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Exposure of lions (*Panthera leo*) to classical rabies virus and Mokola virus in provincial and private game reserves in Mpumalanga province, South Africa

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This study was undertaken to determine the prevalence of neutralising antibodies to two genetically distant lyssaviruses, classical rabies virus (RABV) and Mokola virus (MOKV), the latter being one of the three rabies-related viruses encountered in South Africa, in 160 banked serum samples originating from lions in private and provincial game reserves adjacent to the Mnisi communal area (n = 20) in Mpumalanga and in the greater Kruger National Park (KNP) (n = 140) of South Africa. The KNP lion serum samples were collected between 1995 and 2000, and the rest much later, between 2010 and 2012. The serum samples were tested for neutralising antibodies to RABV and MOKV using the fluorescent antibody virus neutralisation test. Lion sera collected between 1995 and 2000 (n = 140) had a 2.1% and 0.7% prevalence of RABV and MOKV neutralising antibodies, respectively, whereas sera collected between 2010 and 2012 (n = 20) had prevalence values of 65.0% and 36.8%, respectively. Based on these findings, it can be concluded that lions from KNP have been exposed to both RABV and MOKV at low frequencies (≤ 2.0%) between 1995 and 2000, while the sample sizes of the lions from other game reserves were too small to make a valid conclusion.

Conservation implications: These data suggest that lions, like other wild carnivores, are constantly and naturally exposed to viral pathogens, including lyssaviruses, in their natural habitat. These exposures present a risk of population decline of the endangered lion species, exacerbated by intra-group transmission and disruption of predator–prey dynamics, destabilising ecosystems, including the viability of other endangered wildlife species. Other implications include increased human–lion conflict influencing existing conservation efforts because of heightened fear of lions and the potential for co-infection and viral mutation because of concomitant rabies and/or other virus infections complicating disease prevention and control efforts in lion populations.

Keywords: rabies; Mokola virus; African lion; lyssavirus; South Africa; prevalence.

