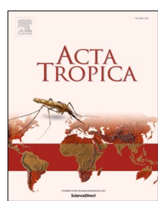
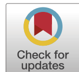
Acta Tropica 268 (2025) 107738

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica





A multi-modal investigation into the prevalence and diagnostic evaluation of vector-borne pathogens and retroviruses in domestic cats throughout Namibia

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A R T I C L E I N F O

Keywords:
Feline
Ehrlichia
Anaplasma
Babesia
Hepatozoon
FIV
FeLV

A B S T R A C T

Neglected, but economically significant, tropical diseases may be prevalent in domestic cats of Namibia. A multi-centre prevalence field study was conducted across Namibia to assess the distribution of vector-borne pathogens and retroviruses from domestic cats. Samples of blood and serum from 280 cats in 15 towns across eight regions (22–51 cats per region) were analysed. Screening for *Ehrlichia, Anaplasma, Babesia*, and *Hepatozoon* species, as well as feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV), was conducted using light microscopy, point-of-care serology, and quantitative real-time polymerase chain reaction (qPCR) assays. Haematology and serum biochemistry analyses were also performed. Several pathogens were identified in Namibian cats using these diagnostic tools. Comparatively, pathogen detection rates varied by modality, namely light microscopy (5 %), serology (42 %), and qPCR (27 %). More specifically, microscopy revealed an overall prevalence (3 %) for inclusions resembling large *Babesia* parasites, serology indicated a seroprevalence for FIV antibodies (4 %) and FeLV antigen (40 %), and an overall prevalence determined by qPCR for *E. canis* (2 %) and *H. canis* (26 %). The investigation also demonstrated the associations between tick presence, pathogen infection, and disease manifestations in Namibian cats. A particular significant positive association was found between *H. canis* infection and FeLV antigen seroprevalence ($P = 0.005$). Overall, the study highlighted the difference of various diagnostic tools for detecting pathogen prevalence in cats. Appropriate diagnostic testing - informed by known associations with disease manifestation - should be key in guiding responsible treatment strategies and evaluating potential zoonotic risks linked to domestic cats of Namibia.

Abbreviations: ALB, Albumin; DNA, Deoxyribonucleic acid; EDTA, Ethylenediamine tetra-acetic acid; ELISA, Enzyme-linked immunosorbent assay; GLOB, Globulin; Hb, Haemoglobin; Hct, Haematocrit; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; MCV, Mean corpuscular volume; qPCR, Quantitative real-time polymerase chain reaction; RBC, Red blood cell; RCC, Red cell count; RDW, Red cell distribution width; RSA, Republic of South Africa; TP, Total protein; US, United States; WCC, White cell count.

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https://doi.org/10.1016/j.actatropica.2025.107738
Received 11 April 2025; Received in revised form 23 June 2025; Accepted 8 July 2025
Available online 10 July 2025
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1. Introduction

Vector-borne pathogens represent an important but underexplored cause of disease in domestic cats, particularly in sub-Saharan countries such as Namibia. Though often neglected, these pathogens may cause economically and clinically significant diseases in feline populations (Noden and Van Der Colf, 2013). Domestic cats can act as incidental hosts for several vector-borne pathogens, many of which are transmitted by arthropod vectors and may cause clinical disease, particularly in immunocompromised animals.

Ticks, especially *Rhipicephalus sanguineus* sensu lato, have been implicated in the transmission of several pathogens of veterinary and zoonotic concern. This tick species is well established as the primary ectoparasite of domestic dogs in Namibia (Horak et al., 2018) and has also been recovered from domestic cats (Madder *et al.*, 2022), indicating potential cross-species vector involvement. In cats, *R. sanguineus* s.l. is suspected to transmit *Ehrlichia canis* (Braga *et al.*, 2017), *Anaplasma platys* (Lima *et al.*, 2010), *Babesia vogeli* (Simking *et al.*, 2010), and *Hepatozoon canis* (Madder *et al.*, 2022). Some of these pathogens not only pose a risk to feline health but may also carry zoonotic implications. Human infections with *E. canis (*Bouza-Mora et al.*, 2017;* Maeda *et al.*, 1987; Perez *et al.*, 2006) and *A. platys* (Arraga-Alvarado *et al.*, 2014; Maggi *et al.*, 2013) have been described, reinforcing the significance of these agents from a One Health perspective.

Although feline vector-borne diseases are of clinical and epidemiological importance, research from Africa remains limited. Notable findings include *E. canis* detection in 3 % of healthy cats in Angola (Oliveira *et al.*, 2018) and reports of large-form *Babesia* species in South African domestic cats (Bosman *et al.*, 2019; Stewart *et al.*, 1980). *Hepatozoon canis* prevalence remains largely undocumented in African felines, with most studies focusing on *H. felis* (Baneth *et al.*, 2013). Retroviral prevalence varies within Africa, with FeLV reported ranging between 15 % (Tchamo *et al.*, 2019) and 41 % (Muchaamba *et al.*, 2014), and low FIV rates documented in the Republic of South Africa (RSA) (Lobetti and Lappin, 2012). Clinically, *E. canis* and *A. platys* infections may present with non-specific signs including lethargy, anorexia, and pyrexia (Lima *et al.*, 2010; Little, 2010; Oliveira *et al.*, 2018). While sub-clinical feline babesiosis is typically attributed to *Babesia felis* and *Babesia leo* (Penzhorn and Oosthuizen, 2020), large-form species like *B. vogeli* (Simking *et al.*, 2010) and *Babesia* species cat Western Cape (Bosman *et al.*, 2019) have also been reported. Feline hepatozoonosis, generally sub-clinical, is most often linked to *H. felis*, though *H. canis* has also been described (Baneth and Allen, 2022). Co-infections with retroviruses such as FIV and FeLV may increase susceptibility to vector-borne diseases, including hepatozoonosis (Baneth *et al.*, 1998), ehrlichiosis (Melo *et al.*, 2023), and babesiosis (Schoeman *et al.*, 2001). Diagnosis of these pathogens typically relies on microscopy, serology, and molecular assays (Madder *et al.*, 2022; Simking *et al.*, 2010).

In Namibia specifically, there is a dearth of data regarding feline vector-borne and retroviral infections. A recent study found no molecular evidence of *B. felis* or seroprevalence of *Ehrlichia* species in cats, though *H. canis* was identified in all tick pools collected from rural feline hosts (Madder *et al.*, 2022). Another study using PCR reported a low FIV prevalence (1 %) among domestic cats (Franzo *et al.*, 2024). Broadly, the prevalence and clinical implications of such neglected pathogens in Namibian domestic cats (*Felis catus*) remain unknown (Noden and Soni, 2015; Noden and Van Der Colf, 2013). This knowledge gap highlights the need for targeted research on the epidemiology and health impact of feline vector-borne pathogens in Namibia. To address this, a multi-centre field study was conducted to determine the prevalence of vector-borne pathogens and retroviruses in domestic cats using a multi-modal diagnostic approach. The study offered crucial baseline data to inform diagnosis, treatment, and prevention strategies in both veterinary and public health contexts.

