

VET

September 2025

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The Monthly Magazine of the SOUTH AFRICAN VETERINARY ASSOCIATION
Die Maandblad van die SUID-AFRIKAANSE VETERINÊRE VERENIGING



THEME

Rabies and Rhinos

CPD

Gastrointestinal disorders of backyard poultry –
Part 2 of 2



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September 2025



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Southern Cape Branch Congress

31 October – 01 November
Venue: Oubaai Hotel Golf & Spa, George
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Dear members, To Progress!

September marks Rabies Month in South Africa, and on the 26th, we join the global community in observing World Rabies Day. Much progress has been made in implementing the National Rabies Strategy, and it is encouraging to note that the South African Veterinary Association, through SAVA-CVC, continues to play a leading role in these efforts. The Battersea Project, primarily in Nelson Mandela Bay, has already enabled approximately 160,000 dog vaccinations since March 2024. The project has also been extended to the Free State and Limpopo provinces. This remarkable achievement is moving South Africa steadily towards the “Zero by 30” target: the elimination of canine-mediated human rabies by the year 2030.

Through collaboration with state veterinary services and community veterinary clinics, the project not only provides mass vaccinations but also strengthens community awareness, facilitates outbreak investigations, and supports access to post-exposure prophylaxis. Together, these efforts bring us closer to the goal of achieving at least 70% vaccination coverage and reducing dog populations responsibly. Let us all, whether in state service or private practice, continue to play our part in securing a human-rabies-free future.

This past month, I had the privilege of attending Faculty Day at the University of Pretoria, where research presentations reflected both high scientific quality and direct relevance to the profession. The calibre of undergraduate presentations was particularly inspiring, demonstrating not only technical skill but also confidence and clarity in communication. A presentation on the role of veterinary telemedicine, especially in rural practice, resonated deeply as it addressed an urgent and practical challenge for our profession.

I also had the pleasure of attending the SASVEPM Congress 2025, where SAVA's collaboration with SASVEPM, particularly through training linked to the HWSETA programme, was reinforced. I extend my sincere thanks to SASVEPM for their hospitality and professionalism.

The recently released report from the Stakeholder Engagement Meeting on Antimicrobial Resistance and Use held in July 2025 highlights several important issues that require SAVA's continued contribution. The National Department of Agriculture emphasised the need to work towards prohibiting the use of growth promoters, not only to align with international trade requirements but also to safeguard local food safety. Many countries and regions have already banned or restricted the use of antibiotics as growth promoters and may impose import restrictions on animal products originating from countries that do not comply.

The meeting further highlighted gaps in surveillance on antimicrobial resistance (AMR) and antimicrobial usage (AMU), noting their impact on compliance with international standards. Concern was raised regarding the continued availability of over-the-counter antimicrobials, which remain a barrier to establishing harmonised veterinary oversight and limit access to high-value global markets. Engagement on this matter will continue, and SAVA remains committed to contributing constructively to national and international discussions.

Looking ahead, an important event for the profession is the upcoming SSC Government-Industry Collaboration Workshop (3–4 September 2025), a joint initiative between South Africa and Denmark. This meeting will provide an opportunity to share experiences in strengthening collaboration between the public and private sectors, with a focus on antimicrobial resistance, biosecurity, and controlled disease outbreak management.



Further to this, as an association, we must remain mindful of the Animal Health Act and prepare to contribute meaningfully to the forthcoming public consultation process on its regulations. We trust that the National Department of Agriculture will provide adequate opportunity for stakeholder engagement, and in the meantime, I encourage all colleagues to re-familiarise themselves with the provisions of the Act.

In October, we look forward to participating in the Agri-SA Atlas at the Future of Agriculture Congress. It promises to be an essential integrative experience, considering the impacts of factors such as climate change on agriculture and a possible way forward. Despite these positive developments, our profession continues to carry the weight of the ongoing absence of a functioning Veterinary Council. This gap has far-reaching implications for governance, oversight, and the regulation of veterinary practice in South Africa.

I urge members to remain patient and to allow due process to unfold, as the South African Veterinary Council (SAVC) and the Minister's office work towards a resolution in the best interests of the profession. I also encourage colleagues to rely only on credible and verified information regarding this matter. SAVA will ensure that all official updates and communications are promptly shared with members.

At the same time, SAVA remains steadfast in its commitment to seeking constructive solutions. We have formally expressed our willingness to assist in facilitating a resolution to this critical issue, and we will continue to engage with all relevant stakeholders to help safeguard the integrity and stability of the profession.

Finally, I wish to acknowledge V-Tech for their generous sponsorship of my attendance at the World Veterinary Congress in Washington. I extend particular thanks to Dr. Oosthuysen and his team, who continue to provide invaluable support to SAVA, especially in stakeholder communication. It is my sincere hope that this partnership will continue to grow from strength to strength and expand into other areas of collaboration.

Colleagues, as we embrace Rabies Month and reflect on our shared achievements, let us also look forward with optimism. May September bring new beginnings—for our profession, for our institutions, and for the communities we serve. **V**

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Editor's notes / Redakteurs notas

In our district, Mopani, Limpopo, I see that rabies vaccination campaigns have already started, and they are being brought to the people. A great initiative to curb this awful disease.

The National Rabies Advisory Group has earmarked the following dates for Rabies updates. Keep an eye out for them and use them in your practice and with your clients:

- 1 Sept – The Start of the program
- 11 Sept – 17 Days to go the celebrate #WorldRabiesDay2025
- 23 Sept – Awareness on which animals can get Rabies
- 24 Sept – The vaccination
- 25 Sept – Areas of Rabies in South Africa
- 26 Sept – What must be done if a person is exposed
- 28 Sept – 19th #WorldRabiesDay!

'September is the ninth month of the Gregorian calendar, has 30 days, and marks the beginning of autumn in the Northern Hemisphere and spring in the Southern Hemisphere. Its name comes from the Latin word for "seven," as it was originally the seventh month in the ancient Roman calendar. People born in September are either Virgos or Libras, and the month's birthstone is sapphire.'

All this may be very true, but September is also a bit sombre as it is the month we focus on Rabies. When I spoke to Dr Didi Claassen about anything new and exciting in the Rabies world in South Africa, she responded that there is nothing new in Rabies. It has been around for so long that we may have exhausted the topic. Yet there is good news. The province of Mpumalanga has managed to curb the animal-human transmission. They have not reported a single case in the foreseeable past. Definitely a step in the right direction toward the goal of a rabies-free world in 2030.

Vetnews also focuses on Rhinos as it is also World Rhino Day, celebrated on September 22, which is for celebrating rhinos and debunking the myth that rhino horn has curative properties.

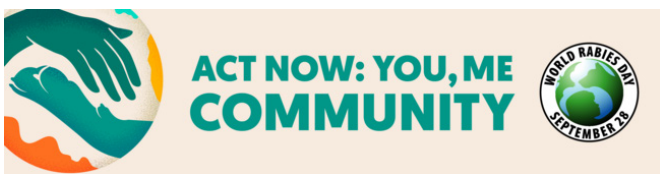
WORLD RHINO DAY 22 September Celebrating the FIVE species of rhinoceros



We apologise for the mistake with the birth date of Dr Bruce Fivaz. His birth year is 1946 and not 1940 as indicated.

Happy Spring Day!!

Andriette



This year on September 28, we are calling on you, me, and our communities to make a difference now! For the first time in its 19-year history, 2025's theme does not include the word "rabies", showing how well-established this movement has become. Whether you are an individual, part of an organization, or a decision-maker, the time to act is today.

- **You** – **Take action in your personal life:** vaccinate your dog, educate yourself about how to prevent rabies and Pre- and Post-Exposure Prophylaxis, or advocate for better policies.
- **Me** – **Lead by example:** inspire others, train professionals, or support rabies elimination efforts in your community.
- **Community** – **Work together:** organize vaccination campaigns, educate learners and their families, and push for stronger rabies elimination programs.

The Global Alliance for Rabies records all events taking place in the world, and it has been reported that South Africa hosts the most events per year. If you are hosting an event, go ahead and log it on their website. <https://rabiesalliance.org/world-rabies-day>

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Insights into the occurrence of rabies viruses in multi-species animals based on diagnostic laboratory submissions

Aurelle Yondo,¹ Ben Enyetornye,¹ Binu T. Velayudhan¹

Editor Hyun Jin Kwun, Pennsylvania State College of Medicine, Hershey, Pennsylvania, USA

ABSTRACT

Rabies is a fatal zoonotic disease caused by the rabies virus (RABV), primarily affecting the central nervous system of mammals. Understanding the epidemiology of animal rabies is critical for developing effective prevention and control strategies. This study aimed to analyse animal rabies cases received at a veterinary diagnostic laboratory in Georgia, USA, over 5 years (2019–2023), focusing on the most commonly infected species, seasonality trends, and geographical distributions. A total of 1,560 rabies-suspect cases, representing 21 species of animals, were tested using a direct fluorescent antibody test (DFAT). Of 1,560 cases, 5.6% (88/1560) were positive across 11 species, with domestic animals accounting for 17% (15/88) of rabies cases, whereas wildlife species exhibited a higher occurrence of 83% (73/88). Among wildlife, the affected species were raccoons (35.2% [31/88]), skunks (25% [22/88]), white-tailed deer (8% [7/88]), foxes (6.8% [6/88]), bats (4.5% [4/88]), bobcats (2.3% [2/88]), and great kudu (1.1% [1/88]). In domestic animals, the affected species included bovine (6.8% [6/88]), feline (5.7% [5/88]), caprine (2.3% [2/88]), and equine (2.3% [2/88]). Positive cases were predominantly detected in submissions from Georgia, with a few additional cases identified in neighbouring states and unknown locations. Furthermore, fall, spring, and summer seasons showed high infection rates compared with winter. Our findings highlight distinct seasonal trends and the significant burden of rabies among wildlife in the Southeastern United States.

IMPORTANCE

Rabies is a fatal zoonotic viral disease that affects the central nervous system of mammals, including humans. It is transmitted mainly through bites or scratches by infected animals such as dogs, bats, raccoons, and other wild animals. The present study analysed data on clinical specimens submitted to a veterinary diagnostic laboratory for the detection of rabies in domestic and wild animals for a period of 5 years. The study examined a total of 1,560 rabies-suspect cases, representing 21 species of animals tested using the standard direct fluorescent antibody (DFA) assay. Out of 1,560 cases, 5.6% were positive across 11 species, with domestic animals accounting for 17% and wild animals accounting for 83% of the total cases. Different species of wild animals showed a significantly higher incidence of rabies, highlighting the importance of wildlife in spreading rabies to domestic animals and the threat it poses to public health.

Rabies is a life-threatening, progressive neurologic viral disease transmitted via the saliva of infected animals, usually through bites or scratches (1–3). It is caused by a bullet-shaped, single-stranded, non-segmented, negative-sense RNA virus belonging to the genus *Lyssavirus* and the *Rhabdoviridae* family (4). The rabies virus (RABV) primarily targets the central nervous system of humans and animals, leading to encephalitis with fatal symptoms, including hyperexcitability, autonomic dysfunction, hydrophobia, and aerophobia after an average incubation period of 20–90 days (3, 5, 6). There are rare cases with longer incubation periods, extending up to years, depending on factors such as exposure site, viral load, and host immune response (7, 8). It is a multiple-host pathogen that affects all warm-blooded animals, but dogs and wildlife serve as significant reservoirs for the virus (9, 10). Rabies represents a significant public health threat on every continent except Antarctica (11), with an estimated 60,000 human cases reported annually (12, 13). Although the global burden of rabies seems to have declined over the past three decades, the disease remains a persistent problem for many countries, including developed nations (14). In wildlife, the rabies virus continues to circulate, frequently exposing unvaccinated domestic animals, especially dogs, making control incredibly challenging (15), underscoring the 2030 dog-mediated rabies elimination goals (16).

In the USA, approximately 4,000 animal rabies cases are reported annually, with over 90% occurring in wildlife such as skunks, bats, raccoons, and foxes (17). In 2020, 4,090 wildlife and 389 domestic animals tested positive for rabies in the country (18). Human rabies cases in the Americas and Caribbean have been linked to sporadic spillover from wildlife, as widespread preventive measures, such as vaccination, have been implemented for companion animals (19, 20). Each year, more than 4 million Americans report animal bites, with approximately 800,000 seeking medical attention

(17). Humans exposed to rabies-positive animals often face long quarantine periods and post-exposure prophylaxis (PEP), causing discomfort and financial burdens to many families (21). Moreover, PEP is expensive and associated with adverse reactions (22). The estimated annual direct and indirect costs of PEP are \$1.7 billion and \$1.3 billion, respectively (23). This suggests that improving rabies control in wildlife through oral vaccination programs, combined with routine vaccination of companion animals and livestock (24, 25) at a lower cost, could alleviate the burden on animal owners.

Given these challenges, constantly updating the epidemiological trends of animal rabies cases submitted to veterinary diagnostic laboratories is crucial to guide the structuring and implementation of preventive and control measures in animals and provide insights

into human exposures to the disease. However, there is limited information on rabies surveillance data in the southeastern United States. This study analysed animal rabies cases submitted to the Athens Veterinary Diagnostic Laboratory (AVDL, University of Georgia, Athens, GA, USA) from 2019 to 2023. We focused on identifying any patterns in rabies cases, such as the occurrence among wild and domestic species, analysing seasonal trends, and mapping the geographical distribution of positive cases. Our findings provide useful insights for long-term policy decisions and improving rabies prevention and control strategies.

MATERIALS AND METHODS

Data collection and analysis

We queried the Athens Veterinary Diagnostic Laboratory (AVDL) database using the Laboratory Information Management System called VetView to retrieve 1,560 rabies-suspect cases submitted to the laboratory from 2019 to 2023. Tissue samples were submitted by various clients from within and outside Georgia, including the Southeastern Cooperative Wildlife Disease Study (SCWDS). Those specimens were tested using the DFAT, and the data were collected in an Excel spreadsheet. Each case was individually reviewed to capture details, including accession number, received date, specimen type, species, geographic locations, seasonality, and diagnostic outcomes. Cases were categorised as positive and negative based on DFAT results. We categorised the positive cases by species, and seasonal trends were analysed by grouping the data into four seasons (winter, spring, summer, and fall). We examined temporal trends over the 5-year period to identify significant patterns in rabies occurrence. Positive cases were mapped for geographic distribution and further classified into cases originating from wildlife and domestic animals for comparative analysis.

Direct fluorescent antibody test

According to the World Health Organisation (WHO), the DFAT is considered the gold standard for rabies testing, designed to detect the presence of rabies virus (RABV) antigens in brain tissue (26). The rabies testing procedure was performed following the Centres for Disease Control and Prevention guidelines and standards (27). Brain tissue samples were collected and sectioned to include identifiable areas of the right and left lateral lobes of the cerebellum, the vermis, and the brainstem. Tissue impressions were prepared on clean glass microscope slides. These slides were then air-dried and fixed in cold acetone (-20°C) for at least an hour to preserve antigen integrity. After the fixation period, the slides were stained with three separate conjugates, including the EMD Millipore Corporation 5100 Light Diagnostics Rabies DFA Reagent (EMD Millipore Corp, Temecula, CA), Fujirebio Diagnostics Inc FITC Anti-Rabies Monoclonal Globulin (FDI,

Malvern, PA), and the Millipore Light Diagnostics Rabies Negative Control, Monoclonal Antibody FITC Conjugate 5102 (EMD Millipore Corp, Temecula, CA) conjugates. Stained slides were incubated in the humid chamber for 30 min, allowing sufficient time for antibody-antigen binding. After incubation, slides were rinsed with rabbit phosphate-buffered saline (PBS) to remove unbound antibodies and examined under a fluorescent microscope's FITC filter. The interpretation of slides was based on fluorescence intensity and antigen distribution. Positive rabies impression smears showed a bright apple-green fluorescence in rabies virus-infected neuronal cells represented by massive intracytoplasmic inclusions of various shapes (dust-like particles, large, round to oval). In all observed fields, samples considered negative displayed no fluorescence and no inclusions, and the tissue appeared as a dull red background.

Before any testing, conjugates were subjected to an initial titration to determine the optimal working dilution for routine use. We prepared serial dilutions of conjugates that will be tested with control material from naturally infected animals. Brain tissues used were from previously submitted accessions, particularly a raccoon strain that was tested and confirmed rabies-positive to ensure the reliability of the results.

Statistical analysis

The statistical analysis was conducted using JMP Pro version software. χ^2 tests were used to evaluate significant associations between the species, seasonality, and rabies occurrence, with significance determined at a P -value < 0.05 .

RESULTS

A total of 1,560 cases were submitted for rabies testing from 2019 to 2023. Out of 1,560 cases, 94.2% [1,470/1,560] were negative for rabies, 5.6% [88/1,560] were positive for rabies across 11 species, and 0.1% [2/1,560] were non-conclusive cases. The occurrence of rabies varied between wildlife (83% [73/88]) and domestic (17% [15/88]) animals, and most rabies-positive cases were coming from Georgia. Over the 5 years, the negative and the total number of rabies submitted cases remained relatively stable until 2021, when there was a decrease followed by an increasing trend starting in 2022.

The number of positives also remained stable, with a slight peak in 2022 (Fig. 1b). Wildlife species significantly accounted for most of the positive cases, with 83% (73/88) representing seven species, whereas 17% (15/88) were from domestic animals (P -value < 0.0001) (Fig. 1b). As shown in Fig. 1c, among wildlife, the affected species were raccoons (35.2%

[31/88], skunks (25% [22/88]), white-tailed deer (8% [7/88]), foxes (6.8% [6/88]), bats (4.5% [4/88]), bobcats (2.3% [2/88]), and great kudu (1.1% [1/88]). In domestic animals, the affected species were bovine (6.8% [6/88]), feline (5.7% [5/88]), caprine (2.3% [2/88]), and equine (2.3% [2/88]) (Fig. 1d).

The positivity rates also differ by species. Skunks (38.6% [22/57]) had the highest positivity rate among wildlife species, followed by great kudu (20% [1/5]), raccoons (12.3% [31/253]), foxes (10.7% [6/56]), bobcats (8.3% [2/24]), bats (7.8% [4/51]), and white-tailed deer (6.5% [7/107]). The domestic species, including bovine (13.3% [6/45]), equine (8.3% [2/24]), caprine (4.3% [2/47]), and feline (4.1% [5/121]), also displayed a difference in positivity rates.

Figure 2 shows the distribution of rabies cases across most Southeastern states and Washington, D.C. Positive cases were predominantly located in Georgia (88.6% [78/88]), with additional cases identified in Washington, D.C. (2.3% [2/88]), Louisiana (1.1% [1/88]), and South Carolina (1.1% [1/88]). The remaining positive cases were reported with unknown locations (6.8% [6/88]). Additionally, we observed a seasonal variation in positive cases throughout the years, with noticeable peaks occurring during certain months. In 2023, specifically, the results showed a very sharp peak characteristic of an increase in positive cases from July until October (Fig. 3). The infection rates during fall (6.9% [26/379]), spring (6% [23/382]), and summer (5.7% [26/456]) were higher than in winter (3.8% [13/343]). The highest proportion of submitted cases was observed during the summer (29.2% [456/1,560]), and 24.3% [379/1,560], 24.5% [382/1,560], and 22% [343/1,560] accounted for submissions received during the fall, spring, and winter, respectively. There is, however, no significant statistical association between seasons and the occurrence of rabies cases (P -value = 0.3387).

