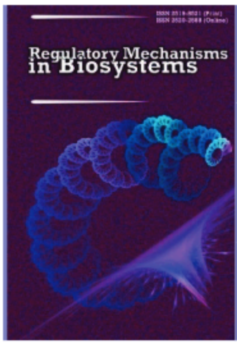

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A pilot study on the impact of parenteral vaccination of free-roaming dogs within the rabies control framework in Ukraine

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This pioneering study is prompted by the imperative to enhance our understanding of a constituent within Ukraine's rabies control strategy, specifically focusing on the vaccination of free-roaming canines against rabies with a local vaccine in certain endemic rabies areas. The cross-sectional study encompassed the capture, sterilization, deworming, and rabies vaccination processes in 160 free-roaming dogs, followed by the collection of blood samples to examine RABV-specific neutralising antibodies in 16 dogs (10% of the vaccinated population), captured from sectors (territories) with a higher density of population and with higher density of previously vaccinated free-roaming dogs. Half of the targeted samples comprised males (n = 8), while the remaining half consisted of females (n = 8). The median of virus-neutralising antibody level was 0.58 IU/mL, with a minimum protective threshold of 0.5 IU/mL. Antibody titers below the 0.5 IU/mL threshold were detected in 25.0% (2/8) of the male group, and in 62.5% (5/8) of the female group. Notably, male dogs exhibited a higher median antibody level of 0.66 IU/mL, compared to females, who presented a median level of 0.26 IU/mL. However, no statistically significant difference was found between the male and female groups (P = 0.36). In general, more than half of the tested population (56.3%) exceeded the 0.5 IU/mL protective threshold 4 months post-vaccination. The inadequate levels of antibodies neutralizing the rabies virus are likely a result of a confluence of factors, including stressors such as nutritional and temperature-related challenges, along with variations in the responses of individual immune systems. Considering the endemic rabies situation and large population of free-roaming dogs in Ukraine, we suggest repeated vaccination for free-roaming dogs against rabies one year after the previous vaccination. In perspective, we suggest conducting large-scale epidemiological studies to assess the impact of animal-related, vaccine-related, and environment-related parameters on the efficacy of rabies vaccines used in Ukraine.

Keywords: FAVN; immunity response; neutralising antibody; One Health; rabies epidemic; stray dogs; vaccination campaigns.

Introduction

Rabies remains endemic in many low- and middle-income countries despite available vaccines and treatments (Taylor et al., 2017; Gibson et al., 2020). The disease claims an estimated 59,000 lives annually, mainly in Asia and Africa, with children under 15 years accounting for 40% of the victims (WHO, TRS, 2018; Rupprecht et al., 2020; Mbilo et al., 2021). Dog bites cause over 99% of these deaths, leading to 3.7 million lost disability-adjusted life years (DALY) and $8.6 billion in annual economic losses (Hampson et al., 2015; Rupprecht et al., 2023; Taylor et al., 2023). With a WOAH estimated population of 700 million domestic dogs worldwide, 75% of which are free-roaming (unowned dog that is without direct human supervision or control), reaching all these animals is posing a huge challenge (Fahrion et al., 2017; Smith et al., 2019).

Mass dog vaccination and improved access to post-exposure prophylaxis (PEP) can prevent human rabies cases and achieve elimination (Lavan et al., 2017; Savadogo et al., 2023). Successful rabies control projects in some regions of Latin America, Africa, and Asia confirm the correlation between reduced dog rabies cases and human deaths (Taylor & Nel, 2015; Rupprecht et al., 2020; Sambo et al., 2022).

Many of the European countries, encompassing nearly all EU Member States, EFTA countries, and the UK, have successfully eliminated rabies (Hampson et al., 2008; Müller et al., 2015). However, some southern and eastern European regions, despite significant efforts to control and eliminate wildlife-mediated rabies, have not achieved complete elimination of the disease at this time (Lojkić et al., 2021; Taylor et al., 2021; Vega et al., 2021). Ukraine has been identified as one of the European countries with a high incidence of rabies, experiencing long-term endemicity in both wild and domestic carnivores (Makovska et al., 2021; Omelchenko et al., 2022; Rudoi et al., 2023).

In the past few decades, Ukraine has consistently reported over 1,500 cases of rabies in animals each year (Kornienko et al., 2019; Polupan et al., 2019). Based on recent estimates, foxes were found to be the main reservoir and vector of rabies and account for 36.5% of the rabies cases, while dogs (19.3%) and cats (25.5%) were additional sources (Makovska et al., 2021). This endemic animal rabies situation poses a constant threat to public health and an economic burden (Makovska et al., 2020; Taylor et al., 2021). Official data from Ukraine's Ministry of Health indicate that between 2007 and 2019 in total 84,148 people sought medical assistance due to animal bites, with 2,155 of them bitten by rabid animals (mostly dogs, cats and foxes). This led to around 23,000 people needing PEP each year. As reported by Antonova et al. (2021), dogs accounted for 77.7% of all animal species attacks, with 28.8% involving rabid dogs. In spite of the PEP endeavors, Ukraine reported 63 human deaths due to rabies from 1996 to 2020 (Antonova et al., 2021).

To address the challenge of rabies, the Ukrainian government has implemented a national rabies control program, which includes oral rabies vaccination campaigns for wild animals and parenteral vaccination of domestic animals including domestic carnivores (free-roaming as well as pet animals) and farm animals (Kornienko et al., 2019; Makovska et al., 2021). Despite these efforts, the increasing population of free-roaming animals (dogs and cats) remains an important issue in the country (www.uaaa.org.ua/uk/stats), especially because these animals may create a link between the wildlife (which acts as a reservoir for rabies virus) and humans (Rupprecht et al., 2020; Makovska et al., 2021). In order to combat this transmission of the virus, vaccination remains the most effective method (Durr et al., 2009; Fahrion et al., 2017).

