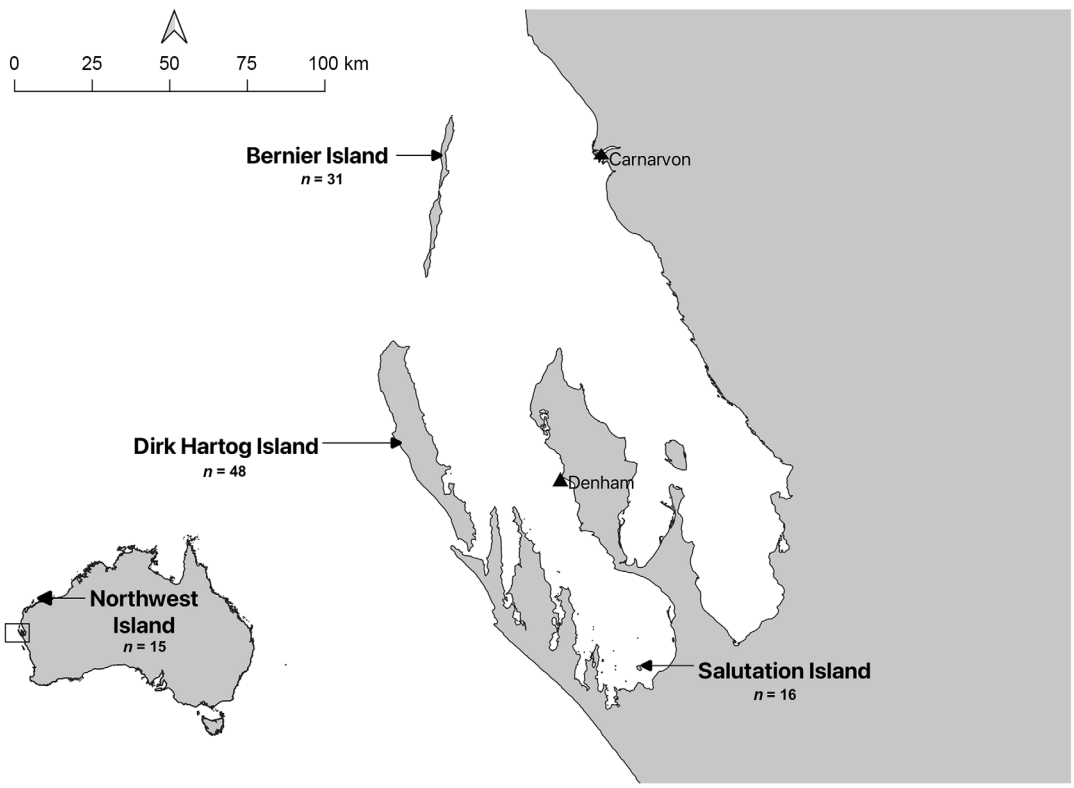

Fig. 1. Sample locations for this study. Four island populations of different rodent species and sympatric wildlife off the Western Australia coast were sampled between March 2020 and May 2021. In total, 110 animals were sampled across Northwest Island (n = 15), Bernier Island (n = 31), Dirk Hartog Island (n = 48) and Salutation Island (n = 16).

and cloacal spaces for marsupials) using sterile flocked swabs (COPAN, Brescia, Italy), followed by physical examination. In 2020, a single swab was used to sample each anatomical site, except for marsupials, from which a combined oral and conjunctival swab and a single cloacal swab were collected. Faeces were collected opportunistically from all animals. Sampling conducted in 2021 was refined to utilise a single swab to swab all anatomical sites. Swabs and tissue were frozen in the field at −20°C, except for Bernier Island swab samples, which were stored at 2–8°C until freezing facilities were available (maximum 6 days duration of storage at 2–8°C). In total, 273 swabs and 22 faecal samples were collected (Table 1).

Chlamydiaceae screening

All Bernier Island swab and faecal samples were tested individually. After initial findings, where separate anatomical swabs were collected, swabs from set pairs of anatomical sites and faeces were pooled for each individual (oral and conjunctival swabs; faeces and rectal swabs), whereas urogenital swabs were analysed as collected.

In 2020, DNA was extracted using a QiaAMP DNA mini-kit according to the manufacturer's instructions (Qiagen, Australia). The DNA was extracted in a total final volume of 70 µL and stored at −20°C until further analysis. DNA quality and concentration was checked using a Qubit™3.0 fluorometer (Invitrogen™, USA). Swabs collected in 2021 were extracted using a PureLink Viral RNA/DNA Mini kit (Invitrogen™, USA) according to the manufacturer's instructions. The change in technique was warranted for both logistical reasons as well as the desire to obtain RNA for a separate research collaboration. Total nucleic acid was extracted in a total final volume of 60 µL, with 30 µL stored at −20°C until further analysis.

Table 1. Total number of individuals (*n*) sampled and total number of swabs or faecal samples collected for each host population in this study by year.

Location	Species	*n*	Live/deceased	2020	2021
Bernier Island	SBM	8	Live	Swabs: 32 Faeces: 7	–
	AGM	18	Live	Swabs: 72 Faeces: 7	–
	SBB	5	Live	Swabs: 10	–
Dirk Hartog Island	AGM	4	Deceased	Swabs: 8	Swabs: 2
	SIM	9	Deceased	Swabs: 19	Swabs: 4
	HM	30	Deceased	Swabs: 75 Faeces: 8	Swabs: 10
	SBB	4	Live	Swabs: 8	–
	Dibbler[A]	1	Deceased	Swabs: 2	–
Salutation Island[B]	GSNR	16	Live	–	Swabs: 16
Northwest Island[B]	SBM	15	Live	–	Swabs: 15

AGM, ash-grey mouse (*Pseudomys albocinereus*); dibbler (*Parantechinus apicalis*); GSNR, greater stick-nest rat (*Leporillus conditor*); HM, house mouse (*Mus musculus*); SBB, Shark Bay bandicoot; SBM, Shark Bay mouse; SIM, sandy inland mouse (*Pseudomys hermannsburgensis*).

[A]The single dibbler (*Parantechinus apicalis*) had been recently released on Dirk Hartog Island as part of translocation of captive-bred animals from Perth Zoo.

[B]Denotes that animals were sampled on arrival at Dirk Hartog Island during translocation within 24 h of capture from source sites.

For detection of *Chlamydiaceae* DNA, a *Chlamydiaceae* family-specific probe-based quantitative polymerase chain reaction (qPCR) targeting the 110 bp fragment of the chlamydial 23S ribosomal RNA (rRNA) gene was performed on all nucleic acid samples (Ehricht *et al.* 2006). The qPCR assays were carried out in a total volume of 15 µL, consisting of 7.5 µL iTaq Universal Probe Supermix (Bio-Rad, Australia), 0.3 µL of 10 µM probe (Sigma Aldrich, Australia), 3.2 µL PCR grade water, 0.5 µL each of 10 µM forward and reverse primer and 3 µL of DNA template (Supplementary Table S1). All samples were run in duplicate, and positive (*C. pecorum* genomic DNA) and negative (MilliQ H_2O) controls were included in each assay. The qPCR conditions were as follows: 95°C for 3 min; 40 cycles of 95°C for 5 s and 60°C for 30 s. In this study, an animal was considered positive for *Chlamydia* spp. if it was detected in duplicate from any single sample and had a quantification cycle (C_t) value ≤ 37 cycles (Kasimov *et al.* 2022).

