ACTA VET. BRNO 2001, 70: 333–338

ORAL RABIES IMMUNIZATION OF SWINE: USE OF VNUKOVO-32/107 VACCINATION STRAIN

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Received November 12, 2000
Accepted August 28, 2001

Abstract

Ondrejka R., A. Ondrejková, Š. Švrček, Z. Beníšek, J. Süli, J. Závadová, M. Maďar, J. Mojžišová*: Oral Rabies Immunization of Swine: Use of Vnukovo-32/107 Vaccination Strain.* Acta Vet. Brno 2001, 70: 333–338.

The apathogenicity and antigenic activity of the live oral rabies vaccine prepared from the strain Vnukovo-32/107 were evaluated in two experiments carried out on non-target species – swine (domestic swine *Sus scrofa domestica* and wild boar *Sus scrofa*). The Vnukovo-32/107 vaccination strain has been used to produce oral rabies vaccine *Kamark* for immunization of free living carnivores. Wild boars compete with foxes for acceptance of baits distributed in the field. The tested vaccination strain for oral application (including very high doses) proved apathogenic to non-target species – swine. Detection and quantification of rabies antibodies were carried out by an immunoenzymatic assay – ELISA, rapid fluorescence focus inhibition test (RFFIT) and virus neutralization test (VNT) on mice on days 30 and 90 post-immunization. The values obtained were expressed in international units (IU/cm^3); the value of 0,5 IU/cm^3 was used as a positivity criterium. Antirabic antibodies were detected in more than 50 % of animals in all three groups of two animal sets on day 30 post-immunization. The results obtained show good antigenic activity of the live oral rabies vaccine prepared from the strain Vnukovo-32/107.

Rabies, vaccination strain Vnukovo-32/107, apathogenicity; antigenic activity

Comprehensive analysis of topical knowledge on rabies immunoprophylaxis indicates that oral vaccination, performed for the first time by Baer et al. in 1971, is the only prospective method of rabies prophylaxis and control in free living carnivores. Many researchers from different countries focused on selection of suitable rabies vaccination strains and determination of their safety, efficacy and stability (Black and Lawson 1970; Baer et al. 1971; Johnston and Voigt 1982; Steck et al. 1982; Wandeler et al. 1982; Švrček et al. 1994).

The vaccine intended for oral immunization of free-living animals should comply with a range of requirements (WHO 1989; Wandeler 1991). Apart from inducing immunity, it should fulfil the second most important requirement, i.e. be apathogenic to target and non-target animal species.

The harmlessness of various oral rabies vaccines was experimentally proved on many free-living animals, captured and held in captivity (Brochier et al. 1989; Guittre et al. 1992; Cliquet et al. 1995; Švrček et al. 1995 a, b). The first experiments were carried out with a SAD strain (Baer et al. 1971; 1975; Debbie et al. 1972). The SAD strain (Street Alabama Dufferin) was originally isolated from a dog in Alabama, the USA, in 1935 (Fenje 1960). Different variants, used for production of oral vaccines, were derived from that strain: SAD-Bern, SAD-B19, ERA, SAG, SAG2, Vnukovo-32. The safety and effectiveness of the strain Vnukovo-32 was confirmed by many experiments (Selimov 1978; 1987). High

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degree of attenuation and good immunogenic activity of the strain Vnukovo-32 on the level of 107th serial passage was proved by many years experience with the field use of live rabies vaccines for wild and domestic animals.

The results of experiments investigating apathogenicity of Vnukovo-32/107 vaccination strain and the antigenic activity of the oral rabies vaccine in immunization of swine are published in this study.

Materials and Methods

Apathogenicity of Vnukovo-32/107 vaccination strain

Experimental animals

Non-target animals-domestic pigs (*Sus scrofa domestica*) and wild boars (*Sus scrofa*), 3-month-old, were tested. Both species of animals came from the school farm in Zemplínska Teplica, and had not been previously vaccinated.

