DOI: 10.11134/btp.2.2026.3

UDC: 57.083.3:578.824.11 Original Article

EVALUATION OF THE IMMUNOBIOLOGICAL PROPERTIES OF AN ORAL BRIQUETTE RABIES VACCINE IN FOXES

Bulatov Y.A.[1], **Abitayev R.T.**[1], **Ussembay A.K.**[1], **Amanova Zh.T.**[1], **Sametova Zh.Zh.**[1], **Turyskeldi Sh.S.**[1,2], **Kondibayeva Zh.B.**[1], **Mazbayeva D.M.**[1], **Kurmasheva A.K.**[1], **Koshemetov Zh.K.**[1], **Renukaradhya J G.**[3], **Toktyrova D.S.**[1,2*]

[1] *Research Institute for Biological Safety Problems, National Holding "QazBioPharm", 25/1, Gvardeiskiy, Korday District, Zhambyl Region, 080409, Kazakhstan*
[2] *Al-Farabi Kazakh National University, Faculty of Biology and Biotechnology, 71 Al-Farabi Avenue, Almaty, 050040, Republic of Kazakhstan*
[3] *The Ohio State University, Department of Animal Sciences, Animal Science Building 2029 Fyffe Road, Columbus, 43210, Ohio State, USA*
** Correspondence: Dariya Toktyrova d.toktirova@biosafety.kz*

ABSTRACT

Rabies remains one of the most dangerous zoonotic infections maintained in natural reservoirs among wild carnivorous animals. Despite the high efficacy of inactivated vaccines in domestic animals, control of rabies in wildlife requires the use of oral vaccines capable of achieving mass immunization of naturally susceptible species. The aim of the present study was to evaluate the immunogenicity, safety, and protective efficacy of an experimental oral briquetted rabies vaccine in naturally susceptible carnivorous animals under pilot conditions. A total of 10 clinically healthy, rabies-seronegative foxes were included in the experiment. Three foxes in the immunogenicity group received a single oral dose of the vaccine formulated as a briquetted bait containing the fixed rabies virus strain *Rabies virus fix/NIIPBB/2024* at a titer of 6.00 log $TCID_{50}$/mL. Five animals received an increased dose of 10 vaccine baits per animal to evaluate safety, while two unvaccinated foxes served as controls. The dynamics of the humoral immune response were assessed using the mouse neutralization test and ELISA. Protective efficacy was evaluated by challenge infection with the CVS test strain. The results showed that the vaccine baits were readily consumed by the animals and did not cause clinical adverse reactions or deviations from physiological norms. ELISA-derived antibody levels exceeded 0.5 IU/mL, corresponding to a sufficient humoral immune response according to the manufacturer's criteria. In parallel, virus-neutralizing antibody titers determined by the mouse neutralization test reached peak values of up to 4.3 $\log_2$ on day 21 post-immunization. Following challenge infection, vaccinated animals survived without clinical signs of rabies, whereas control animals developed the paralytic form of the disease with a fatal outcome. Overall, the findings of this pilot study suggest that the oral briquetted rabies vaccine demonstrates promising immunogenicity, safety, and protective potential, supporting its further investigation for use in oral immunization programs targeting wild carnivorous species and the control of natural rabies foci.

Keywords: rabies, oral vaccination, briquetted vaccine, virus-neutralizing antibodies, wild carnivores.

1. INTRODUCTION

Rabies is caused by infection with lyssaviruses, a group of single-stranded RNA viruses belonging to the family *Rhabdoviridae*. Members of the genus *Lyssavirus* primarily infect the nervous system of mammals, and the disease is almost invariably fatal in both reservoir and incidental hosts following a relatively long incubation period, typically ranging from 1 to 3 months [1,2]. Both cell-mediated and humoral immune mechanisms play important roles in host defense against rabies virus infection [3]. However, in the context of disease prevention, primary emphasis is placed on the humoral immune response. Virus-neutralizing antibodies against rabies virus are a key component of the protective humoral immune response. Their essential role in protection against rabies has been demonstrated in experimental models, and rabies-specific immunoglobulins can neutralize infectious virions and limit viral spread during the early stages of infection. Since most neutralizing antibodies target the rabies virus glycoprotein, which mediates receptor binding and membrane fusion, these antibodies may interfere with viral attachment, entry into host cells, and fusion processes, thereby helping to prevent or limit infection before the virus reaches the central nervous system [4, 5]. Although post-exposure prophylaxis (PEP) is primarily used in humans, preventive vaccination of domestic animals is recommended or mandatory in many regions of the world. In addition, the concept of preventive immunization has been widely implemented in oral rabies vaccination (ORV) programs aimed at controlling virus circulation in wildlife reservoir species and reducing transmission among free-ranging animal populations [6, 7].

Rabies is a vaccine-preventable disease that can be effectively controlled through timely immunization of reservoir animals and the application of PEP in humans. This approach is reflected in the global "Zero by 30" initiative, as well as in current standards for laboratory diagnostics and serological monitoring [8]. One of the key indicators of adequate seroconversion is a virus-neutralizing antibody (VNA/RVNA) level of at least 0.5 IU/mL, determined using validated neutralization assays such as FAVN or RFFIT. However, species-specific differences and the context of mass vaccination campaigns should be taken into account when interpreting serological results [9].

The widespread use of inactivated rabies vaccines in many countries has led to a significant reduction in rabies incidence

DOI: 10.11134/btp.2.2026.3

among domestic animals and, consequently, a decrease in human rabies cases [10]. Inactivated rabies vaccines are generally considered highly effective across a broad range of animal species, including some wildlife species [11-13]. However, in practical terms, such vaccines cannot fully replace large-scale immunization of natural rabies virus reservoirs in wildlife populations. Therefore, oral rabies vaccines – both attenuated and modern recombinant or biotechnological constructs – have become a central component of contemporary strategies for rabies control in wild animal populations [14].

In Kazakhstan, rabies remains an endemic zoonotic and veterinary infection involving both domestic and wild animals. During 2013–2023, 917 animal rabies cases were registered in the country, including 515 cases in farm animals, 352 cases in companion animals, and 50 cases in wild animals [15]. Another recent spatio-temporal analysis reported 926 animal rabies cases in Kazakhstan over the same 10-year period, with livestock accounting for 55.6%, companion animals for 38.8%, and wildlife for 5.6% of cases [16]. These data indicate that rabies continues to circulate among different animal groups in Kazakhstan and that wildlife, although representing a smaller proportion of officially registered cases, remains epidemiologically important for maintaining natural foci of infection and for possible spillover to domestic animals. Therefore, the development and evaluation of oral rabies vaccines targeting wild carnivorous species are particularly relevant for Kazakhstan, where large territories and the presence of wildlife reservoirs complicate conventional rabies control measures [15-17].

In this context, the development of domestic oral rabies vaccines, as well as the optimization of bait delivery systems, is of particular practical importance for countries with large territories where wildlife plays a significant role in maintaining virus circulation. In the present study, an experimental vaccine preparation developed at the Research Institute for Biological Safety Problems was used. The vaccine contains a fixed rabies virus strain *Rabies virus fix/NIIPBB/2024* enclosed in a soft blister and incorporated into an edible briquetted bait composed of fish meal and microcrystalline cellulose. This formulation ensures both the stability of the vaccine and the attractiveness of the bait to target animals. Tetracycline was included as a biomarker of bait consumption for subsequent population monitoring. The use of tetracycline markers is widely applied in oral rabies vaccination (ORV) programs, as it enables detection of bait uptake through characteristic fluorescence in teeth or bone tissues. This approach allows differentiation between cases where the bait was not consumed and situations in which the bait was ingested but seroconversion did not occur.