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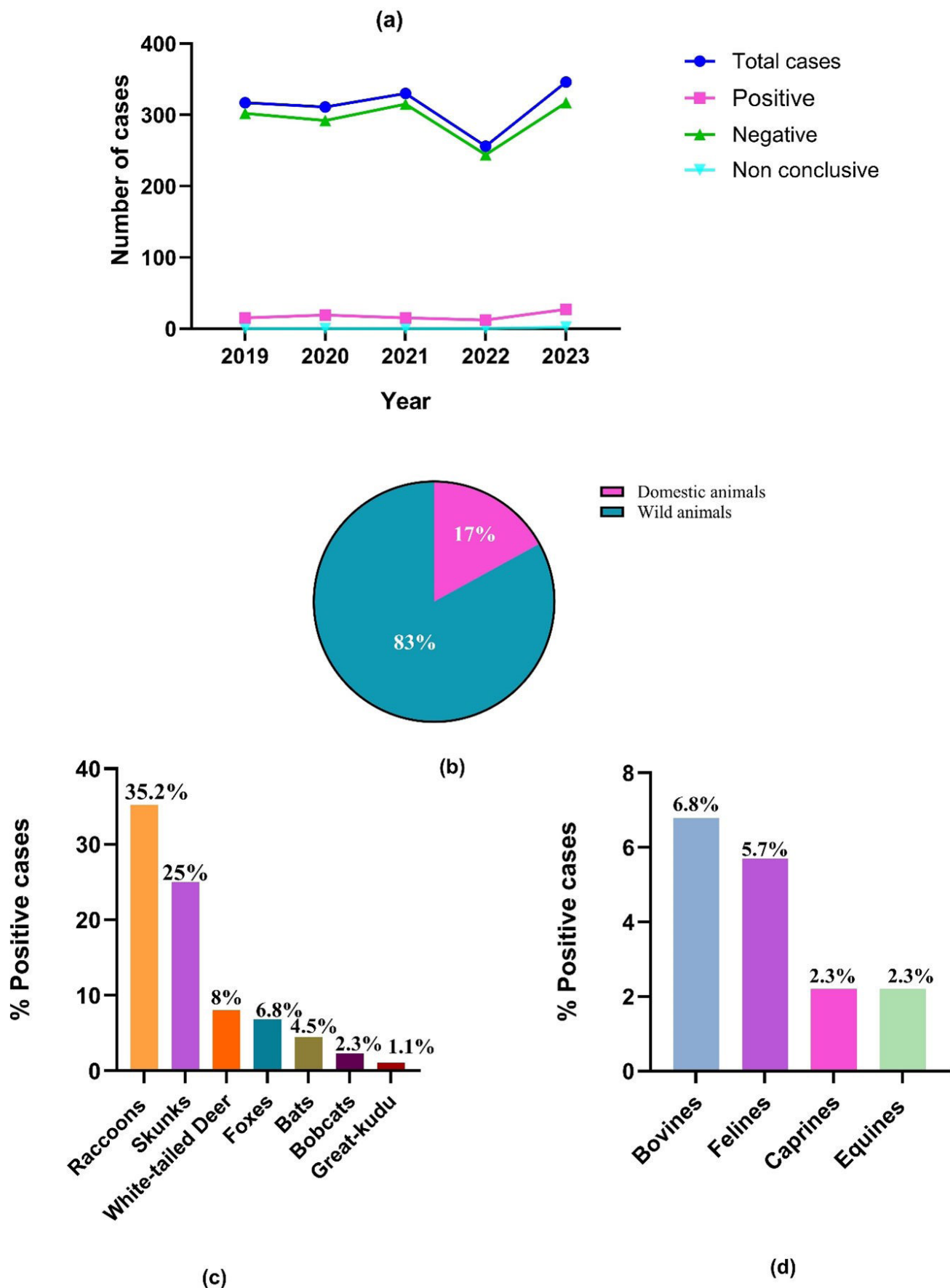


Figure 1

(a) Year-wise distribution of rabies from 2019 to 2023 from cases submitted at Athens Veterinary Diagnostic Laboratory (AVDL)
 (b) Distribution of positive rabies cases among domestic and wildlife animals over 5 years (2019–2023) from cases submitted at AVDL
 (c) Wildlife species distribution of rabies over 5 years (2019–2023) from cases submitted at AVDL
 (d) Domestic species distribution of rabies over 5 years (2019–2023) from cases submitted at AVDL

DISCUSSION

The study investigated the occurrence of rabies among wild and domestic animals between 2019 and 2023, identified the most affected species, mapped the geographic distribution of positive cases, and analysed rabies trends across the years, considering four different seasons (winter, spring, summer, and fall). In recent years, studies conducted in the United States have reported a significant decrease in the number of rabies-positive cases in 2021, followed by an increase in 2022 (28, 29). Our data reveal a comparable trend over the same period, with the decline observed in 2021 potentially related to decreased rabies surveillance activities during the COVID-19 pandemic (28). Our findings also showed that wildlife species exhibit a significantly higher occurrence of rabies than domestic animals, with 83% of cases (73 out of 88 total cases) reported in wildlife. Likewise, studies conducted in the southeastern United States also observed a higher occurrence of rabies in wildlife compared with domestic animals (30, 31).

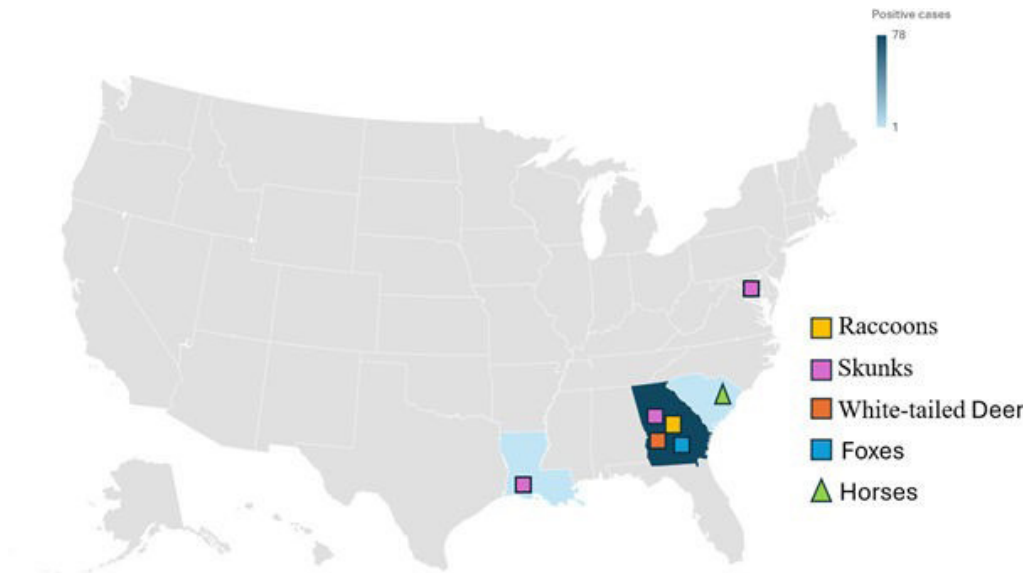


Figure 2:
Geographical distribution of positive rabies cases received across the United States

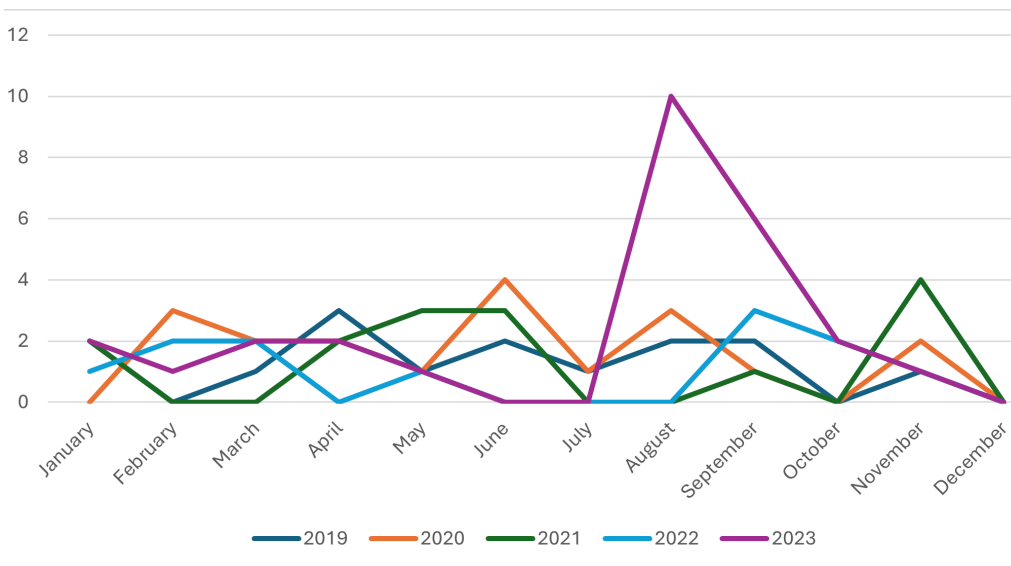


Figure 3:
Seasonal trend of positive animal rabies cases per year (2019–2023) from cases submitted at AVDL. Seasons were defined as Winter (December–February), Spring (March–May), Summer (June–August) and Fall (September–November)

This higher occurrence in wildlife could be attributed to factors such as larger wildlife population densities, increased interactions between wildlife species, and habitat changes (32, 33). The roles of these factors in the occurrence of rabies were not assessed in this study, which is a limitation. Another factor could be a large sample size, especially for raccoons ($n = 253$), and the absence of wildlife-targeted vaccination programs in the southeastern United States. In our study, 79 out of 88 positive domestic and wildlife total cases had unknown vaccination history, which suggests a gap in the surveillance of rabies. Recently, challenges in administering oral rabies vaccination (ORV) in skunk populations have been reported (34). Therefore, further investigations could help in implementing more effective control measures, particularly for species like raccoons and skunks, which are known carriers of rabies. In contrast to our findings, domestic animals such as dogs and cats were reported as the most affected by rabies in Brazil, Ukraine, and South Africa (35–37).

The high occurrence of rabies in Georgia might be due to the proximity of our laboratory, where we may have received more cases within the state of Georgia than outside the state. This finding can also reflect the presence of RABV in this specific region and help inform targeted rabies control strategies. However, although the geographic distribution in our study was predominantly concentrated in Georgia, the additional cases identified in Florida and Alabama in another study suggest a broader regional spread of the disease across the southeastern United States (30).

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Leading Article

Although no statistical significance was observed ($P = 0.3387$), the rabies infection rates during the fall (6.9%), spring (6%), and summer (5.7%) seasons were higher than the winter (3.8%) seasons, aligning with the highest submission rates. It could be linked to a possible connection between increased activity among wildlife and domestic animals during warmer months and the higher occurrence of rabies (32).

During the seasonal peak in the occurrence of rabies that we observed between July and October 2023, the most affected species were raccoons (7.6% [10/131]), whereas tailed deer (2.3% [3/131]), skunks (1.5% [2/131]), bats (0.8% [1/131]), caprine (0.8% [1/131]), and bovine (0.8% [1/131]) were the least affected.

It is unknown whether this increase in rabies occurrence was due to a decrease in surveillance activity. In Brazil, equine rabies cases were consistently reported throughout the year, with no clear seasonality, although peaks were noted in certain months due to increased animal interactions (38).

It is noteworthy that climate change, with its associated rise in temperature, has been associated with increased rabies cases since animals will be more active and be able to move longer distances in warmer temperatures, thereby potentially spreading the virus to other animals and even humans (32, 39).

Moreover, our data confirm that wild animals are more likely to test positive for rabies than domestic animals in the southeastern United States. The most affected species, including raccoons, skunks, white-tailed deer, foxes, bats, and bobcats, further emphasise the significant role of wildlife in the circulation of RABV infection in the region.

Although DFAT is the gold standard for rabies testing (40), it has several limitations, including the high rate of inaccurate results due to the requirement of using high-quality brain samples to perform the test.

Additionally, the interpretation of the results is very subjective and heavily depends on technicians who need to be highly trained before performing the test while following strict biosafety procedures (41).

Access to a necropsy facility is required for proper collection of brain tissues, and a cold chain needs to be maintained to prevent degradation. Such challenges limit DFAT's use, especially in resource-limited settings. Although the quality of submitted samples was not a constant constraint in our DFAT workflow, it is important to highlight these limitations when analysing rabies data, especially collected in remote areas.

Alternative methods, such as real-time polymerase chain reaction (RT-PCR) or LN34 Pan-Lyssavirus RT-PCR assays, could bridge the gap in point-of-care testing as they offer not only a very sensitive and specific diagnostic platform for RABV but also a rapid and cost-effective solution (42, 43).

Overall, our findings highlight distinct seasonal and geographical trends and the burden of rabies among various animal species. We expect these results would add valuable insights into the literature and public health policymakers in the Southeastern United States and contribute to the battle against rabies.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

Aurette Yondo, Conceptualisation, formal analysis, methodology, writing - original draft | Ben Enyetornye, Conceptualisation, formal analysis, methodology, writing - original draft | Binu T. Velayudhan, Conceptualisation, Funding acquisition, Investigation, Resources, Supervision, Writing - review and editing

ETHICS APPROVAL

This study was conducted as a retrospective analysis of specimens submitted to the veterinary diagnostic laboratory and involved no live animals.

All data were obtained from existing laboratory records, and any identifiable information was anonymized before analysis to adhere to integrity and confidentiality standards. **V**

References available on request.



[S6] Captivon 98
Etorphine HCl 9.8 mg/ml (5 ml)

[S6] Thianil
Thiafentanil oxalate 10 mg/ml (10 ml)

[S5] Sedolam 50
Midazolam HCl 50 mg/ml (10 ml)

[S5] Dormadet 40
Medetomidine HCl 40 mg/ml (10 ml)

[S6] Butadyne 50
Butorphanol tartrate 50 mg/ml (5 ml)

[S4] Trexonil
Naltrexone HCl 50 mg/ml (20 ml)

[S4] Zoosedin 20
Atipamezole HCl 20 mg/ml (10 ml)

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Oxidised Mannan: A Novel Adjuvant Candidate for Enhancing Immune Responses in Veterinary Rabies Vaccine

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Rabies continues to pose a serious public health threat worldwide, with vaccination being the most effective means of prevention. However, commercially available inactivated rabies vaccines often require multiple doses and lack potent adjuvants to enhance their efficacy. This study aimed to investigate the coupling of whole inactivated rabies virus to mannan under oxidising conditions to improve immune responses against a standard rabies vaccine. We explored the conjugation of whole inactivated rabies virus with oxidised mannan (Rab-OxMan) to enhance immune responses. Mice were immunised intraperitoneally with 350 µg of the Rab-OxMan formulation on days 1 and 7. Two weeks after immunisation, serum samples were collected to measure levels of IgG, IgM, and TNF-α using ELISA. The vaccine's potency was also evaluated using the National Institutes of Health (NIH) assay. Our findings showed a significant increase in IgG levels and a decrease in IgM levels in the Rab-OxMan group compared to the Alum-adjuvanted vaccine group ($p < 0.05$). Additionally, TNF-α levels were notably higher in the Rab-OxMan group ($p < 0.05$). Statistical analysis revealed that IgG levels had the highest sensitivity and specificity, with a significant correlation between the measured variables. Importantly, the Rab-OxMan formulation provided 1.8 times greater protection in challenge tests compared to the alum-adjuvanted group. This study is the first to demonstrate that oxidised mannan can serve as a novel adjuvant for veterinary rabies vaccines. The results highlight significant improvements in the immunogenicity and efficacy of rabies vaccines, suggesting a promising strategy for enhancing rabies prevention and potentially reducing the incidence of this deadly disease.

INTRODUCTION

Rabies is one of humanity's oldest infectious diseases. The virus claims approximately 60,000 human lives annually and also causes an economic loss of 8.6 billion USD per year globally.¹ An estimated number of 10 million people receive post-exposure treatments each year after being exposed to animals suspected to be infected with rabies.²

The development of the first rabies vaccine by Pasteur successfully reduced the incidence of rabies, but the disease has not been eliminated because it is maintained in many animal reservoirs.³ Many researchers have attempted to produce an affordable and safe rabies vaccine, and the currently recommended inactivated rabies vaccine, adjuvanted with aluminium hydroxide gel, the most common adjuvant, which only induces T helper cell type 2 (Th2) immune responses.⁴ Therefore, new adjuvants are required to increase the immunogenicity of inactivated rabies vaccines. Various carbohydrates such as β -glucan, mannan, and monophosphoryl lipid A (MPLA) can activate the immune system and induce T helper cell type 1 (Th1) immune responses,^{5,6}; therefore, they are attractive immune adjuvant candidates. They may be used alone or in combination with other adjuvants such as Alum. Carbohydrates can be readily metabolised or degraded in vivo and are less likely to generate long-term toxicity.⁷ With their biocompatibility, low toxicity and ease of modification, carbohydrates have been studied as carriers for antigen delivery,⁸ which can often induce immune cell targeting and provide self-adjuvanting activities for a successful vaccination.

Mannan, a polysaccharide derived from the structure of plants as well as the cell wall of yeasts, fungi and bacteria, contains mostly a β -1,4-linked mannose backbone with a small number of β -1,6-linked glucose and galactose side chain residues.⁹ The carbohydrate can be recognised through binding with mannose recognition lectins presented on macrophages and other immune cells, which activates the host immune system via a non-self-recognition mechanism.^{10,11} The recognition initiates a set of signal transduction events leading to cytokine secretion, complement activation and CD8⁺ T cell activation.¹² Although natural carbohydrates can be applied as vaccine components directly,¹³ in many cases, chemical modification of carbohydrates and/or covalent conjugates of antigens and adjuvants is necessary for enhanced efficacy.¹⁴ This can be beneficial in multiple ways, such as prolonged circulation and controlled release, size-induced lymph node targeting, better immune recognition through multivalency, enhanced cell uptake and immune activation. Two strategies (oxidative or reductive) for linking mannan to Antigens have been investigated, which induced drastically different types of immune responses.^{15,16} The conjugation strategy has been applied in many studies, including vaccines against cancer and influenza, such as breast cancer antigen, Mucin1,¹⁷ PCV2 protein of porcine circovirus type 2 virus (PCV2),¹⁸ secreted listeriolysin O (LLO) protein of *Listeria monocytogenes*,¹⁹ and inactivated H1N1 influenza virus²⁰ for investigation of the enhancement of the immune responses. Based on previous reports, our studies focused on the investigation of the inactivated rabies virus conjugated to oxidised mannan to its vaccine efficacy.

MATERIALS AND METHODS

1. Cell, virus, and mice

BHK-21 C13 cells, obtained from the Institute Pasteur (Alborz, Iran), were used in this study. Pasteur strain PV fixed rabies virus, adapted to grow in BHK-21 cells (PV/BHK-21) and provided by the Institut Pasteur (Alborz, Iran), was used throughout this study. All mice were outbred female SW1 sourced from the Research and Production Complex of Pasteur Institute of Iran (Alborz, Iran). All mouse work was conducted at the Animal Laboratory of the quality control department of the Research and Production Complex of Pasteur Institute of Iran (Alborz, Iran), in accordance with an animal ethics application approved by the Iranian Animal Ethics Committee.

2. Cell culture

BHK-21 Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) supplemented with fetal Bovine serum (FBS; 5-10%; Invitrogen, USA) and tryptose phosphate (TP; 0.2-0.3% w/v; Invitrogen, USA).

3. Virus production and inactivation

BHK-21 cells were infected by the rabies virus strain PV/BHK-21 at a cell concentration of $2-3 \times 10^6$ cells/mL, with a multiplicity of infection (MOI) equal to 0.1/cell in a 10-L bioreactor containing 7 L of DMEM (Invitrogen, USA), supplemented with tryptose phosphate (TP; 0.2-0.3% w/v; Invitrogen, USA). For the rabies virus production step, pH was maintained at 7.4, pO₂ at 30% air saturation, agitation rate at 40 rpm and temperature at 37°C. The cell suspensions were centrifuged at 750-850 g for 10 min, and the viral supernatants (harvests) were first clarified by filtration through a 0.8-micron filter and then inactivated by 3 mM of Binary ethyleneimine (BEI) (Sigma, USA).

4. Rabies-mannan conjugate

Whole inactivated rabies was conjugated to oxidised mannan described in Stambas et al.¹⁹ Briefly, mannan (Sigma; USA) (1 mL of 14 mg/mL) in 0.1 M phosphate, pH 6.0, was oxidised with the addition of 0.1 M sodium periodate (100 μ L in water) in the dark at 4°C for 1 hr. The mixture was quenched with 10 μ L ethandiol and reacted for a further 30 min as before. The oxidised mannan mixture was passed through a PD10 column (GE Biosciences) pre-equilibrated with 0.05 M bicarbonate, pH 9.0, to remove by-products. The eluted 2 mL fraction of oxidised mannan (≈ 7 mg/mL) after void volume (2.5 mL) was collected. Each milliliter of the whole inactivated rabies virus bulk was separately reacted with 0.5 mg/mL Al (OH)₃ gel (Croda; Denmark), 100 μ g of filter-sterilized mannan (Sigma; USA), or 350 μ g of filter-sterilized oxidized mannan for 16 h at 20°C in 100 rpm and the resulting preparations were analyzed for its vaccine safety and potency.

According to previous studies, the periodate oxidation condition for mannan was chosen such that aldehyde residues are generated from only a fraction of oxidised mannose units of the mannan, without affecting its C-type lectin binding activity.²¹ For a complex antigen such as a whole inactivated rabies virus, we expect the majority of conjugation of mannan aldehyde groups to take place only at the exposed amino groups, forming Schiff base linkages.

5. Inactivity test

A 0.03 mL of the inactivated bulk rabies vaccine was intracerebrally administered to each of ten SW1 female mice, with body weights ranging from 11 to 15 g. The animals are observed for 21 days. If more than two animals die during the first 48 hours, the test is repeated. The vaccine complies with the test if, from day 3 to day 21 post-injection, the animals show no signs of rabies, and the immunofluorescence test carried out on the brains of the animals shows no indication of the presence of rabies virus.²²

6. Safety test

1 mL of the formulated bulk rabies vaccine was injected intraperitoneally into each of eight female SW1 mice, each weighing 17-22 g. The animals are observed for 21 days. The animals are observed at least daily for 14 days.

