



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

F. Cliquet



Community Reference Laboratory for Rabies
Community Reference Laboratory for Rabies Serology



WHO Collaborating Centre for Research and Management in
Zoonoses Control



OIE Reference Laboratory for Rabies

Afssa Nancy

National reference laboratory on research on rabies and wildlife diseases
Nancy (France)

Epidemiology of sylvatic rabies in Europe

TERRESTRIAL CYCLE

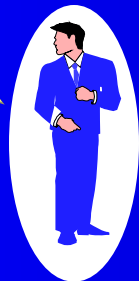
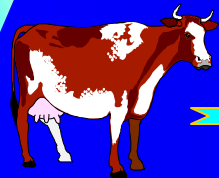
Lyssavirus
Genotype 1



DOG

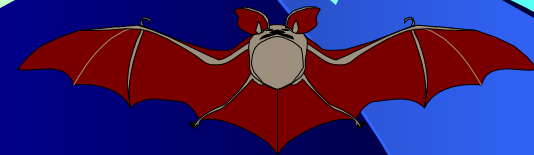


RED FOX



AERIAL CYCLE

Lyssavirus
Genotypes 5 (EBL1) and 6 (EBL2)



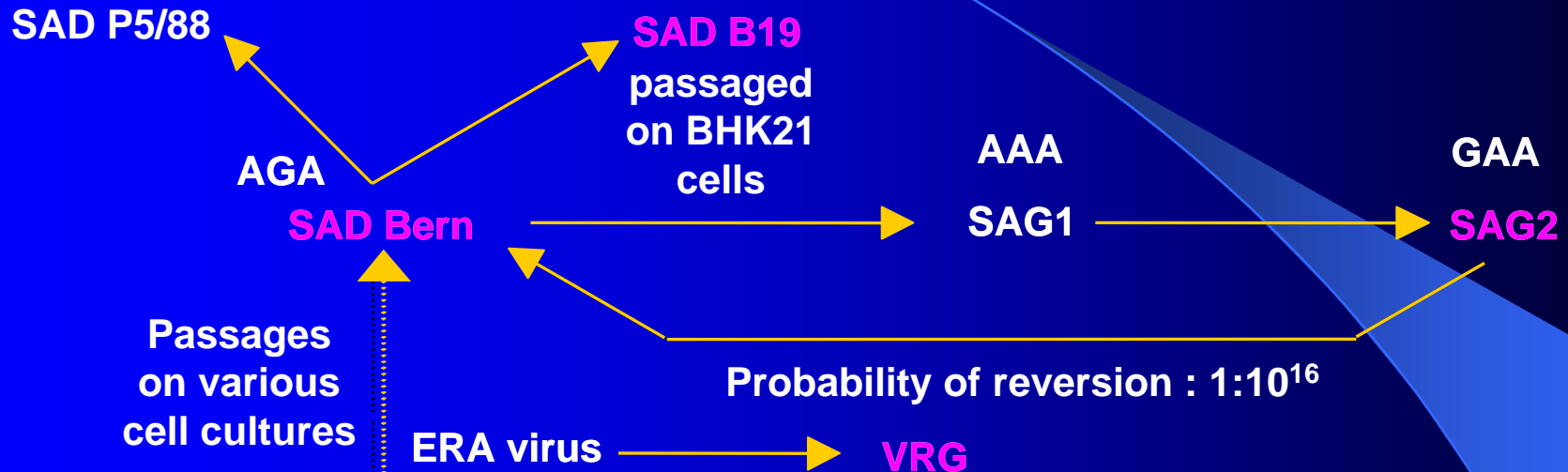
INSECTIVOROUS
BATS



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



Passages on various cell cultures

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

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.

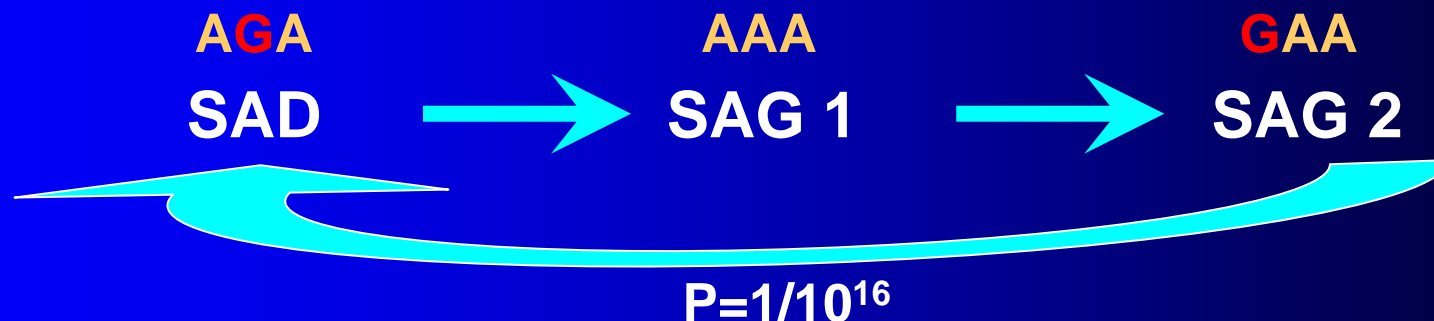
A single mutation on the Arginine 333 codon

SAD_{Bern} → **SAG 1**

Flamand et al., 1980

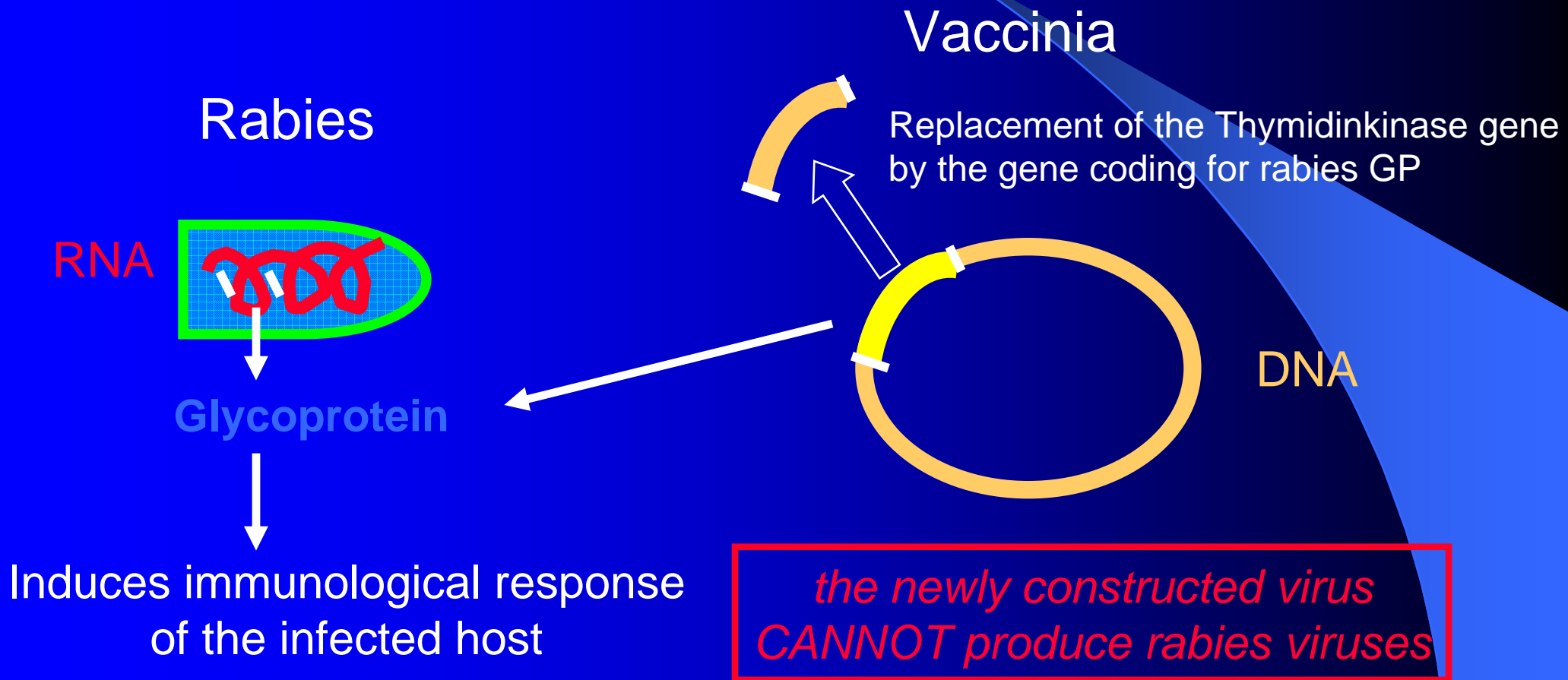
We asked Flamand to select a double mutant.