2. Materials and methods

2.1. Study site and design

Sample collection sites in northern, central, and southern regions are depicted in Fig. 1. These were predominantly rural areas but also included the capital city Windhoek in the Khomas region as the sole major urban site of sample origin. Non-probability convenience sampling was incorporated into routine diagnostic procedures at the Mobile Animal Clinic of the Veterinary Academic Hospital, University of Namibia, from March to October 2022, and stored secondary samples were evaluated. The study population consisted of a total of 280 free-roaming cats, with between 22 and 51 samples analysed per region, based on the availability of stored material.

2.2. Sampling and processing

Domestic pet cats presented at the Mobile Animal Clinic by their owners were included irrespective of health status or age to mitigate selection bias, while exclusion criteria encompassed sample contamination (e.g. blood smears containing material other than blood), inadequate sample quantity or quality (e.g. due to damage), and incomplete patient records. Clinical metadata were extracted from patient records, including signalment, history, tick presence, and clinical signs commonly associated with vector-borne pathogens and retroviruses including pyrexia, lymphadenopathy, splenomegaly, mucous membrane pallor, emaciation, and oculonasal discharge (Baneth and Allen, 2022; Franzo *et al.*, 2024; Lima *et al.*, 2010; Little, 2010; Penzhorn and Oosthuizen, 2020). Blood smears were prepared from peripheral capillary blood collected via ear prick, air-dried, fixed with Kyro-Quick Stain Fixative (Kyron Laboratories, Johannesburg, RSA) for 10 s, and stored for subsequent staining and analysis. Typically, the stored blood slides were evaluated for diagnostic adequacy, including visibility of a suitable feathered edge. The smears were then stained with a Kyro-Quick Romanowsky stain (Kyron Laboratories, Johannesburg, RSA) at standardised intervals, and stored at room temperature until microscopic examination. In addition, 2–4 mL central venous blood was collected for serum and ethylenediamine tetra-acetic acid (EDTA) samples, which varied in amount based on the size of the animal. Serum was allowed to clot for 20–30 min at room temperature prior to storage. Samples were kept cool at −4 °C for haematology and biochemistry analysis (where applicable) within 24 h and then stored at −20 °C until further molecular analyses were conducted.

2.3. Haematology and biochemistry analyses

Haematological analyses, conducted on an IDEXX LaserCyte Dx analyser (version 2.17), utilised a CBC5R test kit (IDEXX Laboratories, Johannesburg, RSA) as per manufacturer guidelines. The complete blood count (CBC) metrics measured included standard red blood cell (RBC) indices, reticulocytes, total and differential white cell count (WCC), and platelets, with reference ranges applied based on manufacturer guidelines. On the other hand, biochemical analyses followed on an IDEXX CatalystOne analyser (version 2.22), assessing biomarkers total protein (TP), albumin (ALB), and globulin (GLOB), also using manufacturer-specified reference ranges. Results from both analyses were processed using IDEXX VetLab workstation (version 5.18), generating reports through the IDEXX VetConnect PLUS platform. Quality control checks on the LaserCyte Dx and CatalystOne analysers were consistently performed according to manufacturer guidelines to ensure result accuracy and reliability.

2.4. Light microscopy

Peripheral blood smears from 280 samples were examined using light microscopy to detect haemoparasites. Initially, the suitability of

each smear was confirmed based on a well-defined feathered edge and appropriate staining. Screening began at a low magnification (10 × objective) using a modified sideways battlement pattern across the entire feathered edge. Thereafter, the entire feathered edge was scrutinised at a higher magnification (100 × objective with oil immersion) to document any inclusions indicative of haemoparasites such as *Ehrlichia, Anaplasma, Babesia*, or *Hepatozoon* species.

2.5. Serology

The SNAP FIV/FeLV Combo Plus Test (IDEXX Laboratories, Johannesburg, RSA) was utilised to perform enzyme-linked immunosorbent assay (ELISA) serodetection of relevant antigens and antibodies from 280 stored whole blood aliquots. Whole blood samples were allowed to thaw at room temperature for 30 min prior to analysis. Then, the SNAP tests were conducted, following manufacturer guidelines. Colour development in the activation circle indicative of antigen or antibody presence was recorded. Chiefly, this test enabled *in vitro* diagnostic detection of the FeLV p27 antigen and antibodies to FIV in feline whole blood.

2.6. Extraction and molecular assays for pathogen detection

Deoxyribonucleic acid (DNA) from all samples were extracted from 200 μL of EDTA-anticoagulated whole blood using the PureLinkTM Genomic DNA Mini kit (Thermo Fisher Scientific, Johannesburg, RSA), following manufacturer guidelines. The extracted nucleic acids were eluted in 50 μL elution buffer and stored at −20 °C until analysis.

Table 1 details the final primer and probe concentrations for the quantitative real-time polymerase chain reaction (qPCR) assays employed for analysis of the whole blood. Each reaction contained 10 μL of 2× TaqMan® Universal Master Mix (Thermo Fisher Scientific, Johannesburg, RSA), optimised primer and probe concentrations, and 2 μL of sample nucleic acid or control material, adjusted to the final volume specified with nuclease-free water *quantum satis*. The primers (Inqaba Biotechnical Industries, Pretoria, RSA) and probes (Integrated DNA Technologies, Whitehead Scientific, Cape Town, RSA) were used as described in previous studies (Madder *et al.*, 2022; Makgabo *et al.*, 2024, 2023; Nkosi *et al.*, 2022; Troskie *et al.*, 2019). However, in some cases, the probes were designed and synthesised with modifications, as demonstrated in Table 1. A supplementary dataset on the optimisation of the qPCR assays is available.

The qPCR assays were run on a Bio-Rad CFX96 thermocycler (Bio-Rad Laboratories, California, US) with cycling conditions tailored for the 2× TaqMan® Universal Master Mix. All assay cycling conditions consisted of an initial incubation at 50 °C for 2 min (one cycle), polymerase activation at 95 °C for 10 min (one cycle), followed by denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min (40 cycles). The *Babesia* species multiplex assay had a modified annealing/extension step at 60 °C for 45 s (45 cycles), as previously described (Troskie *et al.*, 2019). Data analysis was performed using Bio-Rad CFX Maestro 1.1 (version 4.1) for Windows (Bio-Rad Laboratories, California, US). Most assays were standardised for multiplexing to streamline processing and optimise throughput. The quality of the positive controls, consisting of DNA extracted from known, species-specific mono-infected field samples, were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, US) to ensure reliability. Negative controls consisted of molecular-grade water.