To determine the presence of *C. pecorum* DNA, positive samples on the *Chlamydiaceae* qPCR were also screened with species-specific SYBR Green-based qPCR assay targeting a 209 bp fragment of the CpecG_0573 gene (Jelocnik *et al.* 2019*a*). The qPCR assays were carried out in a total volume of 15 µL, consisting of 7.5 µL iTaq Universal SYBR Green Supermix (Bio-Rad, Australia), 3.5 µL PCR grade water, 0.5 µL each of 10 µM forward and reverse primer and 3 µL of DNA template (Table S1). All samples were run in duplicate, and positive (*C. pecorum* genomic DNA) and negative (MilliQ H_2O) controls were included in each assay. The qPCR conditions were as follows: 95°C for 3 min; 35 cycles of 95°C for 15 s, 57°C for 25 s, 72°C for 30 s, and final extension at 72°C for 7 min, followed by high-resolution melt (HRM) analyses by a melt of 77.5 ± 0.5°C. An animal was considered positive for *C. pecorum* if it was detected in duplicate from a single sample and had a C_t value ≤ 33 cycles and HRMs of 77.5°C (Kasimov *et al.* 2022).

For both qPCRs, the C_t cut-off value and the limit of detection were determined using a ten-fold serial dilution from 10^6 to 10^0 copies of quantified *C. pecorum* genomic DNA in triplicate.

To further determine the genetic identity of the infecting chlamydial species, we amplified a 476-bp fragment of the signature Chlamydiales *16S* rRNA gene (Jelocnik *et al.* 2019*b*; Kasimov *et al.* 2022) using two samples from ash-grey mice that exhibited the lowest qPCR cycle threshold (C_t = 36). Positive (*C. pecorum* DNA) and negative (MilliQ H_2O) controls were included in each assay. The PCR reactions were performed in a total volume of 35 µL, consisting of 17.5 µL

Amplitaq Gold mix (ThermoFisher, Australia), 12.5 μL PCR grade water, 1 μL each of 10 μM forward and reverse primer and 3 μL of DNA template (Table S1). The cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 35 cycles of 95°C for 20 s, 60°C for 35 s, 72°C for 45 s and a final extension at 72°C for 7 min (Kasimov *et al.* 2022). PCR products were electrophoresed on a 1.5% agarose gel, followed by transillumination. Bands of the expected size were excised with the amplicons purified and subject to bidirectional Sanger sequencing (Macrogen, South Korea).

Histopathology

All available fixed tissue was trimmed and processed in ethanol and xylene before paraffin embedding. Five μm sections of the paraffin blocks were made and stained with haematoxylin and eosin. Examination of slides was performed by a board-certified veterinary pathologist (NS).

Tissue *C. muridarum* qPCR

For detection of *C. muridarum* in tissue, all available frozen lung, faeces and gastrointestinal tissue from test-positive rodents from Dirk Hartog Island were further subjected to a proprietary *C. muridarum* qPCR at a diagnostic laboratory service for laboratory animal health monitoring, Cerberus Sciences, Adelaide. Briefly, sample was homogenised by bead beating, and 200 μL of centrifuged homogenate was used for nucleic extraction using a NucleoMag VET kit (Macherey-Nagel GmbH and Co., Germany) according to manufacturer's instructions. To detect *C. muridarum*, a set of proprietary primers and probes specifically designed to amplify the *C. muridarum* major outer membrane protein gene (MOMP) was used in a probe-based qPCR (Berry *et al.* 2004). Negative, positive and internal controls were used to validate results.

Statistical analyses

Statistical analysis was performed in R (R Core Team 2021) using the EpiR, stats and car packages. *Chlamydiaceae* 23s rRNA qPCR apparent prevalence was calculated with 95% confidence intervals using the Wilson score interval, with true prevalence calculated assuming 90% sensitivity and 100% specificity. Tests of association were carried out with a statistical significance set at P-value <0.05, using Pearson's Chi-squared tests with Yates' continuity correction or Fisher's exact test dependent on sample sizes. Prevalence ratios for association measures were calculated using Wald 95% confidence limits. A Cramer's V correlation matrix was generated to explore covariance between categorical variables using the creditmodel package.

For continuous variables, normality was tested using Shapiro–Wilk normality test, and equal variances by Levene's test. Associations were determined using Student's *t*-test or Welch two sample *t*-test.

Ethics approval

All sampling was conducted as approved by and in accordance with the Murdoch University Animal ethics committee (RW3307/21, RW3305/21, Cadaver 820, RW3221/20; Cadaver 713; RW3215/20) and Department of Biodiversity, Conservation and Attractions (DBCA) ethics committee (2019-16D; 2020-12B; 2020-20A; 2019-23A; 2021-03A; 2021-08A), and DBCA permits (TFA 2020-0042; TFA 2020-0028; TFA 2019-0182; TFA 2019-0173; FO25000280; FO25000276). Testing and use of swabs was approved under the University of Sunshine Coast Animal Research Ethics Committee (ANE2057).

Results

Chlamydiaceae detection in samples and hosts from this study

In total, 262 samples (including 149 individual anatomical site samples and 113 pooled samples) collected from 110 individuals were tested with the *Chlamydiaceae* 23s qPCR. Of those, 10.3% (27/262; 95% CI 7.2–14.6%) of samples were positive, represented by samples from all anatomical sites. In rodents, there was no statistical difference between anatomical site and test result (Fisher's exact test $P = 0.94$) (Table S2).

The C_t value for all *Chlamydiaceae* 23s rRNA qPCR positive samples was between 36 and 37 cycles. The detection limit of the *Chlamydiaceae* 23s rRNA qPCR used in this study was one copy/μL, with the C_t values from all positives equating to 1–5 copies/μL, indicative of very low infection loads. A conventional *16S* Chlamydiales rRNA PCR, run on samples with highest detected loads ($C_t = 36$ cycles; two ash-grey mice), resulted in faint bands of the expected size, but the chromatogram from bidirectional Sanger sequencing was not resolved.

At an individual level, 20% (22/110; 95% CI 13.6–28.4) of individuals sampled were positive using the family-level *Chlamydiaceae* 23s rRNA qPCR, including 19% (19/100; 95% CI 12.5–27.8) of rodents. Across two locations (Bernier Island and Dirk Hartog Island), positive samples were recorded in five species: ash-grey mice; house mice; sandy inland mice; Shark Bay mice; and Shark Bay bandicoots (Table 2, Fig. 2). All individuals (22/22), and samples (27/27) that were positive on the family-level qPCR, were negative by *C. pecorum* specific qPCR.

For rodents, there were no detectable associations between test result and sex, age or whether the animal was sampled as a live animal or deceased. However, statistical associations with location (Fig. 2) and species were identified (Table 2). It should be noted that Cramer's V coefficients indicated strong correlation among several categorical variables present in the dataset, suggesting risk factor analyses are confounded for these variables (species, year and location) (Fig. S1).

