VacciMonitor 2026;35:e053526
www.vaccimonitor.finlay.edu.cu
Original Article

Evaluation of oral rabies vaccine potency under simulated field temperature conditions

Nermeen Gouda-Shafik[1*] ORCID: https://orcid.org/0000-0002-1792-1629
Heba A. Khafagy[1] ORCID: https://orcid.org/0000-0003-4548-1824
Sara El Sawy-Ahmed[1] ORCID: https://orcid.org/0009-0009-8368-4236
Darwish Mahmoud Darwish[1] ORCID: https://orcid.org/0000-0003-2542-1058
Fady Abd El-Mohsen Shasha[1] ORCID: https://orcid.org/0000-0002-2356-9289
Amal Abd El-Moneim-Mohamed[1] ORCID: https://orcid.org/0000-0001-5494-7169
Mohamed Abdelkhalek Abdrabo[1] ORCID: https://orcid.org/0000-0003-0702-2934
Mohamed Samy Abousenna[1] ORCID: https://orcid.org/0000-0003-2202-9544

[1] Agricultural Research Center (ARC), Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Cairo, Egypt.

Corresponding author: mohamedsamy2020@hotmail.com

Rabies remains a fatal zoonotic disease with a significant burden in developing countries, particularly where free-roaming dogs hinder the success of parenteral vaccination strategies. Oral vaccination using thermostable baits offers a promising solution. This study aimed to evaluate the temperature-dependent stability and immunogenicity of the SERVAC Rabies Vaccine Oral Bait (SERVAC-RVOB), which contains an attenuated Evelyn Rokintniki Abelseth strain, under different temperature conditions simulating field scenarios in rabies-endemic regions. The vaccine baits were stored at 4 °C, 20 °C, 37 °C, and 45 °C for up to 30 days. Virus titers were calculated weekly using the Spearman-Kärber method on BHK-21 cells. An *in vivo* study was conducted using 18 healthy, seronegative dogs divided into five groups based on storage conditions. Each dog received a single bait dose, and serum samples collected on day 28 post-vaccination were analyzed using a commercial blocking ELISA to detect rabies virus-specific antibodies. Results showed that SERVAC-RVOB maintained high titers (7.2 $\log_{10}$ $TCID_{50}$/mL) and induced protective antibody responses when stored at 4 °C and at 20 °C for up to 15 days. However, storage at 37 °C and 45 °C resulted in marked loss of potency and failure to elicit protective immunity. ELISA blocking values dropped significantly under these conditions, indicating a strong correlation between temperature, titer loss, and immunogenicity. These findings support the deployment of SERVAC-RVOB during cooler seasons and recommend the removal of uneaten baits after 2 weeks to maximize efficacy and field monitoring. Maintaining cold-chain logistics or enhancing thermostability is essential for successful rabies control in endemic settings.

Keywords: rabies virus; vaccine potency; ELISA.

Introduction

Rabies is an acute, fatal viral disease that affects the central nervous system. It is classified as a viral zoonosis, primarily transmitted through the bite of infected animals, with domestic dogs serving as the major vectors worldwide.(1)

The causative agent, rabies virus, belongs to the genus *Lyssavirus* within the family *Rhabdoviridae*. Morphologically, the virus is bullet-shaped, enveloped, and measures approximately 180 nm in length and 70 nm in diameter. Its structure consists of a helical nucleocapsid composed of a single-stranded, negative-sense RNA genome tightly associated with an RNA-dependent RNA polymerase. This complex is encased within a matrix (M) protein and enveloped by a lipid bilayer containing knoblike glycoprotein (G) spikes, which are critical for host cell entry.(2)

* Associate professor of virology, PhD of virology, Agricultural Research Center (ARC), Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Cairo, Egypt.

Once clinical symptoms appear, rabies is invariably fatal, leading to death primarily through progressive encephalomyelitis. Globally, rabies causes an estimated 59,000 deaths annually, with approximately 95 % of these fatalities occurring in developing regions of Asia and Africa where access to preventive measures and timely medical care remains limited.[3]

Mitigating the global burden of human rabies is best achieved through effective control of canine rabies.[4] Recognizing this, the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (WOAH), and the Global Alliance for Rabies Control have joined forces to support countries in the global effort to eliminate dog-mediated rabies by 2030.[5]

In the initial phase of this initiative, mass dog vaccination is prioritized as the most cost-effective strategy for controlling and ultimately eliminating rabies.[6] Achieving vaccination coverage of at least 70 % of the dog population in endemic countries is crucial to interrupting virus transmission and substantially reducing human rabies cases.[7]

However, implementing widespread vaccination programs in resource-limited settings poses significant challenges. In countries such as Egypt, a major obstacle is the large population of free-roaming dogs that are not readily accessible for parenteral vaccination. Consequently, devising vaccination strategies that are both effective and cost-efficient becomes critical to achieving high coverage rates.[8]

Oral immunization of wildlife with live attenuated vaccines has been a powerful tool for controlling and eliminating rabies in several countries, particularly across North America and Europe.[9] Building on these successes, oral vaccination strategies have been increasingly assessed for application in dogs, especially free-roaming populations that are difficult to access through traditional parenteral methods.