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



Third family : recombinant vaccine

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



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).



Bioveta website



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Rabigen



Rabidog

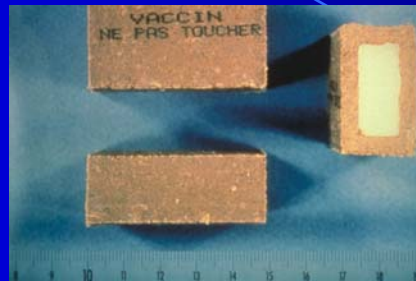


AFSSA-Nancy

Commercially available oral vaccines (2/2)

- VRG baits

Raboral



Merial

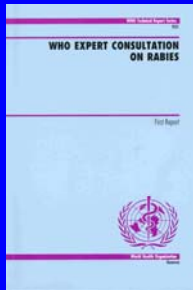
Rectangular Fishmeal polymer VRG bait (France)
for red foxes and raccoon dogs



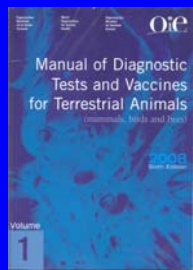
Merial

Square Fishmeal or Chickenmeal Polymer VRG bait
(USA) for raccoons, coyotes and gray foxes

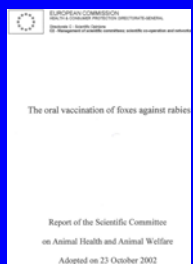
Quality criteria of rabies oral vaccines : Safety, stability, efficacy



WHO Expert Consultation on Rabies, OMS Geneva, WHO Technical Report Series, **2005**, n°931, 87 p.



Rabies chapter (2.1.13) of OIE Manual, **2008**, oral vaccination paragraph.



The oral vaccination of foxes against rabies, Report of the Scientific Committee on Animal Health and Animal Welfare, **2002**, 55 p.



Rabies Vaccine (Live, oral) for foxes, Vaccinum rabiei perorale vivum ad vulpem, European Pharmacopoeia **2007**, p 952-953.

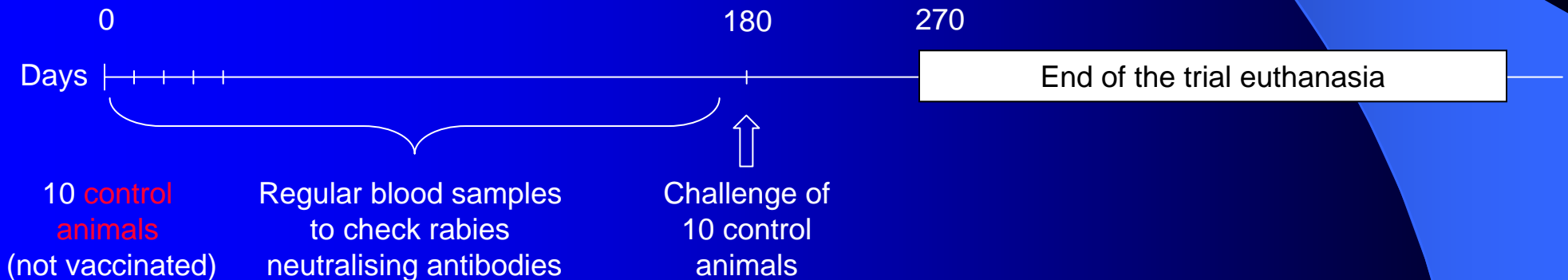
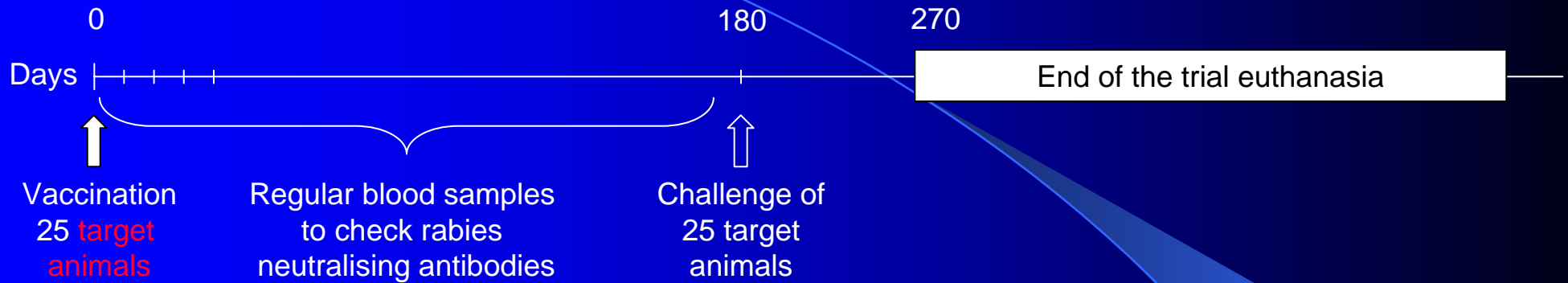
Quality criteria : European Pharmacopoeia, 2005 (1/2)

➤ Safety in target and non target species :

Oral adm	Field dose	10 times dose
Target species	40 foxes ⇒ No sign of rabies for 180 days	10 foxes
Non target species		10 dogs 10 cats ⇒ No sign of rabies for 180 days

➤ Safety in wild rodents : in natural and experimental conditions.

Quality criteria : European Pharmacopoeia, 2005 (2/2)



Quality criteria : Scientific Committee on Animal Health and Animal Welfare (European Commission expert group, 2002)

- 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

Eighth Report of WHO Expert Committee on rabies, W.H.O., Geneva, 1992.

Report of WHO Consultation on requirements and criteria for field trials on oral rabies vaccination of dogs and wild carnivores, Geneva, 1-2 March 1989, doc. WHO/Rab.Res./89-32.

