UDC 619:616.98:578.824.11:615.371-02:591.531.22/29
DOI: 10.29326/2304-196X-2020-1-32-31-37

Testing of Ferarabivac anti-rabies live vaccine for wild carnivores for its immunogenicity and protectivity

A. V. Shishkov[1], D. A. Lozovoy[2], A. V. Borisov[3], D. V. Mikhalishin[4]
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia
[1] *ORCID 0000-0002-9777-1404, e-mail: shishkov@arriah.ru*
[2] *ORCID 0000-0002-5983-7062, e-mail: lozovoy@arriah.ru*
[3] *ORCID 0000-0001-9880-9657, e-mail: borisov@arriah.ru*
[4] *ORCID 0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru*

SUMMARY

Rabies is one of the most important human and animal viral diseases, being one of the most dangerous zoonoses, causing damage to the central nervous system with an inevitable fatal outcome. This disease is of global concern, and it attracts special attention of international organizations (WHO, OIE, FAO, GARC) and of veterinary services in many countries around the world. A variety of anti-rabies vaccines have been used for specific rabies prevention in wild carnivores, however, the safety and effectiveness of some of them is doubtful. New, more advanced products are being developed, one of which is Ferarabivac, a live oral vaccine. The vaccine was tested for its immunogenicity and protectivity in wild carnivores. The optimal immunizing dose was 2.0 cm^3, with the infectivity titre of RV-97 strain of at least 6.00 lg $KKID_{50}/cm^3$. Anti-rabies antibody titres detected in the blood sera of foxes and raccoon dogs 14 days post vaccination, were 0.70 ± 0.18 and 0.73 ± 0.19 IU/cm^3, respectively, which provided protection against rabies virus infection (≥ 0.50 IU/cm^3). Rabies virus neutralizing antibodies in foxes reached their maximum level of 4.30 ± 0.32 IU/cm^3 50 days post vaccination. Antibody titres in vaccinated raccoon dogs also reached their maximum level of 4.53 ± 0.27 IU/cm^3 50 days post vaccination. The minimum protective threshold levels of serum neutralizing antibodies was determined 12 months after the vaccination, and it was 0.62 ± 0.28 and 0.71 ± 0.17 IU/cm^3 in foxes and raccoon dogs, respectively, which proves the necessity to perform booster vaccination one year later. No animals vaccinated against rabies with Ferarabivac live vaccine showed any clinical signs of the disease during the entire observation period following the challenge test carried out 30 days post vaccination.

Key words: rabies of wild carnivorous animals, Ferarabivac anti-rabies live oral vaccine, rabies virus neutralizing antibodies.

For citation: Shishkov A. V., Lozovoy D. A., Borisov A. V., Mikhalishin D. V. Testing of Ferarabivac anti-rabies live vaccine for wild carnivores for its immunogenicity and protectivity. *Veterinary Science Today*. 2020; 1 (32): 31–37. DOI: 10.29326/2304-196X-2020-1-32-31-37.

Conflict of interest. The authors declare no conflict of interest.

For correspondence: Alexander V. Shishkov, Leading Veterinarian of the Laboratory for FMD Prevention FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: shishkov@arriah.ru.*

УДК 619:616.98:578.824.11:615.371-02:591.531.22/29

Исследование иммуногенных и протективных свойств антирабической живой вакцины «Ферарабивак» для диких плотоядных животных

А. В. Шишков[1], Д. А. Лозовой[2], А. В. Борисов[3], Д. В. Михалишин[4]
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия
[1] *ORCID 0000-0002-9777-1404, e-mail: shishkov@arriah.ru*
[2] *ORCID 0000-0002-5983-7062, e-mail: lozovoy@arriah.ru*
[3] *ORCID 0000-0001-9880-9657, e-mail: borisov@arriah.ru*
[4] *ORCID 0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru*

РЕЗЮМЕ

Бешенство имеет первоочередное значение в ряду вирусных болезней человека и животных, являясь одним из самых опасных зоонозов, вызывая поражение центральной нервной системы с неизбежным летальным исходом. Заболевание представляет собой мировую проблему, которой уделяют особое внимание международные организации (ВОЗ, МЭБ, ФАО, GARC) и ветеринарные службы многих стран мира. Для специфической профилактики бешенства среди диких плотоядных животных применяют разнообразные антирабические вакцины, однако существуют сомнения в безопасности и эффективности некоторых из них. Ведутся разработки новых, более совершенных препаратов, одним из которых является антирабическая живая оральная вакцина «Ферарабивак». Проведены исследования по изучению ее иммуногенных и протективных свойств для диких плотоядных животных. Оптимальная иммунизирующая доза препарата составляет 2,0 $см^3$ с титром инфекционной активности вируса бешенства штамма РВ-97 не менее 6,00 lg $ККИД_{50}/см^3$. Через 14 сут после оральной иммунизации данной вакциной антирабические антитела обнаружены в сыворотке крови лисиц и енотовидных собак в титрах

