

Supplementary Material

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

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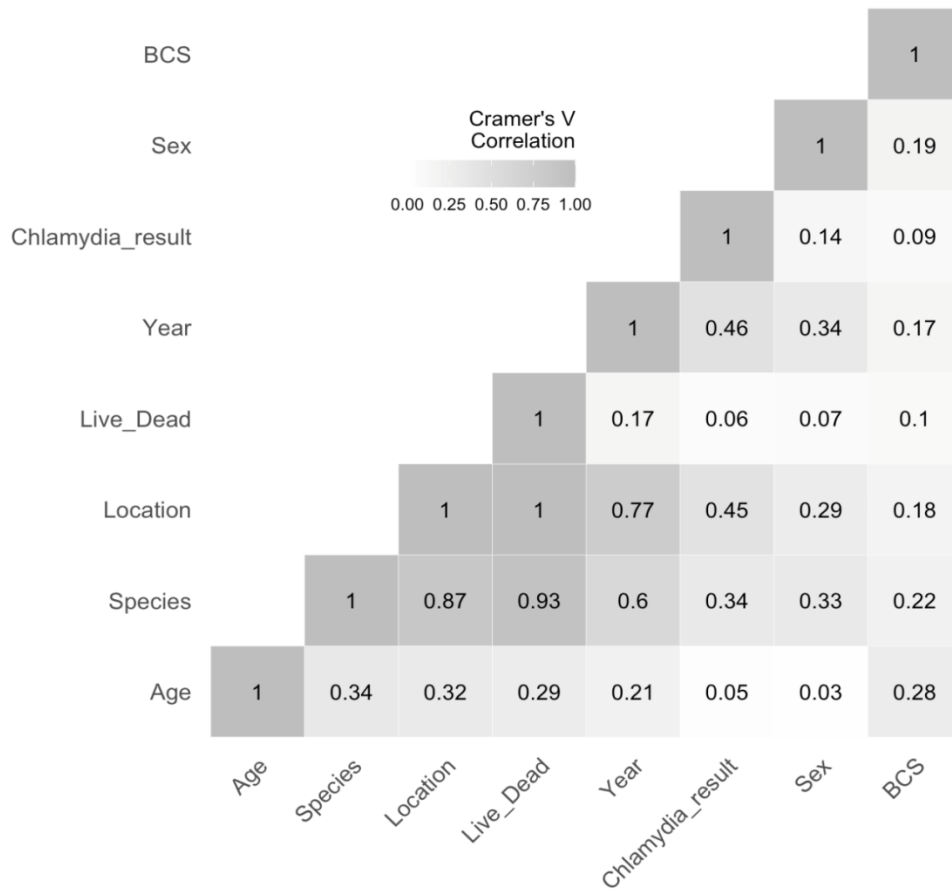
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Supplementary Figure S1: Cramer's V coefficient matrix assessing for correlation between categorical variables for all rodent samples.

Results indicate strong and moderate correlation between several categorical variables for the rodent dataset in this study.

Table S1: Assays and primer sets used in this study for the detection and characterisation of chlamydial DNA.

Assay	Target gene	Primers and probe	Sequence (5' - 3')	Amplicon size (bp)	Annealing Temperature (°C)	Reference
<i>Chlamydiaceae</i> 23S rRNA qPCR	23S rRNA	23S-F 23S-R 23S-Probe	CTGAAACCAGTAGCTTATAAGCGGT ACCTCGCCGTTTAACTTAACTCC FAM-CTCATCATGCAAAAGGCACGCCG-TAMRA	111	60	Ehricht et al. (2006)
<i>C. pecorum</i> qPCR	CpecG_0573	Cpec F3 Cpec B3	ATCGGGACCTTCTCATCG GCTGTTGTAAGGAAGACTCC	209	57	Jelocnik et al. (2019)
Partial Chlamydiales 16S rRNA PCR	16S rRNA	16S F 16S R	GATGAGGCATGCAAGTCGAACG GGAGTTAGCCGGTGCTTCTTTAC	476	60	Kasimov et al. (2022)

Table S2: Influence of sampling site on test-prevalence

No significant difference in test prevalence was detected between anatomical sites. Molecular evidence of *Chlamydiaceae* was detected in rodents from all anatomical sites and faecal samples.