It should be noted that most studies on oral rabies vaccines have been conducted using laboratory models, whereas data on the immunogenicity and protective efficacy of such vaccines in naturally susceptible animals remain limited. Therefore, experimental studies involving natural hosts of the rabies virus, such as foxes and other carnivores, are of particular importance for evaluating newly developed vaccine preparations and their potential application in rabies control programs.

The aim of the present study was to evaluate the immunogenicity, safety, and protective potential of an experimental oral briquetted rabies vaccine in naturally susceptible carnivorous animals.

2. MATERIALS AND METHODS

2.1. Animals and Vaccine

The foxes used in the experiment were obtained from certified breeding facilities. Upon arrival, all animals were individually identified, clinically examined, and placed under quarantine for 14 days. During the quarantine period, routine deworming was performed using albendazole (Albendazole 10%, O.L.KAR, Ukraine).

A total of 10 clinically healthy foxes aged 4-6 months, with a body weight ranging from 3.5-6.0 kg, were included in the study. Both male and female animals were used (n = 3 males, n = 7 females). None of the animals had been previously vaccinated against rabies, as confirmed by veterinary records.

Prior to inclusion in the study, all foxes were tested for the presence of virus-neutralizing antibodies (VNA) against rabies virus. Only seronegative animals were included in the experiment. Animals were allocated into experimental groups (immunogenicity, safety, and control groups) using a random assignment procedure to minimize selection bias. Group allocation was performed according to a predefined randomization scheme prior to the start of the study.

Due to the nature of the experimental design (vaccine administration and clinical observation), blinding was not applied. However, all laboratory analyses, including serological testing, were conducted according to standardized protocols.

During the experimental period, the animals were housed individually in cages equipped with restraining devices. Feeding was performed once daily with a standard commercial antibiotic-free feed at a dose of 300-450 g per animal, depending on individual characteristics and nutritional needs. Water was provided *ad libitum*. The diet was periodically supplemented with vegetables, meat, and boiled eggs.

The oral bait vaccine consisted of a suspension containing the fixed rabies virus strain Rabies virus fix/NIIPBB/2024 with a biological activity of 6.00 $TCID_{50}$/ml. The volume of the vaccine suspension was 2.0 ml. The suspension was filled into flexible polymer containers (“soft blisters”), which were subsequently embedded into edible briquetted baits. The baits were produced using a nutrient matrix composed of fish meal and microcrystalline cellulose (MCC), ensuring attractiveness for animals. Tetracycline was included as a biomarker to monitor bait consumption.

2.2. Immunogenicity and safety assessment

To evaluate the immunogenicity and safety of the oral briquetted rabies vaccine, two experimental groups of foxes were established. For immunogenicity assessment, the experimental group consisted of three clinically healthy foxes. Animals received a single oral dose of the vaccine administered in the form of a briquetted bait, at a rate of one bait per animal. The control group consisted of two clinically healthy foxes of comparable age that did not receive the vaccine and were maintained under identical housing and feeding conditions. Twenty-four hours prior to vaccination, animals from both groups were fasted while maintaining free access to water. Following vaccination, animals were subjected to daily clinical observation with monitoring of behavior, body tempera-

DOI: 10.11134/btp.2.2026.3

ture, general health status, and feed and water intake. Blood samples for serological analysis were collected on days 7, 14, and 21 post-vaccination from the saphenous vein of the hind limb (*V. saphena*). Blood was collected into 6 mL vacuum tubes with clot activator and gel (Avatube, Ecopharm). The samples were centrifuged at 3000 × g for 15 minutes, after which serum was separated and stored at -20 °C until further analysis.

Challenge infection of animals from both the experimental and control groups was performed on day 21 post-vaccination using the reference rabies virus challenge strain CVS. The virus was administered intramuscularly into the masseter muscles at a dose of 1000 LD_{50} in a volume of 1.0 ml, according to the method described by Freuling et al. [13]. After challenge infection, the animals were monitored for 14 days with daily visual assessment of their clinical condition. Contact with animals was restricted in accordance with biosafety requirements.

For safety assessment, a separate group of five clinically healthy foxes aged 4-6 months was used. Animals in this group received the briquetted vaccine orally at an increased dose of ten baits per animal. Following administration, animals were monitored daily for potential adverse reactions, including changes in behavior, body temperature, clinical condition, and feed and water consumption.

2.3. Serological Tests

Mouse Neutralization Test (MNT)

Prior to testing, all serum samples were heat-inactivated at 56 °C for 30 minutes. Serial twofold dilutions of the sera were then prepared and mixed with an equal volume of the reference rabies virus challenge strain CVS containing 50 LD_{50} in 0.03 ml. The resulting mixtures were incubated at 37 °C for 1.5 hours and subsequently inoculated intracerebrally into white outbred laboratory mice weighing 16-18 g in a volume of 0.03 ml. Four mice were used for each serum dilution. Animals were observed for 21 days, and mortality was recorded starting from day 4 post-inoculation. VNA titers were calculated using the Reed-Muench method.

The MNT was used as the primary method for assessing functional anti-rabies immunity.

2.4. Enzyme-Linked Immunosorbent Assay (ELISA)

Specific antibodies against rabies virus were additionally evaluated using a commercial ELISA kit (Hema LLC, Russia), performed in accordance with the manufacturer's instructions.

Optical density (OD) was measured at 450 nm using a microplate reader. Antibody levels were estimated based on the manufacturer's calibration system by comparing OD values of the test samples with those of the provided calibrators and controls.

According to the manufacturer, the assay is intended for use in carnivorous animals; therefore, its application to fox serum samples in this study falls within the intended scope of the test system. The results were interpreted with caution due to potential species-specific variability. It should be noted that ELISA detects binding antibodies and does not directly measure virus-neutralizing activity. Therefore, ELISA results were used as a supplementary indicator of the humoral immune response and interpreted in conjunction with MNT data.

2.5. Bioethics

Animal care, housing, and feeding conditions complied with applicable veterinary and sanitary regulations and standards for the respective animal species, ensuring animal welfare and adherence to biosafety requirements throughout the study period. To minimize unnecessary animal suffering, predefined humane clinical endpoints were established in accordance with the recommendations described by Hartinger et al. [18]. All experimental protocols were reviewed and approved by the Bioethics Committee of the Research Institute for Biological Safety Problems prior to the initiation of the study (Protocol No. 3-03-10-2023). Throughout the study, institutional regulations, standard operating procedures, and guidelines for the care and use of laboratory animals were strictly followed.

2.6. Statistical Analysis

Statistical analysis was performed using GraphPad Prism software (version 8.4.3; GraphPad Software Inc., San Diego, CA, USA).

Virus titers and virus-neutralizing antibody (VNA) titers were calculated using the Reed–Muench method [19].

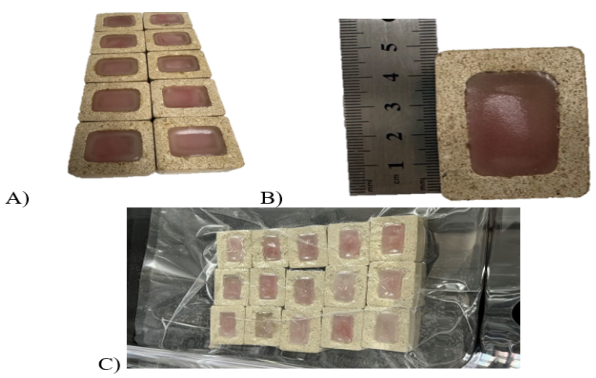
Given the limited sample size, data were primarily analyzed descriptively. Rectal body temperature values were compared between groups using an unpaired Student's t-test. Categorical outcomes, including morbidity and mortality after challenge infection, were summarized descriptively because of the small group size.

Statistical significance was set at $p \leq 0.05$; however, all results should be interpreted with caution due to the small number of animals included in this pilot study.