0,70 ± 0,18 и 0,73 ± 0,19 МЕ/см^3 соответственно, что обеспечивало защиту от заражения вирусом бешенства (≥ 0,50 МЕ/см^3). Спустя 50 сут уровень антирабических вируснейтрализующих антител у лисиц достигал максимальных значений и составлял 4,30 ± 0,32 МЕ/см^3. Титр антител у вакцинированных енотовидных собак достигал максимальных значений также спустя 50 сут и был равен 4,53 ± 0,27 МЕ/см^3. Минимальный пороговый уровень вируснейтрализующих антител определяли через 12 месяцев после иммунизации, он составлял у лисиц и енотовидных собак 0,62 ± 0,28 и 0,71 ± 0,17 МЕ/см^3 соответственно, что доказывает необходимость проведения повторной вакцинации животных против бешенства через год. В результате контрольного заражения через 30 сут после вакцинации все животные, иммунизированные антирабической живой вакциной «Ферарабивак», в течение всего срока наблюдения не проявляли клинических признаков бешенства.

Ключевые слова: бешенство диких плотоядных животных, антирабическая живая оральная вакцина «Ферарабивак», антирабические вируснейтрализующие антитела.

Для цитирования: Шишков А. В., Лозовой Д. А., Борисов А. В., Михалишин Д. В. Исследование иммуногенных и протективных свойств антирабической живой вакцины «Ферарабивак» для диких плотоядных животных. *Ветеринария сегодня.* 2020; 1 (32): 31–37. DOI: 10.29326/2304-196X-2020-1-32-31-37.

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Шишков Александр Валерьевич, ведущий ветеринарный врач лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: shishkov@arriah.ru.*

INTRODUCTION

According to the International Committee on Taxonomy of Viruses, rabies is caused by viruses belonging to the *Lyssavirus* genus *Rhabdoviridae* family of the *Mononegavirales* order [1]. The *Lyssavirus* genus includes the classic rabies virus (RABV), which belongs to phylogenetic group 1 and is widely spread among various animal species around the world [1, 2].

Rabies is of primary importance among human and animal viral diseases, being one of the most dangerous zoonoses, causing damage to the central nervous system with an inevitable fatal outcome [3, 4]. Thus, rabies is a global challenge in the field of infectious pathology, epidemiology, epizootology and ecology [5].

To date, the developed countries, including Russia, where vaccine manufacturing technologies meet modern requirements, have replaced the production of tissue-based anti-rabies vaccines with live and inactivated culture-based rabies vaccines for parenteral and oral use [4].

A variety of anti-rabies vaccines are used for specific rabies prevention in wild carnivores, however, the safety and effectiveness of some of them is doubtful. Currently, new and more advanced vaccines are being developed [4, 6, 7]. WHO, OIE, FAO and GARC experts and specialists constantly highlight the necessity to improve existing anti-rabies vaccines and to develop the newer and safer ones [4].

Anti-epidemic measures implemented in the territory of the Russian Federation in recent years do not result in significant containment of rabies virus spread in animals [2]. In Russia, domestic oral anti-rabies vaccines are used in wild carnivores. The vaccines are based on the attenuated rabies virus strain, which ensures protection against any rabies virus variant belonging to phylogenetic group 1 [8, 9, 10].

The effectiveness of preventive vaccination depends on the route of administration, quality of the produced vaccines, and number of immunized wild carnivores [2, 10]. For each type of target animals, the vaccine efficacy should be demonstrated by statistically reliable studies that include oral vaccination, subsequent challenge test and assessment of vaccine protective efficacy. The most susceptible young animals, for which the vaccine is recommended, were used in the study. Rabies virus neutralizing antibody titers (VNA) should confirm the vaccine efficacy for each target animal species. For this purpose we studied the protective antibody production using VN assay (RFFIT) [8] and the duration of immunity in wild carnivores [6, 7].

The production of anti-rabies vaccines for animals is regulated by the requirements of the World Organization for Animal Health (OIE), according to which the vaccine should provide strong immunity in target animal species (immunogenicity index ≥ 1.0) and induce VNA titer (at least 0.5 IU/cm^3) [4].

The aim of this work was to study immunogenic and protective properties of FGBI "ARRIAH"-manufactured live oral vaccine Ferarabivac in wild carnivores.

MATERIALS AND METHODS

Rabies virus. Rabies virus vaccine strain RV-97 deposited in the Collection of strains of microorganisms of the FGBI "ARRIAH" was used to produce Ferarabivac anti-rabies live attenuated oral vaccine (FGBI "ARRIAH"). Standard control virus strain (CVS-27) was used for challenge.