Several types of recombinant and modified live attenuated vaccines have been evaluated for oral rabies vaccination of dogs (OVD). Among these, the Evelyn Rokintniki Abelseth (ERA) strain, a cell-culture-adapted rabies virus derived from the Street-Alabama-Dufferin (SAD) strain, has been widely studied and characterized as an effective live attenuated vaccine.[10]

The WHO has recognized the potential of OVD for rabies control in canine populations and has supported, since 1988, a series of expert consultations to promote coordination of research on oral vaccination of dogs. These efforts have focused on fostering the development and evaluation of safe and effective oral rabies vaccines and bait systems. WHO guidelines were established for the standardized evaluation of candidate vaccines regarding both efficacy and safety, as well as the development of optimized vaccine baits.[11]

Moreover, additional standardized protocols were formulated to assess baiting systems, baiting strategies, field trial designs, and studies on dog ecology, all critical components for the successful implementation of oral vaccination programs. More recently, the WOAH has demonstrated renewed interest in OVD, reflecting the growing recognition of its importance as a supplementary tool in global rabies control strategies.[12]

The efficacy of oral rabies vaccine candidates should be thoroughly assessed through both direct oral instillation and vaccine-in-bait delivery to caged dogs, evaluating the immune response elicited by each method.[11] In addition, the efficacy of vaccine baits must be verified under field conditions, targeting both owned dogs living in households and free-roaming or ownerless dogs in areas where oral vaccination campaigns are intended to be applied**.** For successful deployment, bait candidates should ideally be produced locally, in large quantities, and as inexpensively as possible. Field trials comparing machine-manufactured and hand-crafted baits are essential to determine production feasibility and operational effectiveness.[11] Standardized protocols for assessing bait preferences have been described.[13]

Several critical parameters must be evaluated and optimized to maximize bait acceptance and ensure effective vaccine delivery, including bait palatability, shape, size, texture of the bait matrix, and the design of the vaccine blister to enable efficient rupture and release of the vaccine into the oral cavity.[14]

Thermostability of the vaccine within the bait under field conditions is another important consideration. Stability of vaccines such as V-RG and SAG2 in baits has been

extensively demonstrated under diverse environmental conditions, including tropical climates.[4,5] However, for OVD, thermostability is relatively less critical than for wildlife vaccination, provided the cold chain is maintained during transport and storage. In OVD programs, baits are distributed directly by hand or placed in selected sites, with unconsumed baits typically recovered within 24 h, limiting environmental exposure.[11]

The aim of this study was to evaluate the stability and immunogenicity of an oral rabies vaccine under laboratory-controlled temperatures simulating field conditions to determine the optimal conditions for its use. This ensures the vaccine's effectiveness when deployed for the immunization of free-roaming and stray dogs, thereby contributing to the reduction of rabies transmission to humans.

Materials and Methods

Rabies vaccine baits

The SERVAC Rabies Vaccine Oral Bait (SERVAC-RVOB),[8] produced by the Veterinary Serum and Vaccine Research Institute (VSVRI), Egypt, contains 3 mL of an attenuated, cell culture-adapted rabies virus vaccine (ERA strain) with a titer of $\geq 10^{7.5}$ $TCID_{50}$/mL. The vaccine was filled in polyethylene sachets and was submitted to the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) Abbassia, Cairo, Egypt, for evaluation.

Virus

The rabies virus ERA strain, adapted to Baby Hamster Kidney (BHK) cells and possessing a titer of $\geq 10^{7.5}$ $TCID_{50}$/mL, was used for virus titration. This strain was supplied by the Strain Bank department at the CLEVB.

Cell line

BHK cells were obtained from the Strain Bank department at the CLEVB for virus titration.

In vitro stability

The intrinsic stability of the SERVAC-RVOB was evaluated under controlled laboratory conditions using temperature regimens selected to simulate field storage and exposure scenarios commonly encountered in rabies-endemic regions, in accordance with international recommendations for oral rabies vaccine evaluation.[15,16]

Vaccine baits were stored at 4 °C (refrigerated conditions), 20 °C (ambient temperature), and at 37 °C and 45 °C to simulate exposure to elevated environmental temperatures that may occur during field distribution and bait deployment. Storage at 4 °C was performed in a laboratory refrigerator, while storage at 20 °C, 37 °C, and 45 °C was conducted in temperature-controlled incubators with continuous monitoring to ensure temperature stability.