Table 2. *Chlamydiaceae* 23s qPCR prevalence recorded in this study across species and demographic characteristics.

	qPCR result	Apparent prevalence (Wilson 95% CI)	True prevalence (Wilson 95% CI)	Prevalence ratio (Wald 95% CI)	*P*-value[A]
All species	22/110	20% (13.6–28.4)	22.2% (15.1–31.6)	–	–
All rodents	19/100	19% (12.5–27.8)	21.1% (13.9–30.9)	–	
Species					**0.04**
Ash-grey mice	9/22	40.9% (23.3–61.3)	45.5% (25.8–68.1)	Ref	Ref
Dibbler	0/1	0% (0.0–94.9)	0% (0.0–100)	–	1.00
Greater stick-nest rat	0/16	0% (0.0–19.4)	0% (0.0–21.5)	–	<**0.01**
House mouse	6/30	20% (9.5–37.3)	22.2% (10.6–41.5)	0.49 (95% CI: 0.20–1.17)	0.18[B]
Sandy inland mouse	1/9	11.1% (0.6–43.5)	12.3% (0.6–48.3)	0.27 (95% CI: 0.04–1.84)	0.21
Shark Bay bandicoot	3/9	33.3% (12.1–64.8)	37% (13.4–71.6)	0.81 (95% CI: 0.28, 2.33)	1.00
Shark Bay mouse	3/23	13% (4.5–32.1)	14.5% (5.0–35.7)	0.32 (95% CI: 0.10, 1.03)	0.08[C]
Rodent demographics					
Sex					
Male	13/54	24.1% (14.6–36.9)	26.7% (16.3–41.1)	1.85 (95% CI: 0.76–4.47)	0.25[D]
Female	6/46	13% (6.1–25.7)	14.5% (6.8–28.5)	Ref	
Age					
Adult	17/86	19.8% (12.7–29.4)	22% (14.1–32.7)	1.38 (95% CI: 0.36–5.35%)	1.00
Subadult	2/14	14.3% (4.0–39.9)	15.9% (4.5–44.4)	Ref	
BCS					
Good	15/76	19.7% (12.3–30)	21.9% (13.7–33.4)	1.38 (95% CI: 0.44, 4.33)	0.76
Poor	3/21	14.3% (5.0–34.6)	15.9% (5.5–38.5)	Ref	
Not recorded[E]	1/3	–	–		

Apparent prevalence and true prevalence adjusted for the sensitivity and specificity of the *Chlamydiaceae* qPCR is provided with Wilson 95% confidence intervals. Risk factors of species, sex, age and BCS were evaluated by significance tests (Fisher's Exact Test or X^2 with Yates continuity correction) and measures of association (prevalence ratio). Statistical testing indicated significant differences (in bold text) in test prevalence among species, accounted for by differences in test prevalence between ash-grey mice and greater stick-nest rats. There was no statistically significant influence of demographic characteristics on test prevalence within rodents in this study.

[A]*P*-value results from Fisher's Exact test unless otherwise indicated.

[B]X^2 (1, $n = 52$) = 1.78, $P = 0.18$.

[C]X^2 (1, $n = 45$) = 3.15, $P = 0.08$.

[D]X^2 (1, $n = 100$) = 1.31, $P = 0.25$.

[E]BCS was not recorded for three individuals, either due to data errors ($n = 2$) or because the state of the carcass prevented BCS assessment ($n = 1$).

Association between *Chlamydiaceae* detection and host health parameters

One of the 22 positive individuals, an SBB from DHI, had abnormalities detected on physical exam that may be consistent with disease associated with *Chlamydia* spp. infection. This individual demonstrated mild serous ocular discharge, although no evidence of conjunctival inflammation was seen (Table 3). Five additional Shark Bay bandicoots during this study demonstrated ocular signs that have previously been associated with *Chlamydia* spp. infection in this species, including serous to purulent discharge, mild to severe conjunctival inflammation, and corneal opacities. All five tested negative for *Chlamydiaceae* by qPCR (Table 2).

No significant associations were detected between *Chlamydiaceae* 23s qPCR result and PCV, TPS or weight in house mice, or weight in ash-grey mice (Fig. 3). Of the seven necropsied qPCR-positive individuals, one house mouse had histopathological findings that could be consistent with known pathology of *C. muridarum* in laboratory mice, demonstrating a bronchopneumonia (Table S3). All available faecal, gastrointestinal and lung tissue (13/13) from these seven individuals, including lung from the house mouse with evidence of bronchopneumonia, were test-negative by *C. muridarum* qPCR (Table S3).

Discussion

Despite suggestions that rodents might be important epidemiological bridges for chlamydial spillover (Eddie *et al.* 1969; Cisláková *et al.* 2004; Burnard and Polkinghorne 2016), there has been little research to elucidate the presence, diversity or health impacts of chlamydial infections in wild rodents. In this study, we used a probe-based 23s rRNA *Chlamydiaceae* qPCR

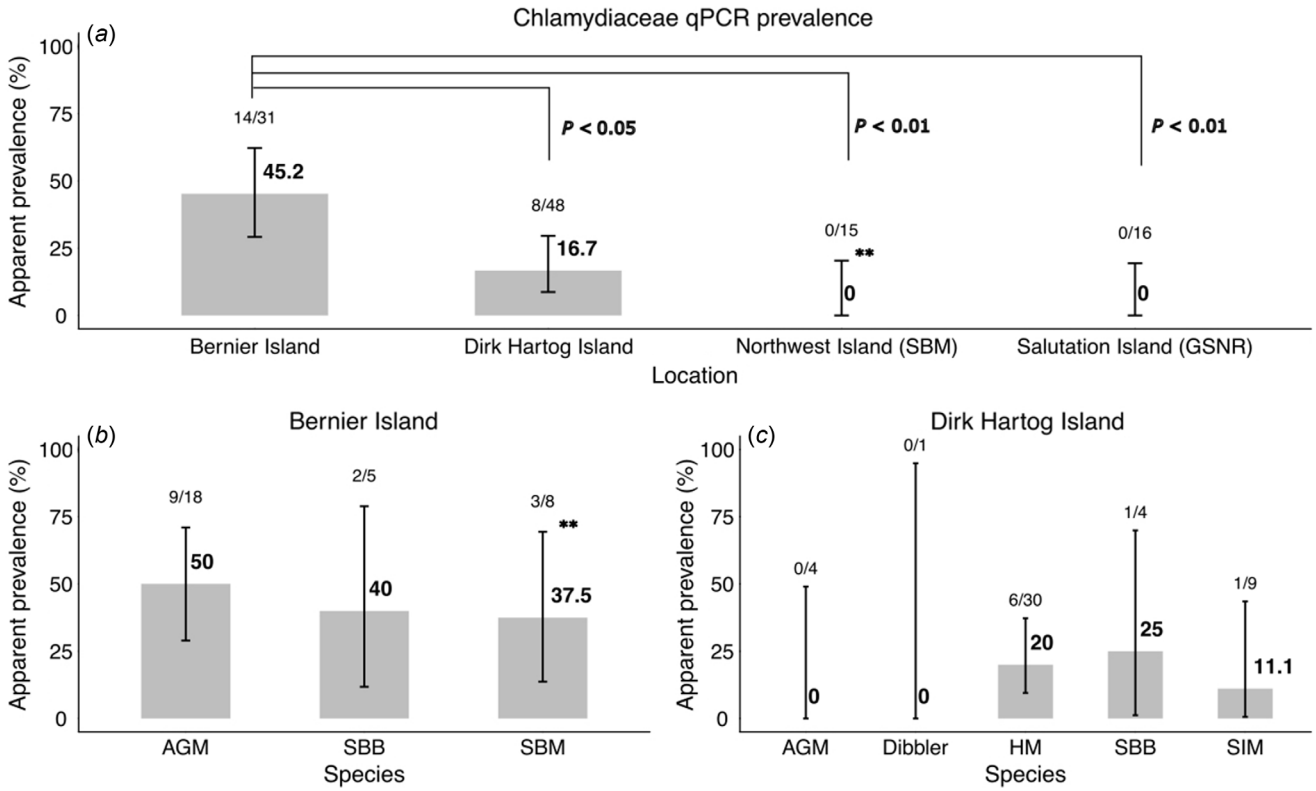

Fig. 2. *Chlamydiaceae* 23s qPCR prevalence recorded in this study by location (*a*), and species stratified by location (*b*, *c*). Apparent prevalence is provided with Wilson 95% confidence intervals indicated by error bars. Statistically significant results are indicated by *P*-values (location differences) or ** (species stratified by location). Prevalence of *Chlamydiaceae* was statistically higher at Bernier Island compared with all other sites (DHI: X^2 (1, n = 79) = 5.83, P = 0.02, prevalence ratio = 2.84 (95% CI: 1.28–6.28); Northwest Island: $P < 0.01$; Salutation Island: $P < 0.01$). There was also a statistically significant difference in prevalence between Shark Bay mice populations from Bernier Island and Northwest Island (P = 0.03). AGM, ash-grey mouse; GSNR, greater stick-nest rat; HM, house mouse; SBB, Shark Bay bandicoot; SBM, Shark Bay mouse; SIM, sandy inland mouse.