Vaccine

Live tissue rabies vaccine – infectious tissue culture medium of the vaccination strain Vnukovo-32/107, cultured in the BHK-21 cell line, was used for the immunization. This vaccine strain was selected for production of oral rabies vaccine by the producer MEVAK Nitra (Slovak Republic), commercial name *Kamark*. Before using the vaccine or the infectious cell culture medium for immunization experiments, the virus titre of the vaccination strain was determined in parallel by intracerebral (i.c.) inoculation test (Koprowski 1973) on mice weighing 6.0 g; the volume of inoculum was 0.03 cm^3 and its decimal dilutions were prepared. Five mice were immunized, each with different dilution, and observed for 21 d post-infection. The specificity of dying was checked by examination of brain imprints of transversal cuts on the level of hippocampus by direct immunofluorescence test (DIFT) (Dean and Abelseth 1973) for detection of rabies antigen. The results were processed by the cumulative method according to Reed and Muench (1938), the rabies tissue culture infectious test RTCIT (Wiktor 1973; Rudd and Trimarchi 1978, modified), on 8 Lab-Tek cell slides, in a BHK-21/138 cell culture, followed by detection of virus multiplication by DIFT.

Immunization of animals

Experiments in domestic swine

Testing of the apathogenicity of the Vnukovo-32/107 vaccination strain was carried out in 3 groups of domestic swine, 8 animals in each.

Group I: animals were immunized orally by a vaccination bait *Kamark*;

Group II: animals were immunized orally by a dose 5× higher than the presumed immunization dose (3 cm^3, 5× concentrated infectious tissue culture medium with 10% addition of stabilizer).

Group III: animals were immunized orally by a dose 15× higher than the presumed immunization dose (3 cm^3 15× concentrated infectious tissue culture medium with 10% addition of stabilizer).

Experiments in wild boars

Animals were divided into 3 groups, 5 animals in each.

Group I: animals were immunized orally by a vaccination bait *Kamark*;

Group II: animals were immunized orally by a dose 5 x higher than the presumed immunization dose (3 cm^3 5 x concentrated infectious tissue culture medium with 10% addition of stabilizer).

Group III: animals were immunized orally by a dose 15x higher than the presumed immunization dose (3 cm^3 15x concentrated infectious tissue culture medium with 10% addition of a stabilizer).

Verification of the apathogenicity

Animals were observed clinically for 90 d. In the observation period, saliva (swabs from oral cavity mucosa) samples were taken at 7-day intervals (days 7-63). Pool samples (2 for each group of domestic swine, 1 for each group of wild boars) were examined by i.c. inoculation test (MICIT) on suckling mice.

On day 180, the experimental animals were killed and partial necropsy was performed to obtain parts of the central nervous system (CNS) and submandibular salivary glands. The material obtained was used to prepare imprints for both DIFT and MICIT.

Antigenic activity

Antigenic activity was determined in animals from the experiments described above (3 groups of domestic swine, 3 groups of wild boars). Blood samples were taken on days 30 and 90 post-immunization and rabies antibodies were detected and quantified simultaneously by 3 following methods: ELISA test (kit developed in our laboratory; Süliová et al. 1988; Beníšek et al. 1989); RFFIT (Wiktor 1973; Bourhy and Sureau 1991) using CVS-11 as a challenge virus; VNT in 12 g mice (Atanasiu 1973) using CVS-11 as a challenge strain at a dose of 50 $MICLD_{50}$.

All sera were examined by ELISA and RFFIT tests. Pool samples (4 pool samples for each group of domestic swine, 2 pool samples for each group of wild boars) were prepared for VNT.

Student's *t*-test was used for statistical analysis. The results obtained from Group 1 immunized by vaccine *Kamark* served as a basis for comparison.

Results and Discussion

Apathogenicity of the vaccination strain Vnukovo-32/107 was verified out in two sets of experiments on non-target species, swine (*Sus scrofa domestica* and *Sus scrofa*). Pool samples were examined by MICIT on suckling mice. No rabies antigen was isolated either from brain imprints prepared from parts of CNS or from submandibular salivary glands of animals killed on day 180 post-immunization.

The results confirmed that the vaccination strain Vnukovo-32/107, when given orally, is apathogenic to the non-target species, swine (including extremely high doses).