The vaccine complies with the test if no animal shows adverse reactions or dies of causes attributable to the vaccine.²²

7. Serological test

In the serological test, ten female SW1 mice, each weighing 13-16 g, are used. Each mouse is vaccinated by an intraperitoneal route using 0.5 mL of 1/5 of the recommended dose volume at days 0 and 7. Blood samples are taken 14 days after the first injection, and the sera are tested individually for IgG, IgM, and TNF α by quantitative enzyme-linked immunosorbent assay.²³

8. Potency test

In addition to Serological assays, potency was analysed according to the National Institutes of Health (NIH) test recommended by the WHO. Mice were immunised on days 0 and 7 with 0.5 mL/dose of all experimental and reference groups via intraperitoneal (IP). Both the experimental vaccines and the international reference standard rabies vaccine were diluted in a serial 5-fold dilution (1/25, 1/125, 1/625). All mice were then challenged on day 14 via an intracerebral administration (IC) of 30 μ L rabies strain CVS11 (Pasteur Institute of Iran, Alborz, Iran) containing 42 LD50. Subsequently, mice were observed for another 14 days, and the mortality of mice was recorded to calculate the ED50 that is normalised with the international reference standard vaccine by using the Spearman–Karber formula to obtain a titer in IU NIH/dose. The Ph. Eur. Biological Reference Preparation (BRP) Batch Number 5 for rabies vaccine (inactivated) for veterinary use (EDQM) was used to calibrate the test. The potency is expressed in International Units/mL (IU/mL).²⁴

9. Statistical analysis

Statistical analysis was performed using one-way ANOVA using GraphPad Prism (version 8.0, GraphPad Software, CA, USA), and statistical analyses were performed using SPSS Statistics software version 26.0 (IBM, USA). All titrations were carried out in triplicate, and titers are expressed as mean values \pm standard deviation (SD). The comparison between groups was considered statistically significant if $p < 0.05$ or 0.001 . Furthermore, receiver operating characteristic (ROC) curves were utilised to evaluate the sensitivity and specificity of the diagnostic test, while multivariable logistic regression analysis was employed to examine the association between the predictor variables and the outcome variable.

RESULTS

1. IgG and IgM assay

As shown in Fig. 1A, mice immunized with the conjugate of inactivated rabies virus and oxidized mannan (Rab-OxMan) exhibited a significantly higher IgG titer (12.09 ± 0.94 mg/mL) compared to those receiving mannan (Rab-Man) (11.01 ± 0.65 mg/mL) and the Alum-adjuvanted inactivated rabies vaccine (Rab-Al) (7.94 ± 0.71 mg/mL), with a p -value of less than 0.05 indicating statistical significance. The elevated IgG response is promising, as higher IgG levels are often correlated with enhanced protection and long-term immunity against pathogens. Conversely, as illustrated in Fig. 1B, the Rab-OxMan group showed a significantly lower IgM titer (0.54 ± 0.05 mg/mL) than both the Rab-Man (1.03 ± 0.10 mg/mL) and Rab-Al groups (0.72 ± 0.07 mg/mL), with p -values < 0.05 . This reduction in IgM, while statistically significant, suggests a potential trade-off, as IgM is typically the first antibody produced in response to infection, reflecting early immune activation.

These results indicate that although the Rab-OxMan formulation effectively induces a stronger IgG response, which is beneficial for long-term efficacy, it is associated with a reduced IgM response compared to other vaccine formulations. Further investigations are needed to evaluate the longevity of the IgG response and its correlation with long-term protection, as well as to understand the implications of the lower IgM response in the context of overall vaccine efficacy.

2. TNF- α assay

In this assay, serum levels of TNF α measured by

ELISA, were significantly higher in mice vaccinated with Rab-OxMan (47.67 ± 13.1 pg/mL) compared to those immunised with Rab-Man (27.0 ± 8.7 pg/mL) and Rab-Al (24.33 ± 6.2 pg/mL), $p < 0.05$. These results, depicted in Fig. 2, demonstrate that immunisation with the Rab-OxMan vaccine leads to an increased TNF- α level compared to the other groups, highlighting its potential effectiveness in eliciting a robust immune response.

3. Sensitivity and specificity analysis of the variables

The specificity and sensitivity of the sera IgG, IgM and TNF- α levels were assessed using a receiver operating characteristic (ROC) curve. Areas under the curve (AUCs) and p -values were obtained as $AUC = 0.895$ and $p < 0.001$ for IgG, $AUC = 0.827$ and $p < 0.001$ for IgM, and $AUC = 0.752$ and $p < 0.001$ for TNF- α (Fig. 3). According to the results, the sera IgG levels demonstrated the highest sensitivity and specificity compared with other variables.

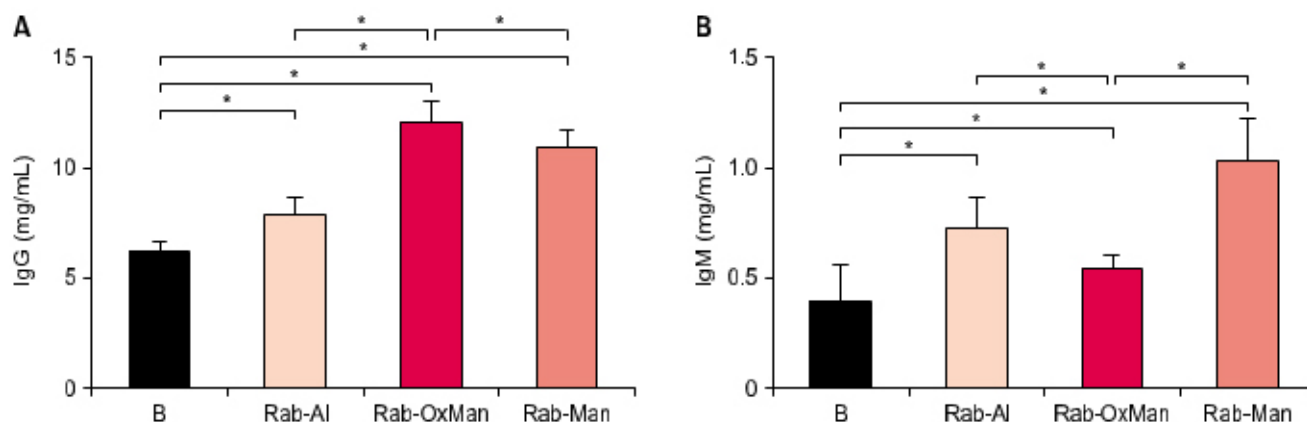


Figure 1:

The IgG (A) and IgM (B) titers in vaccinated mice with three types of inactivated rabies vaccines ($n=10$) were determined by enzyme-linked immunosorbent assay (ELISA). The mean IgG (12.09) and IgM (0.54) titers in the mice sera immunised with Rab-OxMan were significantly higher and lower, respectively, than those in mice immunised with other formulation ($p<0.05$). The IgG (6.29 ± 0.39 mg/mL) and IgM (0.39 ± 0.09 mg/mL) of the inactivated rabies virus bulk without any adjuvant "B" was measured as a control group

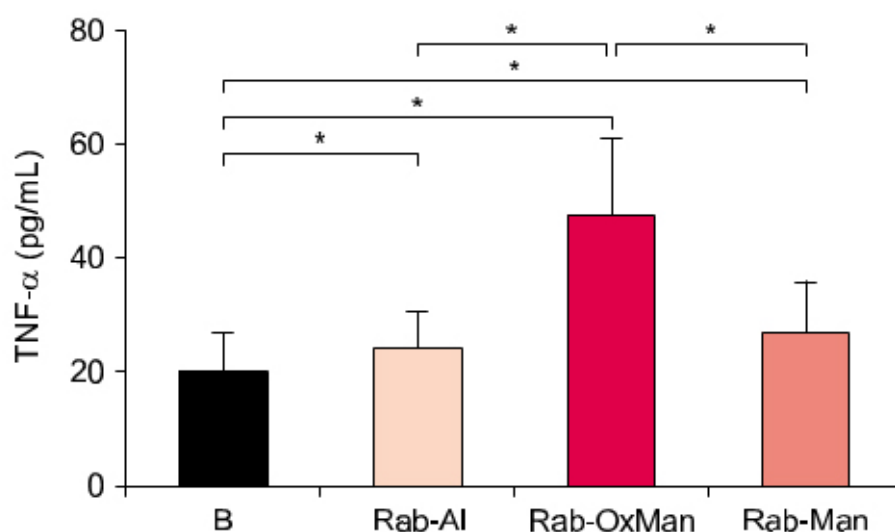


Figure 2:

The amount of TNF- α was measured in the sera of mice from the indicated groups ($n=10$) using a commercial TNF- α ELISA kit at day 14 post-immunisation. The TNF- α level of the inactivated rabies virus bulk without any adjuvant "B" (20.33 pg/mL) was checked as a control group. One-way ANOVA followed by Tukey's HSD test, $*p<0.05$

4. Relationship between immunogenicity variables

The logistic regression analysis revealed significant associations among the immunogenicity variables, including IgG, IgM, and TNF- α serum levels, with p -values <0.001 for all relationships analysed. Specifically, p -values were less than 0.001 in the comparisons of IgG with IgM, IgG with TNF- α , and IgM with TNF- α , indicating robust and statistically significant correlations.

These results suggest that as serum levels of IgG and IgM increase, there is a corresponding rise in TNF- α levels. The p -value of <0.001 indicates a strong likelihood that these associations are not due to random chance (Table 1). Overall, these findings underscore the interconnected nature of immune responses, suggesting that elevated levels of IgG and IgM may influence TNF- α activity, thus playing a crucial role in the overall immunogenicity observed in the study

5. Challenge (NIH) test

The protective activity of inactivated rabies virus samples was determined according to the NIH test. Based on the results, Rab-OxMan vaccine induced the highest potency (6.27 IU/mL) than the Rab-Man (4.21 IU/mL) and Rab-AI counterparts (3.48 IU/mL) (Fig. 4). Significantly, the mice receiving Rab-OxMan and Rab-Man vaccines resulted in an increase of 1.8-fold and 1.2-fold in the vaccine efficacy compared to Rab-AI-vaccinated mice, respectively.

DISCUSSION

Rabies remains a critical public health concern worldwide, with more than 59,000 reported cases of rabies-related deaths annually, predominantly in developing regions.²⁵

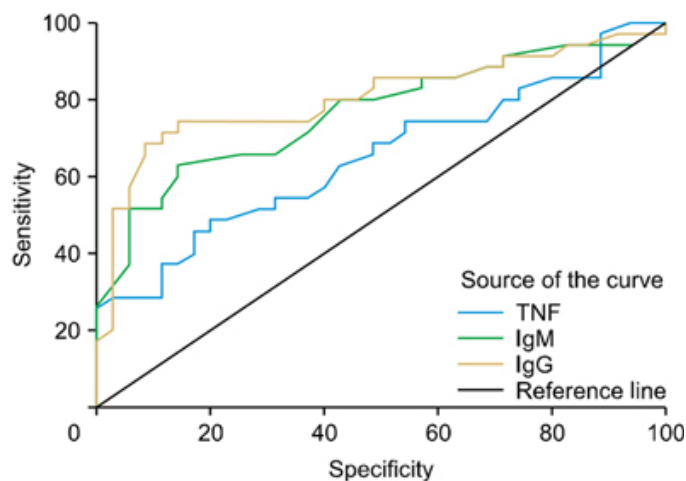


Figure 3:

The ROC curve demonstrated the behaviour of the sera IgG, IgM, and TNF- α sensitivity and specificity

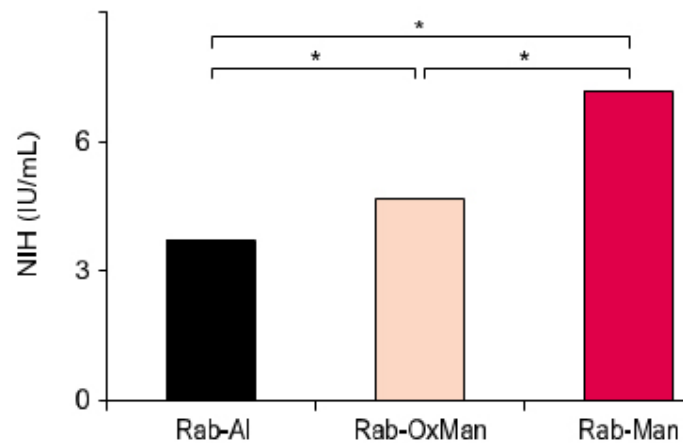


Figure 4:

Potency (NIH) assay results measuring the effectiveness of different rabies vaccine formulations. Values represent International Units per millilitre (IU/mL). Asterisks (*) denote statistically significant differences ($p < 0.05$) between the indicated groups

	B	SE	p-value	OR (95% CI)
IgG×IgM	0.000010	0.000011	<0.001	1.000010 (1.000011-1.000024)
IgG×TNF- α	0.000025	0.000022	<0.001	1.000025 (1.000019-1.000012)
IgM×TNF- α	0.000041	0.000014	<0.001	1.000041 (1.000032-1.000010)

Table 1:

Logistic regression results between the variables

The primary strategy for rabies prevention is vaccination; however, the effectiveness of traditional alum-adsorbed inactivated rabies vaccine is often hindered by the need for multiple doses and the reliance on existing adjuvants, which may not provide optimal immune responses. This calls for improvement of rabies vaccination strategies. Investigation on the other potent and novel adjuvants alone or along with alum has immense potential in this regard. Hence, in the present study, we have explored the immunogenicity effect of the whole inactivated rabies virus conjugated to oxidised mannan as a novel adjuvant and compared its protective efficacy as per the NIH guidelines in SW1 mice.

One of the striking findings of the current study is the significant increase in IgG levels among mice immunised with the Rab-OxMan compared to those receiving the alum-adsorbed vaccine. Elevated IgG levels are indicative of a robust and effective humoral immune response, which is crucial for long-term immunity and protection against rabies virus infection. The concurrent observation of decreased IgM levels suggests a shift toward a more mature immune response, characterised by class switching—an essential aspect of the adaptive immune system’s efficiency in producing high-affinity antibodies.²⁶ This transition is particularly beneficial, as Immunoglobulin G is typically associated with a more specific and durable immune protection, while IgM is often produced earlier in the immune response but provides less effective long-term protection. Moreover, the significant increase in TNF- α levels in the Rab-OxMan group points to enhanced activation of the immune response, as TNF- α is a pivotal pro-inflammatory cytokine that plays critical roles in the activation and proliferation of various immune cells.²⁷ Higher TNF- α levels indicate a more vigorous inflammatory response that can facilitate better antigen presentation and enhance the overall immune reaction. This improved activation could be beneficial for the development of a more potent immune memory, potentially leading to better protection upon re-exposure to the rabies virus. In fact, these data strongly suggest the induction of cellular as well as humoral immunity. This bodes well for the vaccine strategy, as it is widely held that the generation of effective immunity will require induction of a functional T cell response.

The ROC plot also shows that TNF- α shows a strong and consistent increase in sensitivity, which demonstrates its reliability as a marker for. The IgG immune response shows the highest sensitivity, emphasising its role in confirming successful immunisation and long-term protection. IgM also shows reasonable sensitivity but is not as effective as TNF- α and IgG. In general, the analysis suggests that TNF- α and IgG are particularly relevant for assessing rabies vaccination outcomes and provides insights that could enhance diagnostic and vaccination tools. In addition, based on the logistic regression analysis, the interaction among the three variables is statistically significant ($p < 0.001$), indicating a strong correlation between these variables.

In addition to the immunological assessments, the results of the challenge test highlight the tangible impact of the Rab-OxMan formulation on the protective efficacy of the rabies vaccine. Indeed, the oxidised mannan-Rabies conjugate provided 1.8 times greater protection in the challenge test compared to the alum-adsorbed group is particularly noteworthy.

This enhanced level of protection might be attributed to the synergistic effects of the inactivated virus conjugated to oxidised mannan, which may improve the vaccine's ability to elicit stronger and more comprehensive immune responses.²⁸ The Rab-OxMan vaccine was tested for local toxicity in SW1 mice and demonstrated to be safe at doses up to five times the therapeutic level²²

Certainly, future studies should also investigate the potential mechanisms behind the immune enhancements observed with the Rab-OxMan formulation in detail, including the role of innate immune pathways and specific immune cell types involved in the response. Furthermore, the implications of this research extend beyond rabies vaccination. The approach of utilising oxidised polysaccharides as adjuvants could be applicable to other veterinary and human vaccines, potentially leading to broader applications in vaccine development. Overall, these findings suggest that integrating oxidised mannan into rabies vaccine formulations presents a promising avenue for improving vaccination outcomes, enhancing public health responses to rabies, and potentially saving lives.

In conclusion, this study presents compelling evidence that oxidised mannan can serve as a novel adjuvant or co-adjuvant for veterinary rabies vaccines. Significantly enhancing IgG production, modulating IgM responses, and increasing pro-inflammatory cytokine levels of the Rab-OxMan formulation demonstrate substantial improvements in immunogenicity and protective

efficacy of the rabies vaccine. These findings suggest that incorporating oxidised mannan into rabies vaccine formulations could be a promising strategy for improving vaccination outcomes and enhancing public health efforts against rabies. Future studies should focus on further elucidating the mechanisms underlying these immune responses and evaluating the long-term efficacy of this novel vaccine formulation. **V**

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CONFLICT OF INTEREST STATEMENT

None declared.

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Insights into artificial waterhole utilisation patterns by elephants and rhinos: Lessons from a South African Nature Reserve

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ABSTRACT

Artificial water provisioning is a common practice in southern African nature reserves, where different game species exhibit preferences for specific waterhole types. The movement patterns and behaviour of elephants and rhinos are closely linked to water availability, with these mega-herbivores noticeably influencing the environment and other species they interact with at waterholes. Since there is limited research on this topic, understanding preferences for different types of artificial waterholes is crucial, particularly during periods of water scarcity. This knowledge enables reserve managers to effectively manage the numbers and types of waterholes. In this study, we investigate artificial waterhole selection and preferences by elephants and rhinos in the Olifants West Nature Reserve, South Africa. The study area featured various waterhole types, including earth dams, concrete pans, reservoirs, and troughs. By employing camera traps, we analysed visitation frequency, timing of visits, and factors influencing visit frequency. Our findings revealed distinct preferences for specific waterhole types among different social groupings of the study species. Breeding herds of elephants predominantly utilised reservoirs and occasionally visited troughs, while bachelor herds favoured earth dams. Black rhinos showed a preference for earth dams, whereas white rhinos selected troughs and earth dams, with bachelor groups favouring troughs and female rhinos favouring earth dams. The outcomes of this study have significant implications for the development of comprehensive conservation plans in areas where these species reside, and for potential release sites.

Introduction

Artificial waterholes are widely used in nature reserves, and their importance in sustaining wildlife during dry seasons and droughts is often a subject of debate [1, 2]. These waterholes have a significant impact on the environment and on the animal species that depend on them [3]. Herbivores' water requirements influence their movement patterns, behaviour and preferences for certain waterhole types (both natural and artificial) based on the morphology of these waterholes [4–7]. This, in turn, affects vegetation composition, biodiversity, and animal interactions at water sources [3, 7–13]. The presence of artificial waterholes often leads to the congregation of animals that would typically rely on natural water sources, resulting in interspecific and intraspecific competition for water, particularly during periods of scarcity [8].

The design of artificial waterholes, such as high-sided reservoirs, for example, favours certain species like elephants that can access water with their trunks, while excluding smaller animals. Waterholes also vary in shape and lateral extent and may be associated with mud wallows, which are important for thermoregulation and parasite control for species like warthogs (*Phacochoerus africanus*) and rhinos (*Diceros bicornis* and *Ceratotherium simum*) [14– 17].

Other factors influencing waterhole selection include perceived predation risk [18, 19], avoidance of competition [20, 21], species behaviour at waterholes [21, 22], faecal bacterial loads [23], and gregariousness of visiting species [24].

Mega herbivores such as elephants (*Loxodonta africana*) and rhinos (*Diceros bicornis* and *Ceratotherium simum*) have pronounced impacts on other species and the environment [13, 15, 25, 26]. Understanding the waterhole preferences and visitation patterns of these species is crucial for their management and relocation, especially considering the severe poaching pressure they face for their horns and tusks [27–32]. Protecting natural environments and safeguarding vulnerable species like elephants and rhinoceroses are top priorities for protected area managers and wildlife departments.

Effective management of protected areas requires comprehensive plans to guide conservation efforts and ensure the preservation of habitats and their resident species. Managing natural and artificial water sources is a fundamental aspect of such planning, encompassing the selection, placement, utilisation, and impact of artificial waterholes on surrounding vegetation [1–3, 5, 6, 8, 10, 20, 21, 24, 32].