At predefined time intervals, each bait was diluted to prepare a vaccine suspension, which was subsequently titrated as described by WOAH;[16] for this, cytopathic effect (CPE) was monitored in both test suspensions and a reference rabies virus (positive control), and virus titers were calculated using the Spearman–Kärber method and expressed as $\log_{10}$ $TCID_{50}$/mL, with a mean confidence interval of ± 0.5 log units.

Experimental design for *in vivo* evaluation of rabies vaccine oral bait

To evaluate the stability and immunogenicity[15,16] of the SERVAC-RVOB following storage at defined temperatures simulating field-relevant conditions, an *in vivo* trial was conducted using 18 healthy, native-breed adult dogs (aged 6–12 months). All animals were provided by the CLEVB, Abbassia, Cairo, Egypt. Prior to the study, dogs were confirmed to be clinically healthy and seronegative for rabies virus antibodies, as determined by ELISA screening.

The dogs were housed individually in hygienic cages under controlled conditions, with access to balanced nutrition and clean water. They were monitored daily for general health and wellbeing throughout the study period (approximately one month).

Vaccine baits were stored at predefined temperatures (4 °C, 20 °C, 37 °C, and 45 °C) for either 15 or 30 days prior to administration. Based on the storage temperature and duration, animals were allocated into five experimental groups, as described below:

Group 1 (4 °C):

Two dogs received baits stored at 4 °C for 15 days.

Two dogs received baits stored at 4 °C for 30 days.

Group 2 (20 °C):

Two dogs received baits stored at 20 °C for 15 days.

Two dogs received baits stored at 20 °C for 30 days.

Group 3 (37 °C):

Two dogs received baits stored at 37 °C for 15 days.

Two dogs received baits stored at 37 °C for 30 days.

Group 4 (45 °C):

Two dogs received baits stored at 45 °C for 15 days.

Two dogs received baits stored at 45 °C for 30 days.

Group 5 (Control):

Two unvaccinated dogs served as negative controls.

Each vaccinated dog received a single dose of the oral bait corresponding to its assigned group. Blood samples were collected 28 days post-vaccination, centrifuged at 3,000 × g for 30 minutes, and the resulting sera were stored at −20 °C until serological analysis. Seroconversion was assessed using an ELISA to detect rabies virus-specific antibodies.[17]

Serological analysis

A blocking ELISA was employed to monitor the rabies virus-specific antibody titers in vaccinated dogs. Serum samples, collected 28 days post-vaccination, were analyzed using a commercial ELISA kit (BioPro Rabies ELISA, cat# RAB01-02, BioPro, Prague, Czech Republic). The assay was performed according to the manufacturer's instructions. Positive and negative control sera were included in each run, as provided with the kit.

The results were interpreted based on the following criteria:

The optical density (OD) value of the negative control (N) was required to be greater than 0.5 for the assay to be valid.

A sample was considered positive for rabies antibodies if it showed a blocking rate greater than 60 %, which corresponds to a protective antibody level of ≥ 0.5 IU/mL.

Ethical approval

The current study follows the Animal Research: Reporting of In-Vivo Experiments (ARRIVE) guidelines. All procedures involving animal use strictly adhere to the guidelines established by the Institutional Animal Care and Use Committee at the Agricultural Research Center (ARC-IACUC). Ethical approval for this study was obtained from the committee (ARC-IACUC) approval No (ARC-CLEVB-25-28). The manuscript is considered compliant with bioethical standards in good faith. No anesthesia or euthanasia protocols were employed for the animals involved in this study, as all animal-dependent methodological procedures were categorized as either no or low-pain procedures that can be ethically performed on a conscious and alive animal.

Statistical analysis

The All statistical analyses were conducted in Python (SciPy v1. 11). Correlation between viral titer and the immunogenicity was analyzed using Pearson's product moment correlation coefficient for data which were distributed normally. It also was used to evaluate the trends of monotonic data by Spearman's rank correlation. Differences in ELISA blocking percentages measured in vaccinated dogs receiving vaccine baits previously stored at different temperatures were analyzed using the Kruskal–Wallis H test as data were not normally distributed and the sample sizes were small. For dichotomous outcomes (protective vs. non-protective serostatus), Fisher's exact test was applied to determine differences in immunogenicity proportions between vaccine storage conditions.

A p-value < 0.05 was considered statistically significant.

Results

Stability of rabies vaccine oral bait under different temperatures

As shown in Table 1, SERVAC-RVOB exhibited temperature- and time-dependent reductions in viral

titer. Vaccine baits stored at 4 °C showed minimal loss of infectivity, with titers decreasing slightly from 7.6 ± 0.2 to 7.2 ± 0.3 log_{10} $TCID_{50}$/mL over 4 weeks. Storage at 20 °C resulted in a gradual decline, with titers remaining above 6.5 ± 0.3 log_{10} $TCID_{50}$/mL by week 4.