Report of the 3rd WHO consultation on oral immunization of dogs against rabies, Geneva, 21-22 July 1992, Doc WHO/Rab.Res./92.38.

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

Report of the 4th WHO Consultation on oral immunization of dogs against rabies, Geneva, 14-15 June 1993, Doc. WHO/Rab.Res./92.42

Report of the 5th WHO Consultation on oral immunization of dogs against rabies, Geneva, 20-22 June 1994, Doc. WHO/Rab.Res./94.45

Oral immunization of dogs against rabies : report of the 6th WHO consultation, Geneva, 24-25 July 1995, Doc WHO/EMC/ZDI/98.13, 28 p.

Field application of oral rabies vaccines for dogs. WHO-OIE consultation, Geneva, 20-22 July 1998, Doc. WHO/EMC/ZD/98.15, 24 p.

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).

Safety of vaccines

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

<u>Species</u>	<u>Route of inoculation</u>	<u>Reference</u>
Mouse and other rodents	i.c., i.m., p.o.	Steck et al., 1982 Winkler et al., 1976 Wachendörfer et al., 1978 Leblois et al., 1988 Artois et al., 1992 Vos et al., 1999
1 fox cub, 1 cat, 1 stone marten	p.o.	Wandeler, 1988
Skunks (7 / 11)	p.o.	Rupprecht, 1990
Baboons (2 / 4)	p.o.	Bingham et al., 1992

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).
- Four cases (3/4 not notified) of vaccine-induced rabies in Germany in 2001, 2002, 2004 and 2005 (Ottawa, RITA meeting , 2005; FVO inspection, 2004).
- Sixteen cases in Canada from 1989 to 2004, including 4 red foxes, 2 raccoons, 2 striped skunks and one bovine calf (Brasilia, RITA meeting, 2006).

Safety of SAG and VRG vaccines

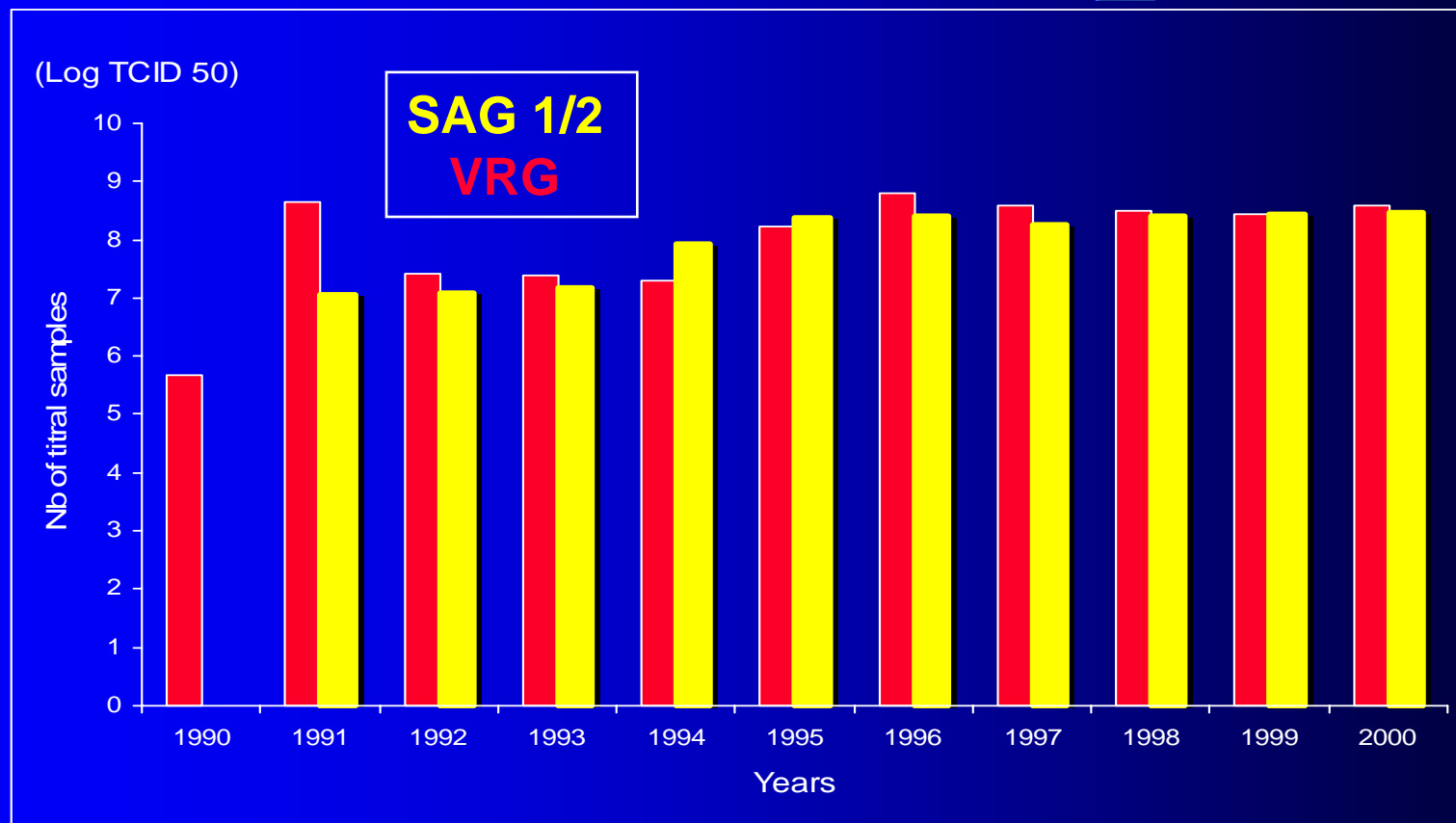
➤ **VRG** : one clinical adverse reaction documented in a woman (Rupprecht et al., 1999) : spontaneously cleared skin lesions as a result of exposure to the vaccine, through a bite while attempting to remove a partially chewed vaccine bait from a dog's mouth.