Based on the estimates provided by WellBeing International, the population of dogs in Ukraine exceeds 7 million with a total of 163 dogs per 1,000 people and 15free-roaming dogs per 1,000 people, which creates a big concern regarding the risk of transmission of zoonotic diseases to humans. Overcrowded shelters are a prevalent issue in cities, and the population of free-roaming dogs and cats continues to grow at a rate surpassing the number of people willing to provide shelter for them. Importantly, alternative methods to reduce the free-roaming dog population such as culling are considered ethically unacceptable according to the Ukrainian legislation (Law of Ukraine No. 3447-IV, "On the protection of animals from cruelty").

The responsibility of vaccination efforts for free-roaming animals primarily lies with local authorities. However, recognizing the need for additional measures, non-governmental organizations (NGOs) as well as national and international volunteering organizations and volunteer groups also contribute significantly to the vaccination programs of free-roaming animals. Nowadays due to many technical challenges, volunteers have tried integrating vaccination programs with sterilization initiatives (Borse et al., 2018). These combined programs usually involve sterilization, deworming, and simultaneous vaccination based on examples from other countries (Fitzpatrick et al., 2016; Berteselli et al., 2021; Smith et al., 2022). However, the effectiveness of these campaigns is unclear due to a lack of national animal identification and monitoring the efficacy of rabies vaccination in Ukraine. In this context, the current preliminary study aims to elucidate a component of Ukraine's rabies control intervention plan, specifically focusing on the parenteral rabies vaccination of free-roaming dogs using a vaccine produced in Ukraine. This involves post-vaccination detection of specific neutralising antibodies to the rabies virus (RABV) as a measure to evaluate the efficacy of the vaccination.

Materials and methods

Study area. The study was conducted in the city of Bila Tserkva, which is located in the central part of Ukraine (Fig. 1). It is the biggest city of Kyiv oblast with an area of 67.84 km^2. In 2019 the estimated human population was about 210 000 people, according to the State Statistics Service of Ukraine.

Fig. 1. Study area in Bila Tserkva (marked in orange)

Between 2012 and 2019, there were 18 rabies cases in cats and five in dogs in Bila Tserkva district, and in Bila Tserkva city, there were six rabies cases in cats and one in a dog (according to the data from the official annual reports of the State Regional Laboratory of Veterinary Medicine of the State Service of Ukraine for Food Safety and Consumer Protection). According to the preliminary data from web resource "animal-id.net", the population of free-roaming dogs in Bila Tserkva city was estimated to be 952 out of which only 18.8% were sterilized in 2019.

Study design. This pilot study was conducted in the framework of the scientific-charitable project between the local government of Bila Tserkva City (Department of Housing and Communal Services of Bila Tserkva City Council), the Faculty of Veterinary Medicine (FVM) of Bila Tserkva National Agrarian University (BTNAU), and the Limited liability company "Four Paws Ukraine" (LLC) which aimed at the humane control of the free-roaming dog population through free of charge sterilization, vaccination against rabies and identification of free-roaming animals. The work was performed by the LLC team (i.e., veterinarians, surgeons, assistants, drivers, specially trained dog catchers), as well as a senior researcher (from FVM of BTNAU) and a junior researcher from FVM of the National University of Life and Environmental Sciences of Ukraine. All aspects related to capturing, deworming, vaccinating against rabies, microchipping and sterilizing were executed by the team of "Four Paws Ukraine".

The fieldwork consisted of two parts. The first part of the program encompassed activities such as capturing, sterilizing, deworming, vaccinating against rabies, implanting the identification chips (transponders) with unique ID numbers (microchip number), and the creation of a database. Before the start of these activities, an awareness campaign and a survey among citizens were organized and conducted to collect information with regard to the most probable places with a high density of free-roaming dogs (e.g., garage cooperatives, industrial zones, city warehouses, areas close to schools, kindergarten areas, bazaars, etc.) which could create hazards for local residents. Finally, the study area was chosen based on the collected information from the surveys in combination with suggestions from the local authorities. To enhance the efficiency of the capturing process, the city was divided into four sectors (A, C, D, F), with a goal to capture approximately 40 dogs per sector. The choice of sectors was guided by topographic boundaries, incorporating preliminary estimates from local authorities and findings from a survey conducted among citizens regarding the locations of free-roaming dogs' habitats. Notably, the sectors were not uniform in size; rather, focus was placed on areas demonstrating the highest concentration of free-roaming dogs. All captured dogs were free-roaming and their vaccination status was unknown. Among the targeted 160 dogs, around 2% were obviously unhealthy dogs (one with cardiac arrhythmia, one with pyometra and one damage to the paw) and 2 were pregnant. Both the unhealthy and pregnant dogs were included for vaccination but were excluded from the second part of the study. The sanitary conditions of the selected dogs corresponded to the conditions of the vast majority of free-roaming dogs in the city. Once captured, the animals were placed in individual cages and transported to a dedicated building (hangar) provided by the local government for this purpose. The sterilization was carried out in a mobile surgical clinic based in a minibus. The technique used was ovariectomy (all genital organs removed) in the case of females and castration (testicles removed) or vasectomy for males. Ultimately, over the course of several days, the dogs underwent sterilization, vaccination, and microchipping procedures and then were safely returned to their respective capture locations. The technology used by the volunteer team involved carrying out all vaccination and sterilization procedures as soon as possible (1.0–1.5 days), and returning the animals to the place of capture to minimize stress. This did not involve any additional treatment or overstaying of the animals and the animals were provided with access to water and regular dry dog food. Overall, this work phase took place from July 22, 2019, to August 2, 2019.