Vaccine. Ferarabivac anti-rabies live attenuated oral vaccine for wild carnivores was used in the study. The vaccine contained rabies virus vaccine strain RV-97 (infectivity titre ≥ 6.0 lg $KKID_{50}$/cm^3) at the dose of 1.0, 2.0 and 5.0 cm^3.

Animals. Red foxes aged 9–12 months (115 animals) and raccoon dogs aged 9–12 months (85 animals) were used to study the vaccine immunogenicity. The animals were purchased from animal farms in the Moscow region. According to the VN assay results, none of the animals had rabies virus VNA.

All the tests were conducted in strict accordance with the Interstate Guide for Care and Use of Laboratory Animals, GOST 33215-2014, adopted by the Interstate Council for Standardization, Metrology and Certification, as well as in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22.09.2012 on the protection of animals used for scientific purposes.

Determination of the rabies virus infectivity titre. Virus infectivity was determined using a monolayer cell line of the newborn Syrian hamster kidneys (BHK 21/2-17), followed by staining with fluorescein isothiocyanate

(FITC)-labeled anti-rabies immunoglobulin G [11]. Virus titers were calculated using Spearman-Kerber method [12] and expressed in lg $KKID_{50}/cm^3$.

Brain and blood sampling. Sampling was performed in compliance with the FGBI "ARRIAH"s Guidelines for brain, blood serum and bone tissue sampling and shipment for rabies diagnosis and assessment of the efficacy of oral vaccination [13].

Fluorescent antibody test (FAT) Smears prepared from a composite sample of brain tissues of target animals were examined by direct FAT, which consists in binding FITC-labeled anti-rabies antibodies to the specific antigen, and further examination for fluorescent antibody-antigen complexes using fluorescence microscopy [14].

Study of vaccine immunogenicity. The duration of the protective immunity in the target animals at the end of the stated protection period was assessed by FAVN using a monolayer BHK-21/2-17 cell line and FITC-immunoglobulin in accordance with the OIE recommendations for rabies [4]. Positive OIE Standard Serum of dog origin (6.7 IU/cm^3) and negative OIE Standard Serum of dog origin were used (ANSES, Nancy, France). Each blood serum was tested in triplicates.

Study of vaccine protectivity. To study the efficacy of vaccination, 25 vaccinated and 10 control animals were used. Challenge was performed on day 30 post vaccination at a dose of 25,000 LD_{50}/cm^3. After the challenge, the animals were observed daily for 90 days. As soon as the animals began to show clinical signs of the disease, they were euthanized and the presence of the virus was confirmed by FAT. At the end of the observation period, all survived animals were euthanized and the brain smears were studied using FAT.

Statistical data processing. The obtained data were statistically processed by calculating arithmetic mean values and the confidence interval of the difference between means computed using the Student's-Fischer equation [12]. The differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Ferarabivac anti-rabies live oral vaccine is designed to prevent rabies in wild carnivores, and is the only effective immunization route in this case is feeding the vaccine to the animals.

At the first stage, the immunizing dose was determined for 30 red foxes. The animals were divided into 3 groups (10 animals per group). Animals in the first group were fed 1 bait containing 1.0 cm^3 of the vaccine, in the second group – 1 bait containing 2.0 cm^3 of the vaccine, in the third group – 1 bait containing 5.0 cm^3 of the vaccine. Blood sera were collected and tested for rabies VNA on days 14, 21, 30 and 60 post vaccination (dpv). The results are shown in Table 1.

The data in Table 1 demonstrate that when foxes ate the bait containing 1.0 cm^3 of the vaccine the anti-rabies VNA titre amounted to 0.30 ± 0.29; 0.90 ± 0.25; 1.25 ± 0.25; 2.00 ± 0.21 IU/cm^3 respectively. Eating of the vaccine at a dose of 2.0 cm^3 resulted in anti-rabies VNA level at 0.69 ± 0.18; 2.20 ± 0.23; 3.20 ± 0.34; 4.20 ± 0.38 IU/cm^3 respectively. Eating of 5.0 cm^3 dose resulted in anti-rabies VNA titres at 0.78 ± 0.25; 2.60 ± 0.35; 4.00 ± 0.28; 4.96 ± 0.30 IU/cm^3 on 14, 21, 30, 60 dpv respectively. According to the obtained data and the OIE requirements as well as in the context of cost-effectiveness the dose of 2.0 cm^3 with infectivity titre being at least 6.00 lg $KKID_{50}/cm^3$ is an effective immunizing dose of the vaccine and it ensures the animal protection against rabies infection in 14 days post immunization. When animals were fed the baits containing 5.0 cm^3 of the vaccine, the comparable anti-rabies VNA levels were induced but it required considerable amount of the vaccine raw material. Administration of the immunizing dose of 1.0 cm^3 on 14 dpv resulted in accumulation of anti-rabies VNA at the amount insufficient for the animal protection against rabies (< 0.50 IU/cm^3).

