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VACCINES AND VACCINATION PROGRAMS USED TO ERADICATION AND CONTROL OF RABIES IN WILDLIFE

Vlad VUTA[1*, 2], Florica BARBUCEANU[1, 2], Gabriel PREDOI[2], Constantin VLAGIOIU[2]

[1] Institute for Diagnosis and Animal Health, Bucharest, Romania
[2] University of Agronomic Sciences and Veterinary Medicine-Faculty of Veterinary Medicine, Bucharest, Romania

*Corresponding author email: vladvuta@yahoo.com

Abstract

Rabies is a fatal viral zoonosis of the central nervous system of mammals. Until recently, rabies was predominantly in domestic dogs, although outbreaks were reports in wildlife. The implementation of dog mass vaccination resulted in the disappearance of dog-mediated rabies in Europe and North America, but the disease unexpectedly re-emerged in wildlife. Oral rabies vaccination (ORV) programs in wildlife are highly effective in control and eradication the disease. After years of successful vaccination campaigns, many previously infected countries in Western, Central and Northern European have become free of rabies. All rabies vaccines used for wildlife immunization are derivatives of the original SAD strain. Currently, five vaccine strains are authorized in Europe and all are derivatives of the original SAD strain: Sad Bern, SAD B19, SAG2, GASGAS and V-RG vaccine (Vaccinia Recombinant Glycoprotein). In our paper, we described and analyzed the main characteristics of available vaccines from Europe market for oral vaccination of wild animals, taking into account the performance and the quality features. In Western Europe, rabies has been eliminated using oral vaccination with all available vaccines. Nevertheless, worries still exist related to the residual pathogenicity of attenuated live vaccines that could induce rabies in certain conditions.

Keywords: *rabies, vaccines, foxes, wildlife.*

INTRODUCTION

Rabies is a Central Nervous System zoonotic disease, with the causative agent Rabies virus, the negative-sense single stranded RNA viruses of the Lyssavirus genus within the family *Rhabdoviridae*, distributed worldwide and found in terrestrial mammals causing between 37,000 and 87,000 human deaths annually (Virus Taxonomy 9th report, 2012; WHO Expert Consultation on Rabies, second report, 2013). In Europe the major reservoir of rabies are wild animals, especially red fox (*Vulpes vulpes*) (Cliquet, 2015). Extensive oral vaccination programs (ORV) with baits for red foxes have reduced the incidence of rabies in many Western European countries (Slate et al., 2009; Zienius et al., 2011).

All rabies vaccines used in oral rabies vaccination (ORV) programs of wild animals are based on live vaccine viruses. In the EU, all rabies vaccines need to fulfill the requirements of European Pharmacopoeia monograph, Rabies vaccine (live, oral) for foxes and raccoon dogs: efficacy, safety and stability and have to be licensed or registered. Therefore, the objective of this paper was to describe and analyze the main characteristics of available vaccines from Europe market for oral vaccination of wild animals.

MATERIALS AND METHODS

Oral Rabies Vaccination Programs

The first fox rabies vaccination field trial using vaccine baits was applied in Switzerland in 1978 (Steck et al., 1982).

The strategy of ORV initially used to red fox proved to be effective in raccoon dog populations as well in several countries (Finland, Baltic countries and Poland) where this animal has a significant role in rabies epidemiology. The first field trial for elimination of rabies in wildlife in areas with a significant raccoon dog population was carried out in Finland in 1988 (EFSA, 2015).

Same time, administrative and political borders may constitute barriers to the movement of foxes, but in most cases vaccination zones need to be clearly defined and vaccination cam-