Table 3. *Chlamydiaceae* 23s qPCR result in Shark Bay bandicoots relative to detection of ocular abnormalities.

Chlamydiaceae qPCR result (and site positive)	SBB ocular exam findings	Location
Positive (conjunctival-oral)	Unilateral serous discharge	Dirk Hartog Island
Positive (cloaca)	None	Bernier Island
Positive (conjunctival-oral)	None	Bernier Island
Negative	Unilateral inflammation and proliferation of conjunctiva impairing visualisation of globe, with mucopurulent discharge	Bernier Island
Negative[A]	Left eye: corneal opacity, mucopurulent discharge, inflammation of conjunctiva and thickening of eyelid margin Right eye: serous discharge	Bernier Island
Negative	Unilateral conjunctival inflammation	Bernier Island
Negative	Unilateral serous discharge	Dirk Hartog Island
Negative	Unilateral conjunctival inflammation with serous discharge	Dirk Hartog Island
Negative	None	Dirk Hartog Island

One test-positive bandicoot demonstrated mild ocular abnormalities. Five bandicoots that were test-negative had detectable ocular abnormalities that were consistent with abnormalities previously associated with *Chlamydia* spp. infection in this species. There was no detectable association between test result and presence of ocular disease in Shark Bay bandicoots (Prevalence ratio 0.4; 95% CI 0.08–2.06).

[A]This individual also had lesions suggesting bandicoot papillomatosis carcinomatosis virus 1 (BPCV1) infection and was subsequently test-positive for BPCV1 by PCR.

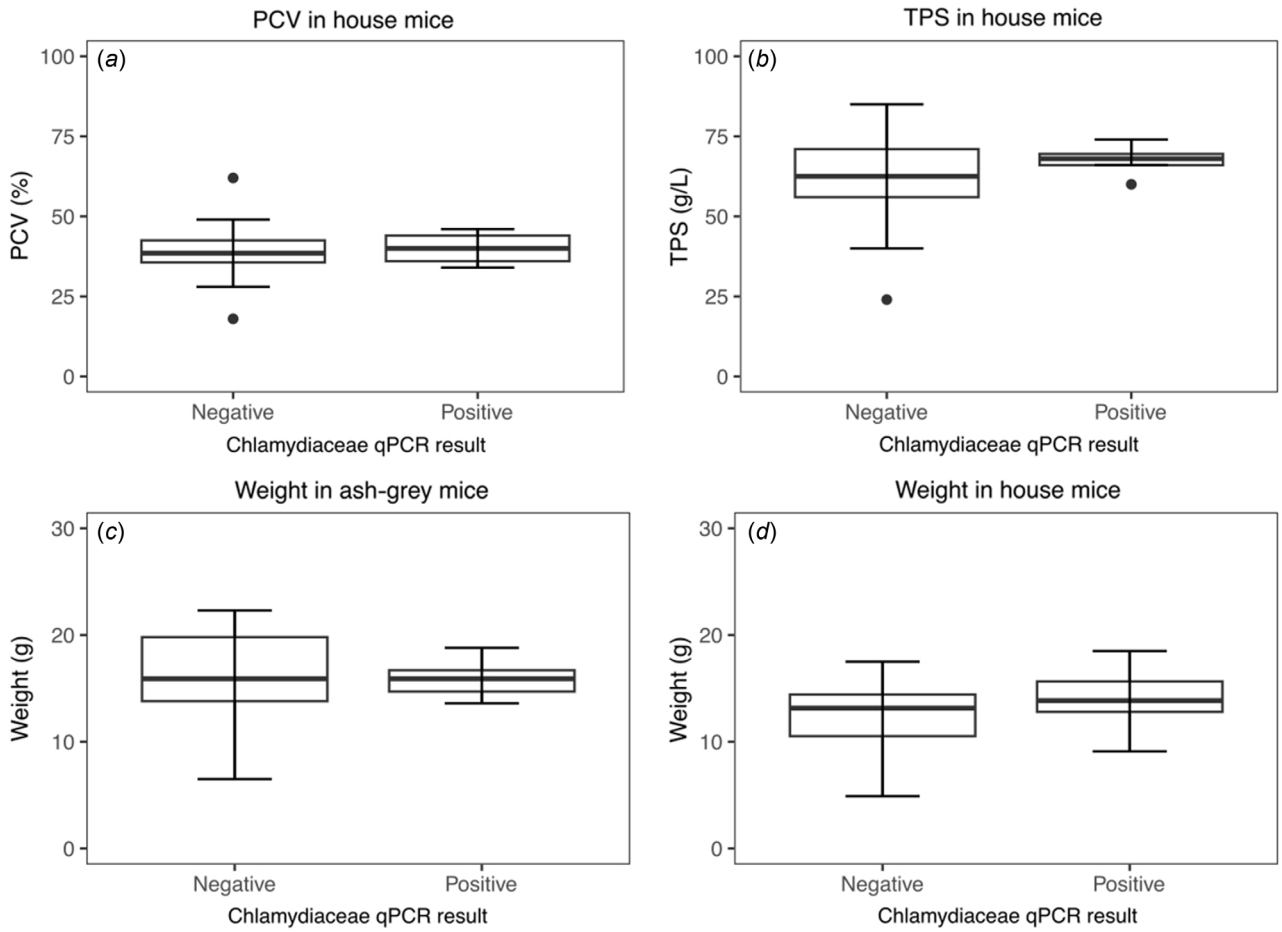

Fig. 3. Influence of *Chlamydiaceae* 23s qPCR result on physical or haematological characteristics. There was no detectable effect of *Chlamydiaceae* qPCR result on (*a*) measured packed cell volume (PCV) in house mice (t (23) = −0.25, P = 0.81), (*b*) measured total plasma solids (TPS) in house mice (t (13.6) = −1.22, P = 0.24), (*c*) weight in ash-grey mice (t (10.8) = 0.85, P = 0.41) or (*d*) weight in house mice (t (28) = 1.04, P = 0.31).

to provide evidence that wild Australian rodents may be subclinically infected with *Chlamydia* spp. However, we found no evidence to indicate that they are significant reservoirs of infection for sympatric wildlife, nor evidence of adverse health associations in the rodents.