During the next stage of the research, we arranged an experiment aimed at the examination of the Ferarabivac vaccine immunogenicity in 25 foxes and 25 raccoon dogs. One vaccine dose containing 2.0 cm^3 of the rabies virus with titre 6.00 lg $KKID_{50}/cm^3$ was orally administered to each animal. In 14, 30, 50, 60, 70, 80 and 90 dpv blood samples were collected from the animals and sera were tested for the virus-specific antibody titres. The test results are shown in Table 2 and Figure 1.

The data shown in Table 2 demonstrate that Ferarabivac anti-rabies live oral vaccine induced anti-rabies

Table 1
Determination of immunizing dose of Ferarabivac anti-rabies live oral vaccine for wild carnivores ($n = 10$, $p < 0,05$)

Таблица 1
Определение иммунизирующей дозы антирабической живой оральной вакцины «Ферарабивак» для диких плотоядных животных ($n = 10$, $p < 0.05$)

Days post vaccination	Anti-rabies VNA titre (IU/cm^3)* after vaccine feeding at various doses		
	1.0 cm^3	2.0 cm^3	5.0 cm^3
14	0.30 ± 0.29	0.69 ± 0.18	0.78 ± 0.25
21	0.90 ± 0.25	2.20 ± 0.23	2.60 ± 0.35
30	1.25 ± 0.25	3.20 ± 0.34	4.00 ± 0.28
60	2.00 ± 0.21	4.20 ± 0.38	4.96 ± 0.30

* VN assay data (FAVN modification).

Table 2
Assessment of the immunogenicity of Ferarabivac anti-rabies live oral vaccine in foxes and raccoon dogs ($n = 25$, $p < 0.05$)

Таблица 2
Оценка иммуногенной активности антирабической живой оральной вакцины «Ферарабивак» на лисицах и енотовидных собаках ($n = 25$, $p < 0,05$)

Days post vaccination	Anti-rabies VNA titre, IU/cm^{3*}	
	foxes	raccoon dogs
14	0.70 ± 0.18	0.73 ± 0.19
30	3.20 ± 0.22	3.50 ± 0.22
50	4.30 ± 0.32	4.53 ± 0.27
60	4.20 ± 0.40	4.30 ± 0.30
70	4.00 ± 0.29	4.09 ± 0.32
80	3.81 ± 0.42	3.90 ± 0.23
90	3.70 ± 0.35	3.80 ± 0.41

* VN assay data (FAVN modification).

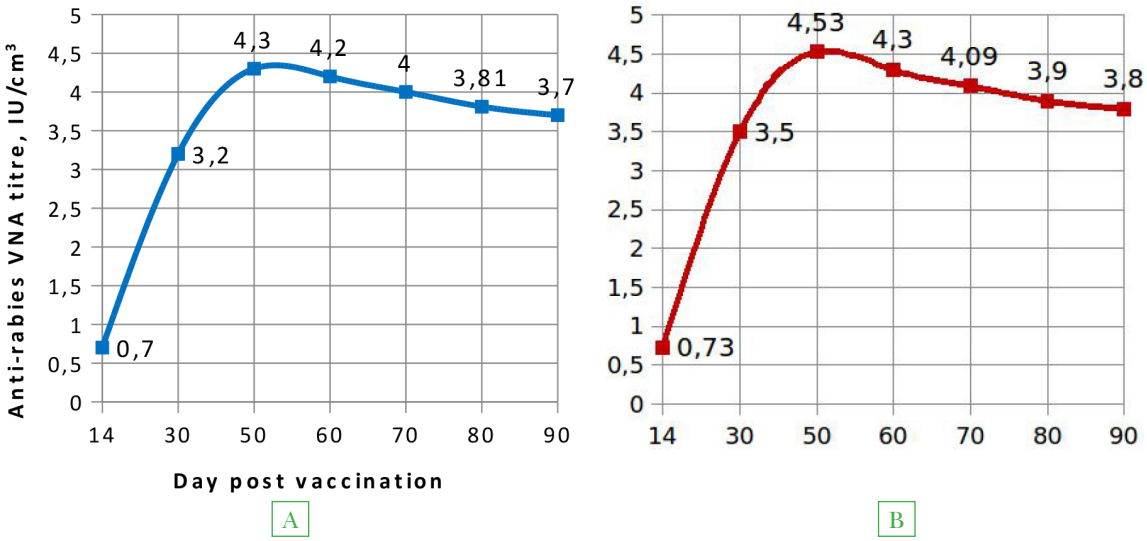

Fig. 1. Immunogenicity of Ferarabivac anti-rabies live oral vaccine in foxes (A) and raccoon dogs (B)

Рис. 1. Иммуногенная активность антирабической живой оральной вакцины «Ферарабивак» при исследовании на лисицах (А) и енотовидных собаках (Б)