In contrast, storage at 37 °C and 45 °C led to pronounced and rapid decreases in viral titer. At 37 °C, titers declined to 5.1 ± 0.4 log_{10} $TCID_{50}$/mL by week 4, while exposure to 45 °C caused a substantial loss of infectivity, reaching 4.1 ± 0.5 log_{10} $TCID_{50}$/mL at the same time point.

In vivo immunogenicity of rabies vaccine oral bait following storage under different temperature conditions

The immunogenicity of SERVAC-RVOB was strongly influenced by storage temperature and duration (Table 2). Dogs receiving baits stored at 4 °C for 15 or 30 days exhibited robust antibody responses, with mean ELISA blocking percentages of 84 % and 81 %, respectively, and all animals achieving protective serostatus.

Baits stored at 20 °C for 15 days elicited protective responses in all animals (mean blocking 77 %), whereas storage for 30 days led to a marked reduction in immunogenicity (mean blocking 49 %), with most dogs failing to reach protective antibody levels.

Storage at elevated temperatures (37 °C) resulted in poor immunogenicity regardless of duration. Dogs receiving baits stored for 15 days had a mean blocking percentage of 37 %, and those receiving baits stored for 30 days had 21 %, with all animals classified as non-protective.

Table 1. Virus titer (log_{10} $TCID_{50}$/mL) over time at different storage temperatures.

Week	4 °C (Refrigerated)	20 °C (Room temperature)	37 °C (Tropical)	45 °C (High heat)
0	7.6 ± 0.2	7.6 ± 0.2	7.6 ± 0.2	7.6 ± 0.2
1	7.5 ± 0.2	7.4 ± 0.2	6.9 ± 0.3	6.2 ± 0.4
2	7.4 ± 0.2	7.1 ± 0.2	6.3 ± 0.3	5.4 ± 0.4
3	7.3 ± 0.3	6.8 ± 0.3	5.7 ± 0.4	4.7 ± 0.5
4	7.2 ± 0.3	6.5 ± 0.3	5.1 ± 0.4	4.1 ± 0.5

Table 2. Mean ELISA (% blocking) at day 28 post-vaccination and serological protection status in dogs receiving rabies vaccine oral bait stored under different conditions.

Group	Storage temperature	Storage duration	Vaccine titer	Mean ELISA (% blocking)*	Serostatus
1	4 °C	15 days	7.4	84 %	Positive (Protective)
1	4 °C	30 days	7.2	81 %	Positive (Protective)
2	20 °C	15 days	7.0	77 %	Positive (Protective)
2	20 °C	30 days	6.5	49 %	Negative (Non-protective)
3	37 °C	15 days	6.0	37 %	Negative (Non-protective)
3	37 °C	30 days	5.1	21 %	Negative (Non-protective)
4	45 °C	15 days	5.0	17 %	Negative (Non-protective)
4	45 °C	30 days	4.1	11 %	Negative (Non-protective)
5	Control (no vaccine)	-	N/A	7 %	Negative (Non-protective)

* Positive (protective): ELISA blocking percentage > 60% (corresponds to ≥ 0.5 IU/mL of rabies antibodies). Negative (non-protective): ELISA blocking percentage < 60%.

Table 3. Summary of statistical analyses evaluating the impact of storage conditions on rabies vaccine potency and immunogenicity.

Analysis	Test used	Statistic	p-value	Interpretation
Vaccine titer vs. ELISA blocking	Pearson correlation	r = 0.967	0.00009	Strong, significant correlation between potency and immunity
Vaccine titer vs. ELISA blocking	Spearman correlation	p= 1.0	< 0.001	Perfect positive monotonic relationship
ELISA blocking across storage groups	Kruskal–Wallis H test	H = 7.73	0.102	No statistical difference, but trend toward reduced response at high temperatures
Protective vs. non-protective status	Fisher's exact test	Odds Ratio = ∞	0.012	Significant drop in protective immunity in high-temp groups

Statistical analysis

The comparison of the vaccine efficacy between various storage conditions (Table 3) indicated that the strength of the immune responses was largely related with the initial viral titers statistically. Pearson's correlation analysis revealed an observable and significant strong positive correlation between vaccine titer and ELISA blocking percentage ($r = 0.967$, $p = 0.00009$) while the research of Spearman's rank correlation added information by demonstrating a perfect monotonic trends of correlation ($\rho = 1.0$, $p < 0.001$) suggesting that a reduction of viral strength was universally reflected by the decrease of its immunogenicity. Although nonparametric pairwise comparisons via the Kruskal–Wallis test showed no significant difference in ELISA blocking percentages in the five storage groups ($H = 7.73$, $p = 0.102$), a strong downward antibody trend was evident with increased temperature and storage time. Moreover, Fisher's exact test determined a significant association between storage temperature and probability to achieve serological protection ($p = 0.012$), where 100 % of dogs vaccinated with the baits stored either at 4 °C or 20 °C for 15 days reaching protective antibody levels, while none of those exposed to higher temperatures or longer storage durations achieved protection.