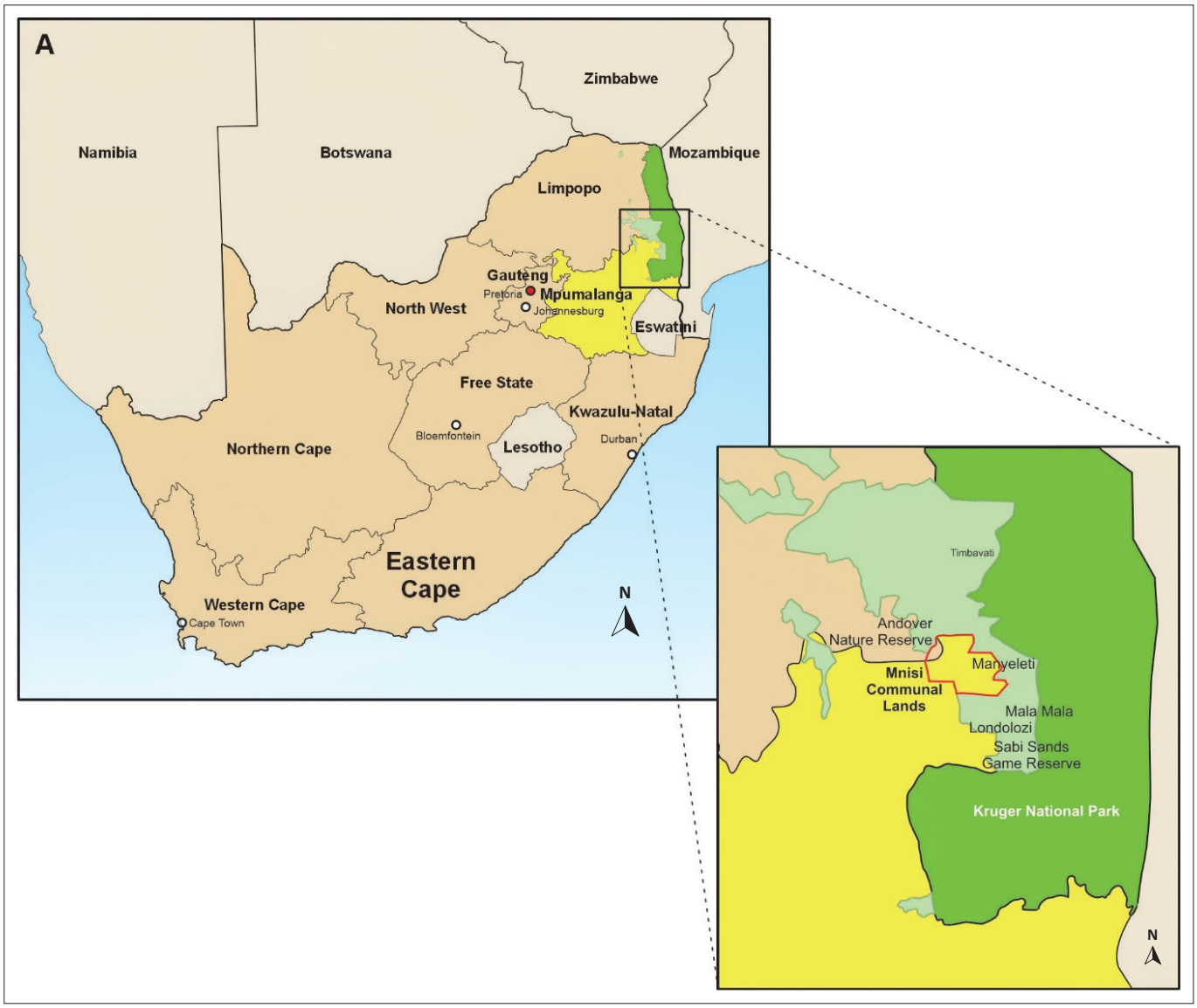
Introduction

According to Bauer et al. (2015), the African lion, *Panthera leo*, is in danger of extinction throughout the greater portion of its host range because of rapid declines in numbers associated with numerous threats, except in four Southern African countries (Botswana, Namibia, South Africa and Zimbabwe). Since 2014, there have been fewer than 40 000 African lions worldwide, with fewer than 50% of these in Africa (Murray 2023). Because of this, Bauer et al. (2015) recommended separate regional assessments of the African lion in the World Conservation Union to ensure correct listing and that protective measures be taken. Bauer et al. (2015) also noted a significant decrease in African lion populations by approximately 43% between 1993 and 2014. The current lion population inhabits approximately 22.0% of their historic range, making some of these populations prone to extinction. The primary threats to this *Felidae* include habitat and prey loss, retaliatory killings resulting from human–lion conflicts, zoonotic diseases including rabies and unsustainable off-take for international trade in lions and lion parts (Born Free USA et al. 2011; Murray 2023).

A 2005/2006 census undertaken by Wray (2012) indicated that the Kruger National Park (KNP) in Mpumalanga province, South Africa, has approximately 2000 African lions. This

is largely because of conservation efforts by the KNP staff and the modifications made within the park to make the environment more conducive for the lion population. However, since 2008, amongst other diseases, there has been a re-emergence of rabies in dogs in rural communities in eastern Mpumalanga, along the western border of the KNP (see Figure 1). A large outbreak of rabies occurred in domestic dogs during the same year, with a spillover into the southern parts of the KNP (Reininghaus 2009). Given the vulnerability of lion populations and their susceptibility to fatal rabies virus infection, proactive risk assessment and management are essential. These measures would help to prevent re-emergence and cross-species transmission within the KNP, surrounding reserves in Eastern Mpumalanga and the broader provincial landscape.

Globally, there are 18 currently recognised lyssavirus species worldwide (International Committee on the Taxonomy of Viruses [ICTV] 2026), More recently, two related and recently discovered tentative species, Taiwan bat lyssavirus (TBLV) and Kotalathi bat lyssavirus (KBLV), were isolated from bats (ICTV 2026; Kokkonen & Gadd 2018; Tu et al. 2018). There are four commonly encountered lyssaviruses in South Africa, namely, classical rabies virus (RABV) and three unusual lyssaviruses (also referred to as rabies-related viruses) – Mokola lyssavirus (MOKV), Duvenhage lyssavirus (DUVV) and Lagos bat lyssavirus (LBV). Within classical RABV, two rabies biotypes, the canid and mongoose biotypes, have been identified in *Carnivora* and *Herpestidae* species, respectively (Swanepoel et al. 1993). In the context of the rabies-related viruses, MOKV was previously identified in a domestic dog (*Canis lupus familiaris*) from Nkomazi (Mpumalanga) and the majority from domestic cats (*Felis catus*) from KwaZulu-Natal and Eastern Cape (Coertse et al. 2017; Sabeta et al. 2007). The aim of this study was to determine the seroprevalence of RABV and MOKV in lions inhabiting privated and provincial game reserves in Mpumalanga, as well as in the greater Kruger National Park.