We found a moderate overall test-prevalence for *Chlamydiaceae* in murid rodents in this study, with possible infection in four of five species sampled, across two geographically isolated locations. To the authors' knowledge, there are only two other targeted molecular-based studies of *Chlamydiaceae* in wild *Muridae* globally. In contrast to our results, neither study found evidence of infection in the 401 individuals sampled (Stephan *et al.* 2014; Mishkin *et al.* 2022). It is important to emphasise that the use of a molecular-based approach combined with the ecological niche occupied by these species increases the likelihood that our results could represent environmental contamination rather than a true infection state. However, our findings are also supported by historical, albeit limited, evidence of *Chlamydiaceae* detection in an ash-grey mouse and a Shark Bay mouse from Bernier Island in the early 2000s (Sims, C., unpubl. data). Combined with the findings from this study, these results suggest that infection of rodents could be endemic in the Shark Bay region. However, further research would be required to confirm these findings. Similar results have been found in *Peromyscus* spp. rodent populations in the USA, where *C. muridarum*, or a closely related species, is thought to be endemic (Ramsey *et al.* 2016). Furthermore, serological studies have indicated exposure of several wild rodent populations globally to *Chlamydia* spp. may be common (Howerth *et al.* 1994; Cisláková *et al.* 2004; Martino *et al.* 2014; Ramsey *et al.* 2016). We also identified significant differences in prevalence among species and locations. However, the marked correlation among species assemblages, location, year and method of recruitment – factors that were not possible to standardise due to both ecological and logistical constraints – limits the inferences that can be made.

A key limitation in this present study is that we were unable to determine the molecular identity of test-positive samples using our methods. Low molecular loads were detected in

all hosts, sequencing was unsuccessful and all animals positive by *Chlamydiaceae* qPCR were subsequently negative by a *C. pecorum* qPCR. A subset was also negative by a *C. muridarum* qPCR, suggesting the presence of another or perhaps multiple *Chlamydia* species in these hosts. Consequently, we were unable to determine species diversity or assess for evidence of cross-species transmission in these populations. The low chlamydial loads in test-positive samples meant that amplifying larger *16S* fragments, the preferred gene marker for unknown chlamydial species, would likely be unsuccessful and as such was not attempted. Screening of multiple gene sites, such as 23S fragments, could have been trialled and may have resolved species identification. However, the finding of a test-positive bandicoot in the establishing population on Dirk Hartog Island indicates chlamydial infections are possibly already present in the population of greatest concern in the context of this study. It is unknown whether this result is indicative of a new infection acquired on Dirk Hartog Island or is a remnant of previous persistent infection following translocation of the infected bandicoot from Bernier Island 13 months prior, because bandicoots were not screened via molecular testing at the time of translocation.

Despite our study limitations, the low molecular loads detected in all hosts would imply these individuals are unlikely to be significant sources of environmental contamination, an important consideration when assessing capacity for spillover (Holzinger-Umlauf *et al.* 1997; Wooters *et al.* 2009; Stokes *et al.* 2020). However, longitudinal studies would be ideal to monitor temporal variation in loads over time. Low extracted nucleic acid concentrations (0.01–2.5 ng/μL) detected in some samples in this study may have partly contributed to this finding and may suggest collection of an inadequate number of host cells, thereby impairing the detection of an obligate intracellular pathogen. This could have been influenced by both storage conditions and sampling technique, although low DNA yields from oral mucosal swabbing of rodents are not unusual (Picazo and García-Olmo 2015; Abusleme *et al.* 2017; Halsey *et al.* 2021). Pooling of anatomical site samples for a single individual improved DNA yields, although this may come at the cost of dilution of total chlamydial loads (Sultan *et al.* 2016; Badman *et al.* 2020). Future studies, particularly of small murids, may wish to examine alternative extraction or PCR protocols or collection methods to increase DNA yields, and to ensure that optimal field storage conditions are available.

Although infection of rodents appeared common in our study, we found no detectable host disease associations. The lack of detection of adverse health associations is perhaps unsurprising. *Chlamydia* infection is not considered a sufficient cause of chlamydiosis in any species, with infections frequently asymptomatic or subclinical in both humans and wildlife (Quigley *et al.* 2018), although the Australian koala (*Phascolarctos cinereus*) is a notable exception (Robbins *et al.* 2019). Complex and currently poorly understood interactions between the agent, environment and host are required in disease pathogenesis, influenced by factors including chlamydial strain (Zhong *et al.* 2020), virulence factors (Zhong *et al.* 2020), infectious doses (Belij-Rammerstorfer *et al.* 2016; Sachse and Borel 2020), inflammatory mediators (Murthy *et al.* 2016), host species (Borel *et al.* 2018; Zhong *et al.* 2020), repeated infections (Belij-Rammerstorfer *et al.* 2016) and co-infections (Quigley and Timms 2020). With such complex pathogenesis, our findings do not negate the potential for current or future pathogenic significance in these hosts, particularly under periods of stress that may lead to opportunistic disease. However, the low loads currently detected in all hosts are consistent with an asymptomatic infection state (Wan *et al.* 2011; Robbins *et al.* 2019).

Detectable health associations were likely limited by the cross-sectional study design and small sample sizes, which risk type II error. Longitudinal studies of chlamydial infections in koalas have shown that two-thirds of infected asymptomatic individuals will progress over time to disease development (Robbins *et al.* 2019). However, there is currently no evidence of adverse effects of natural infection with *Chlamydia* in wild murid rodents, and limited evidence from laboratory murids. In laboratory mice, *C. muridarum* was discovered fortuitously. Natural infection with *C. muridarum* has been linked to exacerbation of respiratory co-infections (Rank 2006), and more recently has been associated with incidental pulmonary lesions in immunocompetent mice and significant respiratory pathology in immunosuppressed mice (Mishkin *et al.* 2022). However, the impact of the incidental lesions on individual health are unknown. In our study, lung pathology that could be consistent with chlamydial disease was identified in a single house mouse, but lung tissue from this animal was negative by *C. muridarum* qPCR, suggesting a different aetiology. In experimental studies, infertility and interstitial pneumonia are recognised sequelae *of C. muridarum* infection (Barron *et al.* 1981; Rank 2006; Jupelli *et al.* 2013). However, such models, typically dependent on hormonal controls or high infectious doses via unnatural routes, may distort our understanding of the pathogenesis of natural infection in rodents (Rank 2006). If infertility were a consequence of natural infection in wild rodents, it could have marked implications for vulnerable fragmented populations that are dependent on fast life histories (Jacob *et al.* 2008). Despite this, the mouse genital tract is not considered a natural site of infection for *C. muridarum* (Wang *et al.* 2016; Cheong *et al.* 2019), and typically *C. muridarum* in laboratory mice is thought to persist as a long-term gastrointestinal tract infection without adverse effect, potentially as a commensal, host-adapted parasite (Rank and Yeruva 2014; Wang *et al.* 2016).

The lack of association of *Chlamydiaceae* DNA detection with ocular disease in Shark Bay bandicoots warrants particular attention. It is important to emphasise that measurement of this association was not a specific objective of our study, and our results do not exclude *Chlamydia* as a potential aetiologic agent of this syndrome. Consequences from chronic tissue remodelling due to *Chlamydia* infection may persist

long after clearance of the inciting infection, complicating diagnostics and measures of association in cross-sectional studies (Ghasemian *et al.* 2018; Hu *et al.* 2018). However, our results accentuate the need for further targeted research of this syndrome to better elucidate aetiologies. Published literature is equivocal as to whether *Chlamydia* spp. have been detected from diseased ocular sites in this species (Bodetti *et al.* 2003; Warren *et al.* 2005; Kumar *et al.* 2007; Kutlin *et al.* 2007). Detection from, and association with, the site of disease expression is an important consideration for potentially ubiquitous organisms. Additionally, there has been limited examination for alternative or co-contributing pathogens, such as *Mycoplasma* spp., herpesviruses or bandicoot papillomatosis carcinomatosis virus 1 (BPCV1). As with other enigmatic disease syndromes in Australian wildlife (O'Dea *et al.* 2016; Eden *et al.* 2017), next-generation sequencing to detect multiple and novel agents of disease may be the most appropriate tool for future investigation of this syndrome, alongside methods to enhance nucleic acid yields.