Table 1
Detection and quantification of rabies antibodies in experiments in domestic swine.
Mean values are expressed in IU/cm^3.

Vaccine	D 30			D 90		
	ELISA	RFFIT	VNT	ELISA	RFFIT	VNT
Kamark	0.52 ± 0.26	0.37 ± 0.16	0.31 ± 0.22	0.28 ± 0.15	0.24 ± 0.14	0.11 ± 0.10
5-times concentr.	0.68 ± 0.11	0.54 ± 0.13^{x}	0.65 ± 0.25	0.39 ± 0.12	0.30 ± 0.07	0.23 ± 0.18
15-times concentr.	0.77 ± 0.17	0.55 ± 0.14^{x}	0.80 ± 0.29	0.61 ± 0.10^{xx}	0.45 ± 0.14^{x}	0.58 ± 0.32
n	8	8	4	8	8	4

n - the number of animals in groups
x - $p < 0.05$
xx - $p < 0.01$

Table 2
Detection and quantification of rabies antibodies in experiments in wild boars.
Mean values are expressed in IU/cm^3.

Vaccine		D 30			D 90	
	ELISA	RFFIT	VNT	ELISA	RFFIT	VNT
Kamark	0.56 ± 0.11	0.44 ± 0.09	0.36 ± 0.15	0.37 ± 0.16	0.27 ± 0.15	0.11 ± 0.11
5-times concentr.	0.60 ± 0.17	0.54 ± 0.13^{x}	0.51 ± 0.0	0.35 ± 0.05	0.34 ± 0.06	0.30 ± 0.09
15-times concentr.	0.71 ± 0.18	0.58 ± 0.14^{x}	0.80 ± 0.29	0.44 ± 0.06	0.37 ± 0.09	0.45 ± 0.07
n	8	8	4	8	8	4

n - the number of animals in groups
x - $p < 0.05$

The results of detection and quantification of rabies antibodies in experiments on swine are shown in Table 1 and 2. Blood sera from both species of animals were used to determine antigenic activity of the vaccines. Detection and quantification of antibodies was carried out on days 30 and 90 post-vaccination using ELISA, RFFIT and VNT tests on mice. The values obtained were expressed in international units (IU/cm^3), in comparison with international reference serum. The value ≥ 0.5 IU/cm^3 was used as a positivity criterium. The virus detection results obtained by different methods were similar and quantitative determination depended on sensitivity of the method. The highest values were obtained by ELISA in both experimental sets (Tables 3 and 4). Rabies antibodies were detected in more than 50 % of animals on day 30 post-immunization in all three groups of animals of both experimental

Table 3
Summarization of animals positive for rabies antibodies after vaccination. Comparison of ELISA and RFFIT tests. Titre of positivity ≥ 0.5 IU/cm^3. Experiments in domestic swine.

Vaccine	D 30				D 90			
	ELISA		RFFIT		ELISA		RFFIT	
	number of posit.	% of posit.	number of posit.	% of posit.	number of posit.	% of posit.	number of posit.	% of posit.
Kamark	5	62.5	2	25.0	1	12.5	1	12.5
5-times concentr.	7	87.5	5	62.5	2	25.0	0	0
15-times concentr.	8	100	6	75.0	5	62.5	4	50.0

Table 4
Summarization of animals positive for rabies antibodies after vaccination. Comparison of ELISA and RFFIT tests. Titre of positivity ≥ 0.5 IU/cm^3. Experiments in wild boars.

Vaccine	D 30				D 90			
	ELISA		RFFIT		ELISA		RFFIT	
	number of posit.	% of posit.	number of posit.	% of posit.	number of posit.	% of posit.	number of posit.	% of posit.
Kamark	3	60	2	40	1	20	0	0
5-times concentr.	4	80	3	60	1	20	0	0
15-times concentr.	4	80	3	60	0	0	1	20

sets. Only the values obtained by ELISA and RFFIT were expressed as percentage proportions as VNT was carried out on pool samples. The levels of rabies antibodies determined on day 90 were lower, except in the group of domestic swine, which were immunized orally by a dose15-times higher ($p < 0.01$). Detection and quantification of rabies antibodies in some animals that accepted vaccination bait *Kamark* provided negative results, because the animals probably swallowed the bait without puncturing the vaccine blister or accepted only a portion of the bait.