Discussion

The effective control of rabies in free-roaming and stray dog populations depends on the availability of oral vaccines that are stable, safe, and immunogenic, even under challenging environmental conditions.[8,16] This study evaluated the thermal stability and immunogenicity of the SERVAC-RVOB, containing an attenuated ERA strain, using laboratory-controlled temperature incubation to simulate storage and environmental conditions commonly encountered in rabies-endemic regions.

By systematically assessing the effects of different storage temperatures (4 °C, 20 °C, 37 °C, and 45 °C) on viral titers and immune responses, we identified optimal conditions that preserve vaccine potency and induce protective seroconversion. These findings support the strategic deployment of SERVAC-RVOB in mass vaccination campaigns, while emphasizing the importance of maintaining appropriate storage conditions during transport and field use. Ensuring vaccine stability under realistic thermal stress contributes directly to the interruption of rabies transmission from dogs to humans.

Our results confirm that SERVAC-RVOB maintains high viral titers when stored at refrigeration temperatures (4 °C), with only a slight reduction from 7.6 to 7.2 $\log_{10}$ $TCID_{50}$/mL over 4 weeks, indicating excellent cold-chain stability. This aligns with the findings from Maki, et al.,[18] who reported that the recombinant oral vaccine RABORAL V-RG® retained potency for up to 18 months at 4 °C with minimal titer loss (<0.4 $\log_{10}$ $TCID_{50}$/mL).

At 20 °C, a moderate decline to 6.5 $\log_{10}$ $TCID_{50}$/mL was recorded, still within the immunogenic threshold (≥6.8 $\log_{10}$ $TCID_{50}$/mL). However, storage at 37 °C and 45 °C resulted in steep declines in viral titer to 5.1 and 4.1 $\log_{10}$ $TCID_{50}$/mL, respectively, by day 28. These

results are consistent with thermostability profiles reported by Pastoret, et al.[19] and Maki, et al.,[18] who documented losses of 1.3 $\log_{10}$ $TCID_{50}$ at 20 °C over 56 days and more than 2 $\log_{10}$ units at 37 °C within one week for RABORAL V-RG®. This temperature sensitivity emphasizes the importance of maintaining cold-chain logistics during storage and transportation, particularly in tropical and subtropical regions. According to WHO[15] and WOAH[16] guidelines, a minimum vaccine potency of 6.8 $\log_{10}$ $TCID_{50}$/mL is critical to induce protective rabies virus neutralizing antibody (RVNA) titers of ≥0.5 IU/mL in vaccinated animals.

The immunogenic performance of oral rabies vaccines under varying environmental conditions is a critical determinant for their field deployment in controlling rabies among free-roaming dogs. In our study, the SERVAC-RVOB demonstrated strong immunogenicity when stored under refrigerated conditions (4 °C), with mean ELISA blocking percentages of 84 % and 81 % after 15 and 30 days of storage, respectively. This correlates with stable viral titers (7.4–7.2 $\log_{10}$ $TCID_{50}$/mL), indicating that cold-chain storage preserves vaccine potency, ensuring protective antibody responses well above the 60 % ELISA blocking threshold (≥ 0.5 IU/mL), in agreement with standards for effective immunization.[17]

Moderate ambient conditions (20 °C) yielded mixed outcomes. Although the vaccine maintained immunogenicity at 15 days (77 % blocking; 7.0 $\log_{10}$), immunoprotection declined significantly by 30 days (49 % blocking; 6.5 $\log_{10}$), that the functional efficacy threshold is likely to be close to 6.8 $\log_{10}$ $TCID_{50}$/mL, consistent with earlier reports[11,16] indicating that effective immunization generally requires titers above this level. In contrast, higher storage temperatures (37 °C and 45 °C) led to substantial declines in immunogenicity. None of the groups stored under these conditions seroconverted above the protective threshold, with blocking ELISA percentages dropping to 21 % or below and corresponding titers falling below 6.0 $\log_{10}$ $TCID_{50}$/mL. This observation is consistent with the findings of Maki et al.[18], who demonstrated that the recombinant oral rabies vaccine RABORAL V-RG® undergoes rapid potency loss under elevated temperatures. Specifically, storage at 37 °C resulted in an approximate 1.5 $\log_{10}$ $TCID_{50}$/mL reduction within 7 days, with even greater declines reported under direct sunlight exposure. These findings highlight the vulnerability of lyophilized and liquid rabies vaccines to thermal degradation, emphasizing the necessity of maintaining cold chain conditions to prevent immunological failure in the field.