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

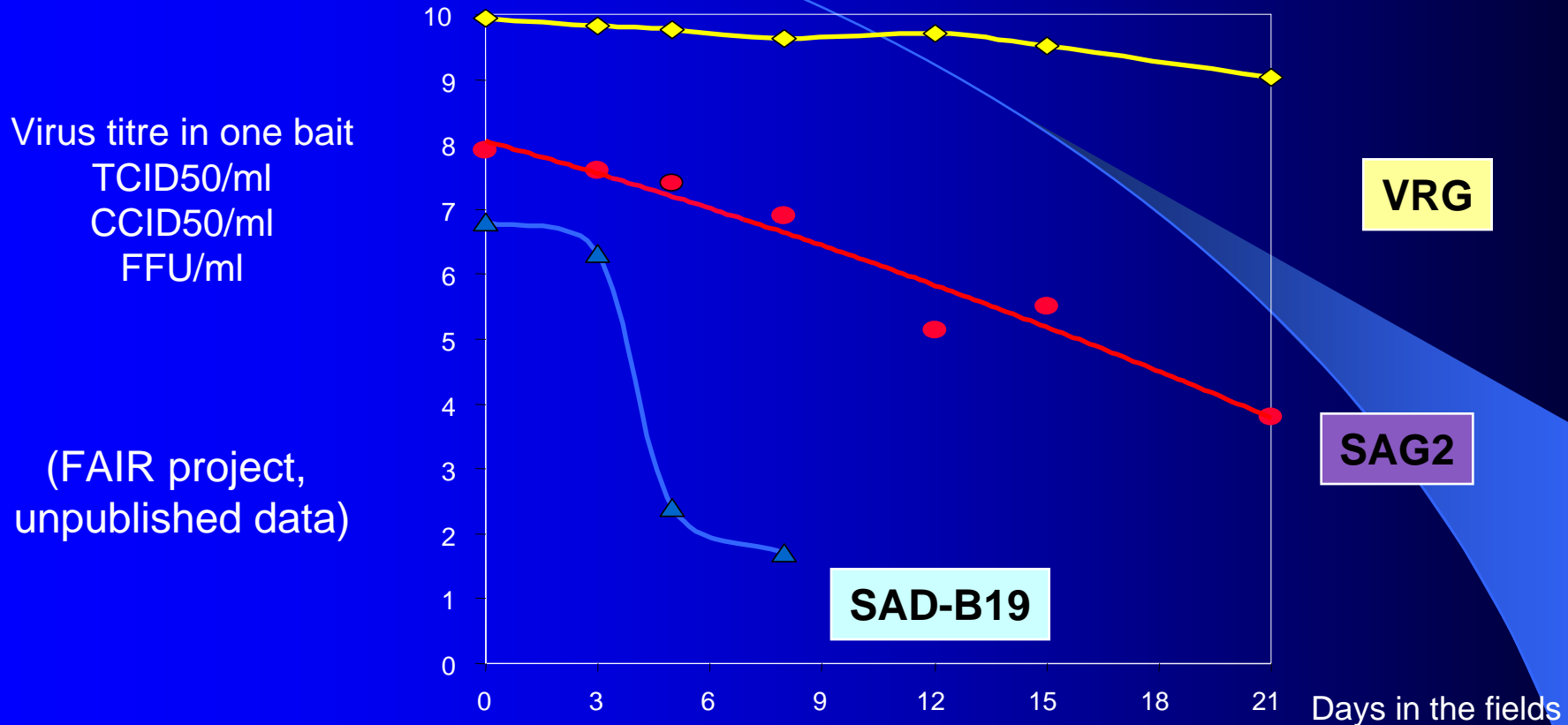
“Preference should be given to vaccines with reduced (non rabies-related) pathogenicity, such as VRG or SAG2, over more pathogenic attenuated live viruses for oral immunisation of wildlife and dogs.” (WHO, 2005 – OIE, 2008).

Control of quality stability before releasing in the field

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.

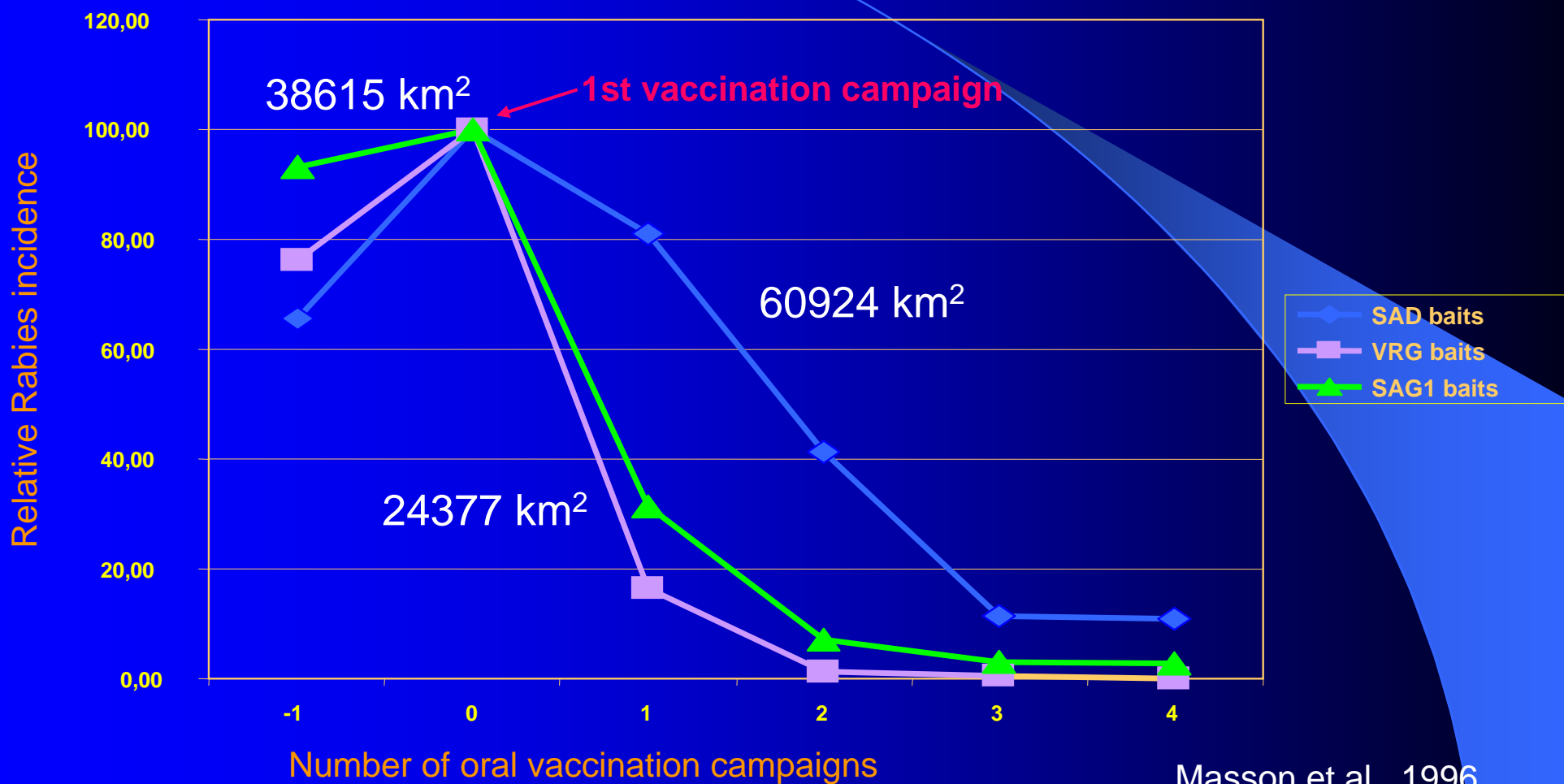


Stability of vaccine baits in the fields



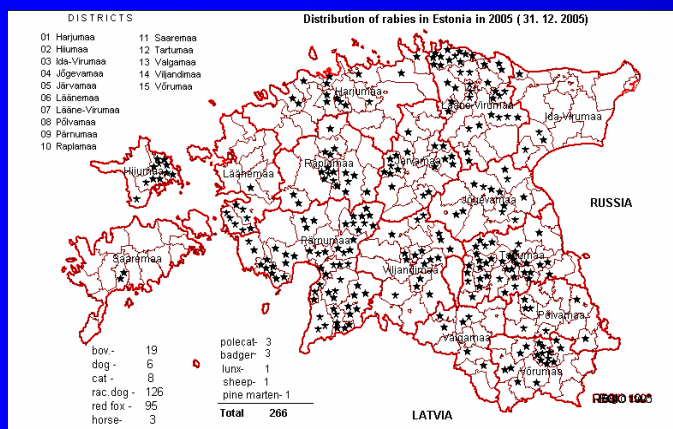
All rabies strains are equally sensitive to temperature
but appropriate preservatives and vials
may create a difference between vaccines