2.7. Statistical analysis

Descriptive statistics were reported as proportions, including patient signalment, history, and clinical features. Pathogen prevalence in whole blood of cats across the regions, assessed using three diagnostic techniques, was tabulated. Associations between pathogen prevalence in whole blood determined by serology and qPCR, and co-infection, tick presence, patient signalment and history, and disease manifestation were assessed using Fisher's Exact test. Generally, all proportions were expressed as percentages, and statistical significance was interpreted at a 5 % level. Statistical analyses were performed using IBM SPSS Statistics (version 29.0) for Windows (IBM Corporation, New York, US).

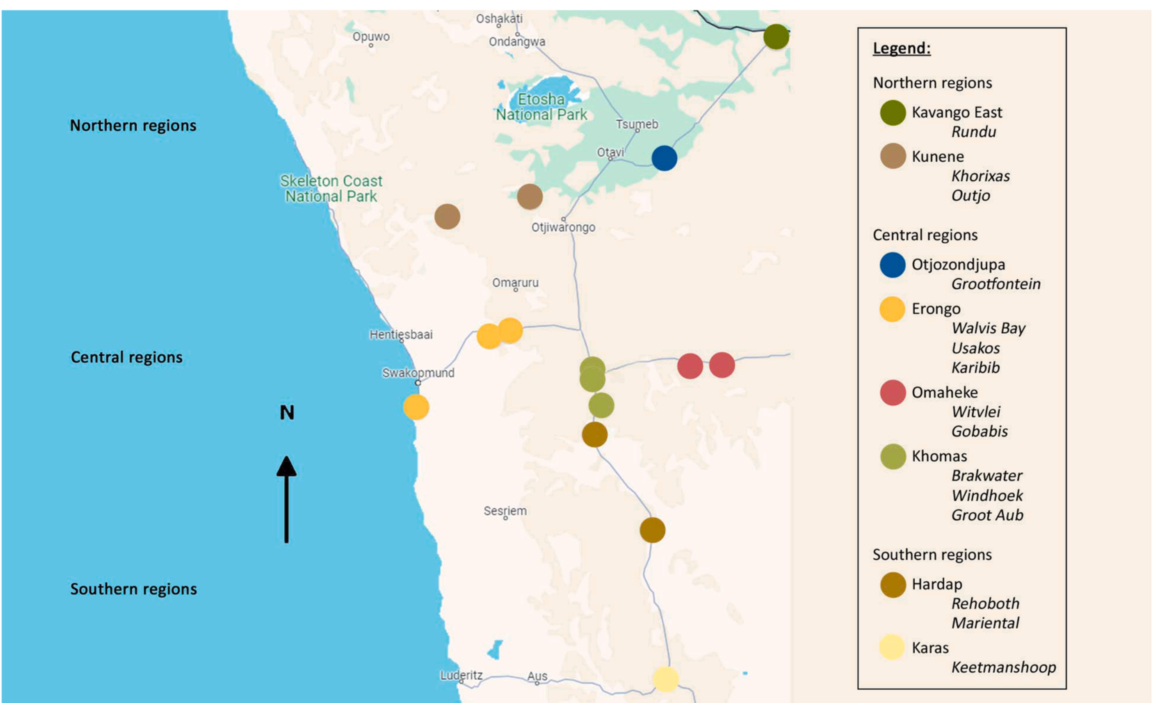

Fig. 1. Feline sample origin sites across eight regions of Namibia.
Map lines delineate study areas and do not necessarily depict accepted national boundaries.

Table 1
qPCR primer and probe concentrations used for screening of vector-borne pathogens in cats.

Target species	Target gene and region	Primer / probe name	Sequence (5′–3′)	Final concentration (µM)	Final reaction volume (µL)
Anaplasma species (Makgabo *et al.*, 2024, 2023)	16S rRNA gene, 64 bp hypervariable V3 region	Ma16S_F	ACAGAAGAAGTCCCGGCAAA	0.80*	20
		Ma16S_R	TTGCCCCCTCCGTATTACC	0.80*	
		Ma16S_P	FAM-CCGTGCCAGCAGC-MGB-NFQ	0.15*	
E. canis (Nkosi *et al.*, 2022)	16S rRNA gene, 289 bp conserved V1 region	AnapEhrlichia_F	AGCYTAACACATGCAAGTCGAA	0.80*	20
		AnapEhrlichia_R	TTACTCACCCGTCTGCCACTAA	0.80*	
		E. canis_P	FAM-AGCCTCTGGCTATAGGA-MGB-NFQ	0.25	
B. vogeli/*B. rossi* multiplex (Troskie *et al.*, 2019)	18S rRNA gene, 450–540 bp conserved V4 region	BDog_F	TGTTGCAGTTAAAARGCTCGTAGTT	0.40*	25
		BDog_R	AGTCTGCTTGAAACACTCTAATTTTCTC	0.40*	
		Bvogeli_P	HEX-AGTTTGCCATTCGTTTGG-MGB-NFQ*	0.25	
		BRossi_P	FAM-TGGCTTTTTGCCTTATTA-MGB-NFQ	0.25	
H. canis (Madder *et al.*, 2022)	Undisclosed	H. canis_F	GGCAGTGACGGTTAACGGGGG	0.40*	20
		H. canis_R	GCACCAGACTTGCCCTCCAATTG	0.40*	
		H. canis_P	FAM-CCGGAGAGGGAGCCTGAGAAACGG-BHQ1 *	0.25	

* Modified.

3. Results

3.1. Feline population description

Table 2 displays the frequencies and percentages of key patient signalment, history, and relevant clinical characteristics at presentation. A supplementary dataset on the patient metadata is available. Most patients were female, mixed breed, and over one year old, with only one purebred cat recorded. Ectoparasite control and up-to-date vaccination were reported in just 11 % and 5 % of cases, respectively. Anorexia (3 %) and weight loss (6 %) were infrequently noted, and ticks were present in only 4 % of cats. Clinical signs suggestive of vector-borne or immunosuppressive disease included pyrexia (12 %), lymphadenopathy (20 %), splenomegaly (6 %), pale mucous membranes (39 %), emaciation (4 %), and oculonasal discharge (16 %).

3.2. Vector-borne pathogen and retrovirus prevalence

As shown in Table 3, haemoparasites of interest were identified through light microscopy of blood smears from cats in four out of eight regions investigated. A supplementary dataset on the diagnostic assessment of the vector-borne pathogens is available. The highest overall haemoparasite prevalence was detected in Khomas (14 %). Neither *Ehrlichia* nor *Anaplasma* species were detected on light microscopy. Interestingly, inclusions akin to large *Babesia* species were noted at a low proportion (3 %) on blood smears from Khomas, Kunene, Kavango, and Hardap, with Kunene showing the highest prevalence (7 %). Moreover, *Hepatozoon* species gamonts were observed on smears only from the Khomas and Hardap regions, with Khomas having the highest prevalence (10 %).