African elephants (*Loxodonta africana*) are mixed feeders that typically drink water daily but can go without it for up to four days [32]. Adult elephants require 150 to 300 litres of water per day [27, 32, 33] and do not have a preferred drinking time [27, 32–34]. In contrast, black rhinoceros (*Diceros bicornis*) are browsers that enjoy wallowing and need to drink water once every four to five days but will drink more frequently (35 litres per day) if water is readily available [32–34]. Adult black rhinos prefer artificial waterholes over natural ones and drink throughout the day and night [32–34]. White rhinos (*Ceratotherium simum*), on the other hand, are grazers that need to drink water daily, and like the black rhino, also wallow [27, 32, 34]. An adult wild white rhino requires up to 72 litres of water a day [32–34], shows no preference for a particular waterhole type [32–34], and drinks at least twice a day [32–34], most often during the late afternoon and after dusk (17:00 to 21:00) [27, 32], but also during the morning and midday periods [32, 33].

Given their dependence on water, elephants, white rhinos, and, to a lesser extent, black rhinos are influenced by water availability [3, 27, 32–34]. Understanding the factors that affect the behaviour and movement patterns of these mega-herbivores is of particular interest from a conservation management perspective, considering their significant impact on the environment, other species, and each other [13, 25, 26]. No comparative regional studies were found that investigated waterhole type preferences among various elephant social groupings. Utilisation patterns of waterholes by elephants influence how other wildlife species use these water sources [20, 35]. As natural surface-water availability diminishes during the dry season, the utilisation of artificial water sources by water-dependent species exhibits seasonal variations, becoming increasingly important [3, 21, 36].

Unfortunately, elephant and rhino populations are facing severe threats due to poaching, habitat fragmentation, and illegal trade of their products [37, 38]. These large herbivores, especially in water-limited habitats, are particularly vulnerable to the impacts of poaching [39]. The demand for ivory, rhino horn, and related products has resulted in significant declines in elephant and rhino populations across Africa [40]. Combined with habitat fragmentation, illegal harvesting has left small, isolated populations of these species, posing further threats to their survival [41]. Acquiring additional information about the water utilisation behaviour of elephants, black rhinos, and white rhinos, including their preferences for specific artificial waterhole types, is crucial for effective management and protection of these animals.

This study focuses on the utilisation of four different waterhole types by elephants, black rhinos, and white rhinos in the Olifants West Nature Reserve (OWNR) in southern Africa.

The study investigates the timing of their visits to waterholes, the frequency of their visitations during different seasons, and their preferences for specific artificial waterhole types. This knowledge is valuable for managing areas that support populations of elephants, black rhinos, and white rhinos, as well as for establishing new areas for their relocation to enhance population conservation efforts. Investigating the utilisation of artificial waterholes by these study species is particularly important considering their declining numbers and the ecological and economic consequences associated with their decline [27–30]. While information on the population densities of these species would contribute to a more comprehensive discussion on waterhole attendance, such data was not provided to the researchers due to the high levels of poaching associated with these species.

Understanding the preferences and utilisation patterns of elephants, black rhinos, and white rhinos for artificial waterholes is important for the effective management of these species. This study provides valuable insights into their visitation patterns, preferences for specific waterhole types, and seasonal variations in waterhole utilisation. Such information can guide conservation strategies, including the optimisation of waterhole design and placement, to reduce competition and support the long-term survival of these iconic and threatened megaherbivores. The findings will assist conservation managers in making informed decisions to protect these species in both current and future protected areas.

Methods

The study was conducted by the first author, who was a student employee of the nature reserve where the research took place. The researcher obtained permission from reserve management to do the study, which involved monitoring waterholes. Ethical clearance for the study (2014/ CAES/037) was granted by the University of South Africa's (UNISA) Ethics Department.

Study site

The OWRN is located within the Balule Private Nature Reserve, forming part of the larger Kruger National Park (KNP) (Coordinates: -24.1987, 30.9090) (Fig 1). The OWRN is approximately 8 800 hm² in size, with open borders into the KNP to the East and South. The western boundary of the OWRN is fenced and represents the furthest point to the West that animals can travel. The Olifants River forms the northern boundary of the OWRN.

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The altitude of the study area ranges from 338 m.a.s.l in the east to 360 m.a.s.l in the west. The study area is arid savannah [42, 43] with granitoid-based geology of the Swazian and Randian age group, derived from the Basement complex [42, 43]. The predominant soil types found in the study area are granitic- and gabbro-derived [42–44].

Climate data was collected from a weather station located at the OWNR research facility at the study site (Fig 2). Climate for the Savannah Biome is characterised by a clearly defined summer-rainfall pattern [45], with most of the rainfall occurring in the warm wet months between November and April. The cool dry months (May to October) are cooler and dryer, characterised by night-time temperatures that drop as low as 0°C in high altitude areas [45].



Figure 1:
Location of the Balule Private Nature Reserve in relation to the Kruger National Park within South Africa.
(Image created by the researcher)

The OWNR is located entirely in the Savannah Biome characterised by a grass and forb dominated ground layer with an upper woody vegetation layer [45]. The vegetation is classified as belonging to the Granite Lowveld (SVI3) vegetation type [45].

Waterhole selection

Four artificial waterhole types were present at the study site and selected for this study, namely, earth dams, concrete pans, reservoirs and troughs (Fig. 3).

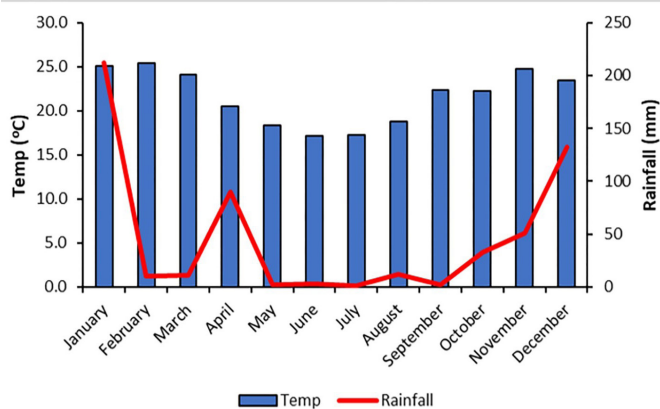


Figure 2:
Temperature and rainfall data for the 2013 study period



Figure 3:
Examples of the different waterhole types in the study area.
(a) is an example of an earth dam, (b) is an example of a pan, (c) is an example of a reservoir, and (d) is an example of a trough.
(Photographs taken by the researcher)

These waterhole types were either already present or were established after the creation of the Balule Private Nature Reserve in the early 1990s. Earth dams did not have a concrete base or sides and were established around natural features.

Earth dams were in woody areas where there was no need for clearing bush to build concrete structures. Reservoirs had a concrete base with high sides and were placed close to boreholes in relatively dense woody vegetation. Concrete pans had a concrete base with low sides and were in relatively open areas dominated by grass.

Troughs were often associated with reservoirs, had a concrete base and were usually placed some distance away from reservoirs in open areas dominated by grass. All waterhole types provided water perennially and were chosen based on their spatial orientation and ease of access from the road network in the reserve.

Eleven waterholes (Table 1) were monitored for the wet (November to April) and dry (May to October) seasons. Days were split into four daily periods, constituting night (18:01 to 06:00), morning (06:01 to 10:00), midday (10:01 to 14:00) and afternoon (14:01 to 18:00).

Waterhole monitoring

Camera traps were used to monitor the selected waterholes 24 hours a day, seven days a week, from January to December 2013.

Camera traps used included Bushnell (model 119466), Tasco (model 9215), Scoutguard (model SG550) and Reconyx Rapidfire (RC55).

All camera brands had similar specifications and were the models available for use by the researcher. Camera settings were standardised for all camera types to take five megapixel photographs every 1.2 seconds once movement was detected. All cameras could take infrared photos at night.

The cameras were placed in the field for a two-week period prior to starting with the data collection to ensure that the correct camera settings and angles were used, and to ensure that all batteries, cameras and SD cards were functional.

Table 1. Olifants West Nature Reserve artificial waterhole types with their local names, waterhole type, capacity, size categories and number of camera traps placed at the waterholes

Artificial Waterhole Characteristics				
Name	Type	Capacity (m ³)	Size category	Nr of camera traps
Ngala	Earth dam	100–1 000	Medium	1
Oxford big dam	Earth dam	> 1 000	Large	2/3 *
Singwe big dam	Earth dam	> 1 000	Large	3/4 *
Leopard's view	Concrete pan	< 100	Small	1
Singwe bush camp	Concrete pan	< 100	Small	1
Toni's dam	Concrete pan	< 100	Small	1
Nyala	Reservoir	100–1 000	Medium	1
Nzulwini	Reservoir	100–1 000	Medium	1
Van Wyk's	Reservoir	100–1 000	Medium	1
Nyala	Trough	< 100	Small	1
Van Wyk's	Trough	< 100	Small	1

*The number of camera traps deployed at Singwe Big Dam and Oxford Big Dam varied depending on the water surface area, which was affected by rainfall.

Doing this could have acclimatised the various species visiting the waterholes to the presence of the cameras, as the researcher did not observe any avoidance behaviour prior to, or during the study, when time was spent at the various waterholes. Camera traps were checked every seven days for damage, to download imagery, clear memory cards, replace batteries, and ensure that they were still properly positioned. Data collected from the camera traps provided information about the number of days each waterhole was effectively photographed. Camera traps were positioned to maximise the percentage of waterhole edge covered, with additional camera traps used to cover larger waterholes. For waterholes that had more than one camera trap, dates and times on the various camera traps were synchronised so that when viewing images from various cameras, it was possible to identify the same animals i.e. images of animals taken within a few minutes of each other on different cameras could be isolated and the animals identified as being the same ones. Also, when there was more than one camera at a waterhole, the cameras were placed in such a way that there was a slight overlap of their field-of-view, resulting in animals moving from one camera's field-of-view to another camera's field-of-view being identified as the same animals. Invariably, there were occasions when camera traps had to be removed for repairs or replacement. To cater for days when waterholes did not have cameras, a sightings-per-day value was calculated for all cameras by dividing the number of sightings at a waterhole by the number of days the waterhole was effectively monitored. This allowed for direct comparison of visitation data between waterhole types. The number of camera traps deployed at Singwe Big Dam and Oxford Big Dam varied depending on available surface water, which was influenced by rainfall. When these dams were very full, more camera traps were placed out than when the dams were empty and had less surface water.

Photographs from the camera traps were digitally dated and time-stamped for accuracy when doing comparisons. Information obtained from the camera traps included the time of day that elephant, black rhino and white rhino utilised the various waterhole types, social grouping types frequenting the waterholes, number of animals, and the duration of stay at waterholes. Elephant group types identified were bachelors, bachelor groups and breeding herds. Black rhino group types were bachelors, bachelor groups, cows, cow groups, bull and cow groups and unknown adults. White rhino groups were the same as for black rhino, except for cow groups, which were not observed during this study, as this group type likely used waterholes without camera traps.

A single visitation to a waterhole was defined as a single photograph or sequence of photographs isolated by a minimum of five minutes from any other photographs according to the timestamp. The five-minute interval is based on direct observations made by the researcher. The timings of visitations (time of day) and duration of stay by elephants and rhinos were used to determine waterhole preferences and utilisation patterns. Duration of stay at a waterhole was calculated by subtracting departure times from associated arrival times at waterholes.

Data analyses

Binomial tests with confidence intervals were used to test for waterhole preference by the different species and their social group types [46]. The rationale for using a binomial test was that it is an exact test, providing a rigorous examination of the observed outcomes against expected probabilities for two distinct possible outcomes, selection (preference) or rejection (no preference) [46]. The precision of the binomial test is particularly beneficial when evaluating waterhole preferences, as it ensures accurate statistical inferences, even in scenarios with small sample sizes or discrete datasets.

The use of binomial tests in this study extends beyond a general preference assessment. By conducting tests across different seasons (wet and dry) and daily periods (night, morning, midday, and afternoon), we capture a comprehensive view of waterhole utilisation patterns. Only significant results with a lower 95% confidence interval above the chance threshold of 25% were reported, ensuring that detected preferences were not due to chance. The 25% threshold was established based on the equal probability of selection among the four distinct waterhole types.

Additionally, a series of Poisson General Linear Models (GLM's) were run to determine the impact of five predictor variables, Season (1 Wet or 2 Dry), Daily Period (1 Morning, 2 Midday, 3 Afternoon, 4 Night), Waterhole type (1 Earth Dam, 2 Concrete 3 Pan, 4 Reservoir, 5 Trough), Waterhole Size (1 Small, 2 Medium, 3 Large), and Social Group Type (1 Bachelor, 2 Bachelor group, 3 Cow, 4 Cow group, 5 Cow and calf, 6 Unknown adult, 7 Breeding herd, 8 Bull and cow) on the frequency of visits to waterholes by the three study species [47]. Poisson GLMs were chosen because the response variable represented count data, characterised by discrete, positive integer values, and this model type accounts for non-normality and variance that is not constant [47].

All predictor variables were treated as fixed effects, with no need to control for random effects, thus eliminating the necessity for generalised linear mixed models [47]. Grouping variables used in the GLMs included Season, Daily Period and Waterhole Type.

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To compare hourly visitation frequencies across all waterhole types for the three study species, the data were square root transformed to stabilise variance and improve the validity of analyses done [46].

All statistical tests were performed using IBM SPSS Statistics 23.

Results

A total of 770 elephant observations (n = 378 wet season, n = 392 dry season), 107 black rhino observations (n = 73 wet season, n = 34 dry season) and 30 white rhino observations (n = 13 wet season, n = 17 dry season) were recorded by the camera traps during this study.

Visitation frequency to waterholes

Seasonal visitation frequencies to all waterhole types by elephants are shown in Fig 4A, for black rhino in Fig 4B and for white rhino in Fig 4C.

Proportional visits to the different waterhole types

Combined seasons, wet season and dry season proportions of visits to the different waterhole types by elephants are depicted in Fig 5A, black rhinos in Fig 5B, and white rhinos in Fig 5C.

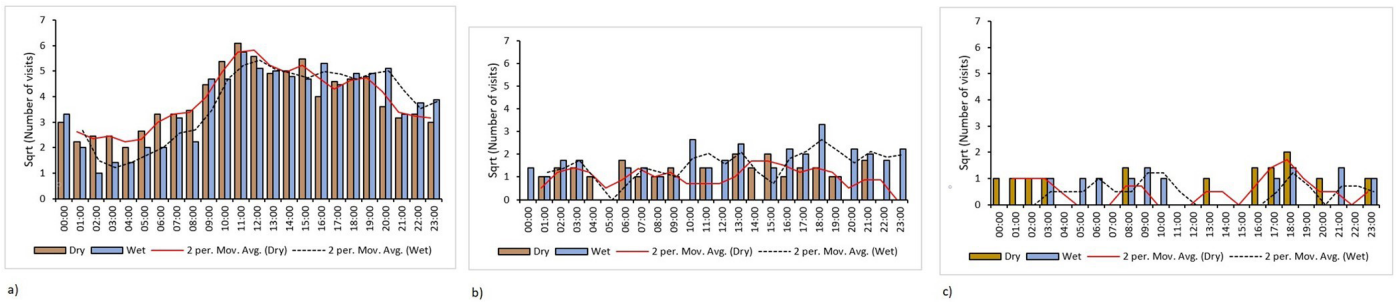


Figure 4: Visitation frequencies to all waterhole types across the hours of a day for the wet and dry seasons with a two-period moving average to show seasonal trends for a) Elephant, b) Black rhino, and c) White rhino

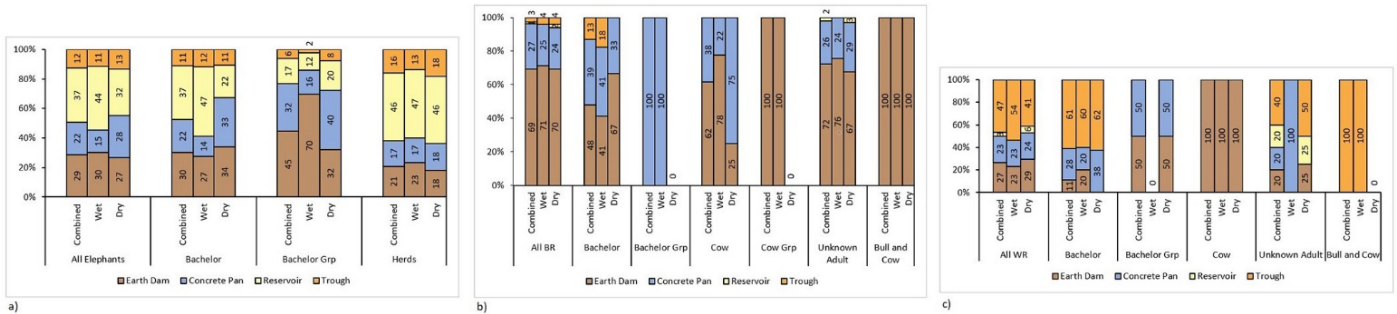


Figure 5: Combined wet and dry season, wet season and dry season proportional use of earth dams, concrete pans, reservoirs and troughs by a) all elephants, bachelors, bachelor groups and herds; b) all black rhinos, bachelors, bachelor groups, solitary cows, cow groups, unknown adults, and bull and cow groups; and c) all white rhinos, bachelors, bachelor groups, solitary cows, unknown adults and bull and cow groups

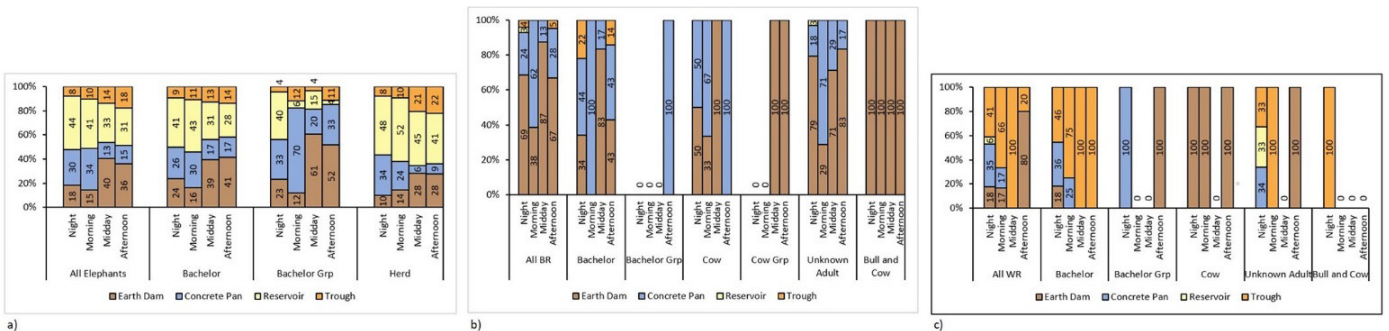


Figure 6: Night, morning, midday and afternoon period proportional use of earth dams, concrete pans, reservoirs and troughs by a) all elephants, bachelors, bachelor groups and herds, b) all black rhinos, bachelors, bachelor groups, solitary cows, cow groups, unknown adults and bull and cow groups, and c) all white rhinos, bachelors, bachelor groups, solitary cows, unknown adults and bull and cow groups

Daily period (night, morning, midday and afternoon) proportions of visits to the various waterhole types by the different elephant social group types are depicted in Fig 6A, black rhino social group types in Fig 6B, and white rhino social group types in Fig 6C.

Waterhole type preferences

Elephant binomial test results indicating seasonal and daily-period waterhole type preferences by the different elephant social group types are shown in Table 2.

Binomial test results showing seasonal and daily-period waterhole type preferences by the different black rhino social group types are depicted in Table 3.

Table 4 shows binomial test results to determine preferences for the waterhole types by the different white rhino social group types.

Effects of season, daily period, waterhole size, waterhole type and social group type on frequency of visits

GLM results for the most significant models with the best fit for the three study species are shown in Table 5.

Discussion

Elephants at OWNR consistently visited waterholes throughout the day, particularly between 09:00 and 20:00 during the dry season, and between 09:00 and 21:00 during the wet season. (Fig 4A). These patterns align with another study conducted in the southern region of Kruger National Park [48]. The midday period, particularly around 11:00 to 12:00, emerged as the time of highest visitation to waterholes, coinciding with peak evapotranspiration and the need for cooling [17].