Efficacy : decrease in rabies incidence observed in France



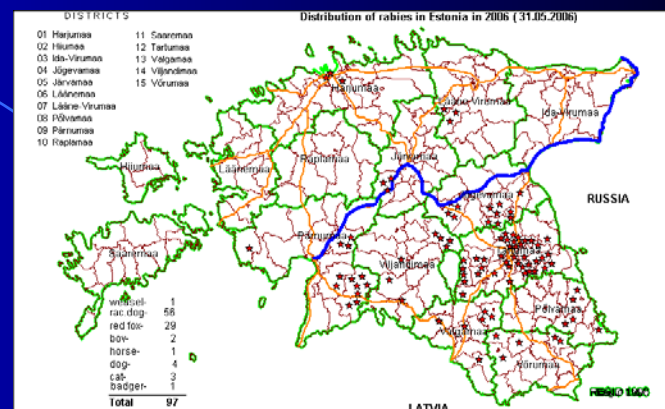
Masson et al., 1996

Results of first oral vaccination campaigns in Estonia (2005 – 2006)



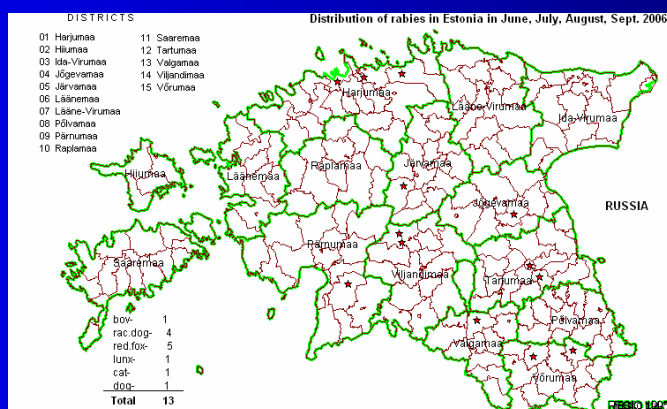
Rabies cases in Estonia in 2005

first oral
vaccination during
autum 2005 (20
baits/km²)



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

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

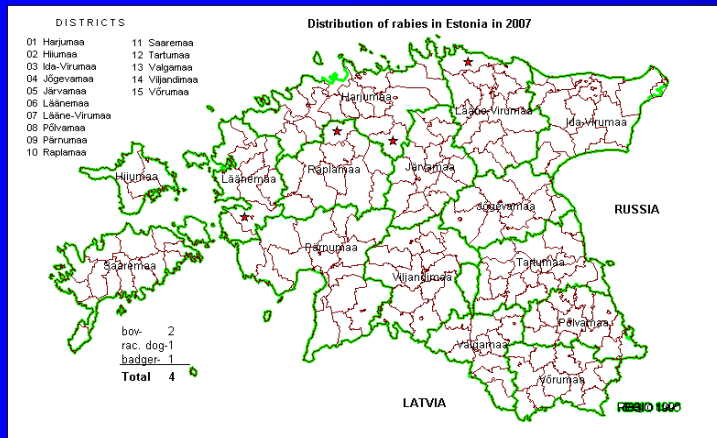


Rabies cases in Estonia
in June-September 2006

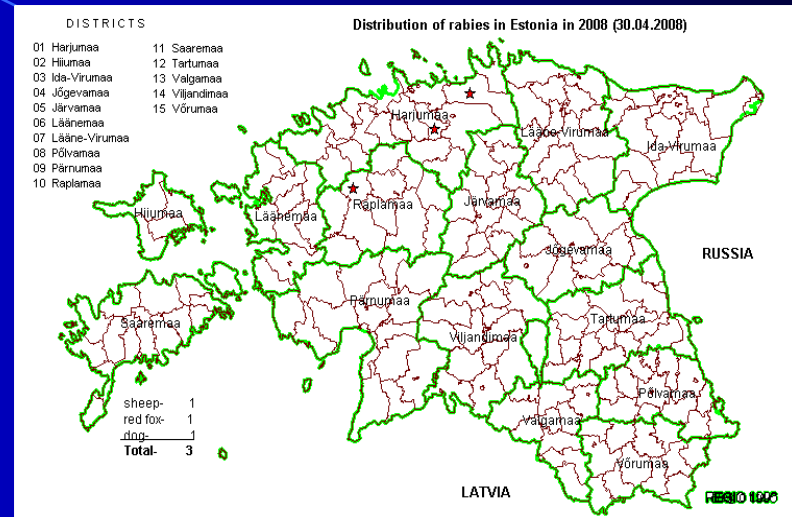
E. Niin, J. Barrat, M. Kristian, J.M. Demerson, F. Cliquet : First oral vaccination of wildlife against rabies in Estonia, in Dev. Biol., 2006, vol. 125, 145-1473

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

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)

Vaccine 26 (2008) 3556–3565

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

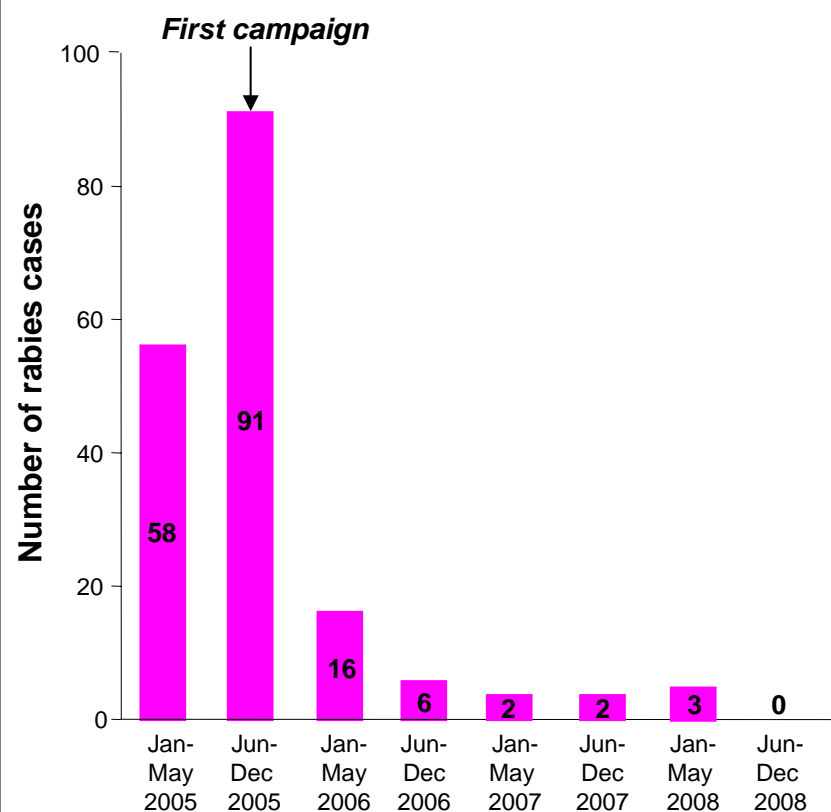
Review

Rabies in Estonia: Situation before and after the first campaigns of oral vaccination of wildlife with SAG2 vaccine bait