3. RESULTS

3.1 Characteristics and acceptability of briquetted baits for oral vaccination

The briquetted vaccine baits used in this study were developed as edible carriers for oral delivery of a vaccine-containing blister to carnivorous animals. The final bait format consisted of a compressed edible matrix containing a soft blister filled with the vaccine suspension. This formulation was selected to provide sufficient structural integrity during handling, accessibility of the vaccine-containing blister after



General appearance of the briquetted bait; B) final bait form containing the vaccine blister; C) vacuum-packed finished vaccine baits before administration.

Figure 1 - Structural characteristics of the briquetted rabies vaccine bait used for oral administration.

DOI: 10.11134/btp.2.2026.3

chewing, and acceptability to target animals.

The bait matrix was produced using an industrial briquetting line. (Figure 1).

The final briquetted form was compact, mechanically stable during routine handling, and suitable for individual oral administration to foxes under experimental conditions. During administration, the foxes showed interest in the bait and consumed it readily. The animals were able to grasp and chew the briquette, which allowed access to the vaccine-containing blister. No refusal of the bait was recorded during administration.

The use of a briquetted bait form was based on previous experimental evaluation of an oral rabies vaccine bait in a seronegative dog model. In that study, the baited briquette demonstrated good tolerability, high bait acceptance, induction of a specific immune response, and protective potential after challenge infection, supporting its further evaluation in naturally susceptible carnivorous species [20]. Therefore, in the present study, the final briquetted bait format was assessed in foxes as a target carnivorous species for oral rabies vaccination.

Overall, the observations obtained in the present experiment indicate that the briquetted bait form was suitable for oral delivery of the vaccine to foxes under pilot experimental conditions.

3.2. Safety and Protective Outcome in Foxes

3.2.1. Clinical safety after oral administration

Clinical safety of the oral briquetted rabies vaccine was assessed in foxes receiving either the standard vaccine dose or an increased dose. Throughout the 14-day observation period, no vaccine-associated adverse reactions were recorded. Vaccinated animals showed no signs of depression, anorexia, abnormal behavior, impaired locomotor activity, or changes in feed and water intake.

Rectal body temperature remained within the physiological range throughout the observation period (Figure 2). The mean body temperature was 39.41 ± 0.16 °C in foxes receiving the standard dose and 39.42 ± 0.30 °C in foxes receiving the increased dose. No statistically significant difference was detected between the groups ($t = 0.11$; $p = 0.91$). These results indicate that oral administration of the briquetted vaccine, including the increased dose, did not induce fever or clinically detectable systemic reactions.

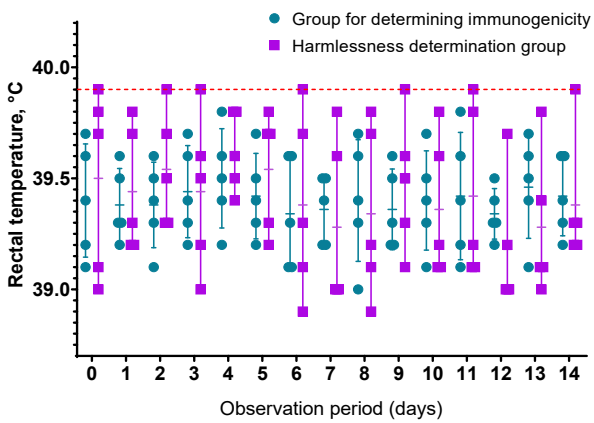
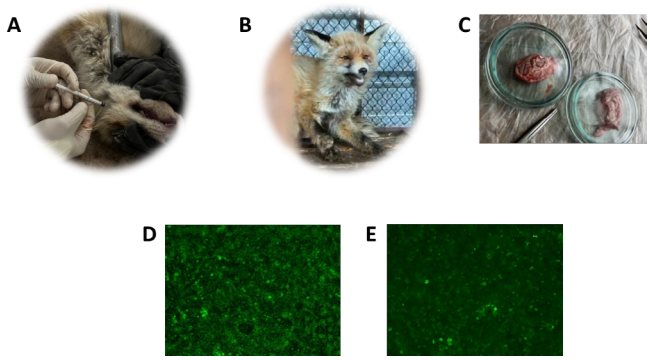


Figure 2 - Dynamics of body temperature in vaccinated animals used for the safety assessment of the oral briquetted vaccine.

3.2.2. Protective outcome after challenge infection

Protective efficacy was evaluated following challenge infection with the reference rabies virus strain CVS. Vaccinated foxes (n = 3) included in the challenge experiment remained clinically healthy throughout the observation period. No clinical signs of rabies, behavioral abnormalities, or deviations in locomotor activity were observed in vaccinated animals.

In contrast, all control animals developed clinical signs consistent with the paralytic form of rabies. Fatal outcomes were recorded on days 16 and 19 post-infection (Figure 3). Brain tissue samples collected during necropsy were positive for rabies virus antigen by the direct fluorescent antibody test. These findings confirm that the challenge infection was effective in control animals and indicate that vaccinated foxes were protected against clinical rabies under the conditions of this pilot experiment.



A) Challenge infection procedure; B) Clinical signs consistent with paralytic rabies in control foxes; D) Brain tissue sampling during necropsy; D and E) detection of rabies virus antigen in brain tissue by direct fluorescent antibody test.

Figure 3 - Clinical observations and laboratory confirmation following challenge infection

Table 1 - Protective outcome after challenge infection with the CVS strain.

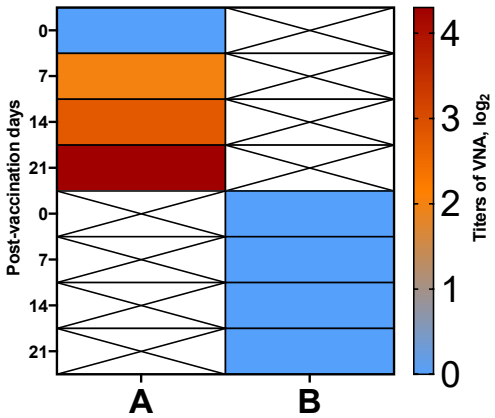
Group	Animals challenged	Clinical signs of rabies	Mortality	Day of death	FAT result
Vaccinated	3	0/3	0/3	Not applicable	Not tested
Control	2	2/2	2/2	16 and 19 dpi	Positive

3.3 Seroprevalence and Dynamics of the Humoral Immune Response

The general post-vaccination condition and behavior of immunized foxes remained within normal physiological limits throughout the study period. No clinical signs of rabies were observed in vaccinated animals during the 21-day observation period.

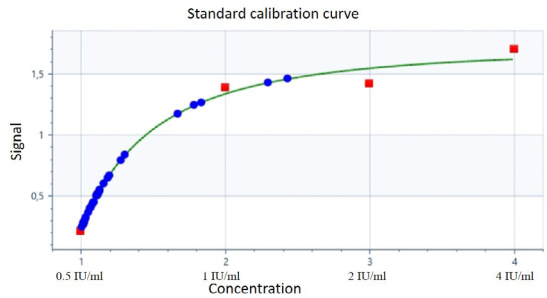
Virus-neutralizing antibody (VNA) testing showed that all animals were seronegative on day 0. By day 7 post-vaccination, neutralizing antibodies were detected in vaccinated foxes, with a mean titer of 2.0 $\log_2$. Antibody titers increased to 2.8 $\log_2$ by day 14 and reached peak values of 4.3 $\log_2$ on day 21 post-vaccination. No rabies virus-specific antibodies were detected in control (non-vaccinated) animals (Figure 4).

DOI: 10.11134/btp.2.2026.3



A) Vaccinated group; B) Control group.

Figure 4 - Virus-neutralizing antibody (VNA) titers in foxes vaccinated with the oral briquetted rabies vaccine.