VNA production in the animals. Thus in 14 days post oral vaccination the anti-rabies antibodies were detected in the sera collected from foxes and raccoon dogs at titres 0.70 ± 0.18 and 0.73 ± 0.19 IU/cm³ respectively, that ensured protection against the rabies virus infection (≥ 0,50 МЕ/см³), and in 30 dpv the titres amounted to 3.20 ± 0.22 and 3.50 ± 0.22 IU/cm³ respectively. On 50 dpv the anti-rabies VNA level in foxes reached its peak and amounted to 4.30 ± 0.32 IU/cm³. VNA titre subsequently decreased but on 90 dpv it was still high and amounted to 3.70 ± 0.35 IU/cm³ that ensured protection against rabies virus infection (≥ 0.50 IU/cm³). VNA titres in Ferarabivac vaccinated raccoon dogs reached their peak level in 50 dpv and amounted to 4.53 ± 0.27 IU/cm³, and hereafter they gradually decreased and on 90 dpv they amounted to 3.80 ± 0.41 IU/cm³.

Ferarabivac anti-rabies live oral vaccine potency in wild carnivores was tested in 35 foxes, which were subdivided in two groups: experimental group (No. 1) – 25 foxes, and control group (No. 2) – 10 foxes; and in 35 raccoon dogs, which were also subdivided into experimental group (No. 3) – 25 animals, and control group (No. 4) – 10 animals. The animals in groups No. 1 and 3 were immunized with the vaccine at a dose of 2.0 cm³. Groups No. 2 and 4 included control non-vaccinated animals. Blood samples were collected from the animals before the infection, and they were tested for anti-rabies VNA titre. In 30 days post the vaccine consumption the animals were challenged with control rabies virus strain CVS-27 at a dose of 25,000 LD_{50}/cm³. The animals' clinical condition was examined daily for 90 days. The results of the experiment are shown in Tables 3 and 4.

After challenge all animals immunized with Ferarabivac anti-rabies live oral vaccine demonstrated no rabies clinical signs. In a month post immunization the mean titres of anti-rabies virus antibodies in foxes and raccoon dogs amounted to 3.24 ± 0.08 and 3.26 ± 0.22 IU/cm³ respectively. The control animals died on day 12–20 post infection. Disease-specific death was confirmed using FAT. The remaining animals were observed for 90 more days after the death of the last control animal. Upon the observation period termination the animals were euthanized and their brains were tested using FAT. No rabies virus was detected in the tested brain samples.

At the final stage of the research, the immunity duration was tested in 25 foxes and 25 raccoon dogs, which were vaccinated with Ferarabivac at a dose of 2.0 cm³ with infectivity titre of the attenuated rabies virus strain RV-97 being at least 6.0 lg $KKID_{50}$/cm³. The animals were observed for a year and blood samples were collected at a regular basis for anti-rabies VNA testing. The test results are shown in Table 5 and Figure 2.

The results demonstrated in Table 5 and Figure 2 indicate that after foxes' immunization with Ferarabivac vaccine the VNA were detected in 0.5 month after single vaccination, and their titres amounted to 0.70 ± 0.31 IU/cm³. Anti-rabies antibody titres reached their peak by month 2 and amounted to 4.30 ± 0.32 IU/cm³. Hereafter, they gradually decreased and amounted to 3.00 ± 0.31 IU/cm³ in six months and to 0.62 ± 0.28 IU/cm³ in 12 months.

After the raccoon dogs' immunization with Ferarabivac vaccine the anti-rabies VNA were detected in 0.5 month after singular feeding with the vaccine and their titres amounted to 0.70 ± 0.29 IU/cm³. Anti-rabies antibody levels reached their peak by month 2 and amounted to 4.51 ± 0.27 IU/cm³. Hereafter, they gradually decreased and reached the value of 3.10 ± 0.43 IU/cm³ in six months and 0.71 ± 0.17 IU/cm³ in 12 months. Otherwise stated, Ferarabivac anti-rabies live oral vaccine provided 12-month protection of wild carnivores against rabies as the titre of the anti-rabies VNA amounted to ≥ 0.5 IU/cm³ that is compliant with the OIE requirements for anti-rabies vaccines [4]. The minimal VNA threshold level was determined in 12 months post immunization that indicates that the animals should be vaccinated against rabies in a year.

CONCLUSION

Immunogenicity and protectivity of the Ferarabivac anti-rabies live oral vaccine for wild carnivores were tested.

The optimal immunization dose of the vaccine was determined to be 2.0 cm³ with infectivity titre of attenuated rabies virus strain RV-97 being at least 6.00 lg $KKID_{50}$/cm³.