The second part involved observing, finding, and re-capturing the free-roaming dogs from sector A on the West (n = 10) and sector F on the East (n = 6) with the biggest dog population and with greater density of previously vaccinated dogs (to increase the chance of finding the same vaccinated (targeted) dogs from the first part) (Fig. 2). These dogs were the same as those vaccinated and sterilized during the first part. They had special ear clips with an individual number, which was compared with the registry of vaccinated dogs. The next steps involved identifying their vaccination status according to our database, collecting blood samples, transporting the samples to the laboratory for rabies antibody testing, and analyzing the results produced by the laboratory. In pursuit of the primary objective to assess the efficacy of vaccination in the free-roaming dog population, only clinically healthy dogs were chosen to create comparable groups of both males and females. This second work phase took place from November 12, 2019, to November 19, 2019.

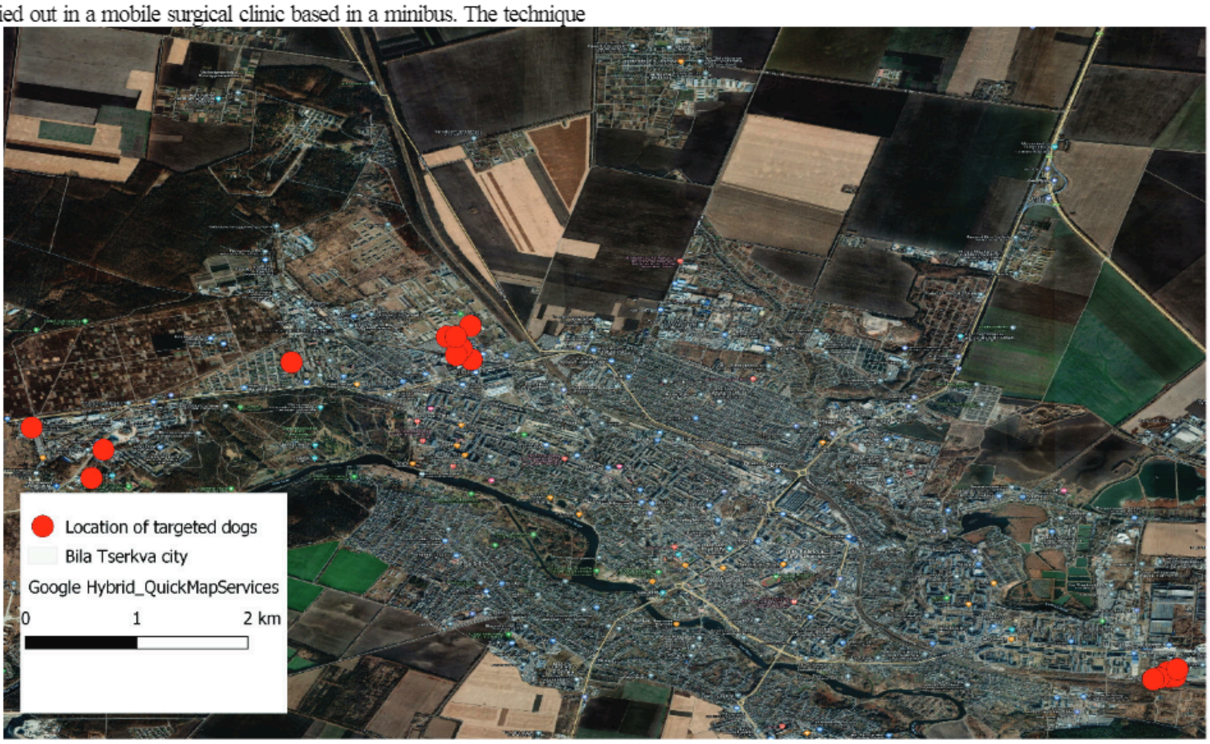

Fig. 2. Spatial distribution of the targeted dogs for blood sampling in Bila Tserkva city: red dots on the West and East of city show the location of the recaptured 16 vaccinated dogs

Rabies vaccination. All dogs received 1 mL dose of vaccine intramuscularly each, as per the manufacturer's instructions. The Rabistar inactivated and adjuvanted vaccine used was tested by the producer Ukrzoovetprompostach (batch number 090818) for potency prior to the assay (vaccine activity not less than 2 IU per dose). During the study, the same batch of vaccine was used and the vaccinations were undertaken in the period from July 22, 2019, to August 1, 2019. During the study, the vaccine was stored at +4 °C and the cold chain was strictly maintained. The protocol included the use of self-contained refrigerators for storing all medicines. Access to the power grid was provided by the local authorities.

Sample collection and serological testing. To analyze the serological response to the rabies vaccine, blood samples were collected from 16 dogs (10% of the vaccinated population), almost 4 months (110–113 days) post-vaccination. The blood samples were randomly collected, however, to ensure a fair comparison between the sexes of the animals, we selected eight males and eight females with similar body constitution and weight, all of whom received the vaccine from the same batch.

