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STABILITY OF RECOMBINANT VACCINIA-RABIES VACCINE IN VETERINARY USE

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ABSTRACT

Wildlife vaccination depends on vaccines which can be orally administered by a baiting system. Therefore only two possibilities exist: either the use of attenuated strains of viruses, or recombinant vector viruses. As far as rabies is concerned, the choice of the recombinant vaccinia-rabies virus was made because it was safer and more stable.

An in vitro stability study of the recombinant product compared to wild rabies virus at different temperatures (4°C, 20°C, 37°C, 45°C) showed that the recombinant virus was more stable. The stability of the recombinant virus was also tested under field conditions; besides natural freezing and thawing cycles, the virus titre remained unchanged in the bait for a month. Taking into account the fact that all baits are eaten by wild animals within this period, one can assume that the vaccine is efficacious for all baiting animals in field conditions. The stability of the recombinant vaccinia-rabies vaccine is of considerable interest in such uncontrolled conditions.

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Introduction

Prophylactic measures taken in the past to control fox rabies, such as the culling of foxes, did not prevent the spread of the epizootic. During recent years, most of the research on the control of fox rabies has concentrated on the development of methods of fox vaccination by the oral route, and this method has already been extensively used in ail infected countries belonging to the European Union. Research has focused on oral vaccination because it is the only means which allows the immunization of a sufficient proportion (75%) of wild foxes through the distribution of vaccine baits. Therefore, the only applicable vaccines were either attenuated strains of rabies virus or live vectored vaccines.

As far as safety and stability are concerned, the use of attenuated rabies virus remains controversial because these virus strains are still pathogenic for laboratory and wild rodents [1], wildlife species such as the chacma baboon (*Papio ursinus*) [2], or target species such as the striped skunk [3]; moreover, these strains may still be pathogenic to man. Thus, humans exposed to SAD-derived attenuated strains of rabies must be treated with a conventional inactivated rabies vaccine. SAD-derived attenuated strains may also be inefficient in certain rabies vectors, such as the raccoon in North America [4]. Because of their residual pathogenicity, the use of attenuated strains of rabies virus for domestic animal vaccination in Western Europe has been discontinued.

The pathogenicity of attenuated rabies virus strains can be abolished by mutating the arginine residue at position 333 of the glycoprotein. This has led to the development of a new attenuated vaccine strain, which is already in use in the field [5]. Another inconvenience of attenuated strains of rabies virus is their heat-sensitivity, which reduces their potential efficacy in field conditions.

Thus, in order to improve both the safety and stability of the vaccines used for fox vaccination in the field, a recombinant vaccinia virus has been developed that expresses the immunizing glycoprotein of rabies virus. This virus vaccine has been tested in the field [6, 7].

Materials and methods

IN VITRO STABILITY

The assays measured intrinsic stability of vaccinia-rabies recombinant virus (VRG) [71 compared to wild rabies virus (G-52 strain). Suspensions of either VRG or rabies virus were maintained at 4°C, 20°C, 37°C or 45°C. Two different suspensions were used for each virus strain. Suspensions were titrated at regular intervals on Vero cells cultivated in microplates. Cythopathic effect was observed either on fresh culture for VRG virus or after immunofluorescent staining for wild rabies virus. Titres were expressed as $\log_{50}TCID_{50}$ / ml with a mean confidence interval of 0.5 log.

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STABILITY UNDER FIELD CONDITIONS

Baits containing VRG were placed in the field within a vaccinated area (Province of Luxembourg, Southern Belgium) to be submitted to environmental temperatures (natural freezing and thawing cycles). Baits were taken at regular intervals and the VRG titrated on Vero cells.

Results

IN VITRO STABILITY

As shown in Tables 1 to 4, stability of the recombinant (VRG) virus is always better than that of wild rabies virus. This is particularly the case at 4°C, that is to say in the conditions to be found in the field during the baiting period (spring and autumn).