Low overall seroprevalence was determined for FIV antibodies (4 %), and a moderate to high seroprevalence for FeLV antigen (40 %), in cats from several regions studied (Table 4). Antibodies to FIV, suggestive of FIV exposure which resulted in previous or current infection, were detected in five regions explored, with the highest seroprevalence in Kavango (11 %). By comparison, no seroprevalence to FIV was detected in Omaheke, Kunene, and Karas. The seroprevalence of FeLV antigen, suggestive of active FeLV infection, was detected in all eight regions explored, with the highest seroprevalence also in Kavango (70 %).

Table 2
Clinical metadata of feline patient signalment, history, tick presence, and clinical features.

	Frequency	Proportion (%)
Signalment		
Sex		
Male	136	49
Female	144	51
Breed		
Mixed	279	100
Pure	1	0
Age		
< 1 year (kittens)	83	30
> 1 year (junior to geriatric)	197	70
History		
Ectoparasite control	31	11
Anorexia	9	3
Weight loss	16	6
Vaccination status	13	5
Tick presence	10	4
Clinical features		
Pyrexia	33	12
Lymphadenopathy	55	20
Splenomegaly	17	6
Pallor	109	39
Emaciation	10	4
Oculonasal discharge	46	16

Total number of individuals (N) = 280 for each variable.

Table 3
Vector-borne pathogen prevalence as determined by light microscopy on blood smear samples from cats across eight regions of Namibia.

	Overall	Northern regions		Central regions				Southern regions	
		Kavango	Kunene	Otjozondjupa	Erongo	Omaheke	Khomas	Hardap	Karas
Individuals (N)	280	46	27	22	42	29	51	36	27
Pathogen (%)									
All pathogens of interest	5	2	7	0	0	0	14	8	0
Ehrlichia species	0	0	0	0	0	0	0	0	0
Anaplasma species	0	0	0	0	0	0	0	0	0
Babesia species*	3	2	7	0	0	0	4	6	0
Hepatozoon species	2	0	0	0	0	0	10	3	0

* Inclusions resembling large *Babesia* species, but molecular confirmation pending.

Table 4
Retrovirus pathogen seroprevalence as determined by ELISA in whole blood samples from cats across eight regions of Namibia.

	Overall	Northern regions		Central regions				Southern regions	
		Kavango	Kunene	Otjozondjupa	Erongo	Omaheke	Khomas	Hardap	Karas
Individuals (N)	280	46	27	22	42	29	51	36	27
Pathogen (%)									
All pathogens of interest	42	72	41	36	36	7	37	47	48
Feline immunodeficiency virus	4	11	0	5	5	0	2	3	0
Feline leukaemia virus	40	70	41	32	31	7	35	44	48

Table 5
Vector-borne pathogen prevalence as determined by qPCR in whole blood samples from cats across eight regions of Namibia.

	Overall	Northern regions		Central regions				Southern regions	
		Kavango	Kunene	Otjozondjupa	Erongo	Omaheke	Khomas	Hardap	Karas
Individuals (N)	280	46	27	22	51	42	29	36	27
Pathogen (%)									
All pathogens of interest	27	4	52	55	12	52	31	28	4
E. canis	2	2	4	5	0	4	0	0	4
Anaplasma species	0	0	0	0	0	0	0	0	0
B. vogeli	0	0	0	0	0	0	0	0	0
B. rossi	0	0	0	0	0	0	0	0	0
H. canis	26	2	52	55	12	52	31	28	0

Table 5 shows the prevalence of haemoparasites of interest across all regions investigated using qPCR screening. The highest overall pathogen prevalence was detected in Otjozondjupa (55 %) and Kunene (52 %). To expand, *E. canis* was present in five of the eight regions, exhibiting a low overall prevalence (2 %) among the pathogens tested by qPCR, with Otjozondjupa showing the highest prevalence (5 %). Neither *Anaplasma* nor any of the *Babesia* species tested for were found in cats in any of the regions. Except for Karas, *H. canis* was identified in all remaining regions, with the highest prevalence in Otjozondjupa (55 %).

3.3. Comparison of diagnostic prevalence at regional level

Fig. 2 illustrates macrogeographic variations in pathogen prevalence determined by different diagnostic methods, with rates averaged across northern, central, and southern regions. In microscopy, overall pathogen prevalence was highest in the northern regions (5 %), followed by the southern (4 %) and central (3 %) regions. No *Ehrlichia* or *Anaplasma* species were detected microscopically. Interestingly, large *Babesia* species piroplasms were found in northern (5 %), southern (3 %), and central (1 %) region samples, while *Hepatozoon* species were observed at low levels in central (3 %) and southern (1 %) Namibia but absent in the north.

Seroprevalence was highest in the north (56 %), with lower rates in the southern (48 %) and central (29 %) regions. Specifically, exposure to FIV was most prevalent in the north (6 %), followed by central (3 %) and southern (1 %) regions, while FeLV seroprevalence was higher in northern (55 %) and southern (46 %) regions than in the central regions (26 %).

Molecular detection patterns differed, with pathogen prevalence highest in the central regions (37 %), followed by the northern (28 %) and southern (16 %) regions. More specifically, *E. canis* showed low prevalence in the north (3 %) and in central and southern (2 %) regions, while *Anaplasma* and *Babesia* species were absent on qPCR. *Hepatozoon canis* detection was higher in central (38 %) and northern (27 %) regions compared to the south (14 %).

Serology showed prominent detection of FeLV antigen (40 %) across all regions, except Omaheke. Across the study regions, qPCR had a significantly higher detection rate (27 %) compared to light microscopy (5 %). *Hepatozoon canis* was the most common protozoal pathogen on qPCR (26 %) in cats across regions. Notably, low prevalence was observed for *E. canis* (2 % on qPCR) and large *Babesia* species (3 % on microscopy) across several regions.