Red square markers indicate the values of calibration sera ranging from 0.5 to 4 IU/ml, respectively, whereas blue circular markers represent the values of the tested samples. A four-parameter logistic (4PL) regression model was used for calibration curve calculation. The standard curve was generated using ScanIt Software 7.0.2 RE for Microplate Readers.

Figure 5 - Standard calibration curve of the ELISA assay.

These findings indicate that the oral briquetted rabies vaccine is capable of inducing virus-neutralizing antibody production in naturally susceptible carnivorous animals.

3.3.1. ELISA-based assessment of anti-rabies IgG antibodies

To further evaluate the humoral immune response, anti-rabies IgG antibodies were assessed using ELISA. Quantitative determination was performed using calibration sera with known antibody concentrations (0.5 and 4.0 IU/mL). Optical density (OD) values of the samples were measured and compared with those of the reference sera.

For initial estimation, antibody concentrations were calculated as the ratio of sample OD to reference serum OD multiplied by the assigned IU/mL value. For more accurate quantification, a standard calibration curve (Figure 5) was constructed using sera with known activity levels (0.5–4.0 IU/mL). Antibody concentrations were determined by interpolation using a four-parameter logistic (4PL) regression model (ScanIt Software 7.0.2 RE).

The ELISA results (Table 2) demonstrate a progressive increase in anti-rabies IgG antibody levels in vaccinated foxes over time. On day 7 post-vaccination, antibody levels ranged from 1.20 to 2.05 IU/mL (mean 1.55 ± 0.25 IU/mL). By day 14, a further increase was observed (1.28–2.86 IU/mL; mean 1.88 ± 0.40 IU/mL). By day 21, antibody levels remained elevated, ranging from 1.03 to 3.39 IU/mL (mean 1.82 ± 0.61 IU/mL).

Overall, these data indicate the development of a sustained humoral immune response following oral immunization. According to the manufacturer's criteria, antibody levels <0.5 IU/mL are considered low, 0.5-4.0 IU/mL indicate a sufficient immune response, and >4.0 IU/mL correspond to high antibody levels. In the present study, all vaccinated animals exhibited antibody levels above 0.5 IU/mL throughout the observation period, corresponding to a sufficient humoral immune response.

However, it should be noted that ELISA measures binding antibodies and does not directly reflect virus-neutralizing activity. Therefore, ELISA results were considered in conjunction with VNA data obtained by the mouse neutralization test.

DISCUSSION

According to current international standards, the efficacy of rabies vaccines is primarily evaluated based on the humoral immune response, particularly the level of VNA, as well as on the survival of animals following challenge infection with a virulent *Rabies virus* (RABV) strain. Virus-neutralizing and antigen-binding antibodies are considered surrogate markers of protection, and titers ≥0.5 IU/ml are generally regarded as indicative of an adequate level of immunity [3, 21]. In addition to serological criteria, survival after challenge infection remains an essential requirement for vaccine licensing and evaluation of protective efficacy [22, 23].

Recent studies indicate that protective immunity against rabies involves both humoral and cellular immune mechanisms, including B- and T-cell responses, particularly in the

Table 2 - ELISA results for the detection of IgG antibodies against rabies virus following immunization with the oral briquetted vaccine.

№	Sample	After 7 days		After 14 days		After 21 days	
		OD	ME/ml	OD	ME/ml	OD	ME/ml
1	№2 VF	0,595	1,40	0,633	1,49	0,446	1,05
2	№3 VF	0,871	2,05	0,545	1,28	0,437	1,03
3	№4 VF	0,510	1,20	1,220	2,86	1,445	3,39
Note: Blanking OD is equal to 0.0505; 0.5 IU/ml is equal to 0.211; 4 IU/ml equals 1.705. *VF - vaccinated fox*							

DOI: 10.11134/btp.2.2026.3

context of vaccine evaluation in dogs [24, 25]. In this regard, assessment of cellular immune responses has been proposed as a complementary approach to traditional in vivo challenge studies [26].

Live attenuated rabies vaccines are known to stimulate both adaptive and innate immune responses, contributing to the development of long-lasting immunity [27-32]. These effects are associated with the activation of antigen-presenting cells and the subsequent induction of both humoral and cellular immune pathways. However, in the present study, the evaluation was limited to humoral immune parameters and challenge infection outcomes. Therefore, the findings should be interpreted within the scope of these assessed endpoints, while the contribution of cellular immunity remains to be further investigated.

The rabies virus strain used in the present study has previously been evaluated in experimental studies in dogs, where it demonstrated acceptable immunogenicity and safety profiles [20]. In the current experiment, foxes vaccinated with the oral briquetted vaccine developed detectable humoral immune responses, with virus-neutralizing antibody titers exceeding the commonly accepted protective threshold of 0.5 IU/ml and reaching mean values of approximately 1.8 IU/ml by day 21 post-vaccination. Furthermore, all vaccinated animals survived challenge infection with a virulent RABV strain, whereas control animals developed clinical disease and succumbed to infection. These findings indicate the development of a protective immune response under the conditions of the present study.

At the same time, the limited number of animals included in this pilot experiment should be taken into account when interpreting the results. Therefore, the obtained data should be considered as preliminary, requiring further confirmation in studies with larger sample sizes.

The results are generally consistent with previously reported data on oral rabies vaccination in wildlife species, where protective immunity has been achieved despite variable levels of seroconversion [33]. This suggests that the developed briquetted oral vaccine has potential for further investigation as a candidate for use in oral vaccination programs targeting wildlife reservoirs.

A limitation of the present study is the relatively small number of experimental animals, which is due to ethical considerations and the complexity of conducting experiments involving a virulent rabies virus strain. Nevertheless, the results demonstrated consistent seroconversion and survival of vaccinated animals following challenge infection, suggesting a protective effect under the conditions of this pilot study. These findings should be considered preliminary and require further confirmation in studies involving larger animal cohorts.

The present findings also support the concept that evaluation of rabies vaccine efficacy should consider an integrated immune response rather than relying on a single immunological parameter. Although the current study was limited to the assessment of humoral immunity, the observed increase in VNA titers may indirectly reflect the involvement of coordinated immune mechanisms. However, cellular immune responses were not evaluated in this study, and therefore any assumptions regarding their contribution remain speculative. Further investigations including cytokine profiling and T-cell response analysis (e.g., IFN-γ production) would be necessary to provide a more comprehensive understanding of the immune mechanisms underlying vaccine-induced protection [34].

In addition to immunological outcomes, the technological characteristics of the vaccine bait represent an important practical aspect. The formulation demonstrated satisfactory stability and handling properties, while its structural features appeared to facilitate consumption by animals. No adverse clinical reactions were observed during the study, and vaccinated foxes maintained normal physiological parameters and behavior. These observations are consistent with previously reported safety data for oral rabies vaccines used in wildlife. Accordingly, the developed formulation can be considered safe under the conditions of this experiment.

From an epidemiological perspective, the induction of virus-neutralizing antibodies above the commonly accepted threshold of 0.5 IU/ml indicates a favorable immune response. The results suggest that a single oral administration may be sufficient to induce seroconversion; however, this conclusion requires further validation under field conditions and in larger populations.

Overall, the findings of the present study indicate that the experimental oral briquetted rabies vaccine is capable of inducing a measurable immune response and providing protection against experimental infection in foxes under controlled conditions. These data support the potential of the vaccine as a candidate for further investigation in the context of oral rabies vaccination programs targeting wildlife reservoirs.