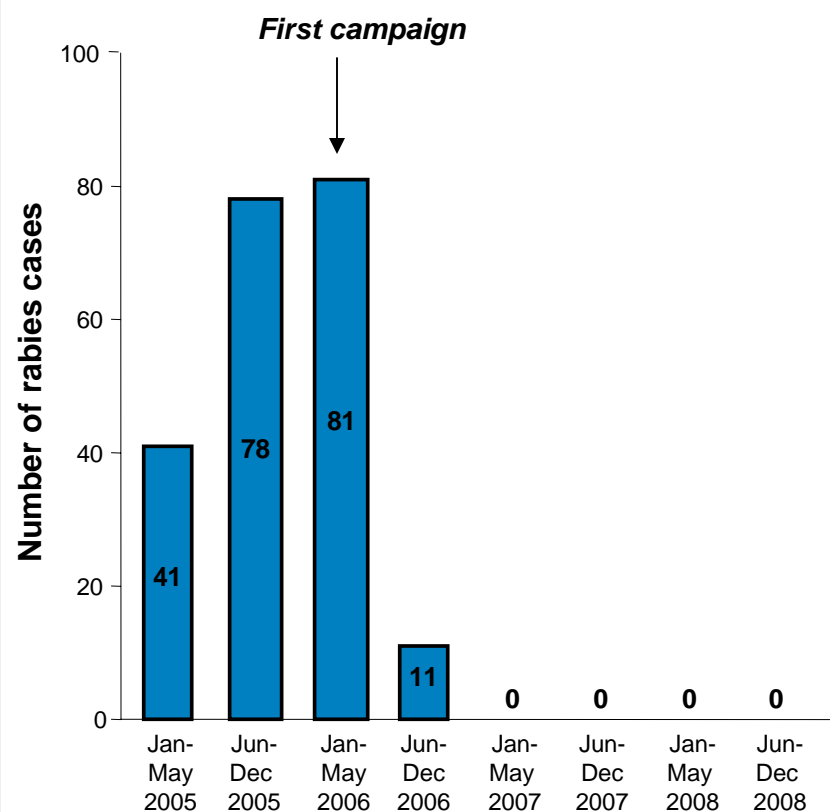
Enel Niin^a, M. Laine^a, A.L. Guiot^b, J.M. Demerson^c, F. Cliquet^{c,*}