paigns synchronized across these administrative lines.
Examples of cross-border re-infections are a lot (Schaarschmidt et al., 2002). These could be prevented by synchronizing control measures on both sides of political or administrative borders and if this is not possible, by the maintenance of an immune belt or buffer zone at the border.
Vaccination programs are required to be conducted and monitored by a scientific team dedicated and deeply involved to this task. The team needs to be trained in field surveys and use validated and accreditated laboratory methods for rabies diagnosis, titration of vaccines, evaluation of bait uptake by the target species, and rabies antibody level. The entire procedure, including bait distribution in the field, needs to be very carefully processed, followed and very well documented. Based on experience in previous oral rabies vaccination campaigns, it is considered crucial that vaccination campaigns must to continue for a period of at least two years after the last reported case of fox-related rabies. The classical pattern of two vaccination campaigns per year, carried out in spring and autumn, has been shown to be successful whatever the fox population density. This two times distribution frequency has been used in all European programs of oral vaccination that resulted in the elimination of rabies (Zanoni et al., 2000; Breitenmoser et al., 2000; Bruyère and Janot, 2000; Brochier et al., 2001; Besch, 2001). Spring distribution is preferably carried out in May or June in order to increase the efficient access of fox cubs to baits. However, early spring campaign carried out in March-April was also shown to be beneficial in Belgium, Luxembourg, and several German Bundesländer (Brochier et al., 1996; Brochier et al., 2001). Where snow is abundant, its melting may degrade the vaccine baits, and in this case is preferably performed before the snow starts to melt. Autumn distribution is organized in September or October (EC, 2002).
There are used 25 baits/ campaign/km2 with a distance between flight lines of 500 meters and 150 meters altitude, by avoiding the territories of localities, water surfaces, highways (Vuta et al., 2016a; Vuta et al., 2016c).
In general, vaccination is not advised to be carried out at temperatures below 0°C, because: frozen vaccine baits do not induce a sufficient immune response and the virus titre may decrease due to freezing-thawing cycles, except for VRG which has been found to remain stable in such conditions (Pastoret et al., 1996). Vaccination using attenuated rabies virus vaccines is not recommended during hot weather. At temperatures above 30°C, melting of the bait occurs and vaccine titer decreases.

Oral animal vaccines against Rabies
In the EU, rabies vaccines used for oral vaccination need to be registered and to comply with the requirements of the European Pharmacopoeia monograph (European Pharmacopoeia, 2005) and with national regulations for veterinary biological products, with particular aspect of efficacy, safety and potency of the vaccine virus and to genetic strain stability (EFSA 2015).
Vaccine baits should contain a biomarker (usually tetracycline) to monitor the bait up-take. Vaccines for ORV currently available and authorized in the EU market are made of the following strains: SAD B19 (live attenuated); SAD Bern (live attenuated); SAG 2 (live attenuated); V-RG (live recombinant); GASGAS (live recombinant).

RESULTS AND DISCUSSIONS

At a 45 days'time following each vaccination campaign, there shall be performed the hunting of foxes in order to assess the efficiency of vaccination, for this purpose, there shall be shot 4 foxes/year/100 km2. For the monitoring of vaccination campaign, there shall be taken samples of thoracic liquids in order to determine post-vaccinal antirabies antibodies and samples of mandible in order to determine vaccinal marker (Tetracycline) (Vuta et al., 2017) (figure 1).
All vaccine strains currently used for oral vaccination programs are modified live-virus vaccines and a live recombinant vaccine.

- SAD family strains vaccines

Modified live-virus vaccines are all derived of the original SAD (Street Alabama Dufferin) attenuated virus (which was isolated from a rabid dog in the Alabama (USA) in 1935), after that it was passaged in mouse brain cells (ERA strain); then it was adapted to BHK cell line by

various passages (SAD Berne). Lysvulpen vaccine (Bioveta, Czech Republic) is an attenuated SAD Berne vaccine. SADB19 and SAD P5/88 vaccines (Impfsoffwerk Dessau-Tornau are produced by several passages on cloned BHK cell line of the SAD Berne strain (Mahl et al., 2014). Those successive and continues selections from the original strain may produce hazardous and uncontrolled results, and variants may remain pathogenic for target and non-target species.

- Vaccine strains selected by monoclonal antibodies

SAG1 and SAG2 vaccines (Street Alabama Gif) (Virbac, France) are selected from the SAD Berne strain after one and two successive mutations of the arginine 333 codon, using specific antirabies glycoprotein monoclonal antibodies, in a site of the genome whose integrity is required for pathogenicity by the oral route (Lafay et al., 1994). SAG2 vaccine is the only rabies oral vaccine registered at the European Medicine Agency, for the moment.

- Live recombinant vaccine

VRG vaccine (vaccinia recombinant glycoprotein) is a vaccinia virus (Copenhagen strain) recombinant coding for the rabies glycoprotein gene from the ERA strain. The already attenuated Copenhagen strain was even more attenuated thanks to the replacement of the thymidine kinase gene by the cDNA of the rabies glycoprotein increasing rabies immunity (Ruprecht et al., 1992).