Elephant

Group type	Season and Daily Period	Preferred waterhole	95% CI lower	95% CI upper	Z-Statistic	P
All	Comb Wet & Dry	Reservoirs	33.72	40.66	7.78	< 0.001
	Wet	Reservoirs	37.81	48.02	8.02	< 0.001
	Dry	Reservoirs	27.05	36.49	3.03	0.002
	Night	Reservoirs	37.79	50.13	7.06	< 0.001
	Morning	Reservoirs	30.72	54.14	3.54	< 0.001
	Midday	Earth dams	33.82	49.98	5.30	< 0.001
	Afternoon	Earth dams	29.13	43.37	3.47	< 0.001
Bachelor	Comb Wet & Dry	Reservoirs	30.77	41.86	4.50	< 0.001
	Wet	Reservoirs	39.45	54.83	6.69	< 0.001
	Dry	Earth dams	26.07	42.84	2.41	0.016
	Night	Reservoirs	32.70	50.15	4.29	< 0.001
	Morning	Reservoirs	27.10	60.51	2.56	0.010
	Midday	Earth dams	28.04	51.75	2.81	0.005
	Afternoon	Earth dams	29.44	54.44	3.08	0.002
Bachelor Grps	Comb Wet & Dry	Earth dams	35.75	53.22	5.16	< 0.001
	Wet	Earth dams	53.88	82.82	6.78	< 0.001
	Dry	Concrete pans	29.81	50.78	3.29	0.001
	Morning	Concrete pans	44.04	89.69	4.34	< 0.001
	Midday	Earth dams	47.44	73.45	6.39	< 0.001
	Afternoon	Earth dams	31.95	71.33	3.22	0.001
Breeding herds	Comb Wet & Dry	Reservoirs	40.50	51.57	8.83	< 0.001
	Wet	Reservoirs	38.79	54.60	6.38	< 0.001
	Dry	Reservoirs	37.65	53.09	6.11	< 0.001
	Night	Reservoirs	38.44	58.67	5.46	< 0.001
	Morning	Reservoirs	36.42	68.00	4.10	< 0.001
	Midday	Reservoirs	34.63	55.28	4.48	< 0.001
	Afternoon	Reservoirs	31.41	52.12	3.69	< 0.001

Table 2:

Elephant binomial test results indicating seasonal and daily-period waterhole type preferences by the different elephant social group types. Only results indicating a preference (lower 95% CI greater than 25% and significant) are shown

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Black rhino

Group type	Season and Daily Period	Preferred waterhole	95% CI lower	95% CI upper	Z-Statistic	P
All	Comb Wet & Dry	Earth dams	59.50	77.73	10.55	< 0.001
	Wet	Earth dams	59.44	81.23	9.12	< 0.001
	Dry	Earth dams	46.49	80.26	5.35	< 0.001
	Night	Earth dams	54.59	81.75	7.18	< 0.001
	Morning	Concrete pans	31.58	86.14	3.04	0.002
	Midday	Earth dams	67.64	97.34	7.07	< 0.001
	Afternoon	Earth dams	43.04	85.41	4.41	< 0.001
Bachelor	Comb Wet & Dry	Earth dams	26.80	69.39	2.53	0.012
	Midday	Earth dams	35.87	99.58	9.92	< 0.001
Cow	Comb Wet & Dry	Earth dams	31.54	86.12	3.04	0.002
	Wet	Earth dams	39.99	97.19	3.66	0.000
	Dry	Concrete pans	28.64	98.14	8.88	< 0.001
	Midday	Earth dams	47.82	100.00	10.95	< 0.001
Cow Group	Comb Wet & Dry	Earth dams	29.24	100.00	3.00	0.003
	Wet	Earth dams	29.24	100.00	3.00	0.003
Unknown adult	Comb Wet & Dry	Earth dams	58.35	83.54	8.01	< 0.001
	Wet	Earth dams	57.79	88.94	6.74	< 0.001
	Dry	Earth dams	43.07	85.44	14.66	< 0.001
	Night	Earth dams	62.10	91.30	22.44	< 0.001
	Morning	Concrete pans	29.02	96.32	9.10	< 0.001
	Midday	Earth dams	29.02	96.32	9.10	< 0.001
	Afternoon	Earth dams	35.87	99.58	9.92	< 0.001
Bull & cow	Comb Wet & Dry	Earth dams	73.54	100.00	6.00	< 0.001
	Wet	Earth dams	66.37	100.00	5.20	< 0.001
	Dry	Earth dams	29.24	100.00	8.49	< 0.001
	Midday	Earth dams	54.07	100.00	12.00	< 0.001
	Afternoon	Earth dams	39.76	100.00	9.80	< 0.001

Table 3:

Binomial test results indicating seasonal and daily-period waterhole type preferences by the different black rhino social group types. Only results indicating a preference (lower 95% CI greater than 25% and significant) are shown

This finding is comparable to that of Hayward & Hayward [21] for their study across various parks and reserves, including Kruger National Park, Pilanesberg National Park, Madikwe Game Reserve, Tembe Elephant Park and Botswana’s Mashatu Game Reserve. In the OWNR daily temperatures are generally high throughout the day, which is common in the lowveld of southern Africa, and only decrease in the evenings.

White rhino

Group type	Season and Daily Period	Preferred waterhole	95% CI lower	95% CI upper	Z-Statistic	P
All	Comb Wet & Dry	Troughs	28.34	65.68	2.74	0.010
	Wet	Troughs	25.14	80.78	2.40	0.016
	Afternoon	Earth dams	28.36	99.59	2.84	0.005
Bachelor	Comb Wet & Dry	Troughs	35.73	82.69	12.36	< 0.001
	Wet	Troughs	26.24	87.84	9.04	< 0.001
Cow	Comb Wet & Dry	Earth dams	39.76	100.00	9.80	< 0.001
	Dry	Earth dams	29.24	100.00	8.49	< 0.001

Table 4:

Binomial test results indicating seasonal and daily-period waterhole type preferences by the different white rhino group types. Only results indicating a preference are shown (lower 95% CI greater than 25% and significant)

Coefficients	β	Z value	CI	Pr(> z)
White Rhino				
Afternoon Period (DailyPeriod*GroupType)	1.003	-1.977	-3.95:1.94	0.05
Dry Season:Afternoon Period (Season*DailyPeriod)	2.043	2.516	0.45:3.63	0.01
Black Rhino				
Unknown Adults (WaterholeType*GroupType)	1.229	3.640	0.57:1.89	< 0.00
Unknown Adults (Period*GroupType)	1.269	3.362	0.53:2.01	< 0.00
Large Waterholes (DailyPeriod*WaterholeSize)	0.700	2.040	0.03:1.37	0.04
Unknown Adults (Season*GroupType)	1.253	2.706	0.35:2.16	0.01
Elephant				
Medium Waterholes (WaterholeSize*GroupType)	0.401	3.046	0.14:0.66	< 0.00
Reservoir (WaterholeType*GroupType)	0.706	5.042	0.43:0.98	< 0.00
Reservoir:Breeding Herd (WaterholeType*GroupType)	0.676	3.379	0.28:1.07	< 0.00
Trough:Breeding Herd (WaterholeType*GroupType)	0.978	3.533	0.44:1.52	< 0.00
Morning Period:Bachelor Groups (Period*GroupType)	1.028	2.859	0.32:1.73	< 0.00
Midday Period:Bachelor Groups (Period*GroupType)	1.247	4.668	0.72:1.77	< 0.00
Medium Waterholes (Period*WaterholeSize)	0.303	2.253	0.04:0.57	0.02
Midday Period:Large Waterholes (Period*WaterholeSize)	1.131	4.202	0.60:1.66	<0.00
Afternoon Period:Large Waterholes (Period*WaterholeSize)	1.090	3.947	0.55:1.63	< 0.00
Midday Period (Period*WaterholeType)	0.490	2.761	0.14:0.84	0.01
Concrete Pan (Period*WaterholeType)	0.996	5.431	0.64:1.36	< 0.00
Reservoir (Period*WaterholeType)	1.385	8.057	1.05:1.72	< 0.00
Wet Season (Season*GroupType)	0.319	2.755	0.09:0.55	0.01
Breeding Herd (Season*GroupType)	0.253	2.181	0.03:0.48	0.03
Wet Season:Medium Waterholes (Season*WaterholeSize)	0.549	3.266	0.22:0.88	< 0.00
Concrete pan (Season*WaterholeType)	0.515	3.784	0.25:0.78	<0.00
Reservoir (Season*WaterholeType)	0.626	4.719	0.37:0.89	< 0.00
Wet Season:Reservoir (Season*WaterholeType)	0.408	2.279	0.06:0.76	0.02

β = beta value, CI = Confidence Interval, Pr(>|z|) = significance

Table 5:
Results for a series of GLM's showing the models with the best fit for White rhino, Black rhino and Elephant

Findings for a study done in Hwange National Park, Zimbabwe [20] indicated peak utilisation of waterholes by elephant at around 20:00, which matches a secondary peak we observed for the wet season. Limited surface water availability in Hwange National Park [20] could be a reason for elephants utilising waterholes less frequently and likely having a greater dependence on alternative thermoregulatory methods [17]. While elephants are generally considered to have no preference for drinking at a specific time [33], our findings suggest that probably due to their large body mass and associated water loss during hotter parts of the day, elephants tend to visit waterholes more frequently between late morning and early evening (09:00 and ~21:00).

In this study, elephants exhibited a clear preference for reservoirs throughout the year. This is likely because reservoirs have high concrete sides and bottoms, are filled with borehole water, and are often out of reach of other game species that could pollute the water. Other waterhole types are more likely to be contaminated with faecal matter and associated bacteria [23]. The avoidance of surface water with high bacteria levels has been substantiated by studies showing that elephants will rather dig for water to drink than use polluted surface water [49, 50]. This preference differs from the broader findings of Du Toit [33], who indicated an overall preference for natural waterhole types like earth dams. We did, however, find that elephants at the study site used earth dams at midday and in the afternoon for bathing or lying down in water to cool down during the hot daily temperatures of the wet season [32]. Reservoirs were preferred at night and in the morning, and earth dams at midday and in the afternoon (Table 2).

The behaviour of bachelor elephant groups differed from that of breeding herds, indicating potential social dynamics. Bachelor elephants preferred reservoirs in the wet season and earth dams in the dry season. Bachelor groups preferred earth dams in the wet season and concrete pans in the dry season. Breeding herds consistently preferred reservoirs throughout both seasons across all daily periods (Figs 5A and 6A). This suggests that female herd members may not tolerate bachelor groups, aligning with documented behaviour in the literature [34, 51]. Bachelor groups, composed of young males testing their strength, could pose a risk of injury to young elephants within

herds [32, 34, 51]. Solitary mature bachelor elephants often linger near herds, waiting for mating opportunities with herd cows [34]. These lone bachelors showed a noticeable preference for reservoirs at certain times of the year and day (Table 2), roughly coinciding with the times that herds visited reservoirs [32, 34]. Although reservoirs were visited mostly at night, test results indicated no preference for a particular waterhole type at night (Table 2). Concrete pans were preferred in the morning, while earth dams were favoured at midday and in the afternoon.

The results of the generalised linear models (GLMs) to determine the effects of Season, Daily Period, Waterhole Size, Waterhole Type, and social Group Type visiting waterholes on frequency of elephant visits to the waterholes revealed significant effects for various of these factors (Table 5). The main waterhole types selected at the study site were reservoirs and concrete pans. Waterholes were used most during the wet season, with a preference for medium-sized waterholes. Breeding herds were the most frequently observed group visiting waterholes, favouring medium-sized reservoirs and their troughs (Table 1). Adult and sub-adult animals that could reach water in reservoirs used these, while juvenile animals used troughs. Bachelor groups were commonly observed at waterholes during morning and afternoon periods. Overall, waterholes were most visited during midday, with reservoirs and concrete pans being the most frequented. Medium-sized waterholes were preferred across all daily periods, with large waterholes being used during midday and afternoon. Breeding herds, consisting of females and their young, may prioritise safety and the availability of clean water sources, leading to a consistent preference for reservoirs. Bachelor groups, composed of young males, may select waterhole types based on factors such as opportunities for interaction with breeding herds or their own social dynamics.

Black rhinos displayed different patterns in their waterhole visits compared to elephants. They visited waterholes across all daily periods, with specific peaks during the dry and wet seasons. Dry season peaks were between 02:00 and 03:00, at 06:00, between 13:00 and 15:00, and again at 21:00 (Fig 4B). Wet season peaks were at 10:00, 13:00, and again at 18:00 (Fig 4B). These results align with the findings of Du Toit [33], who found that black rhinos drink throughout the day and night depending on environmental conditions and water availability, though further research is needed to clarify potential seasonal variations in this behaviour. They also align with previous studies conducted in different arid and semi-arid areas [52, 53]. In arid areas, black rhinos drink when it is cooler, often in the evenings or during the day on overcast days [52, 53]. Kasiringua, Kopij, & Procheş [52] reported that for the arid Waterberg National Park in Namibia, black rhinos drink at night during the dry season, where these authors classified them as evening and night drinkers. Seidel et al. [53] found that for the semi-arid Etosha National Park in Botswana, black rhinos visited waterholes after dusk and before dawn. OWNR is not classified as an arid environment with extreme daily temperatures, resulting in the black rhino on the reserve visiting waterholes throughout the day and night, but with peaks as listed above.

Findings for this study indicate that black rhinos exhibited a preference for earth dams throughout the year (Fig 5B). Du Toit [33] reported a preference for artificial waterhole types, although the specific types were not detailed. Earth dams were favoured at night, midday, and in the afternoon, while concrete pans were preferred in the morning (Fig 6B and Table 3). Bachelor black rhinos showed a preference for earth dams in the dry season and no preference for any waterhole type in the wet season (Fig 5B). They preferred this waterhole type during the midday period, with no preference during other daily periods. Even though black rhino bachelor groups visited concrete pans in the wet season during the afternoon period, binomial test results (Table 3) did not indicate a preference for this waterhole type. There were no recorded visits to any of the waterhole types for the dry season,

leading us to believe that bachelor groups visited alternative water sources that did not have camera traps monitoring them. Solitary black rhino cows preferred earth dams in the wet season and concrete pans in the dry season. There was a preference for earth dams during the midday period, and although they visited concrete pans in the morning and afternoon, binomial test results did not indicate a preference for this waterhole type (Table 3). Black rhino cow groups favoured earth dams in the wet season, with no visits recorded for the dry season. As for black rhino bachelor groups, we speculate that black rhino cow groups visited alternative water sources that did not have camera traps monitoring them. While there were visits to earth dams during the midday and afternoon periods, and results were significant, lower confidence intervals were below the 25% chance threshold. There were no recorded visits to waterholes by cow groups during the night and morning periods. The “unknown adult black rhino” category preferred earth dams across both the wet and dry seasons (Fig 5B). Earth dams were favoured at night, midday and in the afternoon, with concrete pans preferred in the morning (Fig 6B). Black rhino bull and cow groups preferred earth dams across both the wet and dry seasons and across all daily periods. We speculate that bachelor black rhino groups at the study site, often consisting of sub-adult bulls, used concrete pans to avoid confrontation with aggressive territorial bulls who were often with cows at earth dams. GLM results (Table 5) suggest that unknown black rhino adults preferred large ($P = 0.04$) earth dams ($P < 0.00$) in both the wet and dry seasons ($P = 0.01$) for three of the daily periods, midday, afternoon and night ($P < 0.00$).

White rhinos displayed specific patterns in their waterhole visits, with peaks during certain daily periods and variations between the wet and dry seasons. White rhino visits during the dry season peaked at 08:00 and between 16:00 and 18:00 (Fig 4C). Sutherland, Ndlovu, & Pe´rez-Rodríguez [48] recorded peaks in the dry season from 00:00 to 01:00, from 11:00 to 12:00 and a spike in visits between 17:00 and 22:00. Wet season waterhole visits for our study peaked at 09:00, 18:00 and again at 21:00. Wet season visits for the Sutherland, Ndlovu, & Pe´rez-Rodríguez [48] study showed a peak at 17:00 and from 20:00 to 22:00. Du Toit [33] states that white rhinos visit waterholes in the morning, at midday and in the afternoon. Kasiringua, Kopij, & Procheş [52], for the arid Waterberg National Park in Namibia, classified white rhino as evening and night drinkers. In general, our study has similar results to that of Sutherland, Ndlovu, & Pe´rez-Rodríguez [49] for the wet season, in that there are three peaks (Fig 4C) and differs from Du Toit [33] in that we recorded night visits (Fig 6C). Our results also differ from those of Kasiringua, Kopij, & Procheş [52] in that we recorded morning visits (Fig 6C).

The sample size for the white rhino in this study was restricted due to a lack of suitable habitat at the study site, compared to the elusive black rhino, which prefers dense thickets. Although white rhinos visited troughs during both the wet and the dry seasons (Fig 5C), binomial test results only indicate a preference for this waterhole type during the wet season (Table 4). Du Toit [33] found that white rhinos had no preference for a particular waterhole type, whereas we found a preference for troughs and earth dams. Earth dams were preferred during the afternoon, and even though troughs were frequented at night, in the morning and at midday, results did not indicate a preference for troughs during these periods (Table 4). Bachelor white rhinos preferred troughs across both the wet and dry seasons, and although troughs were visited across all daily periods, binomial test results did not indicate a preference for them, or any other waterhole type (Table 4). Bachelor white rhino groups visited earth dams and concrete pans during the dry season, with no visits recorded for the wet season. Test results showed no seasonal or daily period preferences for any waterhole types by bachelor groups (Table 4). Cows visited only the earth dams across the wet and dry seasons (Fig 5C). Earth dams were visited by cows during the night, morning and afternoon periods, with no recorded

visits at midday. Cows showed a preference for earth dams in the dry season. Unknown adults mostly visited troughs during the dry season and concrete pans during the wet season (Fig 5C). Concrete pans were visited at night, troughs in the morning and earth dams in the afternoon (Fig 6C). There were no recorded visits during the midday period. The unknown adult category showed no seasonal or daily preferences for any of the waterhole types. The bull and cow category visited troughs most during the combined wet and dry seasons, and during the wet season. Troughs were visited most at night with no recorded visits to waterholes for the other daily periods (Fig 6C). Binomial test results for bull and cow groups showed no seasonal or daily preferences for any of the waterhole types. No visits to waterholes were recorded for the dry season, or the morning, midday and afternoon periods. As for the black rhino, we believe that white rhino bachelor groups consisting primarily of sub-adults avoided the waterhole type (troughs) that territorial bulls frequented with cows. Territorial bulls are aggressive and do not tolerate other bulls within their territories [32, 34].

GLM results for white rhino (Table 5), indicate that the interaction between the dry season and the afternoon period ($P = 0.01$) led to an increase in frequency of visits to waterholes, and that White rhino in the study area visited waterholes most during the afternoon period ($P = 0.05$). White rhinos frequent open grassland areas and avoid areas with high densities of woody plant species, selecting the predominant waterhole types found in these areas, which are troughs and earth dams [32, 34].

Simultaneous use of waterholes by young elephant bachelor groups, black rhinos and white rhinos in areas where there are no large adult elephant bulls has been problematic in the past [51]. Several white rhino deaths were recorded in Pilanesberg National Park when young elephant bulls intimidated rhinos at waterholes in episodes of what were observed as young bachelor elephants trying to mate with rhinos [51]. This behaviour stopped as soon as large elephant bulls were introduced into the area [51, 54]. In terms of our findings that indicate both elephant bachelor groups and rhinos visit and prefer earth dams and concrete pans, it is important to ensure that a proper mature elephant bull hierarchy is in place for areas that contain bachelor groups of elephants and rhinos.

Although there was spatial and temporal overlap at waterholes in this study, the interaction between the three study species at the different waterhole types for OWNR was limited due to their various social groupings preferring different waterhole types at different times of the day. Elephants spent 37% of their time at reservoirs (Fig 5A) and had exclusive use of this waterhole type, as neither of the rhino species could access water in reservoirs. Black rhinos spent 69% of their time at earth dams (Fig 5B), which could be related to these waterholes being in relatively woody areas, which are the preferred habitat for black rhinos [32, 34]. White rhinos spent 47% of their time at troughs (Fig 5C), suggesting that they, like the black rhino, prefer the waterhole types placed within their preferred habitat, which is relatively open areas dominated by grass. Both black and white rhinos are territorial, which resulted in sub-adult bachelor groups using waterholes at different times than bull and cow groups, to prevent confrontation.

The differences in waterhole preferences between elephants and rhinos can be attributed to their distinct ecological and physiological characteristics. Elephants, with their large body size and high water requirements, are more inclined to select waterholes that provide clean water and opportunities for cooling and bathing. The preference for reservoirs may also be influenced by the presence of other game species that inhabit natural waterholes, making reservoirs a more appealing

option for elephants seeking undisturbed water sources. On the other hand, black rhinos, known for their ability to survive in arid and semi-arid environments, exhibited a consistent preference for earth dams across seasons. This preference may be due to factors such as the availability of water and the avoidance of potential competitors or predators that frequent other waterhole types. White rhinos, being grazers and relying on open grasslands, showed a preference for troughs during the dry season, likely due to the concentrated availability of water in these artificial structures.

Conclusion

Artificial waterholes play an important role in supporting wildlife populations in conservation areas [55]. This study investigated the preferences of elephants, black rhinos, and white rhinos for various artificial waterhole types in the South African savannah. Four waterhole types were examined: earth dams, concrete pans, reservoirs, and troughs. Findings revealed that each species exhibits distinct preferences and utilisation patterns. Elephants favoured reservoirs, earth dams, and concrete pans, with breeding herds showing a particular preference for reservoirs and troughs. Black rhinos preferred large waterholes like earth dams, while white rhinos showed preferences for troughs and earth dams.