Table 3
Challenge of foxes with CVS 27 strain one month post vaccination with Ferarabivac ($n = 3, p < 0,05$)

Таблица 3
Результаты заражения контрольным штаммом вируса бешенства CVS-27 лисиц через месяц после иммунизации вакциной «Ферарабивак» ($n_{иссл.} = 3, p < 0,05$)

Group No.	Animal No.	Anti-rabies VNA titre determined using virus neutralization assay, IU/cm³	Challenge results	
			presence/ absence of rabies clinical signs	brain testing using FAT
1 (experimental)	1	3.20 ± 0.21	–	neg.
	2	3.32 ± 0.20	–	neg.
	3	3.18 ± 0.24	–	neg.
	4	3.25 ± 0.19	–	neg.
	5	3.31 ± 0.20	–	neg.
	6	3.24 ± 0.21	–	neg.
	7	3.42 ± 0.25	–	neg.
	8	3.14 ± 0.18	–	neg.
	9	3.24 ± 0.27	–	neg.
	10	3.20 ± 0.25	–	neg.
	11	3.28 ± 0.21	–	neg.
	12	3.15 ± 0.24	–	neg.
	13	3.25 ± 0.21	–	neg.
	14	3.38 ± 0.25	–	neg.
	15	3.39 ± 0.20	–	neg.
	16	3.18 ± 0.24	–	neg.
	17	3.05 ± 0.19	–	neg.
	18	3.38 ± 0.20	–	neg.
	19	3.08 ± 0.21	–	neg.
	20	3.42 ± 0.27	–	neg.
	21	3.40 ± 0.19	–	neg.
	22	3.24 ± 0.27	–	neg.
	23	3.20 ± 0.27	–	neg.
	24	3.36 ± 0.21	–	neg.
	25	3.19 ± 0.24	–	neg.
	M ± *m*	3.26 ± 0.22		
2 (control)	26	0.00	+	pos.
	27	0.00	+	pos.
	28	0.00	+	pos.
	29	0.00	+	pos.
	30	0.00	+	pos.
	31	0.00	+	pos.
	32	0.00	+	pos.
	33	0.00	+	pos.
	34	0.00	+	pos.
	35	0.00	+	pos.
	M ± *m*	0.00		

«+» – presence of rabies clinical signs and death of the animal;
«–» – absence of rabies clinical signs.

Table 4
Challenge of racoon dogs with CVS 27 strain one month post vaccination with Ferarabivac ($n = 3, p < 0.05$)

Таблица 4
Результаты заражения контрольным штаммом вируса бешенства CVS-27 енотовидных собак через месяц после иммунизации вакциной «Ферарабивак» ($n_{иссл.} = 3, p < 0,05$)

Group No.	Animal No.	Anti-rabies VNA titre determined using virus neutralization assay, IU/cm³	Challenge results	
			presence/absence of rabies clinical signs	brain testing using FAT
1 (experimental)	1	3.45 ± 0.24	–	neg.
	2	3.41 ± 0.21	–	neg.
	3	3.58 ± 0.19	–	neg.
	4	3.35 ± 0.19	–	neg.
	5	3.39 ± 0.22	–	neg.
	6	3.47 ± 0.21	–	neg.
	7	3.61 ± 0.20	–	neg.
	8	3.74 ± 0.26	–	neg.
	9	3.50 ± 0.27	–	neg.
	10	3.40 ± 0.25	–	neg.
	11	3.58 ± 0.21	–	neg.
	12	3.51 ± 0.24	–	neg.
	13	3.39 ± 0.21	–	neg.
	14	3.58 ± 0.25	–	neg.
	15	3.60 ± 0.20	–	neg.
	16	3.48 ± 0.24	–	neg.
	17	3.55 ± 0.28	–	neg.
	18	3.64 ± 0.20	–	neg.
	19	3.48 ± 0.22	–	neg.
	20	3.49 ± 0.27	–	neg.
	21	3.51 ± 0.19	–	neg.
	22	3.49 ± 0.27	–	neg.
	23	3.70 ± 0.27	–	neg.
	24	3.76 ± 0.21	–	neg.
	25	3.69 ± 0.26	–	neg.
	M ± *m*	3.78 ± 0.23		
2 (control)	26	0.00	+	pos.
	27	0.00	+	pos.
	28	0.00	+	pos.
	29	0.00	+	pos.
	30	0.00	+	pos.
	31	0.00	+	pos.
	32	0.00	+	pos.
	33	0.00	+	pos.
	34	0.00	+	pos.
	35	0.00	+	pos.
	M ± *m*	0.00		

«+» – presence of rabies clinical signs and death of the animal;
«–» – absence of rabies clinical signs.

