Vaccines used for rabies control programmes: types, performance in different species, quality control, storage

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Epidemiology of sylvatic rabies in Europe

**TERRESTRIAL CYCLE**

- Lyssavirus
- Genotype 1
- DOG
- RED FOX

**AERIAL CYCLE**

- Lyssavirus
- Genotypes 5 (EBL1) and 6 (EBL2)
- INSECTIVOROUS BATS
Aim of rabies vaccination in animals

- Rabies vaccination in domestic carnivores is intended:
  - to protect individual animals if exposed to rabies virus,
  - to prevent them from transferring rabies virus to other domestic animals or to humans.

- Rabies vaccination in wildlife is intended:
  - to interrupt the transmission from one animal to another one,
  - to eliminate the virus from those reservoirs.
Strains of rabies virus used for production of live oral vaccines

- SAD P5/88
- AGA
- SAD Bern
- SAD B19
- AAA
- SAG1
- GAA
- SAG2
- ERA virus
- VRG

Probability of reversion: 1:10^16

- SAD strain isolated from a naturally infected dog in the USA in 1935
- AGA, AAA, GAA: codons of the rabies strains
- SAD: Street Alabama Dufferin
- ERA: Evelyn (Gaynor), Rokitnicki, Abelseth
- SAG: Street Alabama Gif: name of the laboratory (Gif) that performed the double mutations of the SAD strain
- VRG: Vaccinia Glycoprotein Recombinant
First family: SAD family strains: selection of rabies strains by successive passages in various heterologous hosts

This selection produces hazardous and unpredictable results. A variant may have lost its pathogenicity for a given host species while retaining/developing a new pathogenicity for other species.
2nd family: virulent mutants selected by monoclonal antibodies

Selection of rabies virus carrying mutation in a portion of the genome whose integrity is required for pathogenicity by oral route.

A single mutation on the Arginine 333 codon

\[
\text{SAD}_{\text{Bern}} \rightarrow \text{SAG 1}
\]

Flamand et al., 1980

We asked Flamand to select a double mutant.

A double mutation on the Argininn 333 codon confers to the new strain, compared with SAG1, a lowest probability to revert to the SAD parental strain:

\[
\begin{align*}
\text{AGA} & \quad \text{AAA} & \quad \text{GAA} \\
\text{SAD} & \quad \text{SAG 1} & \quad \text{SAG 2} \\
\end{align*}
\]

\[P=1/10^{16}\]
Third family: recombinant vaccine

Genetic recombination of an (ideally) innocuous carrier virus and a nucleic acid coding for a specific antigen:

Vaccinia

Replacement of the Thymidinkinase gene by the gene coding for rabies GP

Rabies

RNA

Glycoprotein

Induces immunological response of the infected host

DNA

the newly constructed virus CANNOT produce rabies viruses
Commercially available oral vaccines (1/2)

- SAD Bern strain baits (Lysvulpen),
- SAD B19 (Fuchsoral), SAD P5/88 baits,
- SAG baits (n° EU/2/00/021/001 and 002).

Rabigen

Rabidog
Commercially available oral vaccines (2/2)

- VRG baits

Raboral

Rectangular Fishmeal polymer VRG bait (France) for red foxes and raccoon dogs

Square Fishmeal or Chickenmeal Polymer VRG bait (USA) for raccoons, coyotes and gray foxes
Quality criteria of rabies oral vaccines:
Safety, stability, efficacy


Rabies Vaccine (Live, oral) for foxes, Vaccinum rabiei perorale vivum ad vulpem, European Pharmacopoeia 2007, p 952-953.
Quality criteria: European Pharmacopoeia, 2005 (1/2)

Safety in target and non target species:

<table>
<thead>
<tr>
<th>Oral adm</th>
<th>Field dose</th>
<th>10 times dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target species</td>
<td>40 foxes</td>
<td>10 foxes</td>
</tr>
<tr>
<td>Non target species</td>
<td>10 dogs</td>
<td>10 cats</td>
</tr>
</tbody>
</table>

⇒ No sign of rabies for 180 days

Safety in wild rodents: in natural and experimental conditions.

- **Safety in target and non target species**: idem European Pharmacopoeia and WHO criteria.

- **Stability**:
  - **Bait casing**: the melting point of the bait casing should be above 40°C to ensure that the capsule of the vaccine is still covered.
  - **Vaccine titre**: the titre of the final vaccine in the bait should not fall below the indicative 100% protective dose following exposure to 25°C for seven days.
  - The use of the **most stable vaccine** should be preferred in situations where high stability is considered important.
  - **Each vaccine batch** should be tested and approved for titre and stability by an acknowledged quality control scheme according to OIE standards and WHO recommendations.
WHO safety criteria for rabies vaccines


IVth WHO/OIE Concertation meeting on rabies control in Europe, Piestany, 5-7 October 1993.


