

## A NEW RABIES VACCINE WITH AN EXPERIMENTAL ADJUVANT FOR DOMESTIC ANIMALS

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*The authors had developed a new experimental adjuvant based on squalene and its efficacy was tested on target domestic animals – cats and dogs by means of different rabies vaccines. In this experiment the authors compared the immune response on the rabies vaccine without adjuvant and with aluminium adjuvant or experimental squalene adjuvant, respectively. The level of rabies antibodies was determined by rapid fluorescence focus inhibition test on days 14, 30, 90 and 450 (dogs), and on days 14, and 30 (cats), respectively. The rabies antibodies level on day 14 still did not reach the protection level determined by WHO. On day 30 each of the vaccines induced an adequate response, while the most effective seemed to be the vaccine with experimental squalene adjuvant. On day 450 the antibodies level decreased, but the average level was over the protection value, when the experimental squalene-adjuvanted vaccine was used. The level of rabies antibodies did not achieve the protection value in 2 from 10 dogs in this group. There is an open question, if it is sufficient to use only one dose of rabies vaccine for safe primovaccination of young animals.*

*Key words: domestic cat, domestic dog, experimental squalene adjuvant (ESA), rabies vaccination*

### INTRODUCTION

The most important request for human but also veterinary vaccines is their effectiveness to induce an intensive immune response which is able to protect the organism against specific infectious disease. However, very important is also the safety of the vaccines. Inactivated vaccines are preferred at present, though these ones compared with live vaccines are not enough immunogenic. The efficacy of inactivated vaccines may be potentiated by adjuvants. Unfortunately the use of adjuvants is often connected with local and systemic secondary effects. In veterinary medicine mainly aluminium compounds (aluminium hydroxide and aluminium phosphates) are used as adjuvants previously, although their adjuvant activity does not achieve the level of oil adjuvants. In addition the subcutaneous application of aluminium adjuvants can result in some recipients, particularly in cats, in the development of fibrosarcoma (Hendrick and Brooks, 1994; Holst *et al.*,

2001; Carroll *et al.*, 2002). An adjuvant of the oil-in-water type (O/W) (Sülioiva *et al.*, 1999), based on fully metabolisable (Bomford, 1981) isopropyl palmitate (IPP) – isopropylester of palmitic acid was developed in our laboratory. This adjuvant has excellent properties (Beníšek *et al.*, 1999); however, its disadvantage is that it can be used only for resuscitation of lyophilised vaccines and not for potentiation of liquid inactivated vaccines.

The only permitted adjuvant except aluminium compounds in human medicine is the MF59 formulation, an adjuvant of O/W type based on squalene as lipid component and detergents Tween 80 and Span 85. Just squalene and squalane originated by saturation of squalene are the objects of interest of many laboratories which are engaged in the research of new adjuvant formulations. Both lipid compounds occur naturally in higher amounts in the shark liver.

A new experimental adjuvant based on squalene (experimental squalene adjuvant – ESA) was developed in our laboratory; its harmlessness and local reactogenicity was tested on laboratory animals (Beníšek *et al.*, 2004). The ability of ESA to potentiate rabies vaccine was tested also on laboratory animals (Süli *et al.*, 2004). In this work are presented the first results of the verification of the ESA-adjuvanted rabies vaccine effectiveness on target animal species – domestic dogs and cats.

#### MATERIAL AND METHODS

##### *Animals:*

– Young domestic dogs of different breed (age: 3-6 months) and of both sexes, non-vaccinated against rabies before (10 dogs in group total 30 animals) were used in our experiment. The rabies antibodies titres had been attested before the dogs were chosen into the experiment; only dogs without rabies antibodies were selected.

– Domestic cats of different breed (age: 3-24 months) and of both sexes, non-vaccinated against rabies before (7 cats in group, together 21 animals) were used in the experiment. The rabies antibodies titres had been attested before the cats were chosen into the experiment. The cats had been examined also on the presence of antibodies against feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) antigen. All cats were negative, so they were suitable for this experiment.