Blood samples (2.5–5.0 mL) were collected from the conscious dogs from the radial vein in uncoated glass tubes, which after centrifugation, yielded 0.5–1.0 mL of serum for analysis. The samples were transported in a thermal container with a cooler to the authorized reference testing laboratory NeoVetlab Ukraine Ltd, Kyiv City, Ukraine, which is on the list of EC approved laboratories for verifying the effectiveness of rabies vaccination on dogs, cats and ferrets entering an EU country. In addition, this laboratory has been authorized by the State Service of Ukraine for Food Safety and Consumer Protection to test the effectiveness of rabies vaccination in dogs, cats, and ferrets using the Fluorescent Antibody Virus Neutralization (FAVN) test, which is a World Health Organization (WHO) and World Organization for Animal Health (WOAH) recommended test allowing the quantification of sero-neutralising rabies antibodies (Cliquet et al., 1998; WHO, TRS, 2018). Serum samples were stored in the laboratory at +6 to +8 °C for one day and then tested by the FAVN test (all samples were tested in a unique session) using the protocol previously detailed (WOAH, 2023). A positive internal serum control was used with known IU/mL content at 0.5 IU/mL. A serum titer of ≥0.5 IU/mL was considered as the minimum protective neutralising antibody level according to WHO criteria (WHO, TRS, 2018).

Ethical statement. All activities were conducted in strict accordance with ethical component based on the EU regulations, aligned with general animal welfare recommendations and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, (1999). In addition, the policy of "Four Paws Ukraine" has an ethical component according to Policies & Standards of "Four Paws" aligned with the EU legislation. All activities with animals were conducted by "Four Paws Ukraine" with the required permission from the local government. It is important to note that every step taken in this study prioritized the humane and gentle treatment of animals to minimize their stress, which is the key focus of "Four Paws Ukraine" activities.

Data analysis. All collected information such as sector and location of capture, capture date, release date, operation date, doctor's name, vaccination date and label, sex, ID number, ID chip number were recorded in database using Microsoft Excel, 2019 (Microsoft Corp., Santa Rosa, CA, USA, 2019). Statistical analysis was conducted by using free software Jamovi (Australia, 2023, version 2.3.28) (obtained from www.jamovi.org). The Mann-Whitney U test was used to test for statistical differences in serological response between male and female dogs.

Results

In total 160 dogs were captured for vaccination from different sectors: A (n = 50), C (n = 38), D (n = 29), F (n = 43) for the first part of the study. All the animals were of medium size and did not fall into the small or large categories. In the second part, 16 dogs from two sectors: A (n = 10) and F (n = 6) were captured in order to take blood samples and conduct serological testing.

Table 1
Results of virus neutralising antibodies (VNA) testing in vaccinated free-roaming dogs

No.	Sector	Location	ID	Date of vaccination	Date of blood sampling	Sex	Dog's coat	Type of sterilization*	VNA, IU/mL
1	A	Western part of the city	27198	25.07.2019	12.11.2019	female	red	O	0.17
2	A	Western part of the city	27199	25.07.2019	12.11.2019	female	black and red	O	0.17
3	A	Western part of the city	27191	23.07.2019	12.11.2019	female	white	O	0.17
4	A	Western part of the city	27134	25.07.2019	12.11.2019	male	black and red	C	0.17
5	A	Western part of the city	27124	23.07.2019	12.11.2019	female	red	O	0.22
6	A	Western part of the city	27131	25.07.2019	12.11.2019	male	red	C	0.22
7	A	Western part of the city	27138	26.07.2019	12.11.2019	female	black and red	O	0.29
8	A	Western part of the city	27132	25.07.2019	12.11.2019	male	red	C	0.50
9	A	Western part of the city	27130	25.07.2019	12.11.2019	female	black and red	O	0.66
10	A	Western part of the city	27197	25.07.2019	12.11.2019	male	brown and grey	C	0.66
11	F	Eastern part of the city	00007	23.07.2019	12.11.2019	male	black and white	V	0.66
12	F	Eastern part of the city	27129	25.07.2019	12.11.2019	male	white	C	0.87
13	F	Eastern part of the city	27195	25.07.2019	12.11.2019	female	grey	O	1.15
14	F	Eastern part of the city	00006	23.07.2019	12.11.2019	male	white and black	V	1.95
15	F	Eastern part of the city	00005	23.07.2019	12.11.2019	male	grey	V	4.46
16	F	Eastern part of the city	27125	23.07.2019	12.11.2019	female	black	O	7.74

Note: * O – ovariectomy, V – vasectomy, C – castration.

Table 1 details the levels of rabies virus neutralising antibodies (VNA) among the 16 dogs sampled during the study. The titers ranged from 0.17 to 7.74 IU/mL. This indicated that the levels of anti-rabies immunity in dogs can vary significantly, with high individual differences.

Based on the criteria that a titer of antibodies below 0.5 IU/mL is inadequate, 56.3% (9/16) of the free-roaming dogs were found to still have protective levels of neutralising antibodies against rabies. Antibody titers below the 0.5 IU/mL threshold were detected in 25.0% (2/8) of the male group, and in 62.5% (5/8) of the female group (Table 2). The median of neutralising antibody levels in females was observed as 0.26 IU/mL, and the median of antibody levels in males was 0.66 IU/mL (P = 0.36).