Quality criteria: WHO (2005)

- **Safety in target and non target species**: namely wild rodents and other wild and domestic species, and also in non-human primates.
  - Oral administration of the ten times recommended dose in at least 10 young (3 – 6 months old) animals of the target species, or in dogs less than 10 weeks of age.
  - Relevant local wild or domestic animal species that may consume baits should also be administered the field dose of vaccine orally in a volume adapted to body weight.

- **Safety in wild rodents**: at least 10 and if possible 50 of each of the most common local rodent species should be given the field dose of vaccine orally and intramuscularly. If the animals that are vaccinated become sick or die from rabies, the use of the vaccine should be re-evaluated.

- Any rabies virus isolated from animals in vaccination areas should be characterized using monoclonal antibodies or molecular techniques to ensure that no vaccine-induced rabies has occurred.
Quality criteria : OIE (2008)

- **Safety in target and non target species**: similar to the ones of WHO.
  Saliva should be checked for the absence of vaccinal virus.

- **Stability**: similar to the ones of the European Commission expert group and European Pharmacopoeia.

- **Efficacy**: similar to the ones of the WHO:
  - Measuring the bait uptake,
  - Measuring the serological response of target animals after oral vaccination,
  - Measuring the incidence of rabies throughout all the country (vaccinated and unvaccinated areas).
Available vaccines have been more or less extensively tested in different species by different routes of inoculation (cerebral, muscular and oral): puppies, carnivora, avian species, non human primates, rodents and immunocompromised mice.
## Residual pathogenicity of SAD strain: previous data

<table>
<thead>
<tr>
<th>Species</th>
<th>Route of inoculation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse and other rodents</td>
<td>i.c., i.m., p.o.</td>
<td>Steck et al., 1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winkler et al., 1976</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wachendörfer et al., 1978</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leblois et al., 1988</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Artois et al., 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vos et al., 1999</td>
</tr>
<tr>
<td>1 fox cub, 1 cat, 1 stone marten</td>
<td>p.o.</td>
<td>Wandeler, 1988</td>
</tr>
<tr>
<td>Skunks (7 / 11)</td>
<td>p.o.</td>
<td>Rupprecht, 1990</td>
</tr>
<tr>
<td>Baboons (2 / 4)</td>
<td>p.o.</td>
<td>Bingham et al., 1992</td>
</tr>
</tbody>
</table>
Residual pathogenicity of SAD strain: recent data

- One case of rabies induced by vaccinal SAD strain in Austria in 2004 (Rabies Bulletin Europe, 2004) and one case in 2006 (CPCASA, 2006).


- Sixteen cases in Canada from 1989 to 2004, including 4 red foxes, 2 raccoons, 2 stripped skunks and one bovine calf (Brasilia, RITA meeting, 2006).
Safety of SAG and VRG vaccines

- **VRG**: one clinical adverse reaction documented in a woman (Rupprecht et al., 1999): spontaneously cleared skin lesions as a result of exposure to the vaccine, through a bite while attempting to remove a partially chewed vaccine bait from a dog’s mouth.

- **SAG strains**: SAG1 strain is pathogenic for suckling mice by cerebral route (Schumacher et al., 1993).

"Preference should be given to vaccines with reduced (non rabies-related) pathogenicity, such as VRG or SAG2, over more pathogenic attenuated live viruses for oral immunisation of wildlife and dogs." (WHO, 2005 – OIE, 2008).
Laboratories involved in the monitoring and evaluation of rabies programmes are advised to monitor the titre of all batches of rabies virus baits before and during release into the field.
Stability of vaccine baits in the fields

Virus titre in one bait
TCID50/ml
CCID50/ml
FFU/ml

(FAIR project, unpublished data)

All rabies strains are equally sensitive to temperature but appropriate preservatives and vials may create a difference between vaccines.
Efficacy: decrease in rabies incidence observed in France

Masson et al., 1996
Results of first oral vaccination campaigns in Estonia (2005 – 2006)

First oral vaccination during autumn 2005 (20 baits/km²)

Rabies cases in Estonia in 2005

Rabies cases in Estonia in 2006 (until end of May)

Second oral vaccination end of May 2006 (SAG2 vaccines, 20 baits/km²)

Rabies cases in Estonia in June-September 2006

Results of first oral vaccination campaigns in Estonia (2007 – 2008)

Rabies cases in Estonia in 2007
(4 cases)

Rabies cases in Estonia in 2008
(3 cases)

