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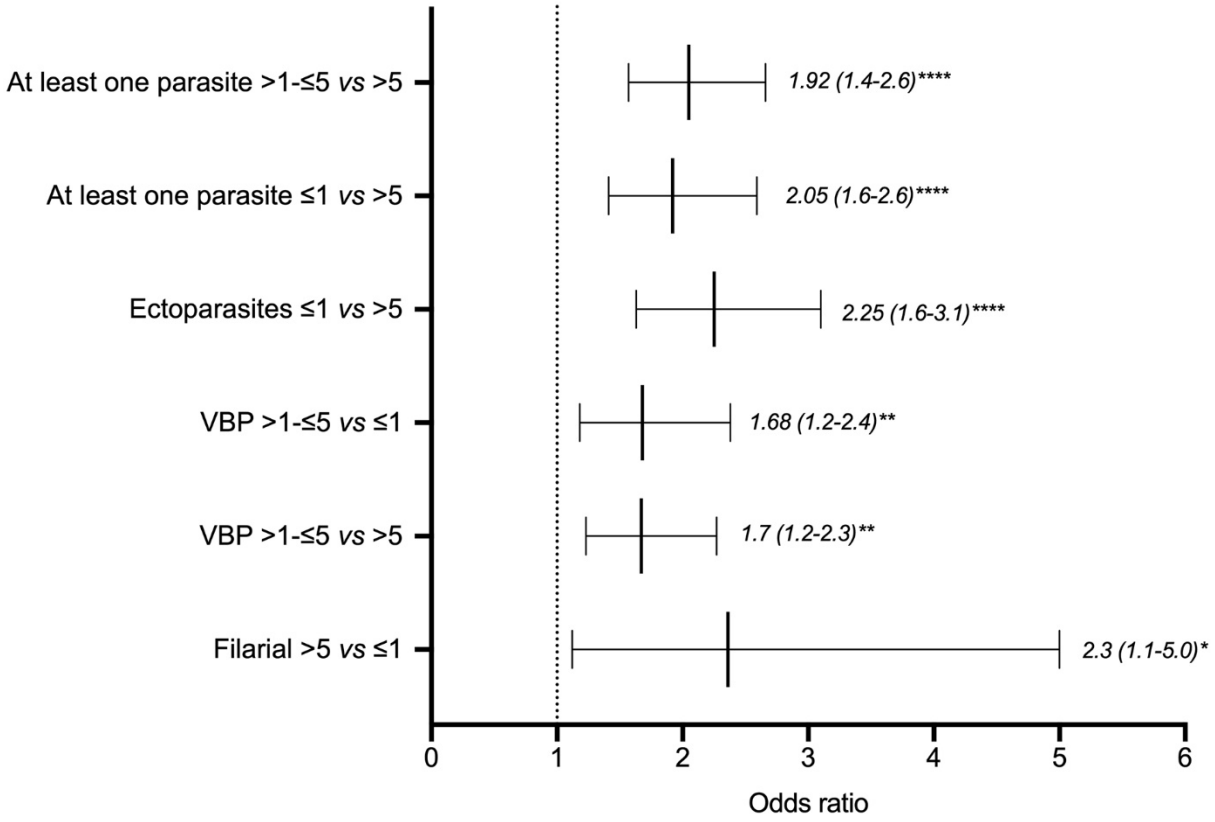
Ectoparasites and Vectorborne Zoonotic Pathogens of Dogs and Cats in Eastern and Southeast Asia, 2017–2018