Rabies incidence decrease in Estonia 2005 - 2008

■ North Estonia 7 campaigns : no more cases



■ South Estonia 3 campaigns : no more cases

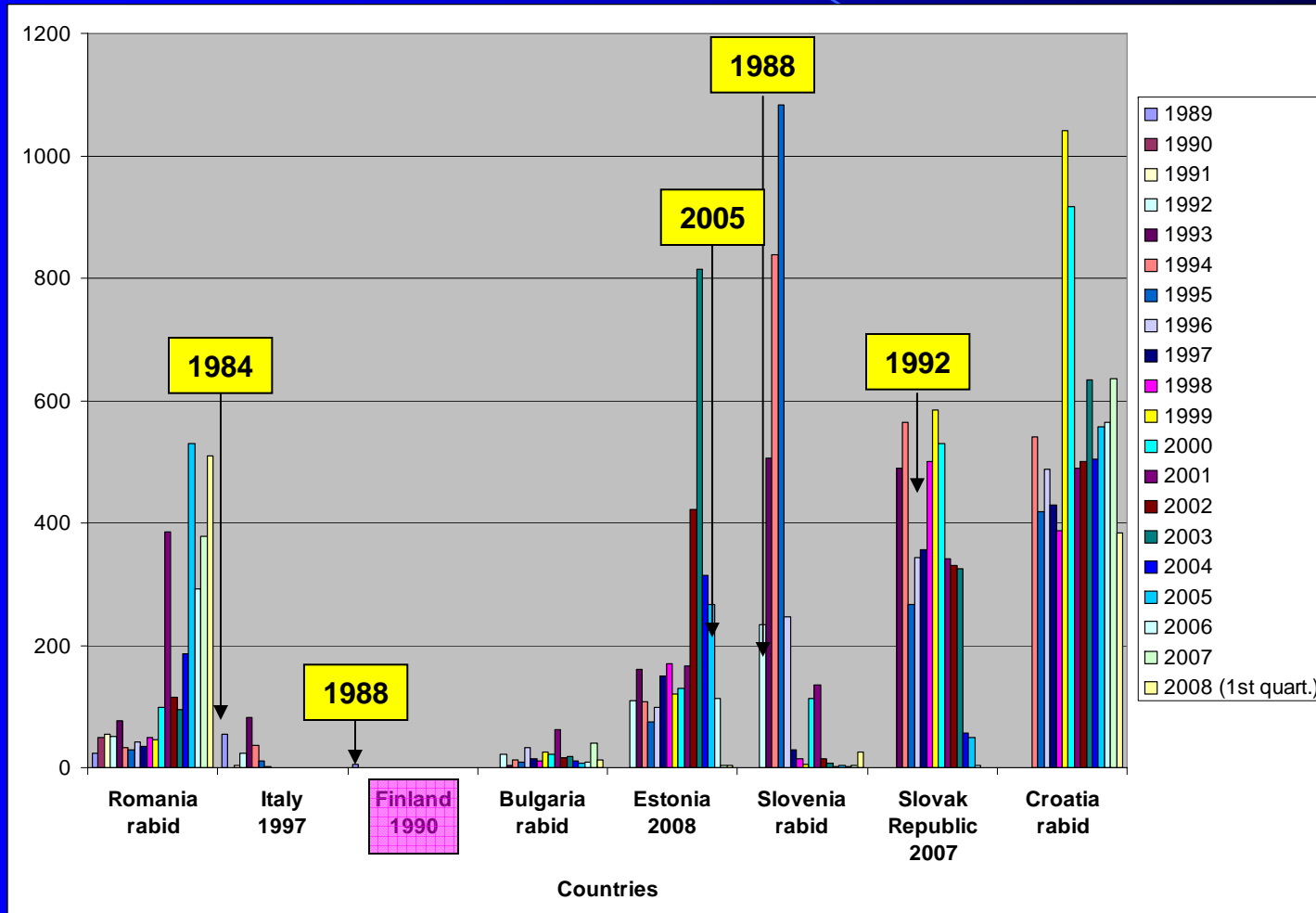


In 2003, 814 cases of rabies

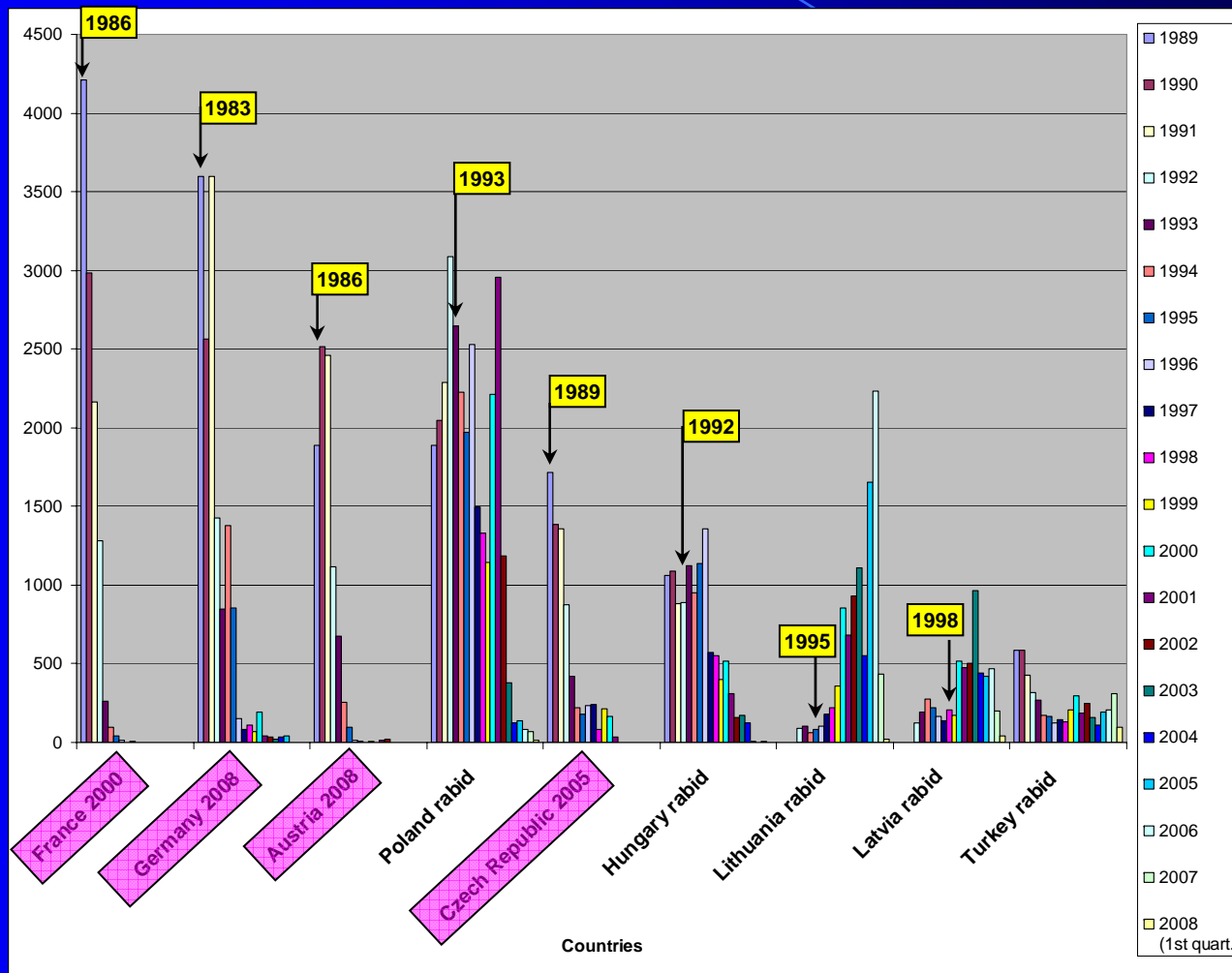
Vaccines used for rabies oral vaccination in Europe

Vaccine	used in
SAD Bern, Lysvulpen	Slovak Rep., Czech Rep., Switzerland, Slovenia, Lithuania, Latvia, Poland
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

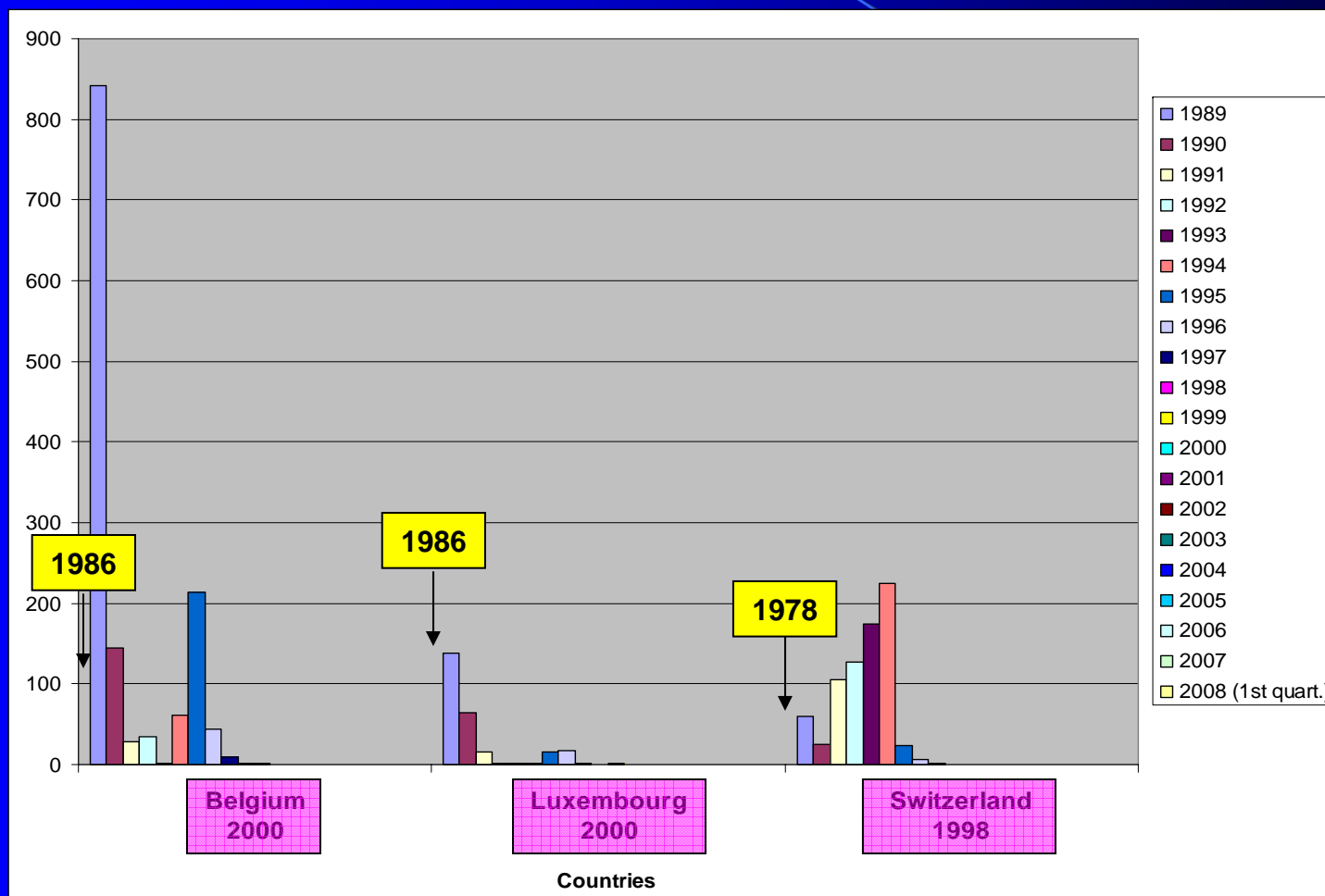
Number of terrestrial rabies cases in several European countries 1989 – 2008 (1/3)



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 $+4^{\circ}\text{C}$.
- 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