Our results corroborate those of Aly, et al.,[8] who demonstrated that oral rabies baits containing the ERA strain retained immunogenicity at titers ≥ 7.0 $\log_{10}$ $TCID_{50}$/mL, inducing seroconversion in 100 % of vaccinated dogs using both ELISA and serum neutralization testing. Similar to our findings, they observed diminished seroconversion when titers fell below protective thresholds, reinforcing the conclusion that prolonged exposure to temperatures above 20 °C compromises vaccine efficacy.

The present study supports the use of SERVAC-RVOB when stored under refrigerated conditions and demonstrates that it retains immunogenicity at ambient temperatures for up to 2 weeks. However, exposure to elevated temperatures (37 °C and 45 °C) led to a significant decline in viral titer and loss of protective antibody responses, indicating reduced efficacy under such conditions. These findings are consistent with previously published data on other oral rabies vaccines, including RABORAL V-RG®, in which thermal exposure led to rapid declines in infective titers and reduced immunogenic performance.[18,19]

Maintaining vaccine stability during storage and transport remains a significant logistical hurdle in rabies -endemic countries, especially where high environmental temperatures and the lack of cold-chain storage and transport are common. For this reason, the development of heat-stable thermostable vaccine formulations is a significant task.[8,18] Promising recent approaches using lyophilization, stabilizing excipients, and encapsulation, among others, should be considered in future studies for SERVAC-RVOB.

While this study employed a blocking ELISA for serological assessment, we recognize the limitation of not performing virus-neutralization assays such as Rapid Fluorescent Focus Inhibition Test (RFFIT) or Fluorescent Antibody Virus Neutralization Test

(FAVN). However, the BioPro Rabies ELISA has been demonstrated to correlate well with neutralizing antibody titers ≥0.5 IU/mL.[16,20,21] Nonetheless, we recommend future studies include RFFIT/FAVN confirmation to strengthen the conclusions.

Statistical analysis revealed a strong correlation between vaccine titer and immune response (Pearson's $r = 0.967$; Spearman's $\rho = 1.0$), confirming that reduced potency leads to diminished immunogenicity. Similar associations were reported by other authors,[8,18] who also used statistical models to link thermal degradation with loss of vaccine efficacy. While the Kruskal–Wallis test showed no significant group differences ($p = 0.102$), Fisher's exact test ($p = 0.012$) confirmed a significant drop in protection at higher temperatures, consistent with findings by Pastoret, et al.[19] These results reinforce the need for cold-chain maintenance to ensure oral rabies vaccine effectiveness.

We acknowledge the limited statistical power due to the small sample size (n=2 per subgroup). This study serves as a pilot-scale evaluation to provide preliminary insights into the thermal sensitivity and immunogenicity trends of SERVAC-RVOB. Further large-scale studies are warranted to validate these findings under field conditions, in accordance with WHO guidance on oral rabies vaccination trials.[11] The observed thermal degradation may be attributed to the absence of specific stabilizers or protective excipients in the vaccine formulation. No cryoprotectants or lyophilizing agents were included in the polyethylene-packaged SERVAC-RVOB. Future work should explore thermostabilizing strategies such as trehalose supplementation, lipid encapsulation, or lyophilized delivery platforms.[18,22]

In summary, SERVAC-RVOB is suitable for storage under refrigerated conditions and for short-term use at ambient temperatures, where it maintains its immunogenicity and ability to induce protective antibody responses. However, prolonged exposure to elevated temperatures significantly compromises vaccine potency and immunogenicity. Therefore, its application in tropical field settings requires either strict temperature management or reformulation strategies to ensure consistent vaccine performance and protective efficacy.

Conclusions

SERVAC-RVOB demonstrates reliable immunogenicity when stored at 4 °C and retains acceptable potency for up to 15 days at ambient temperatures. However, its stability decreases significantly under prolonged heat exposure. Based on these findings, it is recommended to deploy SERVAC-RVOB primarily during cooler seasons, such as winter, to reduce thermal degradation risks. Additionally, removing uneaten baits after 2 weeks may enhance vaccine efficacy, limit environmental exposure, and improve monitoring of vaccination coverage among free-roaming dogs. These measures can contribute to more effective rabies control in endemic areas.

Conflict of interest

The authors declare that there is no conflict of interest.

Author´s contributions

Nermeen Gouda-Shafik: conceptualization, validation and investigation.

Heba A. Khafagy: methodology, formal analysis, and data curation.

Sara El Sawy-Ahmed: methodology, validation, and investigation.