3.4. Pathogen-tick-disease associations

Table 6 presents the associations between pathogen infections detected by serology and qPCR in whole blood and various patient metadata related to signalment and history. The only significant positive association between serological results and patient signalment was the FIV seroprevalence in cats older than one year ($P = 0.036$). Although kittens (< 6 m) and cats at the lower end of the junior stage of life (6 m to 1 y) are at higher risk of FeLV infection, no significant associations were

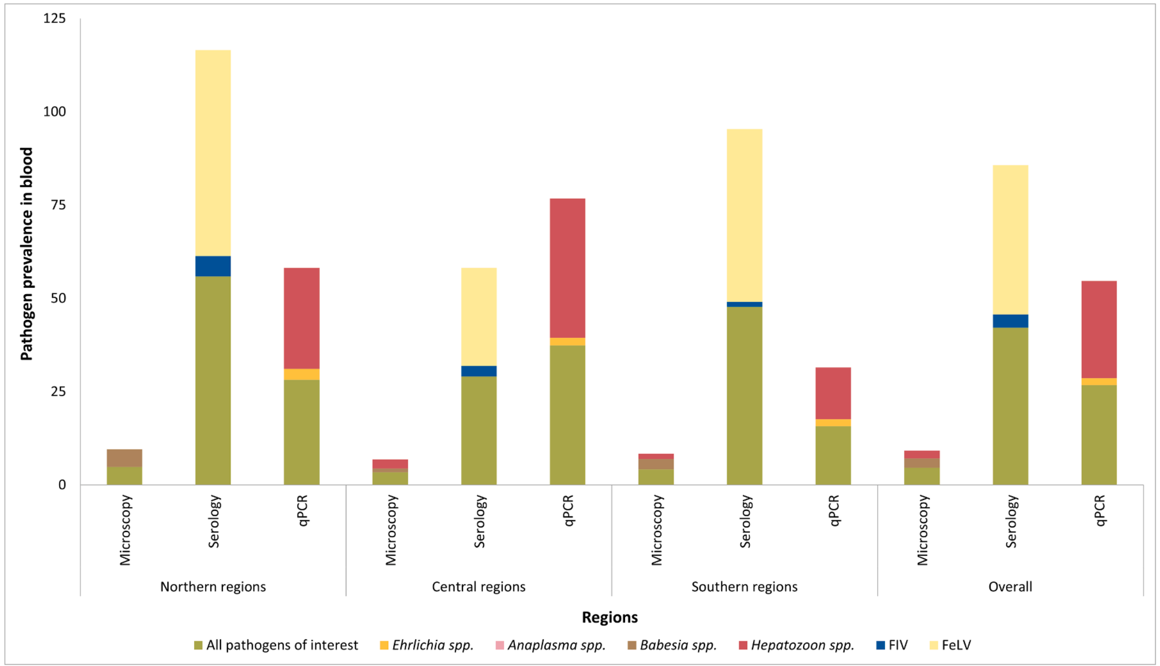

Fig. 2. Macrogeographic variation in vector-borne pathogen prevalence and retroviruses as determined by various diagnostic modalities in whole blood from cats across northern, central, and southern regions of Namibia.

Table 6
Associations of patient signalment, history, tick presence, and clinical features with vector-borne pathogen and retrovirus infections determined by serology and qPCR in whole blood from cats.

	FIV serology			FeLV serology			*E canis* qPCR			*H. canis* qPCR		
	N	%	*P*	N	%	*P*	N	%	*P*	N	%	*P*
Individuals (N)	10			112			5			73		
Signalment												
Sex			0.531			0.903			0.203			1.0
Male	6	60		55	49		4	80		35	48	
Female	4	40		57	51		1	20		38	52	
Breed			1.0			0.40			1.0			1.0
Pure	0	0		1	1		0	0		0	0	
Mixed	10	100		111	99		5	100		73	100	
Age			**0.036***			0.110			0.635			0.103
< 1 year	0	0		27	24		2	40		16	22	
> 1 year	10	100		85	76		3	60		57	79	
History												
Ectoparasite control	1	10	1.0	8	7	0.119	2	40	0.096	5	7	0.277
Anorexia	1	10	0.283	4	4	1.0	0	0	1.0	0	0	0.118
Weight loss	2	20	0.105	3	3	0.113	0	0	1.0	6	8	0.377
Vaccination status	0	0	1.0	6	5	0.773	0	0	1.0	3	4	1.0
Tick presence	n/a	n/a	n/a	n/a	n/a	n/a	1	20	0.167	3	4	0.724
Clinical features												
Pyrexia	3	30	0.101	13	12	1.0	1	20	0.468	6	8	0.398
Lymphadenopathy	2	20	1.0	20	18	0.645	3	60	0.054	17	23	0.393
Splenomegaly	1	10	0.471	7	6	1.0	0	0	1.0	5	7	0.777
Pallor	5	50	0.519	30	27	**< 0.001***	0	0	0.160	33	45	0.212
Emaciation	1	10	0.309	1	1	0.055	0	0	1.0	3	4	0.724
Oculonasal discharge	2	20	0.671	13	12	0.099	1	20	1.0	18	25	**0.042***

Fisher's Exact Significance (2-sided): $P < 0.05$ = statistical significance.

calculated. *Ehrlichia canis* and *H. canis* infections detected by qPCR showed no significant positive associations with patient sex, breed, or age. No significant associations were found between FIV and FeLV serodetection and histories of ectoparasite control, anorexia, weight loss, or vaccination status. Similarly, *E. canis* and *H. canis* infections determined by qPCR showed no significant associations with these histories. However, a notable observation was the high proportion of cats older than one year that were seropositive for FIV (100 %) and FeLV (76 %), as well as those positive for *H. canis* infection on qPCR (78 %).

The relevant associations between tick presence, clinical features, and pathogen prevalence determined by serology and qPCR were scarce, as shown in Table 6. However, significant associations were found, including a significant positive association between FeLV serology and mucous membrane pallor ($P < 0.001$). Only one significant association was observed between pathogens detected by qPCR and clinical features. *Hepatozoon canis* on qPCR showed a significant positive association with oculonasal discharge ($P = 0.042$), which is not a common feature of feline hepatozoonosis.

Table 7
Associations between haematology and serum biochemistry, tick presence, and vector-borne pathogen and retrovirus infections as determined by qPCR in whole blood and serum from cats in Khomas.

	Tick presence			FIV serology			FeLV serology			*H. canis* qPCR		
	N	%	*P*	N	%	*P*	N	%	*P*	N	%	*P*
Individuals (N)	2			1			18			16		
Haematology												
RCC	0	0	1.0	1	100	0.140	3	17	0.692	3	19	0.666
Hct	2	100	0.542	1	100	1.0	10	56	0.352	14	88	0.053
Hb	1	50	0.363	1	100	0.20	3	17	0.730	4	25	0.707
MCV	0	0	1.0	0	0	1.0	0	0	0.544	1	6	1.0
MCH	0	0	1.0	1	100	0.120	2	11	1.0	2	13	1.0
MCHC	0	0	1.0	1	100	0.280	4	22	0.744	8	50	**0.040***
RDW	0	0	n/a	0	0	n/a	0	0	n/a	0	0	n/a
Reticulocytes	0	0	1.0	0	0	1.0	1	6	1.0	2	13	0.237
WCC	1	50	0.363	0	0	1.0	3	17	0.730	3	19	1.0
Neutrophils	2	100	**0.045***	1	100	0.220	3	17	0.724	4	25	0.728
Lymphocytes	0	0	n/a	0	0	n/a	0	0	n/a	0	0	n/a
Monocytes	2	100	**0.002***	0	0	1.0	1	6	1.0	2	13	0.237
Eosinophils	2	100	0.490	1	100	1.0	8	44	0.769	12	75	**0.032***
Basophils	1	50	0.297	0	0	1.0	3	17	1.0	5	31	0.092
Platelets	2	100	0.074	1	100	0.280	3	17	0.211	5	31	0.746
Serum biochemistry												
TP	0	0	n/a	0	0	n/a	0	0	n/a	0	0	n/a
ALB	0	0	n/a	0	0	n/a	0	0	n/a	0	0	n/a
GLOB	2	100	**< 0.001***	0	0	1.0	0	0	0.530	2	13	0.098

Fisher's Exact Significance (2-sided): $P < 0.05$ = statistical significance.