Note: This images was created for this publication by the University of Pretoria, Onderstepoort Campus, IT Services Department October 2025.

FIGURE 1: Private and provincial game reserves in Eastern Mpumalanga bordering the western Kruger National Park and areas adjacent to the Mnisi Tribal Authority.

Research methods and design

Materials and methods

Using a cross-sectional study design, banked serum samples (n = 160) from lions in the private/provincial reserves adjacent to the Mnisi communal area (n = 20) and the KNP (n = 140) were included in this study (Figure 1). Lion serum samples were obtained from both the ARC-OVI (a global reference laboratory for rabies) and the University of Pretoria (Department of Tropical Veterinary Diseases) serum banks. Samples sourced from the University of Pretoria originated from a bovine tuberculosis study conducted in lions within the KNP between 1996 and 2000. In contrast, samples obtained from ARC-OVI were collected from lions inhabiting private and public game reserves bordering the KNP between 2010 and 2012. Neither dataset was stratified by age and sex. The serum samples were assessed for neutralising antibodies using Challenge Virus Standard (CVS), a rabies virus laboratory strain (previously ATCC reference VR 959) and Mokola virus (MOKV) (ARC-OVI laboratory reference number 173/06), previously grown on neuroblastoma cells (CCL-131). Neutralisation levels to both RABV and MOKV of each serum sample were assessed using the method as described by Cliquet, Aubert and Sagne (1998), with slight modifications.

Experimental procedures

Virus neutralisation tests were undertaken using the fluorescent antibody virus neutralisation test as described in the World Organisation for Animal Health (WOAH) Manual of Diagnostic Tests and Vaccines (2018). In brief, threefold serial dilutions of test sera in parallel with an international positive dog reference serum (calibrated to 0.5 IU/mL) were performed. Then, a constant dose of previously titrated laboratory strain CVS or a MOKV field strain was added (in the case of neutralising antibodies against Mokola virus) at approximately 10^2 $TCID_{50}$/mL, and after 1 h of neutralisation, susceptible Baby Hamster Kidney (BHK) cells were added to detect virus infection in the cells by direct fluorescent antibody staining of rabies virus-infected cells.

Observations and/or analytical procedures

The antibody titres were calculated using the Spearman–Karber method (Aubert 1982) and expressed as reciprocals of the dilutions that neutralised 50% of the challenge virus. The antibody titres of the test sera were finally expressed in international units (IU/mL) by comparison to a standard reference control serum with known IU under the same experimental conditions (OIE serum of canine origin).

Statistical analysis by means of the Spearman–Karber method (Ogawa et al. 2008) of the fluorescent antibody virus neutralisation antibody test (FAVNT) results was done to determine the level of exposure of the wildlife carnivores to the two lyssaviruses (RABV and MOKV) and to check if these exposure levels were significantly different. The titre of each serum (test and controls) and the CVS titration were calculated using the Spearman–Kärber formula.

Ethical considerations

Ethical clearance to conduct this study was obtained from the Research Committee of the Faculty of Veterinary Science, University of Pretoria (No. PROTOCOL V054/12).

Results and observations

Of the sera originating from the KNP (n = 140), 2.1% were positive for rabies neutralising antibodies, while 65.0% (n = 20) of the sera from lions from reserves adjacent to the Mnisi study area were positive for rabies antibodies.

Approximately 0.7% of the KNP lions had neutralising antibodies to MOKV, while 36.8% (n = 19) from reserves adjacent to the Mnisi communal area were positive for MOKV antibodies using one in nine dilution as the cut-off value for seroconversion (Table 2). The apparent and true prevalences were, however, found to be exactly the same.

TABLE 1: Summary of results for the neutralisation assays using classical rabies virus as the challenge virus in the fluorescent antibody virus neutralisation antibody test.

Group of animals	Nr negative (Titre < 0.5 IU/mL)	Nr positive (Titre ≥ 0.5 IU/mL)		Total
		Number of samples	%	
Kruger National Park lions	138	3	2.1	**141**
Lions from reserves adjacent to Mnisi communal area	7	13	65.0	**20**
Total	**145**	**16**	**9.9**	**161**

Source: Letsholo, S.L., 2014, *Exposure of wild carnivores to rabies and Mokola viruses in provincial and private game reserves in Mpumalanga province*, unpublished thesis, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

TABLE 2: Summary of test results obtained for the Mokola virus fluorescent antibody virus neutralisation antibody test.