Table 2
Analysis of VNA titers among male and female dogs

Sex	No.	Median	Minimum	Maximum	Dogs with ≥ 0.5 IU/mL titres, %
Male	8	0.66	0.17	4.46	75.0
Female	8	0.26	0.17	7.74	37.5
Overall	16	0.58	0.17	7.74	56.3

Discussion

This pilot study represents the first attempt to analyze the parenteral rabies vaccination practice in Ukraine, which was conducted in a population of free-roaming dogs. It is essential to emphasize that this study was conducted under trouble conditions. Given Ukraine's high number of reported rabies cases, there is considerable interest in evaluating the current operational protocols, management strategies, and efficacy assessment of vaccination programs. The elimination of rabies requires a minimum of 70% of the vaccination coverage of the reservoir population to break the transmission of rabies (Voigt et al., 1985). In Ukraine, the reservoir population of the virus, i. e. the fox population, was subjected to oral vaccination campaigns for more than 20 years (Polupan et al., 2019). However, the entire territory has never been orally vaccinated twice a year every year until the complete elimination of the virus, as recommended (WHO, TRS, 2018; WOAH, 2023). To reduce the risks of rabies transmission to the human population, and in view of the high density of owned and ownerless dogs in Ukraine, additional measures for the control of rabies in Ukraine include vaccination of dogs by the parenteral route.

Our study revealed that 56.3% of the vaccinated dog population demonstrated protective immunity four months post-vaccination with median titer of 0.58 IU/mL. Even though our assessment encompassed just 10% of the total population, equating to a relatively modest sample size of 16 subjects, our findings may provide the basis for determining that this subset adequately reaches a specific target objective as this data aligns with the findings from other researchers. Indeed, the observed decline in titers over the following weeks confirmed a temporal decay in antibody levels, indicating that lower titers observed at four months in primary-vaccinated dogs are attributed to the time elapsed since the peak response of seroconversion, occurring generally between 4 to 6 weeks following the initial vaccination, as observed in other studies (Cliquet et al., 2003; Gunatilake et al., 2023). The free-roaming dogs enrolled in this study may be regarded as primary-vaccinated animals, having potentially never been vaccinated or received timely booster shots. Consequently, their levels of neutralising antibodies are likely to be lower compared to pluri-vaccinated dogs (Berndtsson et al., 2011; Wera et al., 2022; Chuquista-Alcarraz et al., 2023). The percentage of 56.3% of seropositive dogs at Day120 corresponds to values already published (for review see Darkaoui et al., 2016). This percentage is even higher compared to a study where only 40% and 7% of primo-laboratory dogs vaccinated with two different rabies vaccines had a seroprotective titer 4 months after vaccination (Minke et al., 2009). This data could suggest that the local vaccine produced in Ukraine is efficient.

On the other hand, it is also possible that some dogs may have been vaccinated by previous owners (information that was not available to us) which can be the reason for higher levels of antibody titers in some samples in our analysis (4.46, and 7.74 IU/mL). It should also be noted that high individual differences in VNA levels have already been observed in dogs vaccinated by the parenteral route.

In addition to this, stress related factors play a significant role in mounting the immune response. Previous research has shown that malnourished dogs may produce a weaker immune response to vaccines, resulting in lower neutralising antibody titres (Morters et al., 2014; Wait et al., 2020; Wera et al., 2022). The free-roaming dog population we examined could endure a wide range of living conditions and environmental stressors, from extreme temperatures (severe heat in summer during the vaccination time frame), lack of consistent food, absence of a permanent place for living, hazards from wild animals, parasites, and other injuries. In addition, homeless animals often lack guaranteed post-surgery nutrition, which, combined with the stress of surgery, can result in significant variations in the immune responses (Berndtsson et al., 2011; Morters et al., 2014; Wera et al., 2022).

Based on sex differentiation, females may experience more traumatic stress due to ovariectomy, possibly impacting antibody development post-vaccination (Kennedy et al., 2007). While a trend towards lower antibody levels in females was observed, with protective titers in 37.5% (3/8) of females compared to 75% (6/8) of males, there was no statistically significant difference found between the group of males and females ($P = 0.36$) likely due to the small sample size enrolled in the second part of the study.

An important factor influencing the level of the antibody response is the immunogenicity of the vaccine (Minke et al., 2009), or failure to administer the vaccine (Smith et al., 2019, 2022), but in our study it is believed that this effect is of low importance as the vaccination process was performed very carefully and the higher immunological response in some of the vaccinated animals, all receiving the same batch of vaccine, demonstrates the immunogenicity of the vaccine. Furthermore, all our results surpassed the threshold of 0.17 IU/mL, suggesting a potential decline in VNAs after the seroconversion peak and affirming the immunogenicity of the vaccine.

Various factors, including age, breed, and weight, have been identifyed in previous studies as influencing vaccination efficacy (Kennedy et al., 2007; Berndtsson et al., 2011; Pimburage et al., 2017). It is crucial to acknowledge, however, that the current study was unable to affirm this relationship conclusively. This limitation stems from the absence of specific breed and age information for each animal in our dataset. In future investigations, obtaining more comprehensive data on these variables could provide a more detailed and proper understanding of their impact on vaccine effectiveness in the studied canine population. Nevertheless, the lack of antibodies or the existence of antibody levels below the minimum requirement at a particular time does not unequivocally indicate a lack of protection. It is plausible that the animal underwent seroconversion before the blood test date (Cliquet et al., 2003).

In light of Ukraine's endemic status for rabies, we recommend repeated vaccination for free-roaming dogs against rabies one year after previous vaccination to improve the recommended percentage of vaccine coverage of animals in the population. Additionally, whenever feasible, it is crucial to conduct temporal assessments of vaccinated dogs by conducting serological testing during the peak of VNA response, which corresponds to seroconversion of animals, at or around 28 days post-vaccination, as recommended by WHO (WHO, TRS, 2018). In addition, future studies should incorporate a comparative analysis with other vaccines including a specific focus on those presently or previously used in the WOAH vaccine bank. Finally, we recommend a large scale epidemiological study to assess the impact of animal-related, vaccine-related, and environment-related parameters on the efficacy of rabies vaccination. In order to have the ability to assess antibody titers before vaccination and compare them, the serum samples in field conditions can be collected before vaccination or the day of vaccination, stored frozen, and a second blood sampling has to be organized one month after vaccination on the same animals. Finally, the pair samples for each dog are then analyzed for rabies antibody determination.