Research was conducted according to the principles presented in the "Guide for Care and Use of Laboratory Animals", published by the Government of Slovak Republic, No. 289/2003.

*Vaccination:* 1 cm<sup>3</sup> dose of vaccine into the left thigh.

##### *Vaccines used in the experiment:*

*Inactivated rabies vaccine (V)* was obtained from MEVAK Comp. Nitra (Slovak Republic). The rabies virus had been cultivated in cell line BHK-21/13S and was inactivated with  $\beta$ -propiolactone. It was revoked for experimental purposes before production of the commercial vaccine, i.e. before the addition of

aluminium hydroxide which performs as adjuvant in the commercial veterinary rabies vaccine. The virus titer before inactivation was  $10^{6.5}$  MICLD<sub>50</sub>/0.03 mL.

The volume of adjuvant in the vaccine was replaced with equal portion of phosphate buffer of pH = 7.4.

*Inactivated rabies vaccine with aluminium hydroxide adjuvant (V-AI)* was obtained from MEVAK Comp. Nitra (Slovak Republic). It is the commercial *Rabicell* vaccine in which the inactivated rabies antigen is associated with aluminium hydroxide gel (adjuvant). The vaccine was prepared from the same operating batch as the inactivated vaccine (V). The suspension was revoked before the vial filling.

*Inactivated rabies vaccine with experimental squalene adjuvant (V-ESA)* was prepared according to Süli *et al.* (2004) by one step at low temperature. The basis of the adjuvanted vaccine emulsion was the above mentioned inactivated virus suspension from the MEVAK Comp. Nitra (Slovak Republic) from the same operating batch as both previous V and V-AI vaccines. The experimental lipid adjuvant is an oil-in-water type emulsion based on squalene as oil component. The definitive concentration of squalene in the vaccine was 5%. Detergents Poloxamer 105 (definitive concentration 4%) and Abil-care (definitive concentration 2%) were used. The Abil-care detergent allows to prepare the adjuvanted vaccine at lower temperature (37°C). It is important because of the assurance of the maintenance of the rabies antigen integrity in vaccine. The water component was the very inactivated rabies virus suspension (inactivated rabies vaccine – V) in a definitive concentration of 89%. The procedure for preparation is described in detail in the article by Süli *et al.* (2004).

*Blood sampling:*

- Blood samples from *dogs* were obtained before vaccination (day 0), further on days 14, 30, 90 and 450 after vaccination.
- Blood samples from *cats* were obtained before vaccination (day 0), further on days 14 and 30 after vaccination.

*Serological examination of FIV antibodies and FeLV antigen:*

The specific antibodies against FIV and the FeLV antigen were determined by commercial rapid diagnostic test *SNAP COMBO TEST FeLV Ag / FIV Ab* (IDEXX, Canada).

*Determination of rabies antibodies levels:*

Levels of rabies antibodies were determined by *rapid fluorescence focus inhibition test* (RFFIT) according to Smith *et al.* (1973).

*Statistical evaluation* of rabies antibodies titres results was carried out by means of the Student's *t*-test.

## RESULTS AND DISCUSSION

The results were evaluated according to the WHO recommendation (1992), that the vaccination is succesful when the level of rabies antibodies after vaccination is minimum 0.5 international units in 1 cm<sup>3</sup> (IU/cm<sup>3</sup>) of sera.