The vaccine strain SPBN GASGAS (Rabitec, IDT, Germany) is a recombinant rabies virus

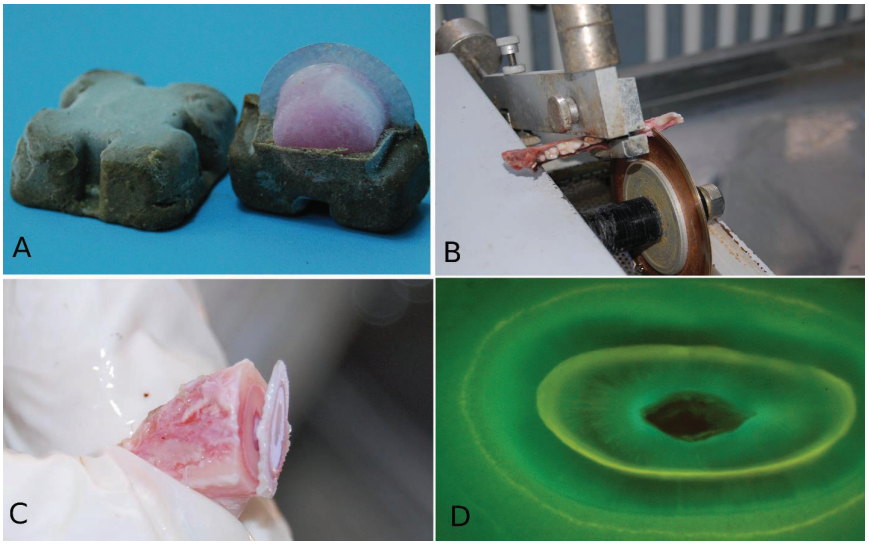

Figure 1. A-vaccine baits. B-special saw used to cut bones/teeth. C-thin bone/teeth section. D-fluorescent tetracycline biomarker deposit, detected by UV light microscopy

which has been derived by site directed mutagenesis from the most widely used oral rabies vaccine for wildlife, the passage attenuated vaccine strain SAD B19. The recombinant virus has been further attenuated compared to the SAD B19 strain by three targeted genetic modifications (EMEA, 2017).

The history of rabies vaccine-strains used in ORV programs is presented in figure 2.

Quality criteria of vaccine baits include stability testing of both vaccine (vaccine titer, genetic and thermo stability) and bait casing (appearance, melting point and temperature stability). As ORV campaigns are also to be conducted nearby human settlements, a number of minimum criteria and precaution measures have to be considered to minimize the risk of humans to come in contact with vaccine baits including mechanical stability of the vaccine bait, warning labels on blisters. Cold chain as specified by the manufacturer (usually -20°C or less) has to be maintained while vaccine baits are stored, during transportation and delivery directly to the customer. Maintenance of the cold chain

has to be documented using calibrated temperature loggers or equivalent equipment and written evidence has to be provided. Storage under any other conditions prior to distribution in the field may have high negative impact on the vaccine titer. The vaccine viral titer of all batches should therefore be verified right before the campaigns by the competent authority and approved laboratory. Such tests should be performed in qualified laboratories, preferably accredited to EN ISO/IEC 17025:2005 with documented, validated methods and standard operational procedures (EFSA, 2015).

The main characteristics of vaccines used in the EU have been summarized in Table 1 and Table 2.