Conclusion

In conclusion, our pilot study gives the first insights into and underlines the importance of rabies vaccination in free-roaming dogs as a preventive measure to ensure human health and animal welfare. In addition, our results reveal that vaccination of free-roaming dogs resulted in a protective immunity rate of 56.3% among the targeted dog population, measured four months after vaccination. This percentage is comparable to those observed in the literature for samples from field dogs collected in similar conditions, i.e., several months after the primary vaccination. The suboptimal levels of rabies virus-neutralising antibodies are also likely due to a combination of stressors, including nutritional and temperature-related challenges, coupled with variations in individual immune system responses.

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References

Antonova, L. O., Makovska, I. F., & Krupinina, T. M. (2021). Istoriya borbi s beshenstvom v Ukraine so vremen Pastera do nashikh dnei [The history of the elimination of rabies in Ukraine from the time of Pasteur to the present day]. Actual Infectology, 9(1), 6–16 (in Russian).

Berndtsson, L. T., Nyman, A.-K. J., Rivera, E., & Klingeborn, B. (2011). Factors associated with the success of rabies vaccination of dogs in Sweden. Acta Veterinaria Scandinavica, 53(1), 22.

Berteselli, G. V., Rapagnà, C., Salini, R., Badagliacca, P., Bellucci, F., Iannino, F., & Dalla Villa, P. (2021). A pilot study to develop an assessment tool for dogs undergoing trap-neuter-release (TNR) in Italy. An overview on the National Implementation of TNR programmes. Animals, 11(11), 3107.

Borse, R. H., Atkins, C. Y., Gambhir, M., Undurraga, E. A., Blanton, J. D., Kahn, E. B., Dyer, J. L., Rupprecht, C. E., & Meltzer, M. I. (2018). Cost-effectiveness of dog rabies vaccination programs in East Africa. PLoS Neglected Tropical Diseases, 12(5), e0006490.

Chuquista-Alcarraz, O., Falcón, N., Vigilato, M. A. N., Rocha, F., Toledo-Barone, G., Amorim-Conselheiro, J., Recuenco, S. E., & Castillo-Neyra, R. (2023). Dog population rabies immunity before a mass vaccination campaign in Lima, Peru: Vulnerabilities for virus reestablishment. The American Journal of Tropical Medicine and Hygiene, 109(2), 420–428.

Cliquet, F., Aubert, M., & Sagné, L. (1998). Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. Journal of Immunological Methods, 212(1), 79–87.

Cliquet, F., Verdier, Y., Sagne, L., Aubert, M., Schereffer, J. L., Selve, M., Wasniewski, M., & Servat, A. (2003). Neutralising antibody titration in 25,000 sera of dogs and cats vaccinated against rabies in France, in the framework of the new regulations that offer an alternative to quarantine. Revue Scientifique et Technique de l'OIE, 22(3), 857–866.

Darkaoui, S., Fassi Fihri, O., Schereffer, J. L., Aboulfidaa, N., Wasniewski, M., Zouine, K., Bouslikhane, M., Yahia, K. I. S., & Cliquet, F. (2016). Immunogenicity and efficacy of Rabivac vaccine for animal rabies control in Morocco. Clinical and Experimental Vaccine Research, 5(1), 60.

Durr, S., Mindekem, R., Kaninga, Y., Doumagoum Moto, D., Meltzer, M. I., Vounatsou, P., & Zinsstag, J. (2009). Effectiveness of dog rabies vaccination programmes: Comparison of owner-charged and free vaccination campaigns. Epidemiology and Infection, 137(11), 1558–1567.

Fahrion, A. S., Taylor, L. H., Torres, G., Müller, T., Dürr, S., Knopf, L., De Balogh, K., Nel, L. H., Gordoncillo, M. J., & Abela-Ridder, B. (2017). The road to dog