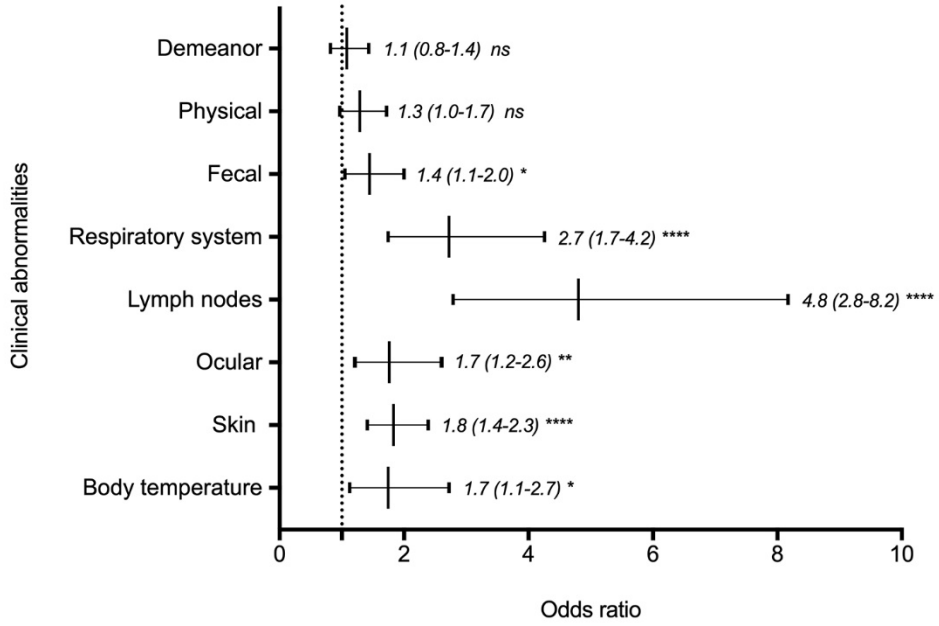
Appendix

Appendix Table. Target genes, primers (sequence and length) and cPCR/qPCR cycling conditions used in this study to detect and characterize parasites from dogs and cats