Table 5
A 12-months' study of immunity duration in wild animals vaccinated with Ferarabivac ($n = 3, p < 0.05$)

Таблица 5
Исследование продолжительности иммунитета в течение 12 месяцев у диких животных, иммунизированных вакциной «Ферарабивак» ($n_{иссл.} = 3, p < 0,05$)

Period post vaccination, month	Anti-rabies VNA titre determined using VN assay, IU/cm³	
	foxes	raccoon dogs
0.5	0.70 ± 0.31	0.70 ± 0.29
1	3.20 ± 0.22	3.50 ± 0.22
2	4.30 ± 0.32	4.51 ± 0.27
3	3.71 ± 0.35	3.80 ± 0.41
4	3.55 ± 0.41	3.62 ± 0.25
5	3.46 ± 0.23	3.46 ± 0.35
6	3.00 ± 0.31	3.10 ± 0.43
8	2.30 ± 0.19	2.63 ± 0.23
9	1.89 ± 0.37	2.01 ± 0.19
10	1.21 ± 0.15	1.53 ± 0.25
12	0.62 ± 0.28	0.71 ± 0.17

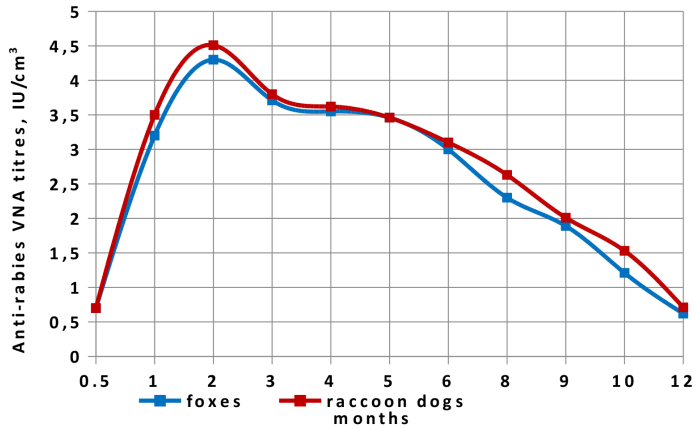

Fig. 2. Immunity duration in foxes and raccoon dogs vaccinated with Ferarabivac anti-rabies live oral vaccine

Рис. 2. Длительность иммунитета против бешенства у лисиц и енотовидных собак, вакцинированных антирабической живой оральной вакциной «Ферарабивак»

It was demonstrated that in 14 days post oral immunization with the vaccine the anti-rabies virus antibodies were detected in sera of foxes and raccoon dogs and their titres amounted to 0.70 ± 0.18 and 0.73 ± 0.19 IU/cm³ respectively, thus providing protection against rabies virus infection (≥ 0.50 IU/cm³). The anti-rabies VNA levels in foxes reached their peak in 50 days, and they amounted to 4.30 ± 0.32 IU/cm³. VNA titres subsequently decreased but, nevertheless, in 90 days they still remained high and amounted to 3.70 ± 0.35 IU/cm³. In the Frearabivac-vaccinated raccoon dogs the VNA titres reached their peak levels in 50 days and amounted to 4.53 ± 0.27 IU/cm³. Hereafter, they gradually decreased, and in 90 days post immunization they amounted to 3.80 ± 0.41 IU/cm³. Minimal VNA threshold level was reported in 12 months post immunization and it amounted to 0.62 ± 0.28 and 0.71 ± 0.17 IU/cm³ in foxes and raccoon dogs respectively, that is indicative of the need of the rabies vaccination of the animals to be repeated in a year.

It was found that in 30 days post challenge all animals immunized with Ferarabivac anti-rabies live oral vaccine demonstrated no rabies clinical signs during the whole observation period. In a month post immunization the mean titres of anti-rabies virus antibodies in foxes and raccoon dogs amounted to 3.24 ± 0.08 and 3.26 ± 0.22 IU/cm³ respectively. The control animals died on day 12–20 post infection. Disease-specific death was confirmed using FAT. The remaining animals were observed for 90 more days after the death of the last control animal. Upon the observation period termination the animals were euthanized and absence of the rabies virus was confirmed by testing their brain tissues using FAT.

REFERENCES

1. Chupin S. A., Doronin M. I., Chernyshova E. V., Shulpin M. I. Genetic characterization of rabies virus isolates detected in the territory of the Vladimir Oblast. *Veterinary Science Today*. 2015; 4 (15): 46–48. eLIBRARY ID: 25596872. (in Russian)

2. Gruzdev K. N., Metlin A. E. Animal Rabies. Vladimir: FGBI "ARRIAH"; 2019. 394 p. (in Russian)

3. Makarov V. V., Lozovoy D. A., Briko N. I. Cheiroptera rabies and man. *Epidemiology and infectious diseases. Current items.* 2015; 6: 46–53. eLIBRARY ID: 25134001. (in Russian)

4. Rabies (infection with rabies virus and other lyssaviruses). In *OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Paris; 2018; Chap. 3.1.17: 578–612. Available at: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.17_RABIES.pdf.

5. Fooks A. R., Banyard A. C., Horton D. L., Johnson N., McElhinney L. M., Jackson A. C. Current status of rabies and prospects for elimination. *Lancet*. 2014; 384 (9951): 1389–1399. DOI: 10.1016/S0140-6736(13)62707-5.