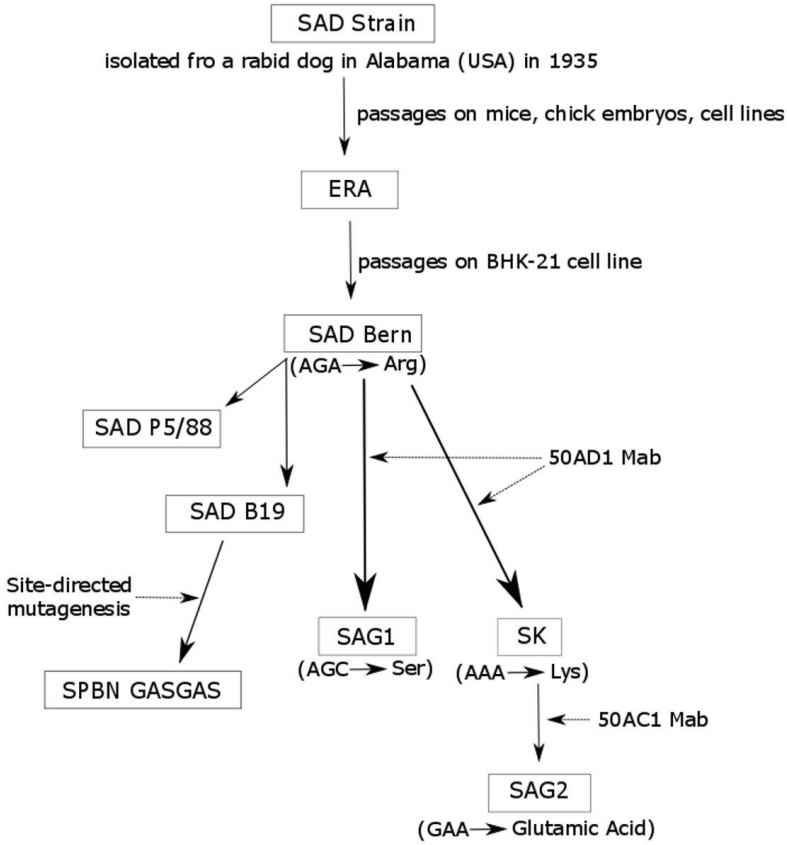

Figure 2. The history of rabies vaccine-strains used in ORV programs. ERA = Evelyn Rokitnicki Abelseth, Mab = monoclonal antibody. The rabies virus SAD strain was isolated from the salivary glands of a rabid dog in the USA during 1935, which was passaged in mice, chick embryos, and various cell lines and was re-named ERA (Evelyn Rokitnicki Abelseth). The SAD Bern strain is a cell line-adapted derivative from the ERA strain. The SAD Bern strain was cultivated using monoclonal antibodies binding specifically to one of the two major antigenic sites (antigenic site III) of the rabies virus glycoprotein, involved in pathogenicity. Under the selective pressure of these monoclonal antibodies, only variants of SAD Bern bearing an amino-acid substitution at the critical position 333 of the rabies virus glycoprotein escaped neutralisation in cell culture. An avirulent mutant, SAG1 (for SAD Avirulent Gif), in which arginine at position 333 was substituted by serine, was isolated from SAD Bern with monoclonal antibody (Mab) 50 AD1. The SAG2 strain was constructed from SAD Bern in a two-step selection procedure using neutralizing monoclonal antibodies. First, a mutant strain (SK) was selected from SAD Bern, where the arginine at position 333 was replaced by lysine. SAG2, a non pathogenic mutant resistant to neutralisation by monoclonal antibody 50 AC1 was selected from SK, where the lysine at position 333 was replaced by a glutamic acid. Therefore, SAG2 can be considered as a double avirulent mutant, since the codon GAA, which codes for glutamic acid, differs from the codon AGA from SAD Bern (coding for arginine) by two nucleotides. The vaccine strain SPBN GASGAS is a recombinant rabies virus which has been derived by site directed mutagenesis from the passage attenuated vaccine strain SAD B19. The recombinant strain has been further attenuated compared to the SAD B19 strain by three targeted genetic modifications.

Table 1. The main features of oral rabies vaccines used in the EU (Data compiled from manufacturers and EMEA)