The examination of rabies antibodies in domestic dogs after vaccination with different vaccines (Table 1) showed that the antibodies production begins on day 14, but its level is still insufficient to protect the animals. The highest level of antibodies was detected on day 30 and on day 90 was only slightly reduced. The protection of dogs after vaccination with the vaccine with no adjuvant was already deficient on day 450. A boundary value of antibody titres after vaccination with commercial aluminium-hydroxide adjuvanted rabies vaccine was detected. The ESA-adjuvanted vaccine ensured the protection of dogs even 450 days after vaccination. Individual intra-group differences were observed which caused a relatively high standard deviation from the average value in the group. These differences might be caused by various factors influencing the vaccination results. According to Mansfield *et al.* (2004) the immune response of dogs against rabies vaccination is influenced e.g. by vaccine selection, by vaccination periods (when more doses are applied or at annual vaccination), by sampling for examination, by the age of dogs, even by the country of the dog's origin. The influence of vaccine choice was eliminated by the fact that all vaccines used in the experiment had been prepared from the same operating batch of the virus suspension, so the difference in the vaccine efficacy might be caused by the presence or by the quality of adjuvant. The vaccination and blood sampling were performed in all experimental groups at the same time. Young dogs of both sexes were chosen into the experiment and they were distributed equally into groups. Several dogs in the group, vaccinated by virus suspension without adjuvant, had achieved the requested protection level not even at day 30 and in the next period the titer of rabies antibodies decreased little by little. There was one such recipient in the group vaccinated by aluminium hydroxide adjuvanted vaccine. The most effective seems to be the vaccine with the experimental adjuvant. The average titres were significantly higher in all examined terms, on day 30 and 90 cca 3.5 times in comparison with the non-adjuvanted vaccine. All animals in the group had been sufficiently immunized and the high titres of rabies antibodies survived up to the 450<sup>th</sup> day. However, there were also 2 from 10 animals with rabies antibodies below the protective level. It is necessary to realise that in this experiment a primovaccination of dogs was performed. It remains an open question if one dose of experimental adjuvanted vaccine is enough to provide the protective level of rabies antibodies the throughout whole year. This question is controvert, according to some researchers a next vaccine dose after primovaccination is needed in order to assure the protection of animals (Sage *et al.*, 1993; Almeida *et al.*, 1997). The usual vaccination scheme – one dose of rabies vaccine per year – offers a better protection to older dogs than young ones (Hogen Esch *et al.*, 2004). However, very old animals can have an already weakened immune system and increased risk of insufficient protection (Mansfield *et al.*, 2004).

The experimental groups of cats were formed using similar principles as in dogs. The cats were distributed equally into the groups according to age and sex. Since it is not compulsory to vaccinate the cats against rabies, older animals could be integrated into the experiment, as well. The cats' immune system may be influenced by immunodeficient diseases i.e. by the presence of feline immunodeficiency virus (FIV) or by feline leukaemia virus (FeLV). As into the

experiment were chosen village cats which are partially free living, all experimental cats had been tested for these diseases before start of the experiment. None of cats were infected.

Table 1. Average titres of rabies antibodies in dogs after vaccination with different rabies vaccines, determined by rapid fluorescence focus inhibition test (RFFIT). Number of animals in groups: n = 10

Vaccine	Titer of rabies antibodies (IU/cm <sup>3</sup> )				
	day 0	day 14	day 30	day 90	day 450
V	0.05 ± 0.03	0.16 ± 0.05	1.27 ± 1.14	1.22 ± 1.14	0.38 ± 0.20
V-AI	0.04 ± 0.03	0.33 ± 0.22	3.29 ± 2.27	2.88 ± 1.96	0.45 ± 0.18
V-ESA	0.04 ± 0.03	0.35 ± 0.18 <sup>x</sup>	4.63 ± 3.01 <sup>x</sup>	4.17 ± 2.21 <sup>xx</sup>	1.02 ± 0.40 <sup>xx</sup>

Legend:

V – inactivated rabies vaccine without adjuvant

V-AI – inactivated rabies vaccine with aluminium hydroxide adjuvant

V-ESA – inactivated rabies vaccine with experimental squalene adjuvant (ESA)

x – p ≤ 0.05; xx – p ≤ 0.01

The basis for comparing were the values from group "V"

The comparing between "V-AI" and "V-ESA" groups did not show any significant differences

The use of non-aluminium adjuvant for cats is especially indicated, because in cats the development of vaccination sarcomas after aluminium-adjuvanted vaccines was observed (Hendrick and Brooks, 1994; Holst *et al.*, 2001; Carroll *et al.*, 2002).