CONCLUSION

Overall, the obtained results indicate that the experimental oral briquetted rabies vaccine is capable of inducing an immune response and was well tolerated in foxes under the conditions of this study. Vaccinated animals developed virus-neutralizing antibody titers exceeding the commonly accepted protective threshold of 0.5 IU/mL, and all vaccinated animals survived challenge infection with a virulent rabies virus strain, suggesting the development of protective immunity. However, given the limited sample size, the results of this study should be considered preliminary. Further investigations involving larger animal groups and field trials are required to confirm the immunogenicity, safety, and protective efficacy of the vaccine. Thus, the experimental oral briquetted vaccine may be considered a promising candidate for further development within oral immunization programs targeting wild carnivore populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

The study was funded by the Ministry of Agriculture of the Republic of Kazakhstan under the program-targeted funding BR22884615 «Scientific provision of epizootic welfare of fish and livestock farms of the Republic of Kazakhstan on infectious and parasitic diseases» 2024-2026 years.

DOI: 10.11134/btp.2.2026.3

AUTHOR CONTRIBUTIONS

Conceptualization, B.Y.E.; methodology, A.R.T., U.A.K., A.Zh.T., S.Zh.Zh., and T.D.S.; statistical analysis, T.Sh.S., K.A.K., M.D.M., and T.D.S.; study design, K.Zh.B., K.A.K., U.A.K., and S.Zh.Zh.; data interpretation, A.Zh.T., T.Sh.S., R.J.G., and T.D.S.; writing-original draft preparation, A.R.T., M.D.M., and T.D.S.; writing-review and editing, B.Ye.A. and T.D.S.; supervision, B.Ye.A. and K.Zh.K.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Ministry of Agriculture of the Republic of Kazakhstan and the staff of the Research Institute for Biological Safety Problems (RIBSP) for their support and assistance in organizing and conducting this research.

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

LITERATURE

1. Dietzschold B., Li J., Faber M., Schnell M. Concepts in the pathogenesis of rabies // *Future Virology.* – 2008. – Vol. 3. – No 5. – *P.* 481-490. https://doi.org/10.2217/17460794.3.5.481

2. Shiraishi R., Nishimura M., Nakashima R., Enta C., Hirayama N. Neutralizing Antibody Response in Dogs and Cats Inoculated with Commercial Inactivated Rabies Vaccines // *Journal of Veterinary Medical Science.* – 2014. – Vol. 76. – 4. – *P.* 605-609. https://doi.org/10.1292/jvms.13-0335

3. Moore S.M., Gilbert A., Vos A., et al. Rabies Virus Antibodies from Oral Vaccination as a Correlate of Protection against Lethal Infection in Wildlife // *Tropical Medicine and Infectious Disease.* – 2017. – Vol. 2. – No. 3. – *P.* 31. https://doi.org/10.3390/tropicalmed2030031

4. Moore S.M., Hanlon C.A. Rabies-Specific Antibodies: Measuring Surrogates of Protection against a Fatal Disease // *PLoS Neglected Tropical Diseases*. – 2010. – Vol. 4. – No 3. – P. 1-6. https://doi.org/10.1371/journal.pntd.0000595

5. Guilherme D.M., Jan H., Rajesh G., Davide C., Hervé B. Monoclonal antibodies against rabies: current uses in prophylaxis and in therap // *Current Opinion in Virology*. – 2022. – Vol. 53. – P. 101204 https://dx.doi.org/10.1016/j.coviro.2022.101204

6. Steck F., Wandeler A., Bichsel P., Capt S., Häfliger U., Schneider L. Oral immunization of foxes against rabies laboratory and field studies // *Comparative Immunology, Microbiology and Infectious Diseases.* – 1982. – Vol. 5. 1-3. – *P.* 165-171. https://doi.org/10.1016/0147-9571(82)90031-5

7. Wandeler A.I., Capt S., Kappeler A., Hauser R. Oral immunization of wildlife against rabies: concept and first field experiments // *Reviews of Infectious Diseases*. – 1988. – Vol. 10 – Suppl 4. – P. 649-653. https://doi.org/10.1093/clinids/10.supplement_4.s649

8. BO3. Zero by 30: the global strategic plan to end human deaths from dog-mediated rabies by 2030. – 2018. https://www.who.int/publications/i/item/9789241513838

9. Zero by 30 the global strategic plan human deaths from dog-mediated rabies by 2030 to end // WOAH – 2018. https://www.woah.org/fileadmin/Home/eng/Media_Center/docs/Zero_by_30_FINAL_online_version.pdf

10. Brown D., Fooks A.R., Schweiger M. Using Intradermal Rabies Vaccine to Boost Immunity in People with Low Rabies Antibody Levels // *Advances in Preventive Medicine.* – 2011. – P. 1-5. https://doi.org/10.4061/2011/601789

11. Rosatte R., Donovan D., Allan M., et al. Rabies in Vaccinated Raccoons from Ontario, Canada // *Journal of Wildlife Diseases.* – 2007. – Vol. 43. – No 2. – *P.* 300-301. https://doi.org/10.7589/0090-3558-43.2.300

12. Rosatte R., Donovan D.M., Allan M., et al. Emergency response to raccoon rabies introduction into Ontario // *Journal of Wildlife Diseases*. – 2001. – Vol. 37 – No 2. – *P.* 265-279. https://doi.org/10.7589/0090-3558-37.2.265

13. Freuling C.M., Eggerbauer E., Finke S., et al. Efficacy of the oral rabies virus vaccine strain SPBN GASGAS in foxes and raccoon dogs // *Vaccine.* – 2019. – Vol. 37 – No 33. – *P.* 4750-4757. https://doi.org/10.1016/j.vaccine.2017.09.093

14. Sterner R.T., Meltzer M.I., Shwiff S.A., Slate D. Tactics and Economics of Wildlife Oral Rabies Vaccination, Canada and the United States // *Emerging Infectious Diseases.* – 2009. – Vol. 15. – No 8. – *P.* 1176-1184. https://doi.org/10.3201/eid1508.081061

15. Mukhanbetkaliyeva A.A., Kabzhanova A.M., Kadyrov A.S., et al. Application of modern spatio-temporal analysis technologies to identify and visualize patterns of rabies emergence among different animal species in Kazakhstan // *Geospatial Health.* – 2024. – Vol. 19. – No 2. https://doi.org/10.4081/gh.2024.1290

16. Gomez-Buendia A., Yessembekova G., Kadyrov A, Mukhanbetkaliyev Y., Cerviño-Luridiana E., Alvarez J., Perez A.M., Abdrakhmanov S.K. A time-space Bayesian regression model of rabies cases in the animal population of Kazakhstan (2013–2023) // *Frontiers in Veterinary Science.* – 2025. – Vol. 12. https://doi.org/10.3389/fvets.2025.1640050

17. Abdrakhmanov S.K., Abenova A.Zh., Mukhanbetkaliyeva A.A., Korennoy F.I., Perez A.M. Animal Rabies in Kazakhstan: Stable Endemicity, Surveillance Pitfalls, and Priority Actions // *Pathogens*. – 2025. – Vol. 14. – No 11. – P. 1079. https://doi.org/10.3390/pathogens14111079

18. Hartinger J., Folz T., Cussler K. Clinical endpoints during rabies vaccine control tests // *ALTEX.* – 2001. – Vol. 18. – No 1. – P.37-40. https://pubmed.ncbi.nlm.nih.gov/11248849/

19. Reed L.J., Muench H. A simple method of estimating fifty per cent endpoints // *American Journal of Epidemiology.* – 1938. – Vol. 27. – No 3. – P. 493-497. https://doi.org/10.1093/oxfordjournals.aje.a118408

20. Bulatov Y., Koshemetov Z., Amanova Z., Abitayev R., Ussembay A., Sametova Z., Kondybaeva Z., Kurmasheva A., Mazbayeva D., Turyskeldy S., Toktyrova D. Safety assessment of an oral rabies vaccine bait for wild carnivores in a seronegative dog model // *Eurasian Journal of Applied Biotechnology.* – 2025. – *Vol.* 3. – *P.* 93-101. https://doi.org/10.11134/btp.3.2025.10

21. Müller T., Freuling C.M. Rabies Vaccines for Wildlife // *In: Ertl, H (eds) Rabies and Rabies Vaccines Springer, Cham.* – 2020. – P. 45-70. https://doi.org/10.1007/978-3-030-

DOI: 10.11134/btp.2.2026.3

21084-7_3

22. Overduin, van Dongen, Visser. The Cellular Immune Response to Rabies Vaccination: A Systematic Review // *Vaccines.* – 2019. – Vol. 7(3). – No 110. https://doi.org/10.3390/vaccines7030110

23. European Medicines Agency (EMA). Rabies Vaccine (Live, Oral) for Foxes and Raccoon Dogs // Monograph 0746 01/2014, 9th ed. – Council of Europe: Strasbourg, France. – 2016.