Molecular diagnostic techniques are increasingly unveiling the ubiquity, diversity and expanding host ranges of chlamydial infections of wildlife. As such, it is not surprising our study has found evidence to suggest wild Australian murids are possible hosts for *Chlamydia* spp. However, in the absence of adverse health associations, low-level shedding in all host species and detection across both source and destination sites, our findings suggest the risk associated with chlamydial infection in murids in the context of the DHI translocations is low. Future research in other geographical settings is still desirable to elucidate the diversity and implications of chlamydial infection in Australian and international murids. These studies should explore techniques to optimise DNA yields and incorporate other species-specific assays following broad-scale screening. Furthermore, with expanding host ranges and increasing evidence of the ubiquity of chlamydial infections in wildlife, future studies should continue to examine sympatric populations to clarify the frequency and importance of cross-species transmission in chlamydial epidemiology.

Conclusions

Based on current evidence, targeted risk-mitigation measures for *Chlamydia* are not warranted for the translocation of Shark Bay mice to Dirk Hartog Island. However, these results do not negate the need for ongoing attention to biosecurity precautions throughout the translocation pathway. Furthermore, disease risk assessments are dynamic. Post-translocation monitoring, incorporating health assessments and necropsy with histopathology in the event of mortalities will be important to refine our assessment. This is particularly the case because chlamydial pathogenesis is complex and may be influenced by future stressors. Overall, our results highlight current knowledge gaps surrounding Australian rodent health and the need to address these to inform WDRAs. A collaborative approach should be considered by all reintroduction programs because translocations and associated monitoring provide a valuable opportunity to obtain this crucial baseline health data.

Supplementary material

Supplementary material is available online.

References

Abusleme L, Hong B-Y, Hoare A, Konkel JE, Diaz PI, Moutsopoulos NM (2017) Oral microbiome characterization in murine models. *Bio-protocol* **7**, e2655. doi:10.21769/BioProtoc.2655

Akter R, Sansom FM, El-Hage CM, Gilkerson JR, Legione AR, Devlin JM (2021) A 25-year retrospective study of *Chlamydia psittaci* in association with equine reproductive loss in Australia. *Journal of Medical Microbiology* **70**, 001284. doi:10.1099/jmm.0.001284

Anstey SI, Kasimov V, Jenkins C, Legione A, Devlin J, Amery-Gale J, Gilkerson J, Hair S, Perkins N, Peel AJ, Borel N, Pannekoek Y, Chaber A-L, Woolford L, Timms P, Jelocnik M (2021) *Chlamydia psittaci* ST24: clonal strains of one health importance dominate in Australian horse, bird and human infections. *Pathogens* **10**, 1015. doi:10.3390/pathogens10081015

Badman SG, Bell SFE, Dean JA, Lemoire J, Coffey L, Debattista J, Redmond AM, Williams OD, Gilks CF, Whiley DM (2020) Reduced sensitivity from pooled urine, pharyngeal and rectal specimens when using a molecular assay for the detection of chlamydia and gonorrhoea near the point of care. *Sexual Health* **17**, 15–21. doi:10.1071/SH19028

Barron AL, White HJ, Rank RG, Soloff BL, Moses EB (1981) A new animal model for the study of *Chlamydia trachomatis* genital infections: infection of mice with the agent of mouse pneumonitis. *The Journal of Infectious Diseases* **143**, 63–66. doi:10.1093/infdis/143.1.63

Beckmann KM, Cromie RL, Sainsbury AW, Hilton GM, Ewen JG, Soorae PS, Kock RA (2022) Wildlife health outcomes and opportunities in conservation translocations. *Ecological Solutions and Evidence* **3**, e12164. doi:10.1002/2688-8319.12164

Belij-Rammerstorfer S, Inic-Kanada A, Stojanovic M, Marinkovic E, Lukic I, Stein E, Montanaro J, Bintner N, Schürer N, Ghasemian E, Kundi M, Barisani-Asenbauer T (2016) Infectious dose and repeated infections are key factors influencing immune response characteristics in guinea pig ocular chlamydial infection. *Microbes and Infection* **18**, 254–262. doi:10.1016/j.micinf.2015.12.001

Berry LJ, Hickey DK, Skelding KA, Bao S, Rendina AM, Hansbro PM, Gockel CM, Beagley KW (2004) Transcutaneous immunization with combined cholera toxin and CpG adjuvant protects against *Chlamydia muridarum* genital tract infection. *Infection and Immunity* **72**, 1019–1028. doi:10.1128/IAI.72.2.1019-1028.2004

Bodetti TJ, Viggers K, Warren K, Swan R, Conaghty S, Sims C, Timms P (2003) Wide range of Chlamydiales types detected in native Australian mammals. *Veterinary Microbiology* **96**, 177–187. doi:10.1016/S0378-1135(03)00211-6

Borel N, Polkinghorne A, Pospischil A (2018) A review on chlamydial diseases in animals: still a challenge for pathologists? *Veterinary Pathology* **55**, 374–390. doi:10.1177/0300985817751218

Borel N, Ciuria S, Flury T, Basso WU, Ruetten M (2023) Zoonotic potential of guinea pigs: outbreak of cryptosporidiosis combined with chlamydiosis in a breeding guinea pig herd. *Schweizer Archiv für Tierheilkunde* **165**, 59–63. doi:10.17236/sat00383

Burnard D, Polkinghorne A (2016) Chlamydial infections in wildlife–conservation threats and/or reservoirs of 'spill-over' infections? *Veterinary Microbiology* **196**, 78–84. doi:10.1016/j.vetmic.2016.10.018

Cheong HC, Lee CYQ, Cheok YY, Tan GMY, Looi CY, Wong WF (2019) *Chlamydiaceae*: diseases in primary hosts and zoonosis. *Microorganisms* **7**, 146. doi:10.3390/microorganisms7050146

Cisláková L, Stanko M, Fricová J, Mosanský L, Trávnicek M, Halánová M, Mardzinová S, Stefanciková A (2004) Small mammals (Insectivora, Rodentia) as a potential source of chlamydial infection in East Slovakia. *Annals of Agricultural and Environmental Medicine* **11**, 139–143.

Dean D, Kandel RP, Adhikari HK, Hessel T (2008) Multiple *Chlamydiaceae* species in trachoma: implications for disease pathogenesis and control. *PLoS Medicine* **5**, e14. doi:10.1371/journal.pmed.0050014

Dean D, Rothschild J, Ruettger A, Kandel RP, Sachse K (2013) Zoonotic *Chlamydiaceae* species associated with trachoma, Nepal. *Emerging Infectious Diseases* **19**, 1948–1955. doi:10.3201/eid1912.130656

Eddie B, Radovsky FJ, Stiller D, Kumada N (1969) Psittacosis-lymphogranuloma venereum (PL) agents (*Bedsonia*, *Chlamydia*) in ticks, fleas, and native mammals in California. *American Journal of Epidemiology* **90**, 449–460. doi:10.1093/oxfordjournals.aje.a121091

Eden J-S, Rose K, Ng J, Shi M, Wang Q, Sintchenko V, Holmes EC (2017) *Francisella tularensis* ssp. *Holarctica* in ringtail possums, Australia. *Emerging Infectious Diseases* **23**, 1198–1201. doi:10.3201/eid2307.161863

Ehricht R, Slickers P, Goellner S, Hotzel H, Sachse K (2006) Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. *Molecular and Cellular Probes* **20**, 60–63. doi:10.1016/j.mcp.2005.09.003

Ghasemian E, Inic-Kanada A, Collingro A, Tagini F, Stein E, Alchalabi H, Schuerer N, Kese D, Babiker BE, Borel N, Greub G, Barisani-Asenbauer T (2018) Detection of *Chlamydiaceae* and *Chlamydia*-like organisms on the ocular surface of children and adults from a trachoma-endemic region. *Scientific Reports* **8**, 7432. doi:10.1038/s41598-018-23887-1

Halsey MK, Stuhler JD, Platt RN, Bradley RD, Stevens RD, Ray DA (2021) Cheeky business: comparing DNA yield of buccal, fecal, and whisker samples for minimally invasive genetic research. *Occasional Papers, Museum of Texas Tech University* **374**, 1–9.