Anatomical site					
Pooled Samples	PCR result	Significance test	Individual samples ^J	PCR result	Significance test
Oral-conjunctival	7/79	$\chi^2 (2, n=193) = 1.69, P = 0.43$	Conjunctival	4/26	Fisher's Exact Test $P = 0.94$
Rectal-faecal:	10/63;		Oral	2/26	
Urogenital	7/51		Rectal	4/26	
			Urogenital	3/26	
			Faecal	2/14	

^J Individual samples were analysed separately from Bernier Island only

Table S3: Histopathology results and tissue *C. muridarum* qPCR results from rodents that had tested positive to *Chlamydiaceae* on Dirk Hartog Island.

One house mouse had histopathological findings that could be consistent with known pathologies of *C. muridarum* in laboratory rodents, demonstrating a subacute suppurative bronchopneumonia and bronchiolitis, but lung tissue from this individual was test negative by *C. muridarum* qPCR. All submitted tissue was test negative by the *C. muridarum* specific qPCR.

Species	Tissue examined	Abnormal tissue findings	Site positive (<i>Chlamydiaceae</i> qPCR)	Tissue subject to <i>C. muridarum</i> PCR
Sandy Inland mouse	Kidney, liver, testis, epididymis, brain, heart, salivary gland and lymph node, lung, adrenal, skin, spleen, oesophagus, stomach, duodenum, pancreas, ileum, colon.	None	Urogenital	Lung
House mouse	Kidney, spleen, lung, uterus, small intestine, liver.	Liver: mild, diffuse, subacute vacuolar hepatopathy (consistent with glycogen).	Rectal	Faeces
House mouse	Brain, liver, heart, skeletal muscle, stomach, salivary gland and lymph node, adrenal, skin, spleen, jejunum, caecum, colon, kidney.	Kidney – marginal, multifocal, chronic lymphoplasmacytic interstitial nephritis.	Rectal and urogenital	Gastrointestinal (GIT) sections (caecum, stomach, small intestine, colon) Lung
House mouse	Salivary gland and lymph node, skeletal muscle, kidney, brain, testis and epididymis, pancreas, skin, lung, small intestine, spleen, heart, liver, caecum.	None	Rectal	GIT sections (caecum, stomach, small intestine, colon) Lung
House mouse	Heart, salivary gland and lymph node, brain, liver, kidney, stomach, skeletal muscle, skin, spleen, lung, colon, small intestine.	Mandibular lymph node lymphofollicular hyperplasia.	Rectal	GIT sections (caecum, stomach, small intestine, colon) Lung
House mouse	Heart, salivary gland and lymph node testis, brain, liver, kidney, skeletal muscle, stomach, skin, lung, pancreas, spleen, ileum, jejunum.	Mandibular lymph node - medullary histiocytosis (drainage reaction).	Conjunctival, oral and urogenital	GIT sections (caecum, stomach, small intestine, colon) Lung
House mouse	Lung, caecum, salivary gland and lymph node, kidney, brain, liver, skin, mammary gland, skeletal muscle, pancreas, spleen, small intestine, cervix, uterine horn, ovary, adrenal, colon, heart, stomach.	Lung – moderate, focally extensive, subacute suppurative bronchopneumonia and bronchiolitis. Caecum – putative oxyuriasis. Mandibular lymph node – medullary histiocytosis (drainage reaction). Kidneys – marginal, focal and bilateral, chronic lymphoplasmacytic interstitial nephritis.	Urogenital	GIT sections (caecum, stomach, small intestine, colon) Lung