24. Bommier E., Chapat L., Guiot A.L., et al. Multivariate analysis of the immune response to different rabies vaccines // *Veterinary Immunology and Immunopathology.* – 2020. – T. 220:109986. https://doi.org/10.1016/j.vetimm.2019.109986

25. Chapat L., Hilaire F., Bouvet J., et al. Multivariate analysis of the immune response to a vaccine as an alternative to the repetition of animal challenge studies for vaccines with demonstrated efficacy // *Veterinary Immunology and Immunopathology.* – 2017. – Vol. 189. – P. 58-65. https://doi.org/10.1016/j.vetimm.2017.06.001

26. Vogt R., Schulte P.A. Evaluation of immune responses // *IARC scientific publications.* – 2011. – Vol. 163. – P. 215-239. https://pubmed.ncbi.nlm.nih.gov/22997865/

27. Wang Z.W., Sarmento L., Wang Y., et al. Attenuated Rabies Virus Activates, while Pathogenic Rabies Virus Evades, the Host Innate Immune Responses in the Central Nervous System // *Journal of Virology.* – 2005. – Vol. 79 – No 19. – P. 12554-12565. https://doi.org/10.1128/jvi.79.19.12554-12565.2005

28. Verena te Kamp, Freuling C.M., Vos A., et al. Responsiveness of various reservoir species to oral rabies vaccination correlates with differences in vaccine uptake of mucosa associated lymphoid tissues // *SCI Rep.* – 2020 – Vol. 10(1). – No 2919. https://doi.org/10.1038/s41598-020-59719-4

29. Ghislat G., Lawrence T. Autophagy in dendritic cells // *Cellular & Molecular Immunology.* – 2018. – Vol. 15(11). – P. 944-952. https://doi.org/10.1038/cmi.2018.2

30. Roy A., D. Craig Hooper. Lethal Silver-Haired Bat Rabies Virus Infection Can Be Prevented by Opening the Blood-Brain Barrier // *J Virol.* – 2007. – Vol. 81(15). – P. 7993-7998. https://doi.org/10.1128/jvi.00710-07

31. Zhang S., Hao M., Feng N., et al. Genetically Modified Rabies Virus Vector-Based Rift Valley Fever Virus Vaccine is Safe and Induces Efficacious Immune Responses in Mice // *Viruses.* – 2019. – T. 11. – Vol. 10. 919. https://doi.org/10.3390/v11100919

32. Lebrun A., Garcia S., Li J., Kean R., Hooper D. Protection Against CNS-Targeted Rabies Virus Infection is Dependent upon Type-1 Immune Mechanisms Induced by Live-Attenuated Rabies Vaccines // *Tropical Medicine and Infectious Disease.* – 2017. – T. 2. – Vol. 3:22. https://doi.org/10.3390/tropicalmed2030022

33. Gnanadurai C.W., Yang Y., Huang Y., et al. Differential Host Immune Responses after Infection with Wild-Type or Lab-Attenuated Rabies Viruses in Dogs // *Rupprecht CE, ed. PLOS Neglected Tropical Diseases.* – 2015. – T. 9. – Vol. 8. https://doi.org/10.1371/journal.pntd.0004023

34. Kiflu A.B. The Immune Escape Strategy of Rabies Virus and Its Pathogenicity Mechanisms // *Viruses.* – 2024. – T. 16. – Vol. 11.1774. https://doi.org/10.3390/v16111774

REFERENCES

1. Dietzschold B., Li J., Faber M., Schnell M. Concepts in the pathogenesis of rabies // *Future Virology.* – 2008. – Vol. 3. – No 5. – *P.* 481-490. https://doi.org/10.2217/17460794.3.5.481

2. Shiraishi R., Nishimura M., Nakashima R., Enta C., Hirayama N. Neutralizing Antibody Response in Dogs and Cats Inoculated with Commercial Inactivated Rabies Vaccines // *Journal of Veterinary Medical Science.* – 2014. – Vol. 76. – 4. – *P.* 605-609. https://doi.org/10.1292/jvms.13-0335

3. Moore S.M., Gilbert A., Vos A., et al. Rabies Virus Antibodies from Oral Vaccination as a Correlate of Protection against Lethal Infection in Wildlife // *Tropical Medicine and Infectious Disease.* – 2017. – Vol. 2. – No. 3. – *P.* 31. https://doi.org/10.3390/tropicalmed2030031

4. Moore S.M., Hanlon C.A. Rabies-Specific Antibodies: Measuring Surrogates of Protection against a Fatal Disease // *PLoS Neglected Tropical Diseases*. – 2010. – Vol. 4. – No 3. – P. 1-6. https://doi.org/10.1371/journal.pntd.0000595

5. Guilherme D.M., Jan H., Rajesh G., Davide C., Hervé B. Monoclonal antibodies against rabies: current uses in prophylaxis and in therap // *Current Opinion in Virology*. – 2022. – Vol. 53. – P. 101204 https://dx.doi.org/10.1016/j.coviro.2022.101204

6. Steck F., Wandeler A., Bichsel P., Capt S., Häfliger U., Schneider L. Oral immunization of foxes against rabies laboratory and field studies // *Comparative Immunology, Microbiology and Infectious Diseases.* – 1982. – Vol. 5. 1-3. – P. 165-171. https://doi.org/10.1016/0147-9571(82)90031-5

7. Wandeler A.I., Capt S., Kappeler A., Hauser R. Oral immunization of wildlife against rabies: concept and first field experiments // *Reviews of Infectious Diseases*. – 1988. – Vol. 10 – Suppl 4. – P. 649-653. https://doi.org/10.1093/clinids/10.supplement_4.s649

8. WHO. Zero by 30: the global strategic plan to end human deaths from dog-mediated rabies by 2030. – 2018. https://www.who.int/publications/i/item/9789241513838

9. Zero by 30 the global strategic plan human deaths from dog-mediated rabies by 2030 to end // WOAH – 2018. https://www.woah.org/fileadmin/Home/eng/Media_Center/docs/Zero_by_30_FINAL_online_version.pdf

10. Brown D., Fooks A.R., Schweiger M. Using Intradermal Rabies Vaccine to Boost Immunity in People with Low Rabies Antibody Levels // *Advances in Preventive Medicine.* – 2011. – P. 1-5. https://doi.org/10.4061/2011/601789

11. Rosatte R., Donovan D., Allan M., et al. Rabies in Vaccinated Raccoons from Ontario, Canada // *Journal of Wildlife Diseases.* – 2007. – Vol. 43. – No 2. – *P.* 300-301. https://doi.org/10.7589/0090-3558-43.2.300

12. Rosatte R., Donovan D.M., Allan M., et al. Emergency response to raccoon rabies introduction into Ontario // *Journal of Wildlife Diseases*. – 2001. – Vol. 37 – No 2. – *P.* 265-279. https://doi.org/10.7589/0090-3558-37.2.265