Hlokwe TM, De Klerk-Lorist L-M, Michel AL (2016) Wildlife on the move: a hidden tuberculosis threat to conservation areas and game farms through introduction of untested animals. *Journal of Wildlife Diseases* **52**, 837–843. doi:10.7589/2015-10-281

Hocking JS, Geisler WM, Kong FYS (2023) Update on the epidemiology, screening, and management of *Chlamydia trachomatis* infection. *Infectious Disease Clinics of North America* **37**, 267–288. doi:10.1016/j.idc.2023.02.007

Holzinger-Umlauf HA-M, Marschang RE, Gravendyck M, Kaleta EF (1997) Investigation on the frequency of *Chlamydia* sp. Infections in tits (Paridae). *Avian Pathology* **26**, 779–789. doi:10.1080/03079459708419252

Howerth EW, Reeves AJ, McElveen MR, Austin FW (1994) Survey for selected diseases in nutria (*Myocastor coypus*) from Louisiana. *Journal of Wildlife Diseases* **30**, 450–453. doi:10.7589/0090-3558-30.3.450

Hu VH, Macleod D, Massae P, Afwamba I, Weiss HA, Mabey DCW, Bailey RL, Burton MJ (2018) Non-chlamydial bacterial infection and progression of conjunctival scarring in trachoma. *Investigative Opthalmology & Visual Science* **59**, 2339–2344. doi:10.1167/iovs.17-23381

International Union for Conservation of Nature (2013) 'Guidelines for reintroductions and other conservation translocations.' (IUCN Species Survival Commission: Gland, Switzerland)

Jacob J, Singleton GR, Hinds LA (2008) Fertility control of rodent pests. *Wildlife Research* **35**, 487–493. doi:10.1071/WR07129

Jelocnik M, Frentiu FD, Timms P, Polkinghorne A (2013) Multilocus sequence analysis provides insights into molecular epidemiology of *Chlamydia pecorum* infections in Australian sheep, cattle, and koalas. *Journal of Clinical Microbiology* **51**, 2625–2632. doi:10.1128/JCM.00992-13

Jelocnik M, Laurence M, Murdoch FR, Polkinghorne A (2019*a*) Detection of *Chlamydiaceae* in ocular swabs from Australian pre-export feedlot sheep. *Australian Veterinary Journal* **97**, 401–403. doi:10.1111/avj.12857

Jelocnik M, Taylor-Brown A, O'Dea C, Anstey S, Bommana S, Masters N, Katouli M, Jenkins C, Polkinghorne A (2019*b*) Detection of a range of genetically diverse chlamydiae in Australian domesticated and wild ungulates. *Transboundary and Emerging Diseases* **66**, 1132–1137. doi:10.1111/tbed.13171

Jupelli M, Shimada K, Chiba N, Slepenkin A, Alsabeh R, Jones HD, Peterson E, Chen S, Arditi M, Crother TR (2013) *Chlamydia pneumoniae* infection in mice induces chronic lung inflammation, iBALT formation, and fibrosis. *PLoS ONE* **8**, e77447. doi:10.1371/journal.pone.0077447

Kasimov V, Dong Y, Shao R, Brunton A, Anstey SI, Hall C, Chalmers G, Conroy G, Booth R, Timms P, Jelocnik M (2022) Emerging and well-characterized chlamydial infections detected in a wide range of wild Australian birds. *Transboundary and Emerging Diseases* **69**, e3154–e3170. doi:10.1111/tbed.14457

Kock RA, Woodford MH, Rossiter PB (2010) Disease risks associated with the translocation of wildlife. *Revue Scientifique et Technique* **29**, 329–350. doi:10.20506/rst.29.2.1980

Kumar S, Kutlin A, Roblin P, Kohlhoff S, Bodetti T, Timms P, Hammerschlag MR (2007) Isolation and antimicrobial susceptibilities of chlamydial isolates from western barred bandicoots. *Journal of Clinical Microbiology* **45**, 392–394. doi:10.1128/JCM.01726-06

Kutlin A, Roblin PM, Kumar S, Kohlhoff S, Bodetti T, Timms P, Hammerschlag MR (2007) Molecular characterization of *Chlamydophila pneumoniae* isolates from Western barred bandicoots. *Journal of Medical Microbiology* **56**, 407–417. doi:10.1099/jmm.0.46850-0

Martino PE, Stanchi NO, Silvestrini M, Brihuega B, Samartino L, Parrado E (2014) Seroprevalence for selected pathogens of zoonotic importance in wild nutria (*Myocastor coypus*). *European Journal of Wildlife Research* **60**, 551–554. doi:10.1007/s10344-014-0805-4

Mishkin N, Ricart Arbona RJ, Carrasco SE, Lawton S, Henderson KS, Momtsios P, Sigar IM, Ramsey KH, Cheleuitte-Nieves C, Monette S, Lipman NS (2022) Reemergence of the murine bacterial pathogen *Chlamydia muridarum* in research mouse colonies. *Comparative Medicine* **72**, 230–242. doi:10.30802/AALAS-CM-22-000045

Murray ES (1964) Guinea pig inclusion conjunctivitis virus: I. Isolation and identification as a member of the psittacosis-lymphogranuloma-trachoma group. *The Journal of Infectious Diseases* **114**, 1–12. doi:10.1093/infdis/114.1.1

Murthy AK, Li W, Ramsey KH (2016) Immunopathogenesis of chlamydial infections. In 'Biology of Chlamydia'. (Ed. G Hacker) pp. 183–215. (Springer International Publishing: Switzerland)

O'Dea MA, Jackson B, Jackson C, Xavier P, Warren K (2016) Discovery and partial genomic characterisation of a novel nidovirus associated with respiratory disease in wild shingleback lizards (*Tiliqua rugosa*). *PLoS ONE* **11**, e0165209. doi:10.1371/journal.pone.0165209

Picazo MG, García-Olmo DC (2015) DNA from tissues of young mice is optimal for genotyping. *Electronic Journal of Biotechnology* **18**, 83–87. doi:10.1016/j.ejbt.2014.12.002

Polkinghorne A, Hanger J, Timms P (2013) Recent advances in understanding the biology, epidemiology and control of chlamydial infections in koalas. *Veterinary Microbiology* **165**, 214–223. doi:10.1016/j.vetmic.2013.02.026

Quigley BL, Timms P (2020) Helping koalas battle disease – recent advances in *Chlamydia* and koala retrovirus (KORV) disease understanding and treatment in koalas. *FEMS Microbiology Reviews* **44**, 583–605. doi:10.1093/femsre/fuaa024

Quigley BL, Carver S, Hanger J, Vidgen ME, Timms P (2018) The relative contribution of causal factors in the transition from infection to clinical chlamydial disease. *Scientific Reports* **8**, 8893. doi:10.1038/s41598-018-27253-z

Ramsey KH, Sigar IM, Schripsema JH, Townsend KE, Barry RJ, Peters J, Platt KB (2016) Detection of *Chlamydia* infection in *Peromyscus* species rodents from sylvatic and laboratory sources. *Pathogens and Diseases* **74**, ftv129. doi:10.1093/femspd/ftv129