Group of animals	Nr negative (Titre < 0.95 log_{10} dilution)	Nr positive (Titre ≥ 0.95 log_{10} dilution)		Total
		Number of samples	%	
Kruger National Park lions	138	1	0.7	**139**
Lions from reserves adjacent to Mnisi communal area	12	7	36.8	**19**
Total	**150**	**8**	**5.1**	**158**

Source: Letsholo, S.L., 2014, *Exposure of wild carnivores to rabies and Mokola viruses in provincial and private game reserves in Mpumalanga province*, unpublished thesis, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

TABLE 3: Table showing serum samples positive for both classical rabies and Mokola virus (cut-off point 0.50 IU/mL and 0.95 log_{10} dilution, respectively).

Item Nr	Lab Nr	Identity Nr	FAVNT titre in Log_{10} dilution (RABV)	FAVNT titre in Log_{10} dilution (MOKV)
1.	143	S748/12	0.95	0.95
2.	151	S1219/11	0.95	1.43
3.	152	S1532/11	1.43	1.43
4.	154	S1957/11	0.95	1.43
5.	157	S2084/11	0.95	1.43

Source: Letsholo, S.L., 2014, *Exposure of wild carnivores to rabies and Mokola viruses in provincial and private game reserves in Mpumalanga province*, unpublished thesis, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

FAVNT, fluorescent antibody virus neutralisation antibody test.

Five serum samples (Table 3) were positive for neutralising antibodies to both classical RABV and MOKV. All five sera were from animals in the reserves adjacent to the Mnisi communal area collected in 2011, except for a single serum sample (S748/12) that was collected in 2012. Specific details of these sera are included in Table 3.

Discussion

The results of this study demonstrated low levels of RABV and MOKV antibodies in lions that populated KNP between 1995 and 2000 (2.1% and 0.7%, respectively) in contrast to the levels of RABV and MOKV antibodies (65.0% and 36.8%, respectively) in lions from private reserves (Table 1; Table 2). The high levels of RABV neutralising antibodies in sera collected between 2010 and 2012 may be because the animals had been previously vaccinated against RABV (in response to the 2008 rabies outbreak). Prior to this outbreak, only a single case of rabies was confirmed in a lion between 1928 and 2006 in South Africa (Bishop et al. 2010). About 50% of serum samples from the study sample population were excluded from analyses because they had previously been vaccinated against RABV.

The amendment of the *Animal Diseases Act No. 35 of 1984* in South Africa (in 2008) facilitated the vaccination of wild carnivore species, particularly jackals, bat-eared foxes, lions, mongooses, leopards, cheetahs and hyenas, which is mandatory as preparedness towards rabies outbreaks in this region. The regulations of this *Act* also made vaccination of wild cats and dogs mandatory for RABV as a prerequisite for animal relocation to reserves/parks/zoos within and even outside South Africa as well as for breeding (Bishop et al. 2010). Vaccination of pets against rabies is a requirement and facilitates international movement of pets to the European Union and other global destinations (Council decision 2000/258/EC version 3 of 03 September 2008), designating a specific institute responsible for establishing the criteria necessary for standardising the serological tests to monitor the effectiveness of rabies vaccines (2000/258/EC). This resulted in the vaccination of wild carnivores against RABV, thereby providing an explanation for the increase in the seroprevalence of circulating antibodies to RABV in these carnivore populations and simultaneously reducing the capacity of these populations to maintain RABV within their populations. However, this does not explain the high levels of seroprevalence of MOKV in animals collected from private and provincial reserves between 2010 and 2012. Cross-neutralisation is known to occur between members within a phylogroup, but not across phylogroups (Badrane et al. 2001; Markotter 2007). The possibility that some of the neutralising antibodies to MOKV could in fact be because of exposure to other rabies-related viruses such as Lagos bat lyssavirus (LBV) or Shimoni bat lyssavirus (SHIBV) cannot be ruled out. Mokola virus, SHIBV and LBV exclusively belong to phylogroup II; hence, antibodies raised against LBV/SHIBV may cross-neutralise antibodies against MOKV (Badrane et al. 2001; Markotter 2007; Wright et al. 2010).