Darwish Mahmoud Darwish: methodology, formal analysis, and investigation.

Fady Abd El-Mohsen Shasha: methodology, formal analysis, investigation.

Amal Abd El-Moneim-Mohamed: methodology, validation, and formal analysis.

Mohamed Abdelkhalek Abdrabo: investigation.

Mohamed Samy Abousenna: conceptualization, methodology, formal analysis, investigation, data curation, writing-original draft preparation, writing-review and editing.

All authors have read and agreed to the published version of the manuscript.

References

1. Khan B, Shrivastava N, Sheikh NP, Singh PK, Jha HC, Parmar HS. Rabies vaccines: Journey from classical to modern era. Vet Vaccine. 2025; 4(1): 100105. doi: https://10.1016/j.vetvac.2025.100105.

2. Zheng J, Sui B, Zhao L. Rabies lyssavirus. In: Wang L, ed. Veterinary Virology of Domestic and Pet Animals. Cham: Springer; 2025. doi: https://10.1007/978-3-031-54690-7_16-1.

3. Scott TP, Nel LH. Lyssaviruses and the Fatal Encephalitic Disease Rabies. Front Immunol. 2021;12:786953. doi: https://10.3389/fimmu.2021.786953.

4. Hampson K, Dushoff J, Cleaveland S, Haydon DT, Kaare M, Packer C, Dobson A. Transmission dynamics and prospects for the elimination of canine rabies. PLoS Biol. 2009;7(3):e53. doi: https://10.1371/journal.pbio.1000053.

5. World Health Organization, Food and Agriculture Organization of the United Nations, World Organization for Animal Health, Global Alliance for Rabies Control. Zero by 30: The global strategic plan to end human deaths from dog-mediated rabies by 2030. Geneva: WHO; 2018. Available from: https://www.woah.org/fileadmin/Home/eng/Media_Center/docs/Zero_by_30_FINAL_online_version.pdf. (Access online: May 6, 2025).

6. Bucher A, Dimov A, Fink G, Chitnis N, Bonfoh B, Zinsstag J. Benefit-cost analysis of coordinated strategies for control of rabies in Africa. Nat Commun. 2023;14(1):5370. doi: https://10.1038/s41467-023-41110-2.

7. Chazya R, Mulenga CAS, Gibson AD, Lohr F, Boutelle C, Bonaparte S, et al. Rabies vaccinations at the rural-urban divide: successes and barriers to dog rabies vaccination programs from a rural and urban campaign in Zambia. Front Vet Sci. 2025;11:1492418. doi: https://10.3389/fvets.2024.1492418.

8. Aly NI, Elnaker YF, Salama ZTS, Diab MS, Saber EA, Sotohy SA, et al. Preparation and the assessed efficacy of oral baits for the vaccination of free-roaming dogs against rabies. Vet World. 2022;15(5):1383-90. doi: https://10.14202/vetworld.2022.1383-1390.

9. Vitasek J. A review of rabies elimination in Europe. Vet Med - Czech. 2004;49(5):171–85. doi: https://10.17221/5692-VETMED.

10. Abelseth MK. Further studies on the use of ERA rabies vaccine in domestic animals. Can Vet J. 1967;8(10):221-7.

11. World Health Organization (WHO). Oral vaccination of dogs against rabies: guidance for research on oral rabies vaccines and field application of oral vaccination of dogs against rabies. Geneva: WHO; 2007. Available from: https://iris.who.int/server/api/core/bitstreams/96167b93-5da6-4cf6-972e-684e7b29858c/content. (Access online: May 6, 2025).

12. World Organization for Animal Health (WOAH). Report of the meeting of the OIE Biological Standards Commission. Paris: WOAH; 2017. Available from: https://www.woah.org/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/BSC/A_BSC_Feb2017.pdf. (Access online: May 6, 2025).

13. Linhart SB, Baer GM, Balderas Torres JM, Engeman RM, Flores Collins E, Meslin FX, et al. Acceptance of candidate baits by domestic dogs for delivery of oral rabies vaccines. Onderstepoort J Vet Res. 1997;64(2):115-24.

14. Bender S, Bergman D, Vos A, Martin A, Chipman R. Field studies evaluating bait acceptance and handling by dogs in Navajo Nation, USA. Trop Med Infect Dis. 2017;2(2):17. doi: https://10.3390/tropicalmed2020017.

15. World Health Organization. International collaborative study to assess the suitability of the candidate 7th WHO International Standard for rabies vaccine (WHO/BS/2018.2335). Geneva: World Health Organization; 2018. Available from: https://cdn.who.int/media/docs/default-source/biologicals/bs-documents-(ecbs)/2019-documents/bs.2018.2335_7th_is_rabies_vaccine.pdf?sfvrsn=a0e738d5_5. (Access online: February 4, 2026).