6. Rybakov S. S., Belik E. V., Metlin A. E., Borisov V. V., Nazarov N. A., Yegorov A. A., Chernyshova E. V., Mikhalishin V. V. Safety and immunogenicity of oral anti-rabies vaccines. *Veterinariya*. 2010; 8: 18–22. eLIBRARY ID: 15198430. (in Russian)

7. Freuling C. M., Eggerbauer E., Finke S., Kaiser C., Kaiser C., Kretzschmar A., Nolden T., Ortmann S., Schröder C., Teifke J. P., Schuster P., Vos A., Mettenleiter T. C., Müller T. Efficacy of the oral rabies virus vaccine strain SPBN GASGAS in foxes and raccoon dogs. *Vaccine*. 2019; 37 (33): 4750–4757. DOI: 10.1016/j.vaccine.2017.09.093.

8. Guidelines for determining the rabies virus neutralizing antibody level in the blood sera of wild carnivorous animals by VNT in BHK-21 cell culture (RFFIT) (MU 37-16) [Metodicheskie ukazaniya po opredeleniyu virusnejtralizuyushchih antitel k virusu beshenstva v syvorotkah krovi dikih plotoyadnyh zhivotnyh v reakcii nejtralizacii v kul'ture kletok BHK-21 (RFFIT) (MU 37-16)]. Vladimir: FGBI "ARRIAH"; 2016. 19 p. (in Russian)

9. Gusev A. A., Avilov V. M., Babak V. A., Puntus I. A., Smailova A. S. Experimental method for immunogenicity evaluation of live vaccines against rabies in wild animals. *Veterinaria Kubani*. 2019; 3: 7–9. DOI: 10.33861/2071-8020-2019-3-7-9. (in Russian)

10. Müller T. F., Schuster P., Vos A. C., Selhorst T., Wenzel U. D., Neubert A. M. Effect of maternal immunity on the immune response to oral vaccination against rabies in young foxes. *Am. J. Vet. Res*. 2001; 62 (7): 1154–1158. DOI: 10.2460/ajvr.2001.62.1154.

11. Metlin A. E., Rybakov S. S., Mikhalishin V. V., Muller T. Oral vaccination of wild carnivorous animals against rabies. *Veterinariya*. 2009; 8: 18–25. eLIBRARY ID: 12930952. (in Russian)

12. Glants S. Medico-biological statistics: translated from English. Dr. of Science (Physics and Mathematics) Yu. A. Danilova; ed. by N. E. Buzikashvili, D. V. Samoilov. M.: Practice; 1999. 459 p. (in Russian)

13. Chernyshova E. V., Sukharkov A. Yu., Metlin A. E., Belik E. V., Nazarov N. A., Chepurkin A. V., Rybakov S. S. Methodological guidelines for the selection and transfer of brain samples, blood sera and bone tissue to diagnose animal rabies and assess the effectiveness of oral rabies vaccines [Metodicheskie ukazaniya po otboru i peresylke prob golovnogo mozga, syvorotok krovi i kostnoj tkani s cel'yu diagnostiki beshenstva zhivotnyh i ocenki effektivnosti oral'nyh antirabicheskih vakcin]. Vladimir: FGBI "ARRIAH"; 2010. 23 p. (in Russian)

14. Guidelines for animal rabies diagnosis using immunofluorescence test [Metodicheskie ukazaniya dlya diagnostiki beshenstva zhivotnyh

v reakcii immunofluorescencii]: approved by the Rosselkhoznadzor 13.09.2017 (MU 33-17). Vladimir: FGBI "ARRIAH"; 2017. 14 p. (in Russian)

Received on 13.01.20
Approved for publication on 14.02.20

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Alexander V. Shishkov, Leading Veterinarian of the Laboratory for FMD Prevention FGBI "ARRIAH", Vladimir, Russia.

Dmitry A. Lozovoy, Doctor of Science (Veterinary Medicine), Associate Professor, Deputy Director for Research and Development of the FGBI "ARRIAH", Vladimir, Russia.

Alexey V. Borisov, Candidate of Science (Veterinary Medicine), Leading Researcher of the Laboratory for FMD Prevention of the FGBI "ARRIAH", Vladimir, Russia.

Dmitry V. Mikhalishin, Candidate of Science (Veterinary Medicine), Head of the Laboratory for FMD Prevention of the FGBI "ARRIAH", Vladimir, Russia.

Шишков Александр Валерьевич, ведущий ветеринарный врач лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Лозовой Дмитрий Анатольевич, доктор ветеринарных наук, доцент, заместитель директора по НИР и развитию ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Борисов Алексей Валерьевич, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Михалишин Дмитрий Валерьевич, кандидат ветеринарных наук, заведующий лабораторией профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.