In nature, wild carnivores and other animal species are exposed to a plethora of bacterial, viral and parasitic agents. Hence, neutralising antibodies to RABV could have resulted from exposures to other lyssaviruses within phylogroup I, such as DUVV or the mongoose RABV. Considering the epidemiology of rabies in South Africa, it is possible that rabies antibodies of mongoose origin could be encountered here, because host species that maintain the mongoose rabies biotype are also found in this province (Malan et al. 2024; Markotter et al. 2006a; Nel et al. 2005; Van Zyl, Markotter & Nel 2010). Exposure of lions to fruit-eating bats at night or during hunting may partly explain the presence of antibodies that cross-neutralise sera from members within and across phylogroups. South African fruit bats, *Epomophorus wahlbergi*, are maintenance host species of LBV (Markotter et al. 2006c), and spillover events to terrestrial mammals are possible.

The high levels of antibodies to MOKV could be explained by the apparently reduced pathogenicity of this lyssavirus, which, on exposure, elicits an antibody response without any observable clinical signs in the host. However, no evidence of such has been noted in literature except in the controversial case of a 3.5-year-old girl in Nigeria in 1968 (Familusi & Moore 1972).

The increase in the seroprevalence of RABV from 2.1% in lions between 1995 and 2000 to 65.0% between 2010 and 2012 may have resulted from mass wildlife vaccination programmes in response to large numbers of unvaccinated dogs in the area. However, MOKV has rarely been isolated from terrestrial animals (Evans et al. 2012; Paweska et al. 2006; Von Teichman et al. 1998), making the high seroprevalence of MOKV in the study sample very difficult to explain.

In general, rabies is transmitted to a susceptible host primarily through the infectious saliva of rabid animals (Bishop et al. 2010). The positive lion sera collected between 2010 and 2012 could have resulted from natural exposures. However, the clinical history provided on the submission forms does not in any way suggest that any of the lions sampled during this time displayed clinical signs compatible with rabies. Ramsden and Johnston (1975) argued that even though ingestion of disease-ridden carcasses can result in lethal infections, as was suspected to be the case with four lions that succumbed to rabies during the Namibian Kudu rabies outbreak (Mansfield et al. 2006), it is possible that some of the exposed animals will only trigger the humoral immune response. It is conceivable that a humoral immune response in the absence of clinical disease may have occured in the large number of seropositive lions sampled between 2010 and 2012, although this is highly unlikely. It is also important to note that as rarely documentated as rabies is in lions including a single case recorded in country between 1928 and 2006 (Bishop et al. 2010), and an additiona four in Namibia (Swanepoel et al. 1993) it was invariably fatal.

In our study, and similar to several studies undertaken by several groups (Almeida et al. 2001; Deem, Davis & Pacheco 2004; Rossouw, Boshoff, Sabeta & Kotzé 2021), the cut-off

point for adequate seroconversion was taken as 0.50 IU/mL, a cut-off value presently recommended by the WOAH and the WHO for the evaluation of animal and human responses to rabies vaccination, but not for natural infection (Jorge et al. 2010). For natural infections, any serological titre above 0.10 IU/mL is considered positive (Jorge et al. 2010). The challenge with this cut-off value, though, is that there are possible non-specific reactions that can occur between 0.10 IU/mL and 0.50 IU/mL (Jorge et al. 2010). Because the cut-off value in this study was taken as 0.50 IU/mL, it must be accepted that some of the samples that had antibodies within this range (>0.10 IU/mL < 0.50 IU/mL) may have been because of natural infections instead of being non-specific reactors (32 such serum samples were found for RABV and six for MOKV). If these cases were considered as positive, the prevalence of rabies antibodies in the study sample in the KNP could rise significantly from 2.1% to 16.3% (n = 140). Using the same argument, the prevalence of MOKV antibodies in the lion population from other reserves would increase from 36.8% to 57.9% (n = 19), considering that the population size of the animals from reserves other than KNP was too small to make any statistically valid conclusions. The prevalence of RABV antibodies in the KNP lions (2.1%) was significantly lower than the 12.3% antibody prevalence that was found in several wild carnivore species in a study involving free-range wild carnivores captured between 2000 and 2006 in Brazilian biomes (Pantanal and Cerrado) by Jorge et al. (2010). Both studies were based on different cut-off values, that is, ≥0.5 IU/mL for this study and ≥0.10 IU/mL for the study by Jorge et al. (2010).