Understanding the preferences of the study species is important for effective waterhole management and conservation. By identifying the specific waterhole preferences of these species, conservationists and reserve managers can optimise the design and placement of waterholes, reducing competition and promoting the well-being of both target and non-target species [20, 35, 36]. Identifying which species prefer which waterhole types can help prevent conflicts and inform strategic waterhole placement [12, 20].

As natural surface water diminishes during the dry season, artificial waterholes become essential for the behaviour and survival of various species [3, 21, 35, 36]. Information from this study can guide water provision and translocation strategies for the study species. Effective management of artificial waterholes is key to enhancing habitat use and ensuring the long-term viability of species utilising these waterholes [51, 54]. Given the poaching pressures the three study species face throughout their range [28–31], implementing efficient conservation plans requires careful consideration of artificial waterhole preferences.

Future research should explore additional factors influencing waterhole selection, including water quality, anthropogenic effects, and seasonal variations. Broader studies across different ecosystems will further refine conservation strategies and enhance our understanding of the study species' needs. Furthermore, investigating waterhole preferences in different ecosystems and regions will enhance our understanding of the study species and contribute to the development of effective conservation strategies. **V**

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References available on request.

Find Rhinos without Finding Rhinos: Active Learning with Multimodal Imagery of South African Rhino Habitats



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ABSTRACT

Much of Earth's charismatic megafauna is endangered by human activities, particularly the rhino, which is at risk of extinction due to the poaching crisis in Africa. Monitoring rhinos' movement is crucial to their protection, but has unfortunately proven difficult because rhinos are elusive. Therefore, instead of tracking rhinos, we propose the novel approach of mapping communal defecation sites, called middens, which give information about rhinos' spatial behaviour valuable to antipoaching, management, and reintroduction efforts. This paper provides the first-ever mapping of rhino midden locations by building classifiers to detect them using remotely sensed thermal, RGB, and LiDAR imagery in passive and active learning settings. As existing active learning methods perform poorly due to the extreme class imbalance in our dataset, we design Multimodal, an active learning system employing a ranking technique and multimodality to achieve competitive performance with passive learning models with 94% fewer labels. Our methods could therefore save over 76 hours in labelling time when used on a similarly sized dataset. Unexpectedly, our midden map reveals that rhino middens are not randomly distributed throughout the landscape; rather, they are clustered. Consequently, rangers should be targeted at areas with high midden densities to strengthen anti-poaching efforts, in line with UN Target 15.7.

Introduction

Vertebrates are going extinct 100x faster than before the Anthropocene, indicating that the Earth is undergoing the sixth mass extinction [Ceballos *et al.*, 2015]. Among the human threats to biodiversity, poaching has endangered myriad species, particularly the rhino. The poaching epidemic is a barrier to achieving the United Nations' Fifteenth Sustainable Development Goal: Life on Land [UN, 2023b]. In particular, Target 15.7 calls for "urgent action to end poaching and trafficking of protected species of flora and fauna" [UN, 2023b]. Poaching, as well as habitat loss, has exterminated African rhinos across much of their historical range [WWF, 2023].

In South Africa, one of the rhino's last strongholds, poaching has driven a 59% decline in Kruger National Park's rhino population since 2013 [Save the Rhino International, 2022]. As rhinos have disproportionately large impacts on ecosystem structure and function due to their role as megaherbivores [Ferreira *et al.*, 2015], it is imperative to strengthen efforts to protect them. Protecting endangered species like the rhino involves studying their patterns of habitat use [Johnson and Gillingham, 2005], but this is challenging due to rhinos being reclusive and dangerous to observe in the wild [Linklater *et al.*, 2013]. Moreover, efforts to study rhinos are often constrained by the limited human and financial resources available in the African savanna landscapes rhinos inhabit, which can be large and inaccessible [Anderson and Gaston, 2013]. Rather than study rhinos directly, we propose for the first time to use rhino middens, depicted in Figure 1, to improve our understanding of rhinos' spatial behaviour. Middens are communal defecation sites used for territorial marking and social communication [Owen-Smith and Smith, 1973]. Thus, understanding midden spatial patterning is a noninvasive means for gaining insight into rhino locations and movement, providing conservation practitioners with spatial information key to effective poaching prevention, management efforts, and reintroduction plans for rhinos.

Unfortunately, rhino midden locations have not yet been mapped because they are distributed throughout huge areas. Manually locating all the middens from the ground is practically impossible, and whether drones can be used for this purpose is untested. Against this background, this paper provides the first results from harnessing remotely sensed data to detect rhino middens along with the first spatial map of middens. Middens are considerably easier to detect in remotely sensed imagery than the elusive rhinos themselves, as the former are stationary and larger.



Figure 1:

Left: A white rhino midden next to a road in iMfolozi [Marneweck, 2013]. Right: A midden in our dataset photographed by a drone. Middens boxed in green

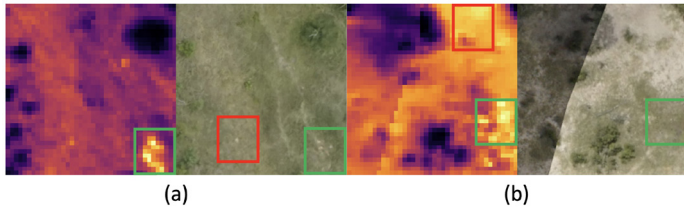


Figure 2:

Each pair shows the thermal (left) and RGB (right) images of the same area containing a midden. Green boxes outline middens. Red boxes outline areas that falsely appear to be middens. In (a), the midden is more obvious in the thermal image than in the RGB image, and in (b), the reverse is true

While remote sensing technology is increasingly being used in ecological research [Marvin *et al.*, 2016], the rate at which large volumes of data are collected often outpaces processing and analysis, preventing crucial insights from being gained rapidly and at scale [Tuia *et al.*, 2022].

To overcome this challenge, we develop machine learning models utilising thermal (heat), RGB (colour), and LiDAR (light detection and ranging) imagery of a site in Kruger National Park with identified middens. We believe all three modalities can play an important role in rhino midden detection. Due to their warm temperature, middens often show up as bright areas in thermal imagery, as seen in the left image of Figure 2(a). In RGB imagery, middens often appear brown, as seen in the right image of Figure 2(b). LiDAR imagery is expected to be more helpful in other sites with high numbers of termite mounds because, although both middens and mounds tend to be warm, the latter often have a higher slope.

First, we consider whether *passive* (i.e., supervised) deep learning techniques are able to detect rhino middens in multimodal imagery. Second, we determine which data modalities and combinations thereof are most informative for automatic midden detection efforts, which is salient because of the limited resources available for conservation and ecosystem monitoring. However, due to geographic differences between ecological sites, a deep learning model that performs well on one site may not perform well on another [Beery *et al.*, 2018], creating a cumbersome labelling burden. Our third contribution is therefore to develop active learning methods that strategically select images to be labelled by an expert in order to find rhino middens in an unlabelled dataset in which most images are empty. The goal is to reach an accuracy that competes with passive learning methods despite having far fewer labelled data points. However, when the dataset has extreme class imbalance, predominant active learning methods [Lewis and Gale, 1994; Kellenberger *et al.*, 2019] are unlikely to query rare positive samples, impeding the model's learning. To overcome this challenge, we introduce the Multimodal active learning system, which leverages information about the signal of interest to rank the instances. We then prioritise querying those most likely to be rare positive samples, accelerating the model's learning. Within Multimodal, we also introduce an ensemble active learning strategy that dynamically weights the predictions from several models to query instances more likely to be positive samples. Our methods apply to the general problem of identifying a rare signal of interest about which we have knowledge in an imbalanced dataset for which complete annotation is impractical.

We train and evaluate our methods using 9,772 images of a site in Kruger National Park captured in three modalities in collaboration with South African National Parks. We perform image classification to identify midden and non-midden images and map middens geographically for the first time. We train a passive neural network on each of our data modalities (thermal, RGB, and LiDAR) as well as on fused combinations of these data types. For the middens in this site, thermal imagery is the most informative, RGB provides a slight boost in accuracy when fused with thermal, and

fusing thermal with LiDAR improves recall. Next, we design and implement a novel multimodal active learning system, Multimodal, that exploits the fact that middens are warm. We compare the performance of our query strategies against several standard baselines. Multimodal achieves statistically identical performance to the best passive learning model with 94% fewer labels, greatly easing the labelling burden on domain experts. Finally, mapping the rhino middens in this site reveals that they are not distributed randomly across the region but rather form clusters, so ranger patrols ought to be targeted at the areas with high midden densities. Thus, we have provided actionable information for rhino conservation as a result of our endeavour to map rhino middens rather than rhinos directly, and our methods facilitate scaling these insights to additional rhino habitats.

Related Work

We discuss related multimodal deep learning and active learning methods in this section. Several studies have fused thermal and RGB data to improve the performance of deep learning models. Alexander *et al.* [2022] and Speth *et al.* [2022] utilised thermal and RGB fusion in a deep learning method to detect cracks in civil infrastructure and locate civilians in disaster zones, respectively. In our setting, we consider fusions of thermal, RGB, and LiDAR imagery. While the above works consider only passive learning settings, we also investigate multimodality in active learning environments. For our active learning models, we both evaluate performance when fusing several types of imagery beforehand and when allowing distinct models trained on different image modalities to form a “committee” for the active learning system. Active learning algorithms are generally distinguished by their strategy for evaluating how informative an unlabelled sample is [Settles, 2009]. One of the most common active learning techniques is uncertainty sampling [Lewis and Gale, 1994], wherein the model requests labels for the images about which it is most uncertain. This method does not explicitly prioritise a particular class, so it is not designed to find extremely rare positive samples in a highly imbalanced dataset. In order to address this mismatch, Kellenberger *et al.* [2019] introduced positive certainty sampling, instead prioritising images for labelling that are likely to be positive samples. Both uncertainty and positive certainty sampling were designed for a single data modality. Zhang *et al.* [2021] developed an active learning algorithm for thermal and RGB data that prioritises images that are classified differently by the separate thermal and RGB models.

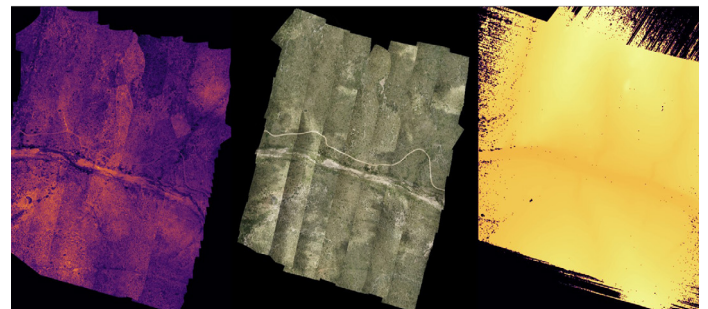


Figure 3:

Thermal (left), RGB (middle), and LiDAR (right) orthomosaics comprising the dataset under study

While this method accommodates two data modalities, like uncertainty sampling, it is not designed for imbalanced datasets. We introduce a form of multimodal positive certainty sampling, which prioritises images that an ensemble of models (one for each modality) determines are likely rare positive samples. Because of the difficulty in identifying these rare positive samples, we need to make the query method as powerful as possible. An example of a method that uses expert knowledge to identify images likely to contain an object of interest is described in De Oliveira and Wehrmeister

Article

[2016], which uses the known human body temperature to identify people in thermal images. This technique is not, however, used within an active learning algorithm. While De Oliveira and Wehrmeister [2016] knew the human body temperature with reasonably high precision, we face the challenge of not knowing the temperature of rhino middens. We do know, however, that they are often warmer than the surrounding ground and vegetation. Thus, in our active learning system, we prioritise images for labelling that have higher pixel values in the thermal band. With this method, the system is able to more quickly learn to distinguish between middens and non-middens.

Setup

Data

The remote sensing data used for this project was collected by a DJI M600 multicopter flown over a 284-hectare site in Kruger National Park in January 2020. This drone was equipped with an animal landscape observatory sensor package, consisting of a FLIR Tau-2 thermal camera, a Sony A6000 camera, and a Riegl VUX-1LR LiDAR scanner, which simultaneously collected thermal, RGB, and LiDAR data, respectively, throughout the drone's flight. The thermal imagery was rectified and mosaicked at a resolution of 0.5 m, the RGB imagery at 0.05 m, and the LiDAR imagery at 0.25 m, yielding the orthomosaics shown in Figure 3.

The ecologists on our team identified candidate middens in the thermal and RGB orthomosaics and confirmed their presence on the ground, yielding a list of the x and y coordinates of the centres of 52 rhino middens. We mapped these middens onto the orthomosaics and then cropped them using an interval of 20 m (40 pixels for thermal, 400 for RGB, and 80 for LiDAR) and a stride of 5 m (10 pixels for thermal, 100 for RGB, and 20 for LiDAR). Each cropped image was assigned a label of 1 if it contained the centre of a midden and 0 otherwise. We downshifted the pixel values of each thermal image such that the cropped thermal images all had a minimum of 0 to enable a meaningful comparison among them. After removing images with all zeros in either the thermal or RGB bands, we were left with 89 images with middens and 9,683 empty images, which means that our dataset has 9,772 images (in each modality), 0.91% of which have a midden. We fused the images using the blend function in the PIL class, yielding fusions of thermal and RGB, thermal and LiDAR, RGB and LiDAR, and thermal, RGB, and LiDAR images, with each data modality weighted equally.

Model

We employ transfer learning with a VGG16 model pretrained on the ImageNet dataset [Simonyan and Zisserman, 2015]. We freeze all the parameters in the model except for those in the classifier. We alter the final linear layer to have a single output feature and then end with a sigmoid function so that the output of the model represents the probability that an image contains a midden. For all our models, we use a batch size of 10, the Binary Cross Entropy loss function in PyTorch, and an Adam optimiser with a learning rate of 0.0001.

Active Learning Methodology

Multimodal Algorithm

Active learning aims to reduce the number of instances that need to be labelled to train a model by requesting labels for those that are most useful for its learning. The general procedure works as follows: (i) a small batch of labelled instances is used to begin training a model, (ii) the model then uses some criteria to select the next batch of instances to be labelled, typically those about which the model is least certain [Lewis and Gale, 1994], (iii) this process continues until a labelling budget is reached, and (iv) the trained model can then be used for inference on the remaining unlabelled instances.

Problematically, however, traditional active learning approaches can have poor performance in the presence of severe class imbalance. To address this challenge, our active learning algorithm, Multimodal, is designed to detect as many of the rare positive samples as possible in each round. To achieve this, rather than have the model predict on the entire training dataset as is done in many typical active learning systems, we propose constraining the set of instances on which the model predicts through a novel technique that exploits some characteristic of the object of interest that can be used for ranking. Furthermore, we propose a dynamic method for combining the outputs of several models trained on different data modalities to further speed up learning.

We first describe our method assuming a single data modality, diagrammed in Figure 4. We assume each instance in the dataset can be assigned a value corresponding to a *metric* (e.g. temperature, colour, etc.) that is associated with the desired rare signal of interest. We then rank all the instances by the distance between their value of the informative metric and a specified *target* value (e.g. human body temperature, colour of grass, etc.) characteristic of the desired signal (top left box of Figure 4). Once the images are ranked, we select a subset to be labelled. Let b be the size of a batch of images selected by the active learning system for labelling. (1) To produce each of the batches, we first compute the output of the model on the sample with the highest ranking out of those remaining unlabelled. To reflect the uncertainty captured in the model's output, we do not simply assign the instance the highest probability class.

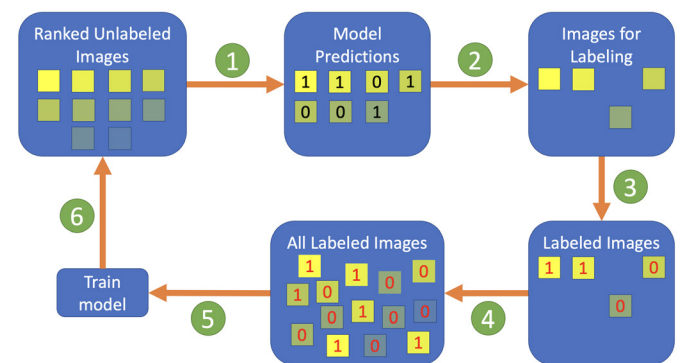


Figure 4:

Active learning cycle where the images are ranked by their brightness. (1) Predict on highest-ranked images. (2) Query images predicted to be positive. (3) Assign labels to queried images. (4) Add the newly labelled images to the set of all labelled images. (5) Train the model on a selection of the labelled images. (6) Restart

Instead, we classify it by randomly sampling a class according to the model's output (i.e., the output specifies the parameters of a multinomial distribution). If the instance is ultimately predicted to be a positive sample, we add it to the batch. We then feed the sample with the next highest ranking to the model and continue this process until we have a batch with b samples predicted by the model to be positive. In this way, we bias the model towards selecting high-ranking images that we know are more likely to be positive instances. (2) Next, the batch is sent to the labeller. (3) The batch is labelled by the annotator and then (4) added to the set of instances queried so far. Because positive samples may be so rare, batches can be imbalanced toward the negative class(es). (5) If all the instances queried so far are negative, then we select all of them for training. If any are positive, we take all of those for training and randomly select an equivalent number of negative instances to get a balanced training set. At this point, the model weights are reset to their initial values to prevent overfitting on a small labelled dataset, and the model is then trained on the selected

instances. (6) This process is repeated until we exhaust the budget on the number of labels that can be provided. Since we are also interested in settings with multiple data modalities, we develop a modification of the above procedure to accommodate an ensemble of models, each of which is trained on its own data modality. Specifically, we modify the way in which the prediction for each instance is calculated. Above, the prediction was simply extracted from the output of a single model, where the output contains the probabilities that the instance belongs to each of the possible classes. In the multimodal setting, we have the outputs of multiple models with possibly differing accuracies, so we want to weight their predictions accordingly.

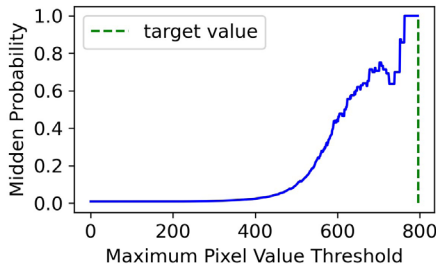


Figure 5: Probability that an image contains a midden tends to increase with its maximum thermal pixel value

In particular, we assign each instance a score for each of the possible classes given by a weighted sum of the models' outputs. If there are M models, then the score for an instance belonging to class i is calculated using Equation 1. Then, as in the unimodal case, the prediction for the instance is obtained by sampling from a multinomial distribution with the class scores as inputs.

$$\text{score}_i = \sum_{m=1}^M \text{weight}_m \times \text{output}_{i,m} \quad (1)$$

$$\text{weight}_m = \frac{\text{correct}_m}{\sum_{n=1}^M \text{correct}_n} \quad (2)$$

After being initialised to $1/M$, the weights are updated in each subsequent round according to the number of queried instances that the models have classified correctly. The weight for model m is given by Equation 2, where correct_m is the number of instances queried so far that were classified correctly by model m . By construction, the weights sum to 1, so the resulting scores can be interpreted as probabilities.

Intuition for Ranking Idea

Having described our active learning algorithm, we now present additional analysis that provides intuition for our choice of ranking metric for our dataset. Note that the following analysis is *not* necessary to use the algorithm and is solely for explanatory purposes.

For our setting, we have chosen the maximum thermal pixel value as the metric to be used for ranking in descending order. This sets the target as the maximum pixel value across all of the thermal images, exploiting our knowledge that the sought-after rhino middens are warm.

To demonstrate that this ranking technique effectively prioritises midden images for labelling, we compute the probability of a thermal image containing a midden given that its maximum pixel value (MPV) is no less than a threshold t .

To calculate this, we first apply Bayes' rule, shown in Equation 3.

$$P(\text{midden} | \text{MPV} \geq t) = \frac{P(\text{MPV} \geq t | \text{midden}) P(\text{midden})}{P(\text{MPV} \geq t)} \quad (3)$$

Let m be the total number of middens and m_t be the number of midden images with MPV no less than t . The first factor in the numerator is then $P(\text{MPV} \geq t | \text{midden}) = \frac{m_t}{m}$. Plugging this into Equation 3 gives Equation 4.

$$P(\text{midden} | \text{MPV} \geq t) = \frac{P(\text{MPV} \geq t | \text{midden}) P(\text{midden})}{P(\text{MPV} \geq t)} \quad (4)$$

We plot Equation 4 in Figure 5, which shows that the probability of an image containing a midden tends to increase as its maximum pixel value nears the target value, demonstrating the utility of the ranking method. More generally, we expect any dataset with an appropriately chosen informative metric and target to obey a similar pattern: the probability of being a positive instance drops with increasing distance from the target value. Our ranking-based active learning query strategy is designed for any such dataset.