13. Freuling C.M., Eggerbauer E., Finke S., et al. Efficacy of the oral rabies virus vaccine strain SPBN GASGAS in

DOI: 10.11134/btp.2.2026.3

foxes and raccoon dogs // *Vaccine.* – 2019. – Vol. 37 – No 33. – *P.* 4750-4757. https://doi.org/10.1016/j.vaccine.2017.09.093

14. Sterner R.T., Meltzer M.I., Shwiff S.A., Slate D. Tactics and Economics of Wildlife Oral Rabies Vaccination, Canada and the United States // *Emerging Infectious Diseases.* – 2009. – Vol. 15. – No 8. – *P.* 1176-1184. https://doi.org/10.3201/eid1508.081061

15. Mukhanbetkaliyeva A.A., Kabzhanova A.M., Kadyrov A.S., et al. Application of modern spatio-temporal analysis technologies to identify and visualize patterns of rabies emergence among different animal species in Kazakhstan // *Geospatial Health.* – 2024. – Vol. 19. – No 2. https://doi.org/10.4081/gh.2024.1290

16. Gomez-Buendia A., Yessembekova G., Kadyrov A, Mukhanbetkaliyev Y., Cerviño-Luridiana E., Alvarez J., Perez A.M., Abdrakhmanov S.K. A time-space Bayesian regression model of rabies cases in the animal population of Kazakhstan (2013–2023) // *Frontiers in Veterinary Science.* – 2025. – Vol. 12. https://doi.org/10.3389/fvets.2025.1640050

17. Abdrakhmanov S.K., Abenova A.Zh., Mukhanbetkaliyeva A.A., Korennoy F.I., Perez A.M. Animal Rabies in Kazakhstan: Stable Endemicity, Surveillance Pitfalls, and Priority Actions // *Pathogens*. – 2025. – Vol. 14. – No 11. – P. 1079. https://doi.org/10.3390/pathogens14111079

18. Hartinger J., Folz T., Cussler K. Clinical endpoints during rabies vaccine control tests // *ALTEX.* – 2001. – Vol. 18. – No 1. – P.37-40. https://pubmed.ncbi.nlm.nih.gov/11248849/

19. Reed L.J., Muench H. A simple method of estimating fifty per cent endpoints // *American Journal of Epidemiology.* – 1938. – Vol. 27. – No 3. – P. 493-497. https://doi.org/10.1093/oxfordjournals.aje.a118408

20. Bulatov Y., Koshemetov Z., Amanova Z., Abitayev R., Ussembay A., Sametova Z., Kondybaeva Z., Kurmasheva A., Mazbayeva D., Turyskeldy S., Toktyrova D. Safety assessment of an oral rabies vaccine bait for wild carnivores in a seronegative dog model // *Eurasian Journal of Applied Biotechnology.* – 2025. – *Vol.* 3. – *P.* 93-101. https://doi.org/10.11134/btp.3.2025.10

21. Müller T., Freuling C.M. Rabies Vaccines for Wildlife // *In: Ertl, H (eds) Rabies and Rabies Vaccines Springer, Cham.* – 2020. – P. 45-70. https://doi.org/10.1007/978-3-030-21084-7_3

22. Overduin, van Dongen, Visser. The Cellular Immune Response to Rabies Vaccination: A Systematic Review // *Vaccines.* – 2019. – Vol. 7(3). – No 110. https://doi.org/10.3390/vaccines7030110

23. European Medicines Agency (EMA). Rabies Vaccine (Live, Oral) for Foxes and Raccoon Dogs // Monograph 0746 01/2014, 9th ed. – Council of Europe: Strasbourg, France. – 2016.

24. Bommier E., Chapat L., Guiot A.L., et al. Multivariate analysis of the immune response to different rabies vaccines // *Veterinary Immunology and Immunopathology.* – 2020. – T. 220:109986. https://doi.org/10.1016/j.vetimm.2019.109986

25. Chapat L., Hilaire F., Bouvet J., et al. Multivariate analysis of the immune response to a vaccine as an alternative to the repetition of animal challenge studies for vaccines with demonstrated efficacy // *Veterinary Immunology and Immunopathology.* – 2017. – Vol. 189. – P. 58-65. https://doi.org/10.1016/j.vetimm.2017.06.001

26. Vogt R., Schulte P.A. Evaluation of immune responses // *IARC scientific publications.* – 2011. – Vol. 163. – P. 215-239. https://pubmed.ncbi.nlm.nih.gov/22997865/

27. Wang Z.W., Sarmento L., Wang Y., et al. Attenuated Rabies Virus Activates, while Pathogenic Rabies Virus Evades, the Host Innate Immune Responses in the Central Nervous System // *Journal of Virology.* – 2005. – Vol. 79 – No 19. – P. 12554-12565. https://doi.org/10.1128/jvi.79.19.12554-12565.2005

28. Verena te Kamp, Freuling C.M., Vos A., et al. Responsiveness of various reservoir species to oral rabies vaccination correlates with differences in vaccine uptake of mucosa associated lymphoid tissues // *SCI Rep.* – 2020 – Vol. 10(1). – No 2919. https://doi.org/10.1038/s41598-020-59719-4

29. Ghislat G., Lawrence T. Autophagy in dendritic cells // *Cellular & Molecular Immunology.* – 2018. – Vol. 15(11). – P. 944-952. https://doi.org/10.1038/cmi.2018.2

30. Roy A., D. Craig Hooper. Lethal Silver-Haired Bat Rabies Virus Infection Can Be Prevented by Opening the Blood-Brain Barrier // *J Virol.* – 2007. – Vol. 81(15). – P. 7993-7998. https://doi.org/10.1128/jvi.00710-07

31. Zhang S., Hao M., Feng N., et al. Genetically Modified Rabies Virus Vector-Based Rift Valley Fever Virus Vaccine is Safe and Induces Efficacious Immune Responses in Mice // *Viruses.* – 2019. – T. 11. – Vol. 10. 919. https://doi.org/10.3390/v11100919

32. Lebrun A., Garcia S., Li J., Kean R., Hooper D. Protection Against CNS-Targeted Rabies Virus Infection is Dependent upon Type-1 Immune Mechanisms Induced by Live-Attenuated Rabies Vaccines // *Tropical Medicine and Infectious Disease.* – 2017. – T. 2. – Vol. 3:22. https://doi.org/10.3390/tropicalmed2030022

33. Gnanadurai C.W., Yang Y., Huang Y., et al. Differential Host Immune Responses after Infection with Wild-Type or Lab-Attenuated Rabies Viruses in Dogs // *Rupprecht CE, ed. PLOS Neglected Tropical Diseases.* – 2015. – T. 9. – Vol. 8. https://doi.org/10.1371/journal.pntd.0004023

34. Kiflu A.B. The Immune Escape Strategy of Rabies Virus and Its Pathogenicity Mechanisms // *Viruses.* – 2024. – T. 16. – Vol. 11.1774. https://doi.org/10.3390/v16111774

DOI: 10.11134/btp.2.2026.3

УДК: 57.083.3:578.824.11

ОЦЕНКА ИММУНОБИОЛОГИЧЕСКИХ СВОЙСТВ ПЕРОРАЛЬНОЙ БРИКЕТИРОВАННОЙ ВАКЦИНЫ ПРОТИВ БЕШЕНСТВА У ЛИС

Булатов Е.А.[1], Абитаев Р.Т.[1], Усембай А.К.[1], Аманова Ж.Т.[1], Саметова Ж.Ж.[1], Турыскелді Ш.С.[1,2], Кондибаева Ж.Б.[1], Мазбаева Д.М.[1], Курмашева А.К.[1], Кошеметов Ж.К.[1], Renukaradhya J G.[3], Токтырова Д.С.[1,2*]