A high level (37.0%) of asymptomatic spotted hyenas with rabies-specific virus-neutralising antibodies was observed in a study in the Serengeti (East et al. 2001). On average, these hyenas lived for 4 years post-sero-assessment, with some of them secreting rabies in their saliva intermittently. On the contrary, none of the serum samples from the Orpen Veterinary Clinic were from animals displaying clinical signs typical of rabies, at least from the accompanying submission forms. However, the levels of rabies-specific virus-neutralising antibodies in the lion population detected in this study (36.8%) were equally as high as those for spotted hyenas in the Serengeti (East et al. 2001). The Serengeti hyenas were also shown to have a substantially different RABV isolate (Sp. *Hyena-X622*, Sp. *Hyena-X518*, Sp. *Hyena-Saliva-S83*, Sp. *Hyena-Saliva-A540*, Sp. *Hyena-Saliva-A503*, Sp. *Hyena-Saliva-S226*, Sp. *Hyena-Saliva-S186* and Sp. *Hyena-X542* isolated sequences) from other canid and viverrid rabies virus isolates obtained in the Serengeti (8.5% sequence divergence on the N-P gene) (East et al. 2001); thus, this may have been a unique case of a less pathogenic variant of RABV.

Concurrent seropositivity to both RABV and MOKV is paradoxical given the absence of clinical rabies signs and documented vaccination history among the sampled animals. This pattern may reflect prior exposure without progression to disease, possibly because of undocumented rabies vaccination. However, this does not account for the presence of MOKV-specific antibodies, which may instead indicate low-dose exposure or species-specific immune modulation, as previously observed by East et al. (2001). Although lions are apex predators, they exhibit opportunistic scavenging behaviour (Amorós et al. 2020), potentially ingesting rotting carcasses of rabid animals with declining viral titres. Such exposure may result in abortive infections that elicit an immune response without clinical manifestation, though unlikely. The findings also raise the possibility of co-circulation or sequential exposure to multiple lyssaviruses within the same phylogroup, although serological cross-reactivity cannot be excluded without further confirmatory diagnostics.

Conclusion

In conclusion, the markedly low RABV and MOKV seroprevalence observed in the KNP lions during 1995–2000 likely reflects limited natural exposure to classical rabies RABV and MOKV strains. In contrast, elevated antibody levels in private reserves post-2008 suggest the influence of vaccination campaigns and possible exposure to attenuated or cross-reactive lyssaviruses. While this study focused on terrestrial lyssaviruses, future investigations incorporating bat-associated lyssavirus exposure, such as those in Markotter, Monadjem and Nel (2013), may yield deeper insights into viral ecology and host–pathogen interactions. Standardisation and validation of hyperimmune sera for LBV, MOKV and related viruses at the ARC-OVI WOAH Rabies Reference Laboratory would significantly enhance diagnostic capacity and facilitate the development of robust serological assays. From a One Health perspective, these findings warrant further investigation into lyssavirus dynamics at the wildlife–livestock–human interface, particularly in endemic zones such as the Mnisi communal area, where the risk of zoonotic spillover remains considerable.

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unpublished and not publicly available. The thesis was supervised by Moritz van Vuuren, Claude Taurai Sabeta and Bjorn Reininghaus. The thesis was reworked, revised and adapted into a journal article for publication. The author confirms that the content has not been previously published or disseminated and complies with ethical standards for original publication.

Competing interests

The authors reported that they received funding from the Research Committee of the Faculty of Veterinary Science, University of Pretoria, Belgian Scholarship Grant and Onderstepoort Veterinary Institute Rabies Department and Botswana Government, Department of Veterinary Science, which may be affected by the research reported in the enclosed publication. The authors have disclosed those interests fully and have implemented an approved plan for managing any potential conflicts arising from their involvement. The terms of these funding arrangements have been reviewed and approved by the affiliated university in accordance with its policy on objectivity in research.

CRediT authorship contribution

Samantha L. Letsholo: Conceptualisation, Methodology, Formal analysis, Investigation, Writing – original draft, Visualisation, Project administration, Data curation, Writing – review & editing. Claude T. Sabeta: Conceptualisation, Methodology, Formal analysis, Investigation, Project administration, Validation, Data curation, Resources, Writing – review & editing, Supervision, Funding acquisition. Moritz van Vuuren: Conceptualisation, Methodology, Formal analysis, Investigation, Resources, Writing – review & editing. Bjorn Reininghaus: Conceptualisation, Methodology, Formal analysis, Investigation, Resources, Writing – review & editing. All authors reviewed the article, contributed to the discussion of results, approved the final version for submission and publication, and take responsibility for the integrity of its findings.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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