rabies control and elimination – what keeps us from moving faster? Frontiers in Public Health, 5, 103.
Fitzpatrick, M. C., Shah, H. A., Pandey, A., Bilinski, A. M., Kakkar, M., Clark, A. D., Townsend, J. P., Abbas, S. S., & Galvani, A. P. (2016). One Health approach to cost-effective rabies control in India. Proceedings of the National Academy of Sciences, 113(51), 14574–14581.
Gibson, A. D., Wallace, R. M., Rahman, A., Bharti, O. K., Isloor, S., Lohr, F., Gamble, L., Mellanby, R. J., King, A., & Day, M. J. (2020). Reviewing solutions of scale for canine rabies elimination in India. Tropical Medicine and Infectious Disease, 5(1), 47.
Gunatilake, M., Pimburage, R., Wimalaratne, O., & Perera, K. (2023). Immunogenicity following dog rabies vaccination: A Sri Lankan experience. In: Slack, V. (Ed.). One Health for dog-mediated rabies elimination in Asia. CABI International. Pp. 128–141.
Hampson, K., Coudeville, L., Lembo, T., Sambo, M., Kieffer, A., Attlan, M., Barrat, J., Blanton, J. D., Briggs, D. J., Cleaveland, S., Costa, P., Freuling, C. M., Hiby, E., Knopf, L., Leanes, F., Meslin, F.-X., Metlin, A., Miranda, M. E., Müller, T., Nel L. H., Recuenco S., Rupprecht, C. E., Schumacher, C., Taylor, L., Vigilato, M.A.N., Zinsstag, J., & Dushoff, J. (2015). Estimating the global burden of endemic canine rabies. PLoS Neglected Tropical Diseases, 9(4), 30–40.
Hampson, K., Dobson, A., Kaare, M., Dushoff, J., Magoto, M., Sindoya, E., & Cleaveland, S. (2008). Rabies exposures, post-exposure prophylaxis and deaths in a region of endemic canine rabies. PLoS Neglected Tropical Diseases, 2(11), e339.
Kennedy, L. J., Lunt, M., Barnes, A., McElhinney, L., Fooks, A. R., Baxter, D. N., & Ollier, W. E. R. (2007). Factors influencing the antibody response of dogs vaccinated against rabies. Vaccine, 25(51), 8500–8507.
Korniienko, L. E., Moroz, O. A., Mezhensky, A. O., Skorokhod, S. V., Datsenko, R. A., Karpulenko, M. S., Polupan, I. M., Dzyuba, Y. M., Nedosekov, V. V., Makovskaya, I. F., Hibaliuk, Y. O., Sonko, M. P., Tsarenko, T. M., & Pishchanskyi, O. V. (2019). Epizootological and epidemiological aspects for rabies in Ukraine for the period from 1999 to 2018. Veterinary Science, Technologies of Animal Husbandry And Nature Management, 3, 90–109.
Lavan, R. P., King, A. I. MacG., Sutton, D. J., & Tunceli, K. (2017). Rationale and support for a One Health program for canine vaccination as the most cost-effective means of controlling zoonotic rabies in endemic settings. Vaccine, 35(13), 1668–1674.
Lojkić, I., Šimić, I., Bedeković, T., & Krešić, N. (2021). Current status of rabies and its eradication in Eastern and Southeastern Europe. Pathogens, 10(6), 742.
Makovska, I., M., Bezymennyi, M., Witkowski, L., Nedosekov, V., Kornienko, L., Karpulenko, M., & Nardi, M. D. (2021). Descriptive spatiotemporal analysis of rabies in domestic carnivores and wildlife in Ukraine in 2012–2018. Medycyna Weterynaryjna, 77(11), 6589–2021.
Makovska, I., Nedosekov, V. V., Kornienko, L. Y., Novokhatny, Y. O., Nebogatkin, I. V., & Yustyniuk, V. Y. (2020). Retrospective study of rabies epidemiology in Ukraine (1950–2019). Theoretical and Applied Veterinary Medicine, 8(1), 36–49.
Mbilo, C., Coetzer, A., Bonfoh, B., Angot, A., Bebay, C., Cassamá, B., De Benedictis, P., Ebou, M. H., Gnanvi, C., Kallo, V., Lokossou, R. H., Manjuba, C., Mokondjimobe, E., Mouillé, B., Mounkaila, M., Ndour, A. P. N., Nel, L., Olugasa, B. O., Pato, P., Pyana, P. P., Rerambyath, G. A., Roamba, R. C., Sadeuh-Mba, S. A., Suluku, R., Richard D. Suu-Ire, R. D., Tejiokem, M. C., Tetchi, M., Tiembre, I., Traor´e, A., Voupawoe, G., & Zinsstag, J. (2021). Dog rabies control in West and Central Africa: A review. Acta Tropica, 224, 105459.
Minke, J. M., Bouvet, J., Cliquet, F., Wasniewski, M., Guiot, A. L., Lemaitre, L., Cariou, C., Cozette, V., Vergne, L., & Guigal, P. M. (2009). Comparison of antibody responses after vaccination with two inactivated rabies vaccines. Veterinary Microbiology, 133(3), 283–286.
Morters, M. K., McKinley, T. J., Horton, D. L., Cleaveland, S., Schoeman, J. P., Restif, O., Whay, H. R., Goddard, A., Fooks, A. R., Damriyasa, I. M., & Wood, J. L. N. (2014). Achieving population-level immunity to rabies in free-roaming dogs in Africa and Asia. PLoS Neglected Tropical Diseases, 8(11), e3160.
Müller, T. F., Schröder, R., Wysocki, P., Mettenleiter, T. C., & Freuling, C. M. (2015). Spatio-temporal use of oral rabies vaccines in fox rabies elimination programmes in Europe. PLoS Neglected Tropical Diseases, 9(8), e0003953.
Omelchenko, H. O., Avramenko, N. O., Kulynych, S. M., Polishchuk, A. A., Petrenko, M. O., Sysa, P. S., & Woźniakowski, G. J. (2022). Rabies virus spread in central and north-eastern regions of Ukraine. Medycyna Weterynaryjna, 78(1), 6605.
Pimburage, R. M. S., Gunatilake, M., Wimalaratne, O., Balasuriya, A., & Perera, K. A. D. (2017). Sero-prevalence of virus neutralizing antibodies for rabies in different groups of dogs following vaccination. BMC Veterinary Research, 13(1), 133.