16. World Organization for Animal Health (WOAH). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris: WOAH; Chapter 3.1.19 Rabies (infection with rabies virus and other lyssaviruses); 2025.p.16. Available from: https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.01.19_RABIES.pdf. (Access online: February 4, 2026).

17. Abousenna MS, El Sawy Ahmed S, Darwish D, Shasha FAE-M, Abd El-Moneim Mohamed A, Khafagy H, et al. Preliminary evaluation of humoral immune response to rabies vaccine using lateral flow immunochromatographic assay. VacciMonitor. 2025;34(1):e053425. Available from: https://vaccimonitor.finlay.edu.cu/index.php/vaccimonitor/article/view/9231. (Access online: February 4, 2026).

18. Maki J, Guiot AL, Aubert M, Brochier B, Cliquet F, Hanlon CA, et al. Oral vaccination of wildlife using a vaccinia –rabies-glycoprotein recombinant virus vaccine (RABORAL V-RG®): A global review. Vet Res. 2017 10;48:57. https://10.1186/s13567-017-0459-9.

19. Pastoret PP, Brochier B, Languet B, Duret C, Chappuis G, Desmettre P. Stability of recombinant vaccinia-rabies vaccine in veterinary use. Dev Biol Stand. 1996;87:245-9.

20. Das LJ, Isloor S, Santosh AK, Bhat A, Sharada R, Rathnamma D, et al. A Comparative Evaluation of the Estimation of Rabies Virus Antibodies among Free-Roaming, Vaccinated Dogs in Bengaluru, India. Viruses. 2022;14(3):484. doi: https://10.3390/v14030484.

21. Fitria Y, Febrianto N, Putri RE, Rahmadani I, Subekti DT. Evaluation of In-House ELISA for Antirabies Antibodies Detection in Domestic Canine. Vet Med Int. 2023;2023:4096258. doi: https://10.1155/2023/4096258.

22. Lankester FJ, Wouters PAWM, Czupryna A, Palmer GH, Mzimbiri I, Cleaveland S, et al. Thermotolerance of an inactivated rabies vaccine for dogs. Vaccine. 2016;34 (46):5504-11. doi: https://10.1016/j.vaccine.2016.10.015.

Evaluación de la potencia de una vacuna antirrábica oral en condiciones de simulación de temperaturas de campo

Resumen

La rabia sigue siendo una enfermedad zoonótica mortal con una carga significativa en los países en desarrollo, especialmente en aquellos en los que los perros callejeros dificultan el éxito de las estrategias de vacunación parenteral. La vacunación oral mediante cebos termoestables ofrece una solución prometedora. El objetivo de este estudio fue evaluar la estabilidad dependiente de la temperatura y la inmunogenicidad de la vacuna oral contra la rabia mediante cebo, SERVAC (SERVAC-RVOB), que contiene una cepa ERA atenuada. La vacuna se evaluó en diferentes temperaturas, que simulan condiciones reales, en regiones donde la rabia es endémica. Los cebos vacunales se almacenaron a 4 °C, 20 °C, 37 °C y 45 °C durante un máximo de 30 días. Los títulos víricos se evaluaron semanalmente en células BHK-21 y se calcularon utilizando el método de Spearman-Kärber. Se realizó un estudio *in vivo* con 18 perros sanos y seronegativos, divididos en cinco grupos en función de las condiciones de almacenamiento. Cada perro recibió una dosis única de cebo y las muestras de suero recogidas el día 28 después de la vacunación se analizaron utilizando un ELISA comercial para detectar anticuerpos específicos contra el virus de la rabia. Los resultados mostraron que SERVAC-RVOB mantuvo títulos elevados (7,2 $\log_{10}$ $TCID_{50}$/mL) e indujo respuestas de anticuerpos protectores cuando se almacenó a 4 °C y a 20 °C durante un máximo de 15 días. Sin embargo, el almacenamiento a 37 °C y 45 °C provocó una pérdida notable de potencia y la incapacidad de provocar inmunidad protectora. Los valores de bloqueo del ELISA disminuyeron significativamente en estas condiciones, lo que indica una fuerte correlación entre la temperatura, la pérdida de títulos y la inmunogenicidad. Estos hallazgos respaldan el despliegue de SERVAC-RVOB durante las estaciones más frías y recomiendan la retirada de los cebos no consumidos después de 2 semanas para maximizar la eficacia y la supervisión sobre el terreno. Mantener la logística de la cadena de frío o mejorar la termoestabilidad es esencial para el éxito del control de la rabia en entornos endémicos.

Palabras clave: virus de la rabia; potencia de la vacuna; ELISA.

Received: October 19, 2025 *Accepted: March 23, 2026*