The efficacy of live vaccine depends in general on both immunogenic and antigenic activity of the selected vaccination strain and the vaccination dose. The latter (at the same inoculum volume) depends on the concentration (titre of the live vaccination virus) in the cellular infectious medium (Wandeler 1991). It is very important to define the residual virulence for target and non-target animals (Švrček et al. 1994, 1995a). Bioveta Ivanovice na Hané (the Czech Republic) has been using the vaccination strain Vnukovo-32/107 for production of live tissue vaccine for all species of domestic animals (Vrzal et al. 1988) and for production of inactivated vaccines for over 20 years.

For the development of oral rabies vaccine the strain Vnukovo-32 on the level of 107th passage was used. This was preceded by extensive many years of experimental work. The experiments were carried out as model experiments on various species of laboratory animals, especially on white mice, but also on target animals – common foxes and related species – farm polar foxes (Švrček et al. 1995b).

The first part of our study dealt with the apathogenicity of the vaccination strain Vnukovo-32/107 for non-target animals – swine, including wild boar, which compete with other animals for vaccination baits distributed in the field (Brochier et al. 1988) and can play an important role in their consumption. No less important is the conclusion that prevention and control of diseases in wild boars can be carried out by distribution of such baits (Aubert et al. 1994).

The results of experiments performed in both species of animals demonstrate that the vaccination strain Vnukovo-32 is apathogenic at extremely high doses to non-target species – swine. Its high degree of attenuation and low residual virulence proves its suitability for the production of rabies vaccine.

The efficacy of rabies vaccines is evaluated by means of target and laboratory animals and detection of seroconversion. The relationship between the level of virus neutralizing antibodies and protection against rabies was described by Bunn (1987). The presence of specific rabies antibodies in the serum of vaccinated animals indicates the performed vaccination. However, one should realize, that also those animals that failed to produce sufficiently high titre of antibodies may be protected against rabies infection. Although the efficacy of correctly performed rabies vaccination is high, induction of immunity in all immunized animals should not be taken for granted (Bourhy et al. 1988). Some animals exhibit genetically inherited inadequate immune reaction (Blancou et al. 1989).

Použitie vakcinačného kmeňa Vnukovo-32/107 pre orálnu antirabickú imunizáciu suidov

Apatogenita a antigénna aktivita živej orálnej antirabickej vakcíny z kmeňa Vnukovo-32/107 bola hodnotená v dvoch pokusoch vykonaných na necieľovom druhu zvierat – suidoch (sviňa domáca *Sus scrofa domestica* a sviňa divá *Sus scrofa*). Vakcinačný kmeň Vnukovo-32/107 sa využíva na prípravu živej orálnej antirabickej vakcíny *Kamark* pre imunizáciu voľne žijúcich karnivorov. Diviačia zver je tiež jedným z konkurentov líšok pri príjme antirabických vakcinačných návnad rozložených vo voľnej prírode. Testovaný vakcinačný kmeň pri orálnej aplikácii (vrátane veľmi vysokých dávok) je pre necieľové druhy – suidy apatogénny. Detekcia a kvantifikácia antirabických protilátok bola vykonaná na 30. a 90. deň po vakcinácii imunoenzymatickým testom ELISA, rýchlym fluorescenčným fokus inhibičným testom (RFFIT) a vírusneutralizačným testom (VNT) na myšiach. Získané hodnoty boli vyjadrené v medzinárodných jednotkách (IU/cm^3); kritéria pozitivity hodnota 0,5 IU/cm^3. Antirabické protilátky boli detekované u viac ako 50 % zvierat na 30. deň po vykonaní imunizácie u všetkých troch skupín zvierat oboch súborov. Získané výsledky svedčia o dobrej antigénnej aktivite živej orálnej antirabickej vakcíny z kmeňa Vnukovo-32/107.

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