Rank RG (2006) Chlamydial diseases. In 'The mouse in biomedical research: diseases'. (Eds JG Fox, MT Davisson, FW Quimby, SW Barthold, CE Newcomer, AL Smith) pp. 325–348. (Elsevier)

Rank RG, Yeruva L (2014) Hidden in plain sight: chlamydial gastrointestinal infection and its relevance to persistence in human genital infection. *Infection and Immunity* **82**, 1362–1371. doi:10.1128/IAI.01244-13

R Core Team (2021) 'R: A language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna, Austria)

Reinhold P, Sachse K, Kaltenboeck B (2011) *Chlamydiaceae* in cattle: commensals, trigger organisms, or pathogens? *The Veterinary Journal* **189**, 257–267. doi:10.1016/j.tvjl.2010.09.003

Rideout BA, Sainsbury AW, Hudson PJ (2017) Which parasites should we be most concerned about in wildlife translocations? *EcoHealth* **14**, 42–46. doi:10.1007/s10393-016-1132-x

Robbins A, Hanger J, Jelocnik M, Quigley BL, Timms P (2019) Longitudinal study of wild koalas (*Phascolarctos cinereus*) reveals chlamydial disease progression in two thirds of infected animals. *Scientific Reports* **9**, 13194. doi:10.1038/s41598-019-49382-9

Sachse K, Borel N (2020) Recent advances in epidemiology, pathology and immunology of veterinary *Chlamydiae*. In 'Chlamydia biology: from genome to disease'. (Eds M Tan, JH Hegemann, C Sütterlin) pp. 403–428. (Caister Academic Press: UK)

Sainsbury AW, Vaughan-Higgins RJ (2012) Analyzing disease risks associated with translocations. *Conservation Biology* **26**, 442–452. doi:10.1111/j.1523-1739.2012.01839.x

Seddon PJ, Strauss WM, Innes J (2012) Animal translocations: what are they and why do we do them? In 'Reintroduction biology: integrating science and management'. (Eds JG Ewen, DP Armstrong, KA Parker, PJ Seddon) pp. 1–32. (Wiley-Blackwell: UK)

Short J (2009) The characteristics and success of vertebrate translocations within Australia: a progress report to Department of Agriculture, Fisheries and Forestry. Wildlife Research and Management Pty Ltd, Kalamunda, WA.

Spalatin J, Fraser CEO, Connell R, Hanson RP, Berman DT (1966) Agents of psittacosis-lymphogranuloma venereum group isolated from muskrats and snowshoe hares in Saskatchewan. *Canadian Journal of Comparative Medicine and Veterinary Science* **30**, 260–264.

Spalatin J, Iversen JO, Hanson RP (1971) Properties of a *Chlamydia psittaci* isolated from muskrats and snowshoe hares in Canada. *Canadian Journal of Microbiology* **17**, 935–942. doi:10.1139/m71-149

Stephan S, Guerra D, Pospischil A, Hilbe M, Weissenböck H, Novotný L, Greub G, Croxatto A, Teifke JP, Ulrich RG, Schlegel M, Ruhl S, Schotte U, Binder A, Sauer S, Borel N (2014) *Chlamydiaceae* and *Chlamydia*-like organisms in free-living small mammals in Europe and Afghanistan. *Journal of Wildlife Diseases* **50**, 195–204. doi:10.7589/2013-08-194

Stockwell M, Clulow S, Clulow J, Mahony M (2008) The impact of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* on a green and golden bell frog *Litoria aurea* reintroduction program at the Hunter Wetlands Centre Australia in the Hunter Region of NSW. *Australian Zoologist* **34**, 379–386. doi:10.7882/AZ.2008.015

Stokes HS, Martens JM, Walder K, Segal Y, Berg ML, Bennett ATD (2020) Species, sex and geographic variation in chlamydial prevalence in abundant wild Australian parrots. *Scientific Reports* **10**, 20478. doi:10.1038/s41598-020-77500-5

Sultan B, White JA, Fish R, Carrick G, Brima N, Copas A, Robinson A, Gilson R, Mercey D, Benn P, Munson E (2016) The "3 in 1" study: pooling self-taken pharyngeal, urethral, and rectal samples into a single sample for analysis for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in men who have sex with men. *Journal of Clinical Microbiology* **54**, 650–656. doi:10.1128/JCM.02460-15

van Grootveld R, Bilsen MP, Boelsums TL, Heddema ER, Groeneveld GH, Gooskens J, de Boer MGJ (2018) *Chlamydia caviae* causing community-acquired pneumonia: an emerging zoonosis. *Vector-Borne and Zoonotic Diseases* **18**, 635–637. doi:10.1089/vbz.2018.2304

Vaughan-Higgins RJ, Vitali SD, Sims C, Page M, Reiss A (2021) Streamlining disease risk analysis for wildlife using the Shark Bay bandicoot as a model. *EcoHealth* **18**, 13–30. doi:10.1007/s10393-021-01521-3

Wan C, Loader J, Hanger J, Beagley KW, Timms P, Polkinghorne A (2011) Using quantitative polymerase chain reaction to correlate *Chlamydia pecorum* infectious load with ocular, urinary and reproductive tract disease in the koala (*Phascolarctos cinereus*). *Australian Veterinary Journal* **89**, 409–412. doi:10.1111/j.1751-0813.2011.00827.x

Wang L, Zhang Q, Zhang T, Zhang Y, Zhu C, Sun X, Zhang N, Xue M, Zhong G (2016) The *Chlamydia muridarum* organisms fail to auto-inoculate the mouse genital tract after colonization in the gastrointestinal tract for 70 days. *PLoS ONE* **11**, e0155880. doi:10.1371/journal.pone.0155880

Warren K, Swan R, Bodetti T, Friend T, Hill S, Timms P (2005) Ocular Chlamydiales infections of western barred bandicoots (*Perameles bougainville*) in Western Australia. *Journal of Zoo and Wildlife Medicine* **36**, 100–102. doi:10.1638/02-067

Waugh C, Khan SA, Carver S, Hanger J, Loader J, Polkinghorne A, Beagley K, Timms P (2016) A prototype recombinant-protein based *Chlamydia pecorum* vaccine results in reduced chlamydial burden and less clinical disease in free-ranging koalas (*Phascolarctos cinereus*). *PLoS ONE* **11**, e0146934. doi:10.1371/journal.pone.0146934

Wildlife Health Australia (2018) 'National wildlife biosecurity guidelines.' (Wildlife Health Australia: Sydney NSW)

Wooters MA, Kaufhold RM, Field JA, Indrawati L, Heinrichs JH, Smith JG (2009) A real-time quantitative polymerase chain reaction assay for the detection of *Chlamydia* in the mouse genital tract model. *Diagnostic Microbiology and Infectious Disease* **63**, 140–147. doi:10.1016/j.diagmicrobio.2008.10.007

Zhong G, Quayle A, Aiyar A, Zhang T (2020) Animal models of *Chlamydia trachomatis* infection. In '*Chlamydia* biology: from genome to disease'. (Eds M Tan, JH Hegemann, C Sütterlin) pp. 459–482. (Caister Academic Press: UK)

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Author affiliations

[A]School of Veterinary Medicine, Murdoch University, Murdoch, WA 6150, Australia.

[B]Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Woodvale, WA 6026, Australia.

[C]Centre for Bioinnovation, University of the Sunshine Coast, Sippy Downs, Qld 4556, Australia.

[D]School of Science, Technology and Engineering, University of the Sunshine Coast, Sippy Downs, Qld 4556, Australia.