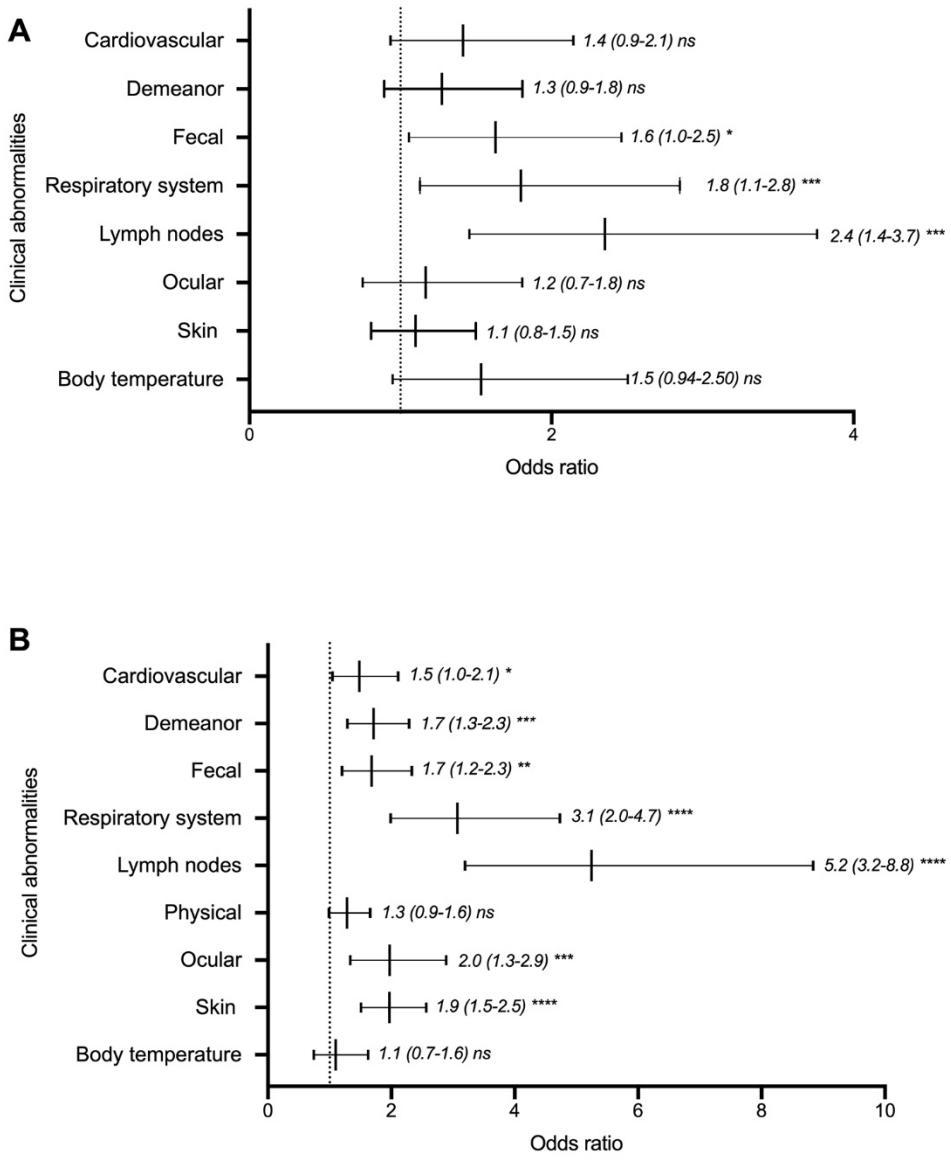
Species/Pathogen	Target gene	Primers	Sequence (5′-3′)	Fragment length (bp)	Reference
Nematodes	*cox*1	NTF NTR	TGATTGGTGGTTTTGGTAA ATAAGTACGAGTATCAATATC	648	(*1*)
Ticks	16S rRNA	RHS16SF RHS16SR	CTGCTCAATGATTTTTTAAATTGCTGT TTACGCTGTTATCCCTAGAG	300	(*2*) Modified as follows: 94°C for 10 min initial denaturation, followed by 35 cycles at 94°C for 45s, 58°C for 45s, 72°C for 60s and 72°C for 7 min for the final elongation.
Fleas, lice, mites	*cox*1	LCO1490 HCO02198	GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA	710	(*3*) Modified as follows: 95°C for 10 min followed by 35 cycles at 95°C for 60 s, 44°C for 60 s, 72°C for 90 s, and 7 min at 72°C for final elongation.
Fleas	*cox*1	LCO1490 Cff-R	GGTCAACAAATCATAAAGATATTGG GAAGGGTCAAAGAATGATGT	601	(*4*) Modified as follows: denaturing at 95°C for 10 min followed by 35 cycles at 95°C for 30 s, 52°C for 30 s, 72°C for 45 s, and 7 5 min at 72°C for final elongation.
Notoedres/ *Lynxacarus*	18S rRNA	Mite18S-F Mite18S-R	ATATTGGAGGGCAAGTCTGG TGGCATCGTTTATGGTTAG	464–490	(*5*)
Babesia/ *Hepatozoon* spp.	18S rRNA	RLB-F RLB-R	GAGGTAGTGACAAGAAATAACAATA TCTTCGATCCCCTAACTTTC	460	(*6*) Modified as follows: 95°C for 10 min initial denaturation, followed by 40 cycles at 95°C for 30s, 52°C for 30s, 72°C for 60s and 72°C for 7 min for the final elongation.
Leishmania spp.	ITS-2	LGITSF2 LGITSR2	GCATGCCATATTCTCAGTGTC GGCCAACGCGAAGTTGAATTC	383–450	(*7*) Modified as follows: 95°C for 10 min initial denaturation, followed by 35 cycles at 95°C for 30s, 60°C for 30s, 72°C for 60s and 72°C for 7 min for the final elongation.
Leishmania spp.	kDNA minicircle	MC-1 MC-2	GTTAGCCGATGGTGGTCTTG CACCCATTTTTCCGATTTTG	447	(*8*)
Leishmania spp.	kDNA minicircle	LEISH-1 LEISH-2 Probe	AACTTTTCTGGTCCTCCGGGTAG ACCCCCAGTTTCCCGCC FAM-AAAAATGGGTGCAGAAAT	120	(*9*)