Vaccine	**VRG**	**SAG2**	**SAD B19**	**SAD P5/88**	**SPBN GASGAS**
Proprietary name	Raboral	Rabigen	Fuchsoral	Rabifox	Rabitec
Company	Merial	Virbac	IDT	IDT	IDT
Quality					
Vaccine titer,	>8 log10 TCID50/dose	>8 log10 TCID50/dose	7 log10 FFU/ml	7 log10 FFU/ml	$10^{6.8}$ – $10^{8.1}$ FFU/1.7 ml
Thermostability, virus titre	Stable (time and temperature details not available)	0.16 log10 reduction after 2 days at 25°	0.4 log10 reduction after 7 days at approx. 25°C	0.26 log10 reduction after 7 days at approx. 25°C	The titer not decrease below $10^{6.6}$ FFU/ml after 5 days at approx. 25°C
Melting point of bait casing	> 50°C	43°C	35°C (new bait casing under development)	35°C (new bait casing under development)	Not mentioned
Safety					
Non-target species tested	**52**	approx. 30	approx. 20	approx. 15	approx. 7
Tested Horizontal transmission	None in foxes (adults and cubs), dogs, cats, cattle, ferrets	None in foxes, may be found in salivary glands of young dogs	None in foxes, rodents, skunks and dogs	None (no information on species)	May be found to the site of vaccine uptake i.e. the *tonsilla palatina*
No Reversion to virulence after	7 backpassages in mice (intracerebral and footpad), 10 backpassages in vero cell cultures, 1 backpassage in fox	5 backpassages in suckling mice	5 passages in foxes and 10 passages in suckling mice	10 passages in suckling mice	5 backpassages in NMRI mice
Efficacy					
Lowest protective dose tested	10^{7} TCID50/dose	$10^{8.1}$ TCID50/dose	$10^{6.0}$ log10 FFU/ml	$10^{6.2}$ log10 FFU/ml	$10^{6.8}$ FFU/dose (i.e. $10^{6.6}$ FFU/ml in 1.7 ml)

TCID-tissue culture infective dose; FFU-focus forming units; EMEA-European Agency for the Evaluation of Medicinal Products

Some modified-live rabies virus oral vaccines may have residual pathogenicity depending on the level of attenuation of the viral strain, as the successive selections from the original strain may produce hazardous and uncontrolled results, and variants may remain pathogenic both in target and non-target species (WHO, 2013).

Different animal species were involved in vaccine-induced rabies cases, such as foxes (Müller et al., 2009; Hostnik et al., 2014), raccoons, striped skunks and even castles (Fehlner-Gardiner et al., 2008; Vuta et al., 2016b; Vuta et al., 2017; Pfaff et al., 2018).

Table 2. The main results from safety trials carried out on target and non-target species using the VRG, SAG2, SAD B19 and GASGAS vaccine

Vaccines	**Carnivora**	**Rodents**	**Immunocompromised mice**	**Non human Primates**
VRG	No pathogenicity	No mortality	No mortality In 40 SCID mice (109 TCID50)	No pathogenicity for 11 chimpanzees (109PFU/ml) 24 Common squirrel monkeys (108PFU/ml) (Rupprecht,1992)
SAG2	No pathogenicity	No mortality	No mortality In 10 SCID mice (108 TCID50)	No pathogenicity for 10 baboons (109 PFU) (Bingham, 1997)
SAD B19	No pathogenicity in several species Pathogenic for Skunk at high doses (109 FFU) (Rupprecht, 1990; Vos, 2002)	Up to 6% mortality in several European wild species (Artois, 1992, Vos, 1999)	No mortality in 10 SCID mice (107.4FFU), mortality in 2/10 nude mice (107.3 FFU)	No pathogenicity for 12 baboons (108.3 FFU) (Vos, 1999)
GASGAS	No pathogenicity	No mortality	No mentioned	No mentioned

TCID-tissue culture infective dose; FFU-focus forming units; PFU-plaque forming units; SCID-severe combined immunodeficient mice

CONCLUSIONS

In Europe, oral vaccination by means of vaccine baits has been found to be successful in eliminating terrestrial wildlife rabies in most cases. However, the ultimate success of ORV campaigns requires a long-term strategy from competent authorities and cross-border cooperation between countries.

Rabies in wildlife was eliminated in those countries where the vaccination campaigns were planned on a national level and coordinated with neighboring countries.

The vaccines authorized in the EU for oral vaccination are Raboral (V-RG strain), Rabigen (SAG2 strain), Fuchsoral (SAD B19 strain), Lysvulpen (two dominant sub-populations of SAD Bern and SAD B19-'like' viruses) and a new vaccine, Rabitec (SPBN GASGAS) The efficacy of the existing vaccines is one of the factors that has contributed substantially to the success of rabies monitoring, control and elimination in several European countries. Very few vaccine-associated cases of rabies in the field have been reported and published so far. Nevertheless, worries still exist related to the residual pathogenicity of attenuated live vaccines that could induce rabies in certain conditions.

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