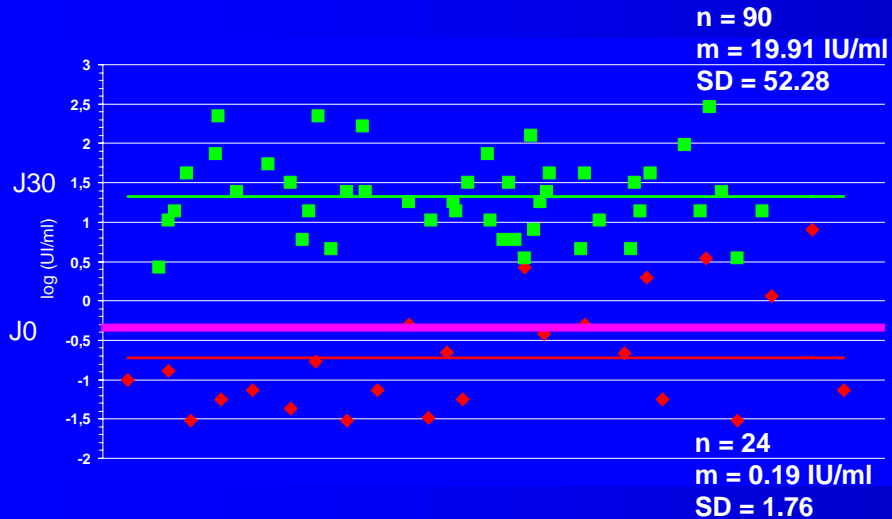
Injectable vaccines

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

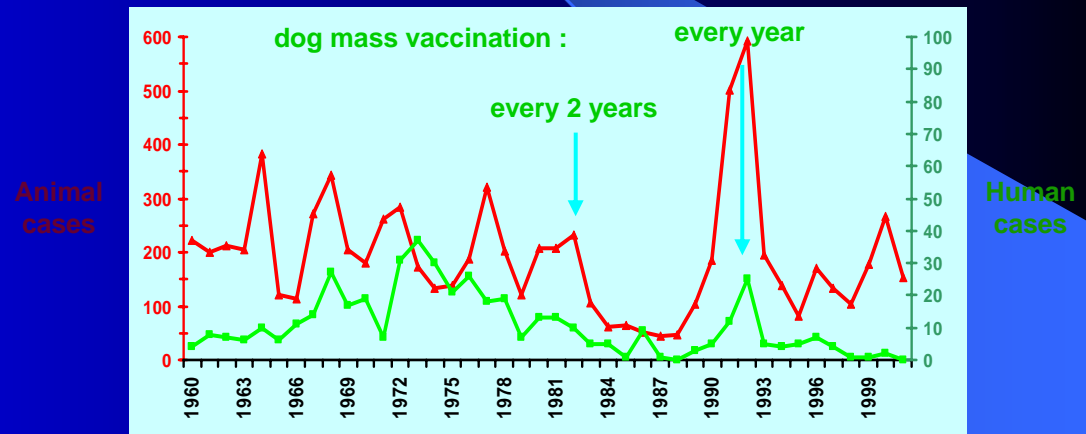
 - Quality controls have to be performed to guarantee :
 - Safety : control of the inactivated process
 - Stability : during long storage and under liquid or lyophilised forms
 - Efficacy :
- Potency test : NIH test or Pharmacopeia test (Rabies vaccine (inactivated) for veterinary use, 2008, 451).
- Immunogenicity 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



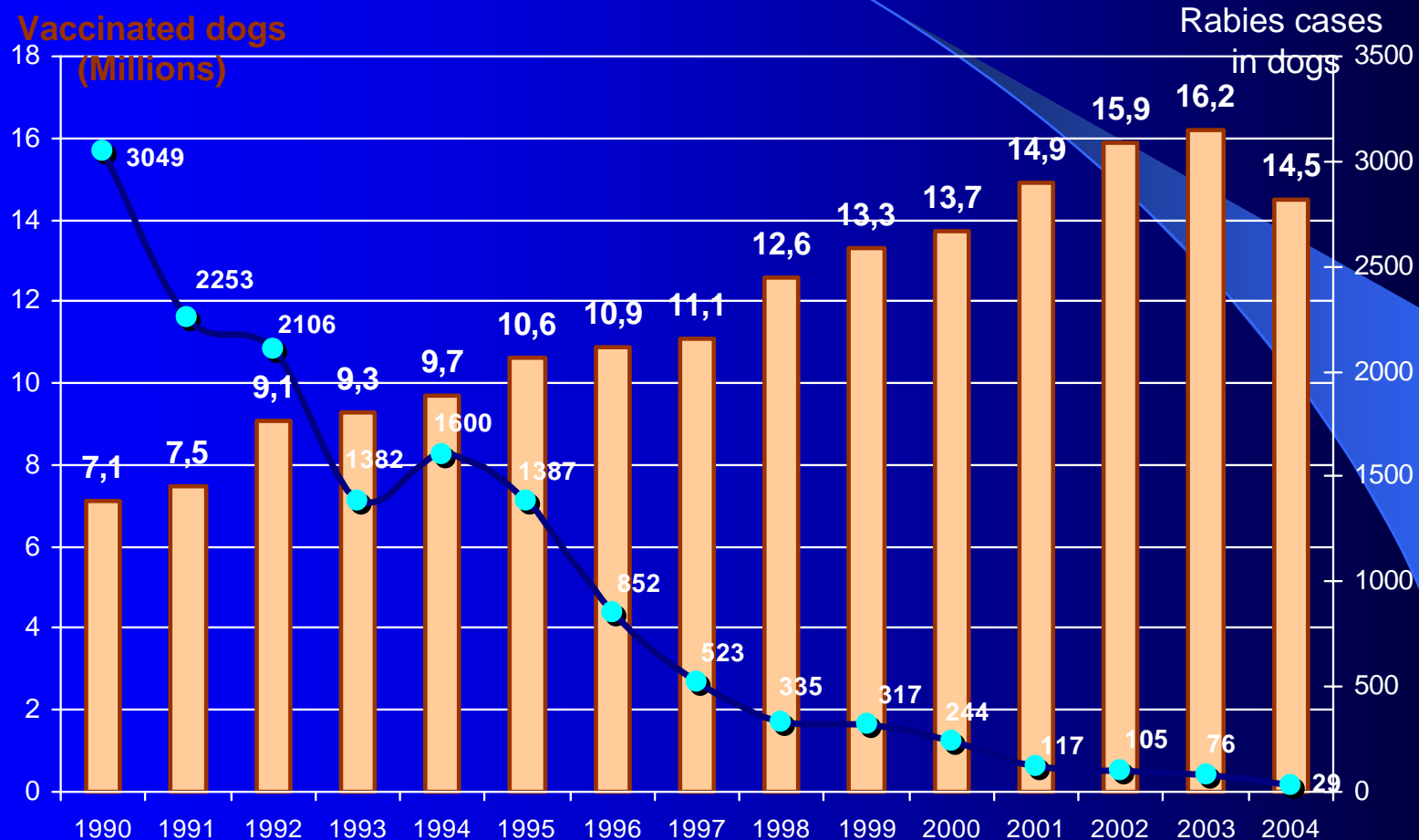
Rabies vaccination of dogs

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.