The cats from our experiment were watched long-time after the experiment. The owners did not observe any changes on them. The results in Table 2 show that the adjuvanted vaccines are more effective also for cats as for dogs. A significant increase of vaccine efficacy (p ≤ 0.01) was caused only by the ESA, but the level of rabies antibodies necessary for the protection of animals against rabies (over 0.5 IU/cm<sup>3</sup>) was achieved also by other vaccines used in the experiment. Since the average titer value of rabies antibodies after application of ESA-adjuvanted vaccine was higher compared to the commercial aluminium-adjuvanted vaccine, the difference was not significant.

Table 2. Average titres of rabies antibodies in cats after vaccination with different rabies vaccines, determined by rapid fluorescence focus inhibition test (RFFIT). Number of animals in groups: n = 7

Vaccine	Titer of rabies antibodies (IU/cm <sup>3</sup> )		
	day 0	day 14	day 30
V	0.04 ± 0.02	0.19 ± 0.05	1.02 ± 0.32
V-AI	0.03 ± 0.02	0.34 ± 0.16 <sup>x</sup>	2.24 ± 1.82
V-ESA	0.04 ± 0.03	0.44 ± 0.16 <sup>xx</sup>	3.93 ± 1.50 <sup>xx</sup>

Legend: same as for Table 1.

Sex, eventually sterilisation of an animal can play an important role in cats. The fertile cats have greater risk of insufficient protection, particularly the tomcats (Mansfield *et al.*, 2004) because testosterone may suppress the immune system (Rife *et al.*, 1990).

Blood sampling in cats is a procedure often hindered because of the nature of these animals (vagancy, aggression). The owners did not agree with repeated sampling after longer intervals. This is the reason why further examination of rabies antibodies after vaccination was impossible. Literature data are also incomplete because the cats vaccinated against rabies are mostly bred closely, i.e. they are non-stray animals and they are watched. The experiments are hindered also by juridical regulations, even though the result of our experiment was in fact rabies immunization of animals.

The experimental rabies vaccine with squalene adjuvant was developed for domestic animals (first of all for dogs and cats). Testing on laboratory animals showed sufficient efficacy of this vaccine, better than the commercial ones (Süli *et al.*, 2004). It is needed to continue in verification of the properties of the experimental adjuvanted rabies vaccine on a greater number of domestic animals, but the first results presented in this work suggest suitable properties of the vaccine.

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#### NOVA VAKCINA PROTIV BESNILA DOBIJENA KORIŠĆENJEM EKSPERIMENTALNOG ADJUVANSA

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#### SADRŽAJ

U ovom ogledu autori su ispitivali imunski odgovor pasa i mačaka posle vakcinacije protiv besnila vakcinama bez adjuvansa, sa aluminijumom kao adjuvansom i sa novim skvalenskim adjuvansom. Koncentracija antitela protiv virusa besnila je određivana brzim testom inhibicije fluorescentnog fokusa i to 14-og, 30-og, 90-og i 450-og dana kod pasa, a 14-og i 30-og dana kod mačaka. Koncentracija antitela 14-og dana nakon vakcinacije nije dostizala zaštitni nivo propisan od strane WHO. Tridesetog dana posle vakcinacije, sve vakcine su dale zadovoljavajući imunski odgovor, a najefikasnija je bila vakcina sa eksperimentalnim skvalenskim adjuvansom. Posle 450 dana, koncentracija antitela je bila smanjena, ali je srednja vrednost njihove koncentracije bila iznad nivoa protekcije kada je korišćena eksperimentalna vakcina. Samo kod dva psa (od 10), antitela nisu dostizala zahtevani nivo protekcije. Ostaje otvoreno pitanje da li je protiv besnila dovoljno vršiti samo jednokratnu vakcinaciju mladih životinja.