Table 7 highlights relevant associations between haematological and biochemical profiles, tick presence, and pathogen infections specifically in Khomas cats. A supplementary dataset on the bloodwork analysis is available. Significant positive biomarker associations with tick presence included neutrophilia ($P = 0.045$), monocytosis ($P = 0.002$), and hyperglobulinaemia ($P < 0.001$). No significant associations were recorded between serological results and haematology or serum biochemistry biomarkers. However, is a worthwhile to acknowledge that more than half of patients seropositive for FeLV in Khomas, and the one patient seropositive for FIV in the same region, in most cases had a decreased haematocrit (Hct), indicative of anaemia. Similarly, a high proportion of Khomas patients infected with *H. canis* on qPCR (88 %) had a decreased Hct, which may be related to anaemia. Further, 50 % of *H. canis*-infected cats based on qPCR had a positive association with mean corpuscular haemoglobin concentration (MCHC) ($P = 0.040$). *Hepatozoon canis* infection detected by qPCR was also associated with a high proportion of eosinophilia (75 %) and a significant positive association observed ($P = 0.032$).

3.5. Diagnostic modality associations

A strong and significant positive association was found between *H. canis* infection and FeLV antigen seroprevalence ($P = 0.005$). *Ehrlichia canis* infection showed no positive association with the pathogens tested by serology. Similarly, no associations could be calculated for *Anaplasma* and *Babesia* species infection, because both were negative on qPCR.

4. Discussion

This study was the first to extensively screen for vector-borne pathogens and retroviruses in domestic cats across various regions of Namibia, using multiple diagnostic methods to evaluate pathogen prevalence and assess disease manifestations. Few studies have explored haemoparasites in Namibian cats, and there has been a lack of a comprehensive overview of vector-borne diseases in the country. This research provided new insights into pathogen distribution, particularly in northern and southern Namibia, estimating regional infection prevalence and linking it to clinical presentations, thereby guiding appropriate diagnostic methods, and informing on potential zoonotic risks of vector-borne diseases in Namibian cats. Notably, significant associations were found between pathogen infection, tick presence, and disease manifestation. Overall pathogen prevalence rates were reported: 5 % using light microscopy, 42 % in seroprevalence by in-house ELISA, and 27 % with qPCR, highlighting the importance of careful diagnostic choices.

The patient population included an almost equal number of male and female mixed-breed cats, mostly older than one year. While no significant association was found between signalment (sex, breed, age) and pathogen infection, a positive association between FIV seroprevalence and older cats was expected, since FIV typically affects adults who can remain persistently infected for life (Bezerra *et al.*, 2024). The high prevalence of immunosuppressive diseases, such as FeLV, in older cats likely contributed to increased *H. canis* infection rates in this group. Despite the fastidious grooming habits of cats, reducing observed tick presence, only 11 % had adequate ectoparasite control, raising their risk for vector-borne infections. In particular, only 7 % of *H. canis*-infected cats had sufficient ectoparasite control, implying a higher risk of tick exposure and ingestion during grooming. Few cats had a history of anorexia or weight loss, and no significant associations were found between these conditions and *H. canis, E. canis*, FIV, or FeLV. The lack of association between vaccination status and FIV or FeLV may have reflected vaccination practices in Namibia, and vaccination remains a key factor in analysing these pathogens (Little *et al.*, 2020).

Few cats presented with ticks, likely due to their grooming habits, and none were recorded from the Omaheke and Kavango regions. The low number of cats with a history of ectoparasite control, combined with the risk of tick ingestion during grooming, may have contributed to the 26 % *H. canis* infection rate detected by qPCR. No significant association was found between tick presence and *H. canis* infection, likely because tick ingestion is the primary transmission route (Baneth and Allen, 2022). No significant associations were found between haemoparasite species of interest and known related clinical features (Baneth and Allen, 2022; Little, 2010; Simking *et al.*, 2010). However, FeLV was associated with mucous membrane pallor, possibly anaemia-related, as indicated by lower Hct counts (Hartmann, 2012). Cats with FIV/FeLV infections are susceptible to opportunistic infections, such as respiratory diseases (Hartmann, 2012), but no association was found between oculonasal discharge and FIV/FeLV, suggesting limited exposure to respiratory pathogens due to the free-roaming history of these cats. No significant clinical features were associated with *E. canis* infection, while *H. canis*

was positively associated with oculonasal discharge, likely due to FeLV-related immunosuppression (Baneth *et al.*, 1998). Associations between pathogen infection and clinical signs should invariably be interpreted with caution, as undetected pathogens not investigated in this study may have contributed to the observed disease manifestations.

The lack of significant associations between serological results and haematology or serum biochemistry likely reflected the disease state in seropositive cats, since FIV and FeLV can remain asymptomatic for extended periods (Hartmann, 2012). More than half of the seropositive cats from Khomas had reduced Hct counts, indicating possible anaemia due to bone marrow suppression, with no association to reticulocyte counts, since FeLV-related anaemia is typically non-regenerative (Hartmann, 2012). Likewise, many *H. canis*-infected cats also showed decreased Hct, potentially suggesting FeLV-associated anaemia (Baneth and Allen, 2022). A significant positive association with MCHC might have reflected artefactual haemoglobin (Hb) value misestimations. Additionally, the association between eosinophilia and *H. canis* infection indicated potential parasitism or infection. Immunosuppressive diseases, such as FeLV or FIV, often lead to secondary infections, neoplasia, and anaemia (Hartmann, 2012). Accordingly, the connections between *H. canis* infection and haematological/biochemical findings related to underlying immunosuppression should be considered. Associations between tick presence and neutrophilia, monocytosis, and hyperglobulinemia should be interpreted cautiously, since only two tick-carrying cats were from Khomas.

Ehrlichia or *Anaplasma* species were not detected by light microscopy in feline blood smears, suggesting that most rickettsial infections would likely be missed using this method alone, since these inclusions are challenging to identify. However, *E. canis* was detected at a low prevalence (2 %) by qPCR, representing the first confirmed record of *E. canis* in Namibian cats across five regions, likely linked to the widespread presence of *R. sanguineus* s.l. (Horak *et al.*, 2018). This result was consistent with another African study reporting 3 % prevalence (Oliveira *et al.*, 2018), and aligned with low rates < 1 %) in Brazilian cats (Braga *et al.*, 2012; Guimarães *et al.*, 2019). In this case, none of the *E. canis*-positive cats were seropositive for FIV/FeLV, although immunocompromise has been linked to feline infections (Melo *et al.*, 2023).