Polupan, I., Bezymennyi, M., Gibaliuk, Y., Drozhzhe, Z., Rudoi, O., Ukhovskyi, V., Nedosekov, V., & De Nardi, M. (2019). An analysis of rabies incidence and its geographic spread in the buffer area among orally vaccinated wildlife in Ukraine from 2012 to 2016. Frontiers in Veterinary Science, 6, 290.
Rudoi, O., Drozhzhe, Z., Chechet, O., Ukhovskyi, V., & Kovalenko, V. (2023). Spread of rabies in the Kyiv Oblast during 2020–2022. Scientific Horizons, 26(8), 117.
Rupprecht, C. E., Abela-Ridder, B., Abila, R., Amparo, A. C., Banyard, A., Blanton, J., Chanachai, K., Dallmeier, K., De Balogh, K., Del Rio Vilas, V., Ertl, H., Freuling, C., Hill, R., Houillon, G., Jakava-Viljanen, M., Kasemsuwan, S., Léchenet, J., Nel, L., Panichabhongse, P., & Mueller, T. (2020). Towards rabies elimination in the Asia-Pacific region: From theory to practice. Biologicals, 64, 83–95.
Rupprecht, C. E., Mshelbwala, P. P., Reeves, R. G., & Kuzmin, I. V. (2023). Rabies in a postpandemic world: Resilient reservoirs, redoubtable riposte, recurrent roadblocks, and resolute recidivism. Animal Diseases, 3(1), 15.
Sambo, M., Ferguson, E. A., Abela-Ridder, B., Changalucha, J., Cleaveland, S., Lushasi, K., Mchau, G. J., Nanai, A., Nonga, H., Steenson, R., Johnson, P. C., & Hampson, K. (2022). Scaling-up the delivery of dog vaccination campaigns against rabies in Tanzania. PLoS Neglected Tropical Diseases, 16(2), e0010124.
Savadogo, M., Dahourou, L. D., Ilboudo, A. K., Ilboudo, S. G., Zangré, H., Tarnagda, G., Souli, Z., Combari, A. H. B., Diarra, R., Bidima, M., Traoré, M. G. B., Mandé, C. D., Sondo, K. A., & De Balogh, K. (2023). The rabies free Burkina Faso initiative: An example of how one health-oriented civil society organizations can contribute towards the achievement of the rabies zero by 30 goal. One Health Outlook, 5(1), 9.
Smith, L., Goold, C., Quinnell, R., Munteanu, A. M., Hartmann, S., Dalla Villa, P., & Collins, L. M. (2022). Population dynamics of free-roaming dogs in two European regions and implications for population control. PLoS One, 17(9), e0266636.
Smith, L., Hartmann, S., Munteanu, A. M., Dalla Villa, P., Quinnell, R. J., & Collins, L. M. (2019). The effectiveness of dog population management: A systematic review. Animals, 9(12), 1020.
Smith, Q. R. J., Hartmann, A. S., Villa, D. P., & Collins, L. (2022). Attitudes towards free-roaming dogs and dog ownership practices in Bulgaria, Italy, and Ukraine. PLoS One, 17(3), e0252368.
Smith, T., Millien, M., Vos, A., Fracciterne, F. A., Crowdis, K., Chirodea, C., Medley, A., Chipman, R., Qin, Y., Blanton, J., & Wallace, R. (2019). Evaluation of immune responses in dogs to oral rabies vaccine under field conditions. Vaccine, 37(33), 4743–4749.
Taylor, E., Del Rio Vilas, V., Scott, T., Coetzer, A., Prada, J. M., Alireza, G., Alqadi, N. A., Berry, A., Bazzal, B., Barkia, A., Davlyatov, F., Farahtaj, F., Harabech, K., Imnadze, P., Mahiout, F., Majeed, M. I., Nedosekov, V., Nel, L., Rich, H., & Horton, D. (2021). Rabies in the Middle East, Eastern Europe, Central Asia and North Africa: Building evidence and delivering a regional approach to rabies elimination. Journal of Infection and Public Health, 14(6), 787–794.
Taylor, E., Prada, J. M., Del Rio Vilas, V., Undurraga, E. A., Wallace, R., & Horton, D. L. (2023). Cost-effectiveness analysis of integrated bite case management and sustained dog vaccination for rabies control. The American Journal of Tropical Medicine and Hygiene, 109(1), 205–213.
Taylor, L., & Nel, L. (2015). Global epidemiology of canine rabies: Past, present, and future prospects. Veterinary Medicine: Research and Reports, 5(6), 361–371.
Taylor, L. H., Wallace, R. M., Balaram, D., Lindenmayer, J. M., Eckery, D. C., Mutonono-Watkiss, B., Parravani, E., & Nel, L. H. (2017). The role of dog population management in rabies elimination – a review of current approaches and future opportunities. Frontiers in Veterinary Science, 4, 109.
Vega, S., Lorenzo-Rebenaque, L., Marin, C., Domingo, R., & Fariñas, F. (2021). Tackling the threat of rabies reintroduction in Europe. Frontiers in Veterinary Science, 7, 613712.
Voigt, D., Tinline, R., & Broekhoven, L. (1985). A spatial simulation model for rabies control. In: Bacon, J. (Ed.). Population dynamics of rabies wildlife. Academic Press, London. Pp. 311–349.
Wait, L. F., Dobson, A. P., & Graham, A. L. (2020). Do parasite infections interfere with immunisation? A review and meta-analysis. Vaccine, 38(35), 5582–5590.
Wera, E., Warembourg, C., Bulu, P. M., Siko, M. M., & Dürr, S. (2022). Immune response after rabies vaccination in owned free-roaming domestic dogs in Flores Island, Indonesia. Frontiers in Veterinary Science, 9, 868380.
WHO, TRS (2018). WHO expert consultation on rabies: Third report. World Health Organization.
WOAH (2023). WOAH Terrestrial Manual (Chapter 3.1.18. – Rabies (infection with rabies virus and other lyssaviruses)).