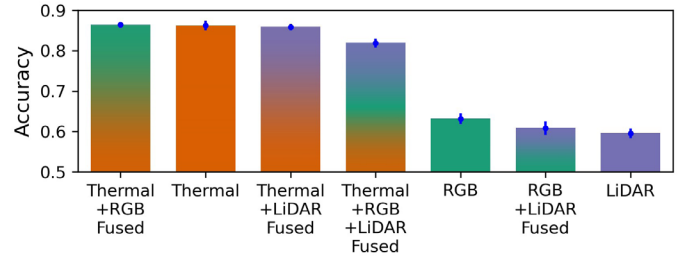


Figure 6: Mean accuracy for the passive models across 30 trials after training for 10 epochs. Models are ranked in descending order by accuracy

Results

In this section, we present the performance of models passively trained on thermal, RGB, LiDAR, and fused imagery and show that the trained models are able to detect middens in a held-out test set with high accuracy. We also display the performance of our MultimodalAL active learning algorithm in comparison to several baselines. We demonstrate our method efficiently selects images for labelling and achieves fast midden retrieval in a large, imbalanced, and initially unlabelled dataset. All error bars in Subsections 5.1 and 5.2 show one standard error of the mean in each direction. All models are trained for 10 epochs, and a threshold of 0.5 is used on the models' sigmoid output for test image classification. Each experiment is run 30 times.

Detecting Middens with Passive Learning

We passively train models with images in different modalities to establish that neural networks are capable of accurately detecting rhino middens in remotely sensed imagery. To train our passive learning models, we assume that the system has access to all of the images' labels from the start. We split this labelled data into training and test sets with the following random selections. First, we add 80% of the midden images (71) to the training set and leave 20% (18) for the test set. We then add 18 empty (non-midden) images to the test set, yielding a balanced test set of 36 images. Of the remaining empty images, we randomly sample 71 and add them to the training set to balance it, yielding a balanced training set of 142 images. For each trial, we train the model on the thermal, RGB, LiDAR, or fused imagery and then record the accuracy on the test set at the end, graphed in Figure 6, where each trial has a different random assignment of images to the training and test sets. We also report the mean and standard errors of the accuracy, precision, recall, and F1 score across the passive trials in Table 1. We observe that the Thermal+RGB Fused model achieves the best performance on accuracy and precision, and the Thermal+LiDAR Fused model achieves the best recall and F1 score. Among the three data modalities, the Thermal model significantly outperforms the RGB and LiDAR models on our dataset.

Thus, based on our results, if resources on the ground are very constrained, it could be helpful to prioritise thermal sensors for midden detection.

Efficient Querying with Active Learning

The passive learning systems achieve high accuracy but require 9,736 labelled images, so such systems *cannot* be used to map middens in other sites where we do not have any of their coordinates beforehand. Moreover, differing topography among sites makes transfer learning challenging. Labelling enough images for passive learning systems to be accurate would take domain experts dozens of hours. In this subsection, we demonstrate that our MultimodalAL algorithm, described in Subsection 4.1, is able to match the best passive learning performance with 94% fewer labelled images by employing an efficient query strategy. While we do have the ground-truth midden locations for this site, enabling us to test various detection methods, we use the results hereinafter as a proof of concept for our system.

	T+R Fused	Thermal	T+L Fused	T+R+L Fused	RGB	R+L Fused	LiDAR
Accuracy	.864± .008	.862± .012	.858± .008	.818± .011	.632± .014	.608± .016	.595± .012
Precision	.875± .010	.846± .014	.826± .011	.823± .012	.629± .015	.616± .020	.632± .023
Recall	.855± .016	.894± .016	.917± .011	.818± .017	.670± .023	.678± .028	.576± .041
F1	.861± .009	.866± .012	.866± .007	.817± .012	.642± .015	.631± .015	.565± .024

Table 1:

Passive learning statistics. T=Thermal, R=RGB, and L=LiDAR. The Thermal+RGB (T+R) Fused model achieves the best performance on accuracy and precision, and the Thermal+LiDAR (T+L) Fused model achieves the best recall and F1 score

For active learning, we use the same dataset and perform the same train-test split procedure as for passive learning, except that we do not balance the training set, so all the empty images not set aside for testing (9,665) are available to the system during training. We test two versions of MultimodalAL. First, MultimodalAL: Thermal+RGB Fused uses the version of our proposed algorithm for a single model trained on the pre-fused thermal+RGB images, and second, MultimodalAL: Thermal + RGB maintains separate models trained on the thermal and RGB images and uses the version of the algorithm that accommodates multiple modalities. In addition to our proposed MultimodalAL algorithm, we implement several baselines: (1) Random: randomly chooses images to be labelled in each round, (2) Uncertainty: requests labels for the images with model outputs closest to 0.5, a very common active learning strategy [Lewis and Gale, 1994], (3) Positive Certainty: requests labels for the images with outputs closest to 1, an active learning strategy more tailored to imbalanced datasets like ours [Kellenberger *et al.*, 2019], and (4) Disagree: asks the user to label the images with the greatest difference in output between the thermal and RGB models [Zhang *et al.*, 2021]. We train and evaluate the single-model systems on the thermal+RGB fused data since that model performed the best in the passive setting. For the multi-model systems, we use separate thermal and RGB models. We train each of these on the images in its modality. In evaluating the system as a whole, images in the test set are classified by weighting the models' outputs by their weights learned during training, calculated using Equation 2.

We graph the accuracy of our proposed methods in comparison to the baselines as the labelling budget increases in Figure 7. We find that both versions of MultimodalAL outperform all of the baselines by a statistically significant amount ($p < 0.05$) at 500 labels. This is despite the fact that Positive Certainty is statistically significantly better ($p < 0.05$) than Random, demonstrating it is a strong baseline. Positive Certainty outperforming the other baselines is consistent with our hypothesis that prioritising images the system thinks are positive samples is more suitable for highly imbalanced datasets than prioritising images about which the system is

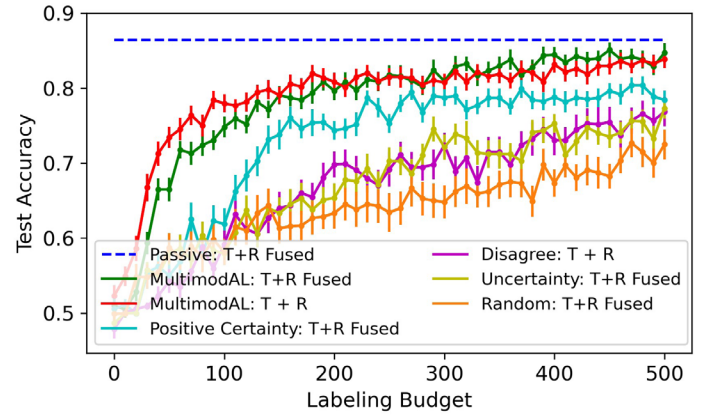


Figure 7:

Mean accuracy on the test set for the active learning methods with up to 500 images labelled across 30 trials. Both versions of MultimodalAL outperform the baselines and, with 500 labels, are statistically indistinguishable from the best passive method

uncertain. Even so, it is the two variants of MultimodalAL that get within 0.026% of the best passive learning accuracy by 500 labels, which is *not* a statistically significant difference ($p > 0.05$). Hence, there is a negligible difference in performance despite the 94% difference in the number of labelled images provided to the passive system and MultimodalAL.

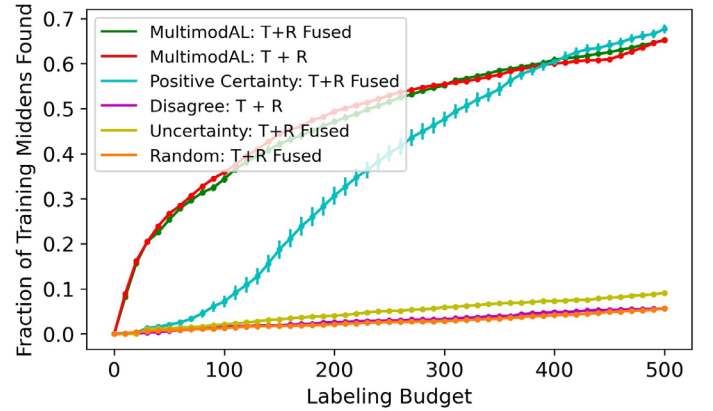


Figure 8:

Mean fraction of middens found in the training set for the active learning methods with up to 500 images labelled across 30 trials. Both versions of MultimodalAL find more middens more quickly than the baselines with up to 400 labels

Interestingly, MultimodalAL: Thermal + RGB outperforms MultimodalAL: Thermal+RGB Fused for small numbers of labels (< 200), despite the opposite pattern holding for larger numbers of labels. Because MultimodalAL: Thermal + RGB combines the scores across two modalities, it will more strongly prioritise images that are very clearly middens in both imagery types than the single-model MultimodalAL: Thermal+RGB Fused, which will assign a high score to images that are clear in *either* data type. This could result in more informative midden images being selected in the former case and leading to improved performance when the number of discovered middens is low. Overall, the advantages of MultimodalAL are greatest when the labelling budget is very small (< 150). For example, we see that for up to 50 images, labelled only MultimodalAL does better than Random. Furthermore, we see diminishing returns in accuracy for MultimodalAL after around 200 labels once the system has been trained on enough middens (≈ 33) to serve as a reasonable detector. To dig deeper into the origin of MultimodalAL's performance gains over the baselines, we consider another important evaluation metric: the fraction of middens discovered during querying by the active learning system out of all those present in the training set as more labels are provided. Figure 8 shows that both versions of the MultimodalAL system are able to find more middens more quickly than the baselines. At around 400 labels, the Positive Certainty model is accurate enough to compete with MultimodalAL in terms of midden finding despite maintaining a lower test set accuracy, but for smaller numbers of labels, its midden retrieval is significantly inferior.

This suggests that Positive Certainty is finding less informative middens than MultimodalAL, which is biased towards selecting the warmest middens. A comparison of Figures 7 and 8 indicates a positive relationship between the accuracy achieved by the active learning system and the number of positive samples it is able to find. In addition, the fact that both versions of MultimodalAL find roughly the same number of middens throughout the active learning process, despite MultimodalAL: T + R achieving higher accuracy at the beginning, is further evidence for it finding more informative middens than MultimodalAL: Thermal+RGB Fused. Figure 8 also demonstrates that MultimodalAL is successfully addressing the class imbalance issue in our dataset. Both versions of MultimodalAL find over 65% of the middens by 500 images labelled. This is quite remarkable given that only 71 out of the 9,736 images in the training set are middens. Hence, although less than 1% of the images given to the active learning system are middens, our active learning algorithm is able to discover 46 of them by the time 500 labels have been requested. This 9% positivity rate is 12x larger than the overall positivity rate of middens in the training set. Perhaps more impressively, after just 100 queries, MultimodalAL has found over 34% of all the middens, whereas none of the baselines manage to find more than 8%. The number of middens found is significant because these are confirmed by humans, so we are highly confident that these are indeed middens. Once the labelling budget has been exhausted, the remaining unlabelled images are classified using the trained model, but because the model is not 100% accurate, we have more uncertainty as to whether the images classified as middens are truly middens.

Mode	Test Accuracy	Labelling Budget	Labelling Time
Passive	0.86 ± 0.01	9,736	81 hours
Active	0.84 ± 0.01	500	250 mins
Active	0.81 ± 0.01	200	100 mins
Active	0.73 ± 0.01	50	25 mins

Table 2:
Comparison of passive (Thermal+RGB Fused) and active (MultimodalAL: Thermal + RGB) test accuracy, labelling budget, and labelling time

By achieving competitive accuracy and quickly identifying middens in an unlabelled dataset, MultimodalAL drastically alleviates the labelling burden, as quantified in Table 2. If we assume each image takes 30 seconds for a domain expert to label, then labelling every image in the dataset would require over 80 hours, but labelling 500 images would take under 4.2 hours, which is a significant amount of time saved in a resource-constrained domain — all while identifying 65% of all the middens in the dataset. This demonstrates the power of an active learning system that employs a domain-inspired ranking technique to find rare positive samples in a highly imbalanced dataset.

Midden Mapping

From the locations of the middens in the site, we create the first-ever midden map, presented in Figure 9, and extract actionable insights. We emphasise that we are not showing landscape features on our map to protect rhino territories from poachers. Because rhino middens are used for territorial marking, by mapping these middens, we can also figure out where the rhinos' territories are. We can then target ranger patrols and ecological monitors at the middens to better protect the rhinos from poachers. Unexpectedly, the midden map reveals that the middens are not distributed randomly across the region but instead form clusters. Using K-means, we have identified six such clusters on our site. We recommend that antipoaching efforts prioritise those areas of high midden density to increase their efficacy.

Furthermore, we find that the rhino middens are often located along animal paths. Ecological monitors can thus focus their efforts along these paths since they are likely being used by rhinos. Additionally, knowing where the middens are is useful because monitors can visit them to more quickly find rhinos or check for fresh dung as a way of confirming the presence of rhinos in the area. This is crucial to demonstrating that the rhinos are being effectively managed and that the area should continue to be funded and resourced. Thus, by more effectively targeting scarce resources, we expect MultimodalAL to have a significant impact on rhino conservation.

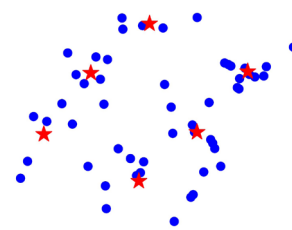


Figure 9:
Midden map for a 2x2-km site in Kruger National Park. Blue dots are middens. Red stars are centres of midden clusters

Future Work

In advancing a decade-long collaboration with South African National Parks, we will use MultimodalAL to map middens across other sites in Kruger National Park. To do this, we will use a model pre-trained on the labelled dataset studied herein to warm-start an active learning system, further lessening the labelling burden. Unique terrain features, such as termite mounds present in some other sites, could increase the advantages of multimodality. To support this work, we hope to develop an interactive user interface for MultimodalAL to allow domain experts to label images queried by the model(s) without needing to work directly with computer scientists. We also present two ideas for building on the MultimodalAL system. First, the algorithm could be extended by dynamically weighting the maximum thermal pixel values and the model(s)' output. Second, we could define the midden detection task as one of image segmentation rather than classification and use the model from Kirillov *et al.* [2023]. We also encourage the evaluation of the MultimodalAL system on imagery from other domains (e.g., wildfire or animal detection). To facilitate this future work, we make our code publicly available at <https://github.com/lgordon99/rhino-middendetector>. In contributing to the UN's Fifteenth Sustainable Development Goal, we encourage protected area managers to use the MultimodalAL system to map rhino middens across their landscapes. However, we must keep our dataset private due to its sensitive nature with respect to the rhino poaching crisis. In accordance with the "Leave no one behind" principle [UN, 2023a], we believe rhino midden maps can empower park rangers and ecological monitors in carrying out their indispensable work in conserving the planet's last wild places.

Conclusion

We make several contributions in this paper. In collaboration with South African National Parks, we map rhino middens for the first time and display where anti-poaching patrols ought to focus their efforts. Thus, we have demonstrated a way of finding and protecting rhinos without directly tracking them. We find that models passively trained on thermal, thermal+RGB fused, or thermal+LiDAR fused imagery achieve high accuracy in distinguishing between midden and empty images. To alleviate the labelling burden intrinsic to passive learning, we introduce MultimodalAL, a novel active learning methodology for highly imbalanced datasets that is applicable when the instances can be ranked according to an informative metric. In addition to exploiting domain knowledge, this method also accommodates multimodal data. We demonstrate that the multi-model instantiation of the algorithm outperforms a single-model system on our dataset for a small labelling budget. Overall, both MultimodalAL systems outperform baseline query strategies and find more middens than the baselines for up to 400 labels provided. Furthermore, only MultimodalAL achieves an accuracy statistically indistinguishable from that of the best passive model with 94% fewer labels, saving domain experts dozens of hours in data annotation efforts. By introducing this scalable method for mapping middens, we present new insights to support conservation practitioners in advancing rhino conservation across southern Africa. **□**

Acknowledgments

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References available on request.

The Contribution of Veterinary Science in Rhino Conservation

Dr Hendrik Hansen



South African veterinarians are at the forefront of innovation, driving the development of science-based solutions to address uniquely African conservation challenges. The veterinary team at **Embryo Plus** has demonstrated this leadership through groundbreaking work in **White Rhino conservation**, applying advanced reproductive technologies and veterinary protocols to strengthen population growth and long-term survival of this iconic species.

At a time when the Southern White Rhino population faces critical threats from poaching and habitat loss, and when **optimal reproduction is essential for survival**, veterinary innovation has become a cornerstone of conservation. Below are key projects and protocols developed by this team, all of which are already making a tangible contribution to rhino and broader wildlife conservation.

1. Advanced Reproductive Technologies (ART): In Vitro Fertilisation (IVF) and Ovum Pick-Up (OPU)

Over a 7-year research program and more than **300 procedures**, Embryo Plus developed reliable techniques and purpose-built equipment for **oocyte collection** from anaesthetised White Rhino cows.

- **ICSI & IVF:** Oocytes are fertilised using **intracytoplasmic sperm injection (ICSI)** at Embryo Plus' IVF laboratory in Brits, South Africa, followed by incubation in specialised IVF systems.
- **Scientific Value:** These innovations enable applications across **other endangered species**, opening possibilities for genetic rescue and advanced reproductive management worldwide.

- **Global Relevance:** The protocols and expertise developed in rhinos can be transferred to other rare wildlife species facing reproductive bottlenecks.

2. Pregnancy Diagnosis and Term Prediction

Accurate pregnancy diagnosis is **vital for translocation and breeding programs** due to the rhino's long gestation (16 months).

- **Risk Reduction:** Prevents calving in holding bomas or transport crates, a major cause of calf and cow losses.
- **Technology Used: Transrectal ultrasound** provides far more reliable results than traditional blood progesterone tests.
- **Management Impact:** Enables safe translocation, better population planning, and early detection of reproductive pathologies.

3. Treatment of Non-Cyclic and Infertile Rhino Cows

A significant number of White Rhino cows suffer from **cystic ovarian conditions**, leading to prolonged inter-calving intervals and reduced population growth.

- **Diagnostic Innovation:** Ultrasound allows accurate identification of ovarian and uterine pathology.
- **Therapeutic Protocol:** OPU (Ovum Pick-Up) is used not only for IVF but also as a **treatment procedure**, aspirating cysts and stimulating new cycles.
- **Impact:** Increases reproductive efficiency in both wild and intensive populations, addressing one of the major hidden threats to rhino population recovery.

4. Stimulation of Reproductive Cycles in Post-Puberty Heifers

In captive or orphaned populations, young females often fail to initiate cycles due to disrupted social structures.

- **Veterinary Intervention:** OPU stimulates dormant follicles, successfully triggering reproductive cycles.
- **Results:** Pregnancies achieved within **34 days post-procedure**; subsequent natural breeding cycles continue without further intervention.
- **Population Benefit:** These interventions have already resulted in **multiple live calves**, increasing conservation success.

5. Semen Collection, Freezing, and Genetic Banking

Embryo Plus veterinarians developed a **specialised semen collection probe** and freezing protocol unique to White Rhino bulls.

- **Genetic Preservation:** Allows cryobanking of semen from a wide genetic pool of bulls for future use.
- **Breeding Soundness Exams:** Subfertile bulls can be removed from breeding groups, ensuring optimal mating.
- **Translocation Benefit:** Positive fertility screening improves breeding success after relocation.

6. Anaesthetic Protocols for Safe Immobilisation

The demands of long reproductive procedures required **novel anaesthetic induction and maintenance protocols**.

- **Innovation:** Development of concentrated butorphanol and fentanyl formulations, enabling reliable dosing via 2 ml darts.
- **Impact:** Increased safety and effectiveness of immobilisation during lengthy procedures such as OPU, translocation, and dehorning.
- **Conservation Utility:** These drug protocols can be applied more widely across rhino management operations.

Conclusion

The contribution of veterinary science, and specifically the work of Embryo Plus veterinarians, represents a **transformational step in rhino conservation**. These protocols—ranging from IVF and pregnancy diagnosis to genetic banking and safe anaesthetic techniques—address both immediate conservation needs and long-term sustainability of the species. This work is a **true testimony to the dedication of South African veterinarians**, whose science-driven solutions are not only helping to safeguard the **Southern White Rhino** but are also laying the groundwork for the conservation of endangered species worldwide. **U**



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Limpopo Rabies team

Inanlé CVC was established in March 2025 when the principal veterinarian, Dr Ina Visser, moved from Centurion to Magoebaskloof. Previously known as Doringkloof CvC, the name changed, but not the mission. Inanlé CVC was extremely fortunate to become part of the Battersea Rabies project in April 2025.

The rabies team went operational on 2 April 2025. Armed with a branded vehicle, rabies vaccine and educational material, dr Visser and Collen Mononela did our first vaccinations in a village called Polaseng, Collen's home town. Little did we know of all the challenges that were ahead of us. Being a vet from a city small animal clinic, these rural canines were something else. The best to describe the situation between a rural dog and its owner is cohabitation. Very few of these animals know a kind human interaction. We had our job cut out for us. It was strange for us to learn that some owners have never really touched their own dogs. Proper handling and restraint to do a vaccination was quite a mission.