[1] Научно-исследовательский институт проблем биологической безопасности Национального холдинга «QazBioPharm», 25/1, Гвардейский, Кордайский район, Жамбылская область, 080409, Казахстан
[2] Казахский национальный университет имени аль-Фараби, Факультет биологии и биотехнологии, проспект аль-Фараби, 71, Алматы 050040, Казахстан
[3] Университет штата Огайо, кафедра зоотехнических наук, здание Animal Science Building, 2029 Fyffe Road, Колумбус, 43210, штат Огайо, США
** Автор для корреспонденции: d.toktirova@biosafety.kz*

АННОТАЦИЯ

Бешенство остается одной из наиболее опасных зоонозных инфекций, поддерживаемых в природных резервуарах среди диких плотоядных животных. Несмотря на высокую эффективность инактивированных вакцин у домашних животных, контроль бешенства в дикой фауне требует применения пероральных вакцин, обеспечивающих массовую иммунизацию естественно восприимчивых видов. Целью настоящего исследования являлась оценка иммуногенности, безопасности и защитной эффективности пероральной брикетированной вакцины против бешенства, испытанной на естественно восприимчивых плотоядных животных в пилотных условиях. В эксперимент было включено 10 клинически здоровых лисиц, серонегативных по антителам к вирусу бешенства. Три лисицы группы иммуногенности получили однократную пероральную дозу вакцины, сформулированной в виде брикетированной приманки, содержащей фиксированный штамм вируса бешенства *Rabies virus fix/NIIPBB/2024* с титром 6,00 log $TCID_{50}$/мл. Пять животных получили повышенную дозу, составлявшую 10 вакцинных приманок на животное, для оценки безопасности, тогда как две невакцинированные лисицы служили контролем. Динамику гуморального иммунного ответа определяли с помощью теста нейтрализации на мышах и ИФА. Защитную эффективность оценивали путем заражения контрольным штаммом CVS. Полученные результаты показали, что вакцинные приманки охотно поедались животными и не вызывали клинических побочных реакций или отклонений от физиологических норм. Уровни антител, определенные методом ИФА, превышали 0,5 МЕ/мл, что соответствует достаточному гуморальному иммунному ответу согласно критериям производителя. Параллельно титры вируснейтрализующих антител, определённые в тесте нейтрализации на мышах, достигали максимальных значений до 4,3 $\log_2$ на 21-й день после иммунизации. После контрольного заражения вакцинированные животные выживали без клинических признаков бешенства, тогда как у контрольных животных развивалась паралитическая форма заболевания с летальным исходом. В целом результаты данного пилотного исследования свидетельствуют о том, что пероральная брикетированная вакцина против бешенства обладает перспективной иммуногенностью, безопасностью и защитным потенциалом, что обосновывает необходимость дальнейших исследований для её применения в программах пероральной иммунизации диких плотоядных животных и контроля природных очагов бешенства.

Ключевые слова: бешенство, пероральная вакцинация, брикетированная вакцина, вируснейтрализующие антитела, дикие плотоядные.

ӘОЖ: 57.083.3:578.824.11

ТҮЛКІЛЕРДЕ ҚҰТЫРУҒА ҚАРСЫ ПЕРОРАЛЬДЫ БРИКЕТТЕЛГЕН ВАКЦИНАНЫҢ ИММУНОБИОЛОГИЯЛЫҚ ҚАСИЕТТЕРІН БАҒАЛАУ

Булатов Е.А.[1], Абитаев Р.Т.[1], Үсембай А.Қ.[1], Аманова Ж.Т.[1], Саметова Ж.Ж.[1], Турыскелді Ш.С.[1,2], Кондибаева Ж.Б.[1], Мәзбаева Д.М.[1], Курмашева А.К.[1], Кошеметов Ж.К.[1], Renukaradhya J G.[3], Тоқтырова Д.С.[1,2*]

[1] Биологиялық қауіпсіздік проблемаларын ғылыми-зерттеу институты «QazBioPharm» Ұлттық холдингі, 25/1, Гвардейский, Қордай ауданы, Жамбыл облысы, 080409, Қазақстан
[2] Әл-Фараби атындағы Қазақ Ұлттық Университеті, Биология және биотехнология факультеті, әл-Фараби даңғылы, Алматы, 050040, Қазақстан
[3] Огайо штаты университеті, Жануарлар ғылымдары кафедрасы, Animal Science Building ғимараты, 2029 Fyffe Road, Колумбус, 43210, Огайо штаты, АҚШ
** Корреспондент авторы: d.toktirova@biosafety.kz*

ТҮЙІН

Құтыру – жабайы етқоректі жануарлар арасында табиғи резервуарларда сақталатын аса қауіпті зооноздық инфекциялардың бірі болып табылады. Үй жануарларында инактивтелген вакциналардың тиімділігі жоғары болғанымен,

DOI: 10.11134/btp.2.2026.3

жабайы жануарлардағы құтыруды бақылау табиғи сезімтал түрлерді жаппай иммундауды қамтамасыз ететін пероральды вакциналарды қолдануды талап етеді. Осы зерттеудің мақсаты пилоттық жағдайларда табиғи түрде сезімтал етқоректі жануарларда эксперименттік пероральды брикеттелген құтыруға қарсы вакцинаның иммуногендігін, қауіпсіздігін және қорғаныш тиімділігін бағалау болды. Тәжірибеде құтыру вирусына қарсы антиденелері жоқ, клиникалық тұрғыдан сау 10 түлкі енгізілді. Иммуногендік тобындағы үш түлкі құрамында титрі 6,00 log $TCID_{50}$/мл болатын *Rabies virus fix/NIIPBB/2024* бекітілген құтыру вирусы штамы бар брикеттелген приманка түріндегі вакцинаның бір реттік пероральды дозасын алды. Қауіпсіздікті бағалау үшін бес жануарға әр жануарға 10 вакциналық приманкадан тұратын жоғарылатылған доза берілді, ал екі вакцинацияланбаған түлкі бақылау тобы ретінде пайдаланылды. Гуморальды иммундық жауаптың динамикасы тышқандардағы нейтрализация тесті және ИФТ әдістері арқылы анықталды. Қорғаныш тиімділігі CVS тест-штамымен жұқтыру арқылы бағаланды. Нәтижелер жемдік вакциналардың жануарлар тарапынан жақсы қабылданғанын және клиникалық жағымсыз әсерлер мен физиологиялық нормалардан ауытқулар туғызбағанын көрсетті. ИФТ әдісімен анықталған антидене деңгейлері 0,5 ХБ/мл-ден жоғары болды, бұл өндіруші критерийлері бойынша жеткілікті гуморальды иммундық жауапқа сәйкес келеді. Сонымен қатар, тышқандардағы нейтрализация тесті арқылы анықталған вируснейтрализдеуші антиденелер титрі иммундаудан кейінгі 21-күні 4,3 log_2 дейін жетті. Жұқтырудан кейін вакцинацияланған жануарлар құтырудың клиникалық белгілерінсіз тірі қалды, ал бақылау тобындағы жануарларда аурудың салдану түрі дамып, өліммен аяқталды. Жалпы алғанда, бұл пилоттық зерттеудің нәтижелері пероральды брикеттелген құтыруға қарсы вакцинаның перспективалы иммуногендігін, қауіпсіздігін және қорғаныш әлеуетін көрсетеді, бұл оны жабайы етқоректі жануарларды пероральды иммундау бағдарламаларында және құтырудың табиғи ошақтарын бақылауда қолдану үшін әрі қарай зерттеуді негіздейді.

Түйін сөздер: құтыру, пероральды вакцинация, брикеттелген вакцина, вирус-бейтараптандырушы антиденелер, жабайы етқоректілер.