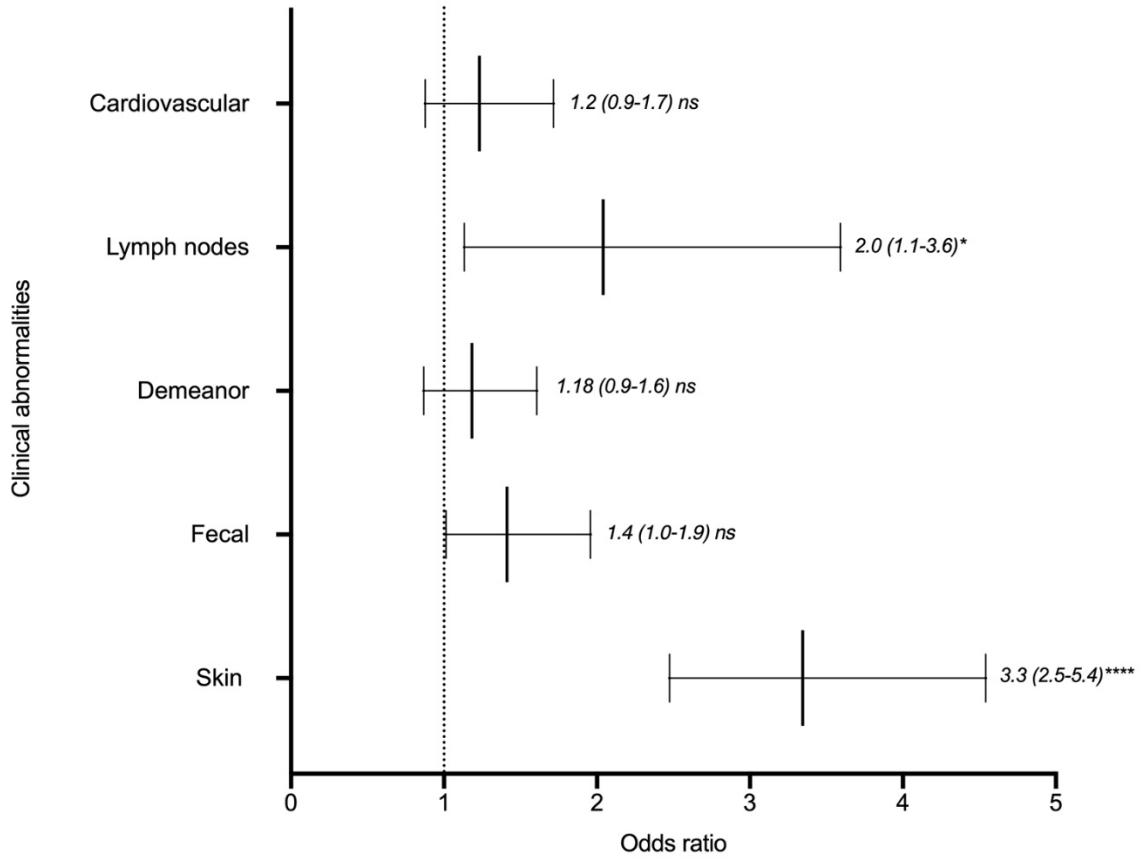
Appendix Figure 1. Box plot of odds ratio (median and range) of the detection/exposure to at least one vector-borne pathogen or ectoparasite, ectoparasites- or vector-borne pathogens-only, and to filarial parasites in dogs aging ≤1, >1-≤5 and >5 years. Odds ratio and CI 95% in brackets. **** $p < 0.0001$, ** $p < 0.001$, * $p < 0.01$, ns not significant.



Appendix Figure 2. Box plot of odds ratio (median and range) of selected clinical abnormalities associated with overall detection/exposure to at least one parasite in dogs. Odds ratio and CI 95% in brackets. **** $p < 0.0001$, ** $p < 0.001$, * $p < 0.01$, ns not significant.



Appendix Figure 3. Box plot of odds ratio (median and range) of selected clinical abnormalities associated with the detection/exposure to VBPs (**A**) or ectoparasitic infestation in dogs (**B**). Odds ratio and CI 95% in brackets. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.002$, * $p < 0.01$, ns not significant.



Appendix Figure 4. Box plot of odds ratio (median and range) of selected clinical abnormalities associated with the detection of ectoparasitic infestation in cats. Odds ratio and CI 95% in brackets. **** p < 0.0001, * p < 0.01, ns not significant.

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