The feline populations in the rural areas are very small and mostly feral, but interestingly we found a huge feral population in a township to the east of Tzaneen.


Our focus is to vaccinate as many dogs as possible. We do the house to house approach where we drive up and down with a load hauler to get the attention of the owners.

Afternoons and school holidays are more productive when we have a bakkie load of youngsters riding along and showing us the dogs of the village. We also educate school children about bite prevention and proper wound care after a dog bite. If we can change the mindset of one child every day, we have made a huge impact on the wellness of the dogs in the rural areas.

A few months past and our team has another member and a small vehicle with a big heart. A young lady - Xalati Rigigisi, a recently qualified Animal Health technician joined our team in July. On 14 July the new team took off in their little Jimny to start their rabies journey. They have already covered multiple villages to the east of Tzaneen and also helped the state vets with a rabies campaign in Vembe district.

Collen and Xalati had the privileged to travel to the Eastern Cape to meet the awesome teams under the leadership of dr Renee and Dr Emelina. They spent 2 weeks with the Eastern Cape teams and gained a tremendous amount of experience. They were taught how to take brain samples with the straw technique for rabies testing and did a lot of vaccinations as well as school visits with the eastern Cape teams.

They different provincial teams discussed their common issues and their differences. An interesting difference between the 2 provinces is- that in the Eastern Cape most rural village homes are not fenced off, whereas in Limpopo, almost every house is fenced off and sometimes our team can not gain access to animals due to property boundaries.

We would like to thank SAVA and Battersea for the wonderful opportunity to be part of the rabies solution in South Africa. 



On the left is Collen Sello Mononela (kennel assistant) and on the right is Xalati Rigigisi (Animal Health Technician)



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Susan Heine

Farewell Tribute to Susan Heine

SAVA bids farewell to Susan Heine, our accountant, who officially retired at the end of 2023 after 22 years' service; however, she continued to assist in the accounts department until recently despite personal challenges, which highlights her loyalty and commitment.

Her contributions to the VetHouse team have been invaluable, and her sharp mind and quick wit will be deeply missed.

Susan, SAVA wishes you a well-deserved retirement.

Enjoy this new chapter in your life, knowing that your legacy will live on.

SAVA Management and Staff 🇷🇺

After 22 years of dedicated service, we bid farewell to Susan Heine, SAVA's beloved accountant and a pillar of our organisation. Susan's commitment, precision, and quick wit have shaped the heart of SAVA's financial operations and left a legacy that will be felt for years to come.

I had the privilege of calling Susan my mentor for nearly eight years, a time filled with learning, growth, and deep admiration. Her guidance was always thoughtful, her support unwavering, and her presence a source of calm and confidence. Even after her official retirement at the end of 2023, Susan's dedication to SAVA didn't end.

In 2025, she graciously returned to assist the accounting department, once again offering her expertise and steady hand when it was most needed. This act alone speaks volumes about her character and her deep care for the organisation and its people. Susan's place in the office and within SAVA is truly missed. Her legacy is not only in the work she did, but in the relationships she built and the example she set. As she now steps into a well-deserved chapter of rest and new adventures, we, in particular, I, thank her... for everything.

With heartfelt gratitude,

Sonja Ludik 🇷🇺

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THE WARMEST PLACE TO BE

Steve Wimberley



Like most children, I learned from a very young age that the milk we drink comes from cows. It was a simple fact, and being a city boy, I never questioned it. It was only when I reached my second year of veterinary school that the implications of this fact hit me. For a cow to have milk, it must fall pregnant and give birth to a calf. Cows don't just simply have milk!!

The farmer needs to know whether his cows are pregnant, and this is where the vet comes in. Pregnancy determination is done by rectal examination as early as 6 weeks after conception. As students, we were taught to feel the ovaries and uterus via the rectum, so as vets we can quickly pick up an early pregnancy.

It was a bitterly cold winter morning when we set off to the farm for our first encounter with rectal examinations in dairy cows. An arctic wind swept across the veld, and the frost lay white on the ground. The sun shone from a bright blue sky, but it was too early to be of any comfort. We were wrapped up in our anoraks, gloves, scarves and beanies.

Underneath, we wore the veterinary students' standard "large animal" attire – sleeveless green overalls and white boots. We were very proud to be wearing this outfit for the first time.

We arrived at the farm to find the cows all lined up eating their concentrate of maize and other delicious nutrients. This kept the front end occupied while we were busy at the rear. There was a corrugated iron roof but no walls to protect us from the freezing wind. We were very reluctant to strip down and start work. My friend Garth was the first to pluck up the courage. He took off all his wrappings and approached the first cow. We all watched with amusement. He lifted the tail and inserted his right arm.

"It's so WARM", he shouted!!

That was all the rest of us needed to get going.

Next to me, another friend, Herman, was ready to go. He stripped off his outer layers to reveal a pristine green overall and boots so white they were hard to look at in the sunlight. He was German and very particular about his appearance. With confidence, he stepped forward and lifted his cow's tail. With that, the cow grunted, arched her back and let fly with a stream of projectile green diarrhoea. The green liquid landed perfectly between Herman's shin and boot and filled it to the brim.

"Aaaaargh", he cried and bent to inspect the damage.

The second phase of diarrhoea then caught him on the back of the head and ran down his neck and under his collar.

Poor Herman had to clean himself up as we were laughing too much to be of any assistance.

Herman taught us a very important lesson, and from that moment on, we all approached our cows with respect, lifted the tails and stood to one side for a moment before plunging our arms into the glorious warmth!!!! **V**

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I hope this article finds you and your families well and safe!!! Last month, we looked at "devotion". In this edition, I would like to discuss the ability to shift one's state of mind from a negative one to a positive one within minutes. I enjoy watching reality shows, with a particular interest in human behaviour and performance. Inevitably, amongst the challenges that contestants are put through, you see individuals get knocked out and get up again within a challenge. It is interesting to think about what allowed those people to do that and suddenly change their mindsets. Every now and then, you may feel anxious, scared, overwhelmed, annoyed, disheartened, and I have experienced that on many occasions. What we will discuss next is using some potential tools to shift your energy within minutes (\pm 20 minutes or a bit more). If I weren't aware of these, I would've easily slipped into negative thought/pattern loops. I'm hoping to share the easiest to implement and the most effective from my experience, but ultimately, you need to find the ones that work for you. I will also include some resources at the end, as there are more techniques.

1. Working Out/Stretching

Even if you don't feel like moving, just commit to a warmup. Then, when you're warming up, you get the energy moving, and you'll feel more motivated to work out. The intention is to transform your energy. The alternative is simple stretches and mobility work. Oftentimes, we carry trapped energy in our bodies and stretching and/or mobility exercises can help unblock it.

2. Dance

When I was in high school, I was actively involved in traditional Greek folk dancing, and I would spend 15 hours a week just practising the various dances I was learning. Dance is one of the simplest and most powerful ways to constructively shift your energy. Choose an uplifting song, dance like mad for a few minutes, and you will feel a BIG shift in your energy, guaranteed. I strongly recommend dancing every day, even just for a few minutes. It would always elevate my mood and shift my energy.

3. Walking Outside/Sunlight/Grounding

I have often taken a simple walk outside, whether at home or at work, and I have found that to be a very powerful way to shift my energy. Please do not take your phone with you and ensure that you are present as you walk, making it a cognitively purposeful exercise. Some people well known in history who practised this habit are Albert Einstein, Aristotle, Charles Darwin and Ludwig Van Beethoven. In addition, another elemental force of transformation is sunlight, especially on your face. I personally love the nourishing sensation of the sun on my face, and I remember doing that a lot in winter in high school. Take the time to pause and really focus on the sensation it provides on your body, or areas of your body that are exposed.

Furthermore, you can experience even more benefits when you put your bare feet on our earth (soil or grass). The purpose and benefit are thought to come from realigning your body's electrical energy with that of the earth, thus creating a physical connection with our planet. You can stand or sit in your garden (or a local park) barefoot, and start feeling the connection.

4. Good Music/singing/humming

Music is a universal language for a reason. Everyone knows music can shift your energy. I have used music many a time for getting unstuck. It is possible to forget about your favourite song when you're not in the best mood, which is why I have put mine on Spotify™ and it shows up on the opening screen of the app – "The Bongo Song" by Safri Duo.

We can also add singing and humming to this approach. If you have ever sung, without inhibition, you might remember just how transformative singing can be. It's not about being a great singer; it's about expression and changing the frequency of your body.

You don't even have to sing words; you can just hum along to your chosen tune. Any activity where we are creating vibrations in our bodies has the potential to be incredibly powerful at shifting our energy.

5. Laughing

In my opinion, laughter really is the best medicine, and it is one I use a lot lately for myself. Find ways to laugh more often. I like to look up jokes, watch comedy video clips or watch comedy movies. Sometimes, just the act of smiling at yourself in the mirror can be a good start.


6. Crying

Crying can be huge for release as well as creating space for you to shift into higher levels of consciousness. I often see this with people experiencing high levels of frustration and anger. Don't be afraid to cry. And when you do, just let it out fully. Let your body collapse and shake without resisting it. On the other side of that release is a lot more serenity. One that applies to me is allowing tears of joy to flow through as well when they arise. Don't be afraid of that either. Love watching people succeed and do well.

Additional resources

Journaling, expressing gratitude, breathing exercises, yoga, taking a shower, essential oils, affirmations, meditation, etc. Some links:

- 4-7-8 Breathing
- Alternate Nostril Breathing
- Box Breathing
- Healthline
- 1,132 Positive Affirmations
- 80 Powerful Affirmations
- Meditation
- Heart Centered Leadership

Next month, we will continue looking at more ideas for improving our quality of life and overall performance both at work and at home 

When a Rabies Scare Hits Home: Your Legal Blueprint for Reporting and Responding

Trudie Prinsloo (Legalvet Services)



This article is intended to provide information and educate veterinarians on relevant aspects of the law. It is not intended as personal legal advice. SAVA is not responsible or liable for any advice or other information provided herein.

It is a call that sends a shiver down every veterinarian's spine. A panicked client on the phone, a dog with hypersalivation and "strange behaviour," or a cat that's suddenly aggressive and attacking its owner. For a moment, clinical training kicks in and differential diagnoses flash through your mind. But then it hits you: this isn't just another clinical case. This is a potential rabies case. In that instant, your role shifts from clinician to public health protector, and your legal and ethical obligations take centre stage.

As veterinarians, we are custodians of animal health and, by extension, public health. Our legal and ethical framework is mainly based on the Animal Diseases Act, the Animal Disease Regulations published in terms of this Act, the SAVC's Rules Relating to the Practising of Veterinary Professions, and the SAVC's Ethical Guidelines. We must use this framework to navigate the situation. Knowing these rules is not just good practice; it's a non-negotiable professional requirement.

The Animal Diseases Act

Rabies is a controlled animal disease under the Animal Diseases Act and its Regulations. The moment you, as a veterinarian, find a clinical case or even suspect the presence of rabies, you have an immediate and non-negotiable legal duty. This isn't an option; it's a mandatory legal requirement.

The Act, specifically Section 11(2), stipulates that any veterinarian or person who has knowledge of, or suspects the presence of, a controlled animal disease shall immediately report it to the nearest State Veterinarian. Note the use of the words "**shall immediately**"—this trumps client confidentiality and cannot be delayed or put off until a convenient time.

The Act is also clear that suspicion is enough. You don't need a lab report or a definitive diagnosis to trigger your legal duty. If the clinical picture, history, or epidemiological context makes you even suspect rabies, you must report it. Failing to do so can have serious consequences, not only for public safety but also for your own legal liability and professional standing.

Once you've made that call, the legal authority shifts. The State Veterinarian now has a wide range of powers under the Act, including the authority to:

- Place the animal in isolation.
- Order the destruction of the animal.
- Mandate the vaccination of other animals on the premises or in the area.
- Issue control measures to prevent the disease from spreading.

Your role at this point becomes one of full cooperation. You must adhere to the State Vet's directives, and it's your job to explain to the client that these are not your recommendations, but rather legally binding orders. This can be a tough conversation, but it's essential to manage client expectations and ensure compliance.

The Ethical Minefield: Public Health vs. Client Relationships

This is where the law and professional ethics converge, often uncomfortably. Our SAVC Ethical Rules require us to act in the best interest of our patients and clients, but they also place a paramount duty on us to protect public health and safety. Consider the ethical dilemma. A client may plead with you to "wait and see," to treat the animal symptomatically. They may refuse to believe that their beloved pet could be a carrier of such a horrific disease. Our natural empathy pulls us to support the client, to give them comfort. But our ethical and legal duty is clear: public safety must prevail, including that of the owner and family. The potential for human death is a risk we simply cannot take. Our obligation is also to the broader community, and that means we must, at times, make difficult and emotionally charged decisions that a client may not understand or agree with. Another ethical consideration is client confidentiality. While we are bound by a duty of confidentiality, it is not absolute. In a rabies case, the legal duty to report a controlled disease explicitly overrides any professional obligation of secrecy.

You are legally obliged to provide the State Veterinarian with all relevant information, including the client's name and address.

Do not overlook the importance of dealing with the human contacts of a suspect rabies case. You have to inform your clients that they must contact their medical service provider. Unfortunately, most medical doctors are not equipped to deal with this and often downplay the seriousness and importance of immediate preventative treatment for human contacts.

Make sure the authorities at the Department of Health are contacted. Contact the National Institute for Communicable Diseases (NICD) hotline for Rabies (0800-212-552) if appropriate steps are not taken by the Department of Health or the attending physician.

A Step-by-Step Guide

So, when that call comes in, what do you do? Here's a practical, actionable blueprint to follow:

- 1. Immediately Isolate the Patient.** Your priority is to prevent any further exposure. Isolate the suspect animal in a secure, quiet area. Advise the client not to handle the animal or allow it to interact with other people or animals.
- 2. Contact the State Veterinarian.** Do this **immediately**. Have a clear, concise history ready: the clinical signs, the date of onset, any potential exposure, and the client's details. Don't waste time on a definitive diagnosis; the suspicion is what matters. The State Vet will guide you on the next steps, including quarantine protocols and sample submission.
- 3. Manage the Client Relationship with Authority and Empathy.** This is the hardest part. Explain that rabies is a controlled animal disease and that you are legally required to report it. Be firm but compassionate. Explain that the decision to quarantine or euthanise is not yours, but a legal requirement under the Animal Diseases Act, designed to protect the public, including the owners themselves. Explain the seriousness of the situation without inciting panic, and refer any human exposure concerns to a medical doctor immediately.

- 4. Practice Meticulous Record-Keeping.** This is crucial. From a legal standpoint, if it was not documented, it did not happen. Document every conversation with the client, all clinical observations, the time and date of your call to the State Veterinarian, and the details of the State Vet you spoke to. This paper trail is your best defence should there ever be a complaint or legal claim against you.
- 5. Secure and Submit the Specimen.** Rabies diagnosis in South Africa is primarily a post-mortem process, involving the submission of brain tissue. Do not perform the post-mortem and decapitation yourself unless you are requested by the State Veterinarian and you have the correct personal protective equipment. The State Vets usually take control and do the post-mortems themselves. If you are tasked with the transportation of the samples to the Onderstepoort Veterinary Research Institute, make sure to use the provided triple-packaging to protect yourself and others from exposure.

Shared Responsibility

Handling a rabies scare is a stressful and emotional experience. It forces us to confront the grim realities of our profession. But by adhering to the legal and ethical blueprint, we fulfil a higher purpose. Our knowledge and adherence to the law are our most powerful tools. They protect not only the public from a deadly disease but also our own professional integrity and legal standing.

By following the rules, we ensure that what could be a tragic public health disaster remains a contained, well-managed event. We are the last line of defence, and it's our legal and ethical duty to be ready when that call comes in.

If you have any questions, please feel free to contact me at trudie@legalvetservices.co.za 

Animal Diseases Act, No. 35 of 1984.



Managing Refractory Cases in Feline Chronic Gingivostomatitis

By Dr. Mareli van de Wetering
Onderstepoort Veterinary
Academic Hospital



In a previous VetNews column, we provided an overview of feline chronic gingivostomatitis (FCGS) and the general therapeutic strategies available. In this article, we turn our focus to the management of refractory cases—patients who fail to respond adequately to standard surgical treatment. These cases often require complex, multimodal, and long-term medical intervention.

Surgical Treatment: The Gold Standard

Partial-mouth or full-mouth dental extractions remain the gold standard for FCGS management. The decision between the two is based on the anatomical distribution and severity of inflammation. However, despite surgical intervention, some cats do not respond or only show partial improvement. In a 2015 study by Jennings et al., 28% of cats achieved complete resolution post-extraction, 39% showed substantial improvement, and 33% had minimal or no improvement. Typically, clinical improvement occurs within 33 to 49 days post-operatively (Soltero-Rivera et al., 2023). Cats showing little to no improvement within this period are classified as refractory and require ongoing medical management to control inflammation and pain.

Medical Management of Refractory Cases

When addressing refractory FCGS, treatment must be tailored to the individual patient, taking into account comorbidities, concurrent medications, and drug administration feasibility.

A combination of analgesics, immunosuppressants, and in select cases, immunomodulating therapies, may be used.

Analgesia: A Cornerstone of Therapy

Pain is a significant concern in refractory FCGS patients, often leading to anorexia, weight loss, and reduced grooming behaviour.

Effective analgesia is therefore critical.

- NSAIDs (e.g., Meloxicam):

Widely used in clinical practice. At doses of 0.01–0.05 mg/kg q24h, long-term meloxicam use in IRIS Stage 1–2 chronic kidney disease cats did not show adverse renal effects (Monteiro et al., 2019).

Nonetheless, hydration status should be carefully monitored in cats with poor oral intake.

- Amantadine:

At 3–5 mg/kg q24h, this NMDA antagonist has shown benefits for chronic pain and quality of life in cats with osteoarthritis. Sedation is a possible side effect (Shiple et al., 2021).

- Gabapentin:

Effective for neuropathic and musculoskeletal pain. Administer at 5–10 mg/kg q8–12h (Siao et al., 2010). Useful as an adjunct.

- Buprenorphine:

Administered via the buccal mucosa, though bioavailability may be compromised in stomatitis patients. A study by Stathopoulou et al. (2018) found decreased absorption in cats with oral inflammation.

Immunosuppressive Therapy

- Corticosteroids:

Commonly used but should be approached with caution. Long-term use can induce diabetes mellitus, and the clinical remission rate is low (Hennet et al., 2011).

- Cyclosporine:

An effective option for true refractory cases, administered at 2.5 mg/kg. Its use is best reserved for patients unresponsive to dental extractions.

Lommer (2013) reported a 45.5% clinical cure rate after ≥3 months of treatment.

Therapeutic monitoring is essential; plasma concentrations >300 ng/mL are associated with better outcomes. Due to the risk of opportunistic infections and systemic toxoplasmosis (Last et al., 2004), treated cats should be kept indoors and not fed raw meat.

Immunomodulating Therapy

For advanced, refractory cases, two promising therapies have been explored:

- Recombinant feline interferon-omega
- Mesenchymal stromal cell (MSC) therapy

Multiple studies (Arzi et al., 2016; 2017; 2020; Hennet et al., 2011) have reported positive outcomes. Unfortunately, these options are not currently available in South Africa.

Antimicrobial Therapy: Use with Caution

Antibiotics are frequently used but rarely justified in the long-term management of FCGS. Evidence suggests that their benefits are transient and less effective than immunosuppressants (Soltero-Rivera et al., 2023). Moreover, a study by Tsang et al. (2021) highlighted the prevalence of antimicrobial resistance genes in the oral microbiota of affected cats, underscoring the importance of antimicrobial stewardship.

Monitoring Disease Progression

The Stomatitis Disease Activity Index (SDAI) offers a structured method for monitoring both clinical and at-home progress. It incorporates:

- Owner-reported assessments of appetite, activity, grooming behaviour, and overall comfort
- Veterinary assessments of body weight and site-specific inflammation (scored 0–3)

This tool facilitates early identification of refractory cases, timely therapeutic interventions, and standardised tracking of patient response over time.



One of the cases that required a full mouth extraction, severe inflammation can be seen on the gingiva, caudal oral cavity, as well as the soft palate on day 0 on the day of procedure, and the significant improvement on follow-up oral examination on day 30 and day 60 post procedure.

Looking Ahead

At the Onderstepoort Dental and Maxillofacial Surgery Clinic, we are conducting a study on FCGS, especially on the inflammatory response and resolution after treatment.

Initial findings are encouraging, and we invite veterinarians to reach out for more information, to discuss challenging cases, or to refer patients to be included in the study.

Contact us: 012 529 8276 or vandewetering.mareli@up.ac.za (References available upon request). 



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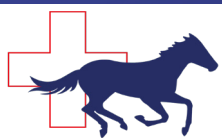
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