Review
Rabies in Estonia: Situation before and after the first campaigns of oral vaccination of wildlife with SAG2 vaccine bait
Enel Niin*, M. Laine*, A.L. Guiot¹, J.M. Demerson¹, F. Clignet¹,

Workshop on rabies: regional cooperation towards eradicating the oldest known zoonotic disease in Europe, 04-05 December 2008, Antalya (Turkey)
In 2003, 814 cases of rabies

*Workshop on rabies: regional cooperation towards eradicating the oldest known zoonotic disease in Europe, 04-05 December 2008, Antalya (Turkey)*
## Vaccines used for rabies oral vaccination in Europe

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Used in</th>
</tr>
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<tbody>
<tr>
<td>SAD Bern, Lysvulpen</td>
<td>Slovak Rep., Czech Rep., Switzerland, Slovenia, Lithuania, Latvia, Poland</td>
</tr>
</tbody>
</table>
| SAD B19  
SAD P5/88       | all European countries that vaccinate(d), today mainly in Germany, Poland, Austria, Finland, Latvia, Slovenia |
| SAG 1/2         | France, Switzerland, Austria, Hungary, Lithuania, Estonia                |
| VRG            | Belgium, Luxembourg, France, Ukraine                                    |
Number of terrestrial rabies cases in several European countries 1989 – 2008 (1/3)
Number of terrestrial rabies cases in several European countries 1989 – 2008 (2/3)
Number of terrestrial rabies cases in several European countries 1989 – 2008 (3/3)
Storage of rabies vaccines

- All modified live-virus vaccines (SAD B19, SAD P5/88, Lysvulpen, SAG1 and SAG2) are stored at –20°C, in plastic bags or in carton boxes in dark conditions.

- VRG is stored at +4°C.
- The vaccines should not be used after the indicated expiry date.
- During the campaigns, it is highly recommended to maintain the vaccines at those temperatures until dropping and to have a system for registration of temperature.
Cost-benefit of vaccination

A cost-effectiveness study of rabies eradication in Switzerland (Zanoni et al., 2000) and in France (Aubert, 199):

- In Switzerland, evaluation that only 3.1% of the direct costs of rabies in 1993 were for the vaccine baits (SAG2).

- In France, the benefit of oral vaccination was obtained after the fourth year of the programme, and the costs of baits (SAG2 and VRG vaccines) were negligible compared to the costs of preventive vaccination of pets and prevention in humans.
Rabies vaccines for dogs
Injectable vaccines

- Cell culture vaccines are recommended:
  - Inactivated
  - Adjuvanted
  - Possibly combined with other antigens
  - Potency: 1.0 IU/dose

- Quality controls have to be performed to guarantee:
  - Safety: control of the inactivated process
  - Stability: during long storage and under liquid or lyophilised forms
  - Efficacy:

  → Potency test: NIH test or Pharmacopeia test (Rabies vaccine (inactivated) for veterinary use, 2008, 451).

  → Immunogenicity on 35 animals (serological survey and challenge study).
Examples of injectable vaccines for dogs produced locally

Pilot parenteral vaccination programme in Morocco

Incidence of rabies in humans and animals in Tunisia

Human cases

Dog mass vaccination:
- every year
- every 2 years

Workshop on rabies: regional cooperation towards eradicating the oldest known zoonotic disease in Europe, 04-05 December 2008, Antalya (Turkey)
Rabies vaccination of dogs

National programmes for the control of rabies in animals including

→ adequate surveillance,
→ mass parenteral vaccination campaigns (house to house visits, fixed vaccination posts or mobile teams). Can be followed by destruction of unmarked dogs (Malaysia) in pilot areas prior to large geographical application,
→ dog population studies allowing dog population management and ABC programmes.

Need of increasing amount of resources invested in infected areas
Cases of rabies in dogs and number of vaccinated dogs in Mexico
1990 – 2004

(Slide kindly given by Dr. F. Meslin, WHO)
Future trends: oral vaccination of dogs combined to parenteral vaccination

- The major obstacle in canine rabies is the accessibility to vaccination of ownerless dogs.
- In India, this method presents many advantages as stray dogs (75.2%, Sudarshan et al., Int. J. of Inf. Dis., 2007) are responsible of human deaths.
Conclusions

- Available oral vaccines have allowed elimination of rabies in a number of European countries: existing rabies vaccines are efficient on the main vectors (foxes and raccoon dogs).

- However serious concerns exist about residual pathogenicity of certain vaccines in wild and domestic carnivores and in rodents.

- Quality control for rabies oral and injectable vaccine batches (batch release) through European Pharmacopoeia and EC is in place since one year (OCABR, Articles 81 and 82 procedures).

- Successful experiences should be adapted and tested in those areas still infected despite intensive oral vaccination programmes.