Erythrocyte inclusions resembling large *Babesia* species were found in 3 % of feline blood smears. This prevalence was similar to prior reports of *B. vogeli* in cats (1 %) (Simking *et al.*, 2010). However, all samples in this study tested negative on qPCR for large canine-associated *Babesia*, including *B. vogeli* and *B. rossi*. Plausibly, these inclusions may therefore represent a known or novel large *Babesia* species in cats (Bosman *et al.*, 2019; Stewart *et al.*, 1980) not tested for molecularly in this study. Although feline *Babesia* species infections have been noted by Namibian practitioners, previous studies did not detect *B. felis* commonly associated with feline babesiosis (Madder *et al.*, 2022). *Babesia felis* infection has, however, been documented in cheetahs from Namibia (Bosman *et al.*, 2007), suggesting that the potential for *B. felis* infection in naturally infected domestic felids cannot be ruled out. Further research is needed to molecularly characterise the large *Babesia*-like inclusions observed on microscopy and determine the cause of feline babesiosis in Namibia.

Hepatozoon species were found in 2 % of cats by light microscopy and 26 % by qPCR, highlighting an increased effectiveness of qPCR in detecting *H. canis*, which was confirmed in cats from seven regions. Low parasitaemia levels on smears, consistent with previous findings (Baneth and Allen, 2022), suggested microscopy alone may miss many *H. canis* cases. This study marked the first confirmed detection of *H. canis* in Namibian cat blood, aligning with prior research showing a 32 % prevalence using molecular methods (Jittapalapong *et al.*, 2006). Arguably, the absence of significant clinical disease associations supported the notion that *H. canis* infections are often sub-clinical (Baneth and Allen, 2022). Co-morbidity with FeLV and FIV was observed, similar to earlier findings (Baneth *et al.*, 1998). Given the presence of potential tick vectors, such as *R. sanguineus* s.l. (Baneth and Allen, 2022; Madder *et al.*, 2022), further research is needed to explore the relationship between feline and canine hepatozoonosis and underlying immunosuppressive conditions in Namibia.

This study represents the first large-scale screening of FIV and FeLV seroprevalence in Namibian domestic cats. The investigation demonstrated low FIV seroprevalence (4 %) across multiple regions, with PCR testing from a related study confirming only four FIV-positive cases (Franzo *et al.*, 2024). Interestingly, the associated PCR-positive samples were seronegative for FIV antibodies, suggesting antibody testing may be unreliable in early or late infection stages. Conversely, seropositive but PCR-negative results could imply low viraemia or an unknown sequence variant. These findings mirrored the low FIV seroprevalence reported in South Africa (Lobetti and Lappin, 2012). In comparison, FeLV seroprevalence (40 %) highlighted the risk of virus spread, because serological tests do not differentiate between progressive and regressive states. This aligned with a previous African study reporting 41 % FeLV prevalence (Muchaamba *et al.*, 2014). Diagnosing progressive FeLV-infected cats is vital, since they can spread the virus while asymptomatic. In addition, since only 5 % of the feline population had a history of being fully vaccinated, it is likely that the FeLV antigen detected did not relate to vaccine reactions but were indicative of active infections. Importantly, a positive association between *H. canis* infection and FeLV seroprevalence was noted, with 22 % of *H. canis*-infected cats being under one year old, lacking age-related resistance to FeLV infection (Hartmann, 2012). The immunosuppressive nature of FeLV likely increased susceptibility to active or re-activated *H. canis* infection (Baneth and Allen, 2022), underscoring the importance of careful diagnostic interpretation.

The overall pathogen prevalence was highest in central regions by qPCR, but lowest by microscopy, reflecting differences potentially due to environmental, host-vector, or socio-economic factors. Overall prevalence trends on microscopy may have been influenced by the large *Babesia* species observed, aligning regionally with *Babesia* species detection. In contrast, overall prevalence trends on molecular analysis aligned with *H. canis* detected on qPCR at regional level. *Hepatozoon* species prevalence showed regional alignment between qPCR and microscopy in the central regions but diverged in the north, where qPCR detected *H. canis*, but microscopy did not, possibly due to the comparative insensitivity of microscopy. Overall, FeLV-driven seroprevalence was elevated in the north and south, likely due to increased disease exposure from limited access to veterinary services in remote areas. High FIV and FeLV seroprevalence in the north suggested immunocompromise in local cats, potentially linked to associated *H. canis* infection on qPCR. The low *E. canis* and *Babesia* species prevalence across several regions indicated these pathogens may be of concern in selected cases.

This study faced several challenges and limitations. Non-probability sampling may have introduced bias, impacting the generalisability of the results. Limited secondary samples and regional disparities affected statistical representativeness and regional interpretations. Metadata collection was challenging, with only two operators collecting data on signalment, history, and clinical presentation. Haematological and biochemical analyses were restricted to Khomas due to logistical restrictions, limiting broader evaluation. Blood smear analysis was focused only on parasite detection, excluding cell morphology, and automated leukocyte and thrombocyte counts were not manually confirmed. Serological testing covered only viral diseases, since funding constraints prevented broader protozoal screenings. Additionally, qPCR testing targeted canine pathogens instead of feline haemoparasites due to budget and logistical restrictions, limiting molecular screening scope. The *Anaplasma* assay used was originally developed as genus-specific but later shown to potentially cross-react with *Ehrlichia* species (Makgabo *et al.*, 2024, 2023). No positive samples were detected with this test, although 2 % of samples were *E. canis* positive, suggesting that the *Anaplasma* assay does not cross-react with *E. canis*. The absence of sequencing to confirm the molecular identity of *H. canis* and *E. canis* in

Namibian cats, as well as exploration of the large *Babesia*-like inclusions observed on microscopy, should be addressed by future research investigations.

This study presents several key recommendations. Practitioners should not rely solely on clinical signs for diagnosing pathogens, since many cases are sub-clinical, making confirmatory pathogen testing essential. The impact of concurrent immunosuppressive diseases must also be considered when diagnosing, treating, and predicting outcomes for vector-borne diseases. Given the prevalence of *R. sanguineus* s.l. in Namibia, diseases associated with this vector should be included in diagnostic evaluations, and cats with ticks should undergo haematological and serum biochemistry screening, even if asymptomatic. In-house diagnoses based solely on presentation and blood smears can lead to misdiagnoses and inappropriate treatments, highlighting broader industry challenges such as emerging pathogens, antimicrobial resistance, zoonotic risks, and limited access to advanced diagnostic techniques, such as qPCR. The authors recommend referring cases for appropriate diagnostic tests, especially when clinical disease manifestations are present. Expanding diagnostic testing across regions and including other reservoirs of vector-borne disease infections such as fleas for analysis, could enhance diagnostic insights in future research.

Diagnosing *Ehrlichia* species infections based solely on clinical signs poses challenges, particularly with the availability of molecular tests, such as qPCR, which support more responsible diagnostic practices. This study found no significant associations between *E. canis* infection and disease manifestation, likely due to its low prevalence and absence in cats from Khomas subjected to further analysis. Given the relevance of canine ehrlichiosis in Namibia, the authors recommend caution in diagnosing and treating *E. canis* infections in cats, emphasising the importance of accurate diagnosis before initiating long-term antimicrobial treatment. Unconfirmed diagnoses risk contributing to antimicrobial resistance, particularly with doxycycline. Meanwhile, the absence of *Anaplasma* species on both microscopy and qPCR suggests it does not significantly contribute to feline disease in Namibia, although further sequence analyses are recommended to explore its relevance. Both veterinarians and pet owners should stay vigilant about the zoonotic risks linked to *E. canis* and *A. platys* infections.

This study lacked sufficient data to draw definitive conclusions about feline babesiosis in Namibia, though large *Babesia*-like inclusions were observed on blood smears. Practitioners should exercise caution when treating symptomatic patients with *Babesia*-like inclusions, because the specific *Babesia* species responsible remains unidentified. Further research is needed to classify these inclusions and inform appropriate treatment, which differs for large and small *Babesia* species. While *H. canis* infection is typically sub-clinical, significant associations with haematology biomarkers were noted, and a CBC is recommended when *H. canis* gamonts are found. Treatment should focus on severe parasitaemia cases, considering antimicrobial stewardship and the risk of relapse in co-infections or immunocompromised cats. The study also found a significant association between *H. canis* infection and FeLV seropositivity, highlighting the need to investigate co-morbid immunosuppressive diseases in cases of *H. canis* infection in Namibian cats. Furthermore, future studies should further explore pathogens commonly associated with cats, including feline *Mycoplasma, Babesia, Hepatozoon*, and *Cytauxzoon* species.

The study findings of low FIV and moderate to high FeLV seroprevalence across Namibia highlighted the presence of these viral pathogens and underscored the need for accurate diagnosis through routine in-house screening and PCR testing, as indicated. Reliable vaccination histories are crucial for interpreting diagnostic results, since FeLV vaccines should only be administered to FeLV-negative cats, and FIV vaccination, which is less accessible in Africa, complicates FIV status interpretation due to vaccine-induced antibodies. Given the high prevalence of FeLV in Namibia, it is advisable that FeLV vaccination be considered a core vaccine for cats. Therefore, this study suggests reviewing FeLV vaccination practices in Namibia and emphasises the importance of FeLV screening in understanding disease risks related to *H. canis* infection. Further investigation into the relationship between feline hepatozoonosis and immunosuppressive diseases, such as FeLV, is recommended.

5. Conclusions

This study addresses a critical gap in the global understanding of neglected, yet economically significant, tropical diseases affecting feline populations in sub-Saharan Africa. Despite the increasing recognition of companion animals as sentinels for zoonotic pathogens, systematic investigations into feline haemoparasites remain extremely limited in many developing regions. This multi-centre prevalence study is the first of its kind to comprehensively assess the distribution of vector-borne pathogens and retroviruses in domestic cats across eight regions of Namibia. By evaluating and comparing three key diagnostic modalities - light microscopy, serology, and qPCR - this research offers a subtle appraisal of their relative effectiveness in resource-variable settings. Moreover, the study provides the first confirmed detection of *E. canis* and *H. canis* in Namibian cats across multiple regions and presents the first large-scale seroprevalence data for FIV and FeLV in the country. The identification of significant associations between pathogen prevalence, tick presence, and clinical disease manifestation adds further weight to the importance of accurate diagnostics in guiding treatment strategies and assessing zoonotic risk. Given the growing interest in global veterinary health, One Health perspectives, and the surveillance of emerging infectious diseases, this study contributes novel and regionally significant data with direct relevance to the international parasitology and veterinary research communities. It provides a foundation for future comparative studies and informs both local veterinary practices and global disease surveillance frameworks.

Funding sources

This work was supported by the Agricultural Sector Education and Training Authority (De Villiers L, no. N02001, 2022–2024); the Health and Welfare Sector Education and Training Authority (De Villiers L, no. A0Y104, 2021); the IDEXX Laboratories South Africa (De Villiers L, 2022); the Faculty of Health Sciences and Veterinary Medicine (De Villiers L, no. 2901, 2022); the University of Pretoria Pathobiology research theme (De Villiers L, no. N18344, 2022); the University of Pretoria postgraduate bursary (De Villiers L, 2021–2023); the South African Veterinary Foundation (De Villiers L, 2022).

Research data

Supplementary research datasets associated with this article can be found at https://doi.org/10.25403/UPresearchdata.28350473.

Declarations

Ethics approval and consent to participate

The study was performed in accordance with the conditions of the Animal Ethics Committees of the Faculty of Veterinary Science, University of Pretoria (no. REC171–21), and the Faculty of Health Sciences and Veterinary Medicine, University of Namibia (no. NEC0001). Exemption to conduct research under Section 20 of the *Animal Disease Act* 35 of 1984 was granted by the Department of Agriculture, Land Reform and Rural Development, South Africa (no. 12/11/12 (2144NC)).

Consent for publication

Not applicable.

CRediT authorship contribution statement

Lourens de Villiers: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Barend L. Penzhorn:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Johan P. Schoeman:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Umberto Molini:** Supervision, Resources, Methodology, Investigation, Conceptualization. **Mari de Villiers:** Resources, Investigation, Data curation. **Charles Byaruhanga:** Methodology. **S. Marcus Makgabo:** Writing – review & editing, Methodology. **Nicola E. Collins:** Writing – review & editing, Methodology. **Samantha Zealand:** Investigation. **Ian J.M. Baines:** Resources. **Wilhelm H. Stoltsz:** Methodology. **Peter N. Thompson:** Formal analysis. **Marinda C. Oosthuizen:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The corresponding author wishes to thank the support staff who contributed to the study through provision of training, and technical and resource support, including Dr G. Nkuekam, Dr K. Du Preez, Ms L. Coetzee, Prof S. Khaiseb, Ms I. Vorster, Ms M. Troskie, Ms F. Nkosi; the administrative staff at Department of Veterinary Tropical Diseases, University of Pretoria; the Mobile Animal Clinic, School of Veterinary Medicine, University of Namibia; all project-associated staff at the Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; and the Faculty of Health Sciences and Veterinary Medicine, University of Namibia, Windhoek, Namibia for the research support.

Data availability

Data will be made available on request.

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