Table 1. Stability at 4°C.*Titre de la figure*

Recombinant VRG		
Days	**Log titre***	**Difference**
0	8.6	0
3	8.6	0
7	8.5	0.1
14	8.4	0.2
28	8.3	0.3
56	8.2	0.4
90	8.3	0.3
150	8.4	0.2
240	8	0.6
547	8.2	0.4

Rabies virus		
Days	**Log titre***	**Difference**
0	5.7	0
1	5.7	0
2	5.07	0.63
4	5.15	0.55
7	5.15	0.55
14	6	0.3
28	5	0.7
35	4.65	1.05
60	2.47	3.22

Légende de la figure. (*) Mean titre of assays made on two different suspensions, expressed as $\log_{10}$ $TCID_{50}$ / ml; mean confidence interval at 5% : 0.6 $\log_{10}$

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Table 2. *Stability at 20°C*

Recombinant VRG		
Days	**Log titre***	**Difference**
0	8.6	0
3	8.6	0
7	8.2	0.4
14	8	0.6
28	7.3	1.3
56	6.2	2.4
90	5.3	3.3
150	2.3	6.3

Rabies virus		
Days	**Log titre***	**Difference**
0	5.7	0
1	5.32	0.37
2	5.07	0.62
4	4.9	0.3
7	4.57	1.12
14	4,07	1.62
28	<1.5	>4.2

Légende de la figure. Mean titre of assays made on two different suspensions, expressed as $\log_{10}$ $TCID_{50}$ / ml; mean confidence interval at 5% : 0.6 $\log_{10}$.

Table 3. *Stability at 37°C.*

Recombinant VRG		
Days	**Log titre***	**Difference**
0	8.6	0
3	8.3	0.3
7	7.1	1.5
14	5.1	3.5
28	1.9	6.7

Rabies virus		
Days	**Log titre***	**Difference**
0	5.7	0
1	5	0.7
2	4.15	1.55
4	3.5	2.2
7	2.9	2.8
14	<0,5	>5.2

Légende de la figure. *Mean titre of assays made on two different suspensions, expressed as $\log_{10}$ $TCID_{50}$ / ml; mean confidence interval at 5% : 0.6 $\log_{10}$.

Table 4. *Stability at 45°C*

Recombinant VRG		
Days	**Log titre***	**Difference**
0	8.6	0
3	7.1	1.7
7	4.2	4.4
14	1.7	6.9

Rabies virus		
Days	**Log titre***	**Difference**
0	5.7	0
1	3.82	1.37
2	2.32	3.37
4	<1.5	>4.2

Légende de la figure. *Mean titre of assays made on two different suspensions, expressed as $\log_{10}$ $TCID_{50}$ / ml; mean confidence interval at 5% : 0.6 $\log_{10}$.

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STABILITY IN FIELD CONDITIONS

Despite important variations of environmental temperatures (occurrence of natural freezing and thawing cycles) (Fig.l), the VRG titre remained stable (Table 5) over a period of one month, that is to say the period of time within which nearly 100% of the bait is taken by wild animals in field conditions [8].

Conclusions

As shown by the results obtained both in vitro and under field condition, the recombinant vaccinia-rabies vaccine (VRG) is highly stable. This is particularly true, as shown by in vitro studies, at 4°C; that is to say the temperature encountered by the vaccine in field conditions during the periods of use (spring and autumn). Even in the presence of naturally occurring freezing and thawing cycles, the VRG titre remains stable, under field conditions, over a period of one month; that is to say the period of time within which nearly 100% of vaccine baits are taken by wild animals. It can therefore be concluded that VRG vaccines are still efficacious even when taken after staying a prolonged period in the field. Another practical consequence of VRG stability is the fact that this vaccine does not need to be frozen before use and is therefore easier to dispatch.

Figure 1. *Environmental temperatures during 30 days (09.03.95-08.04.95) - Range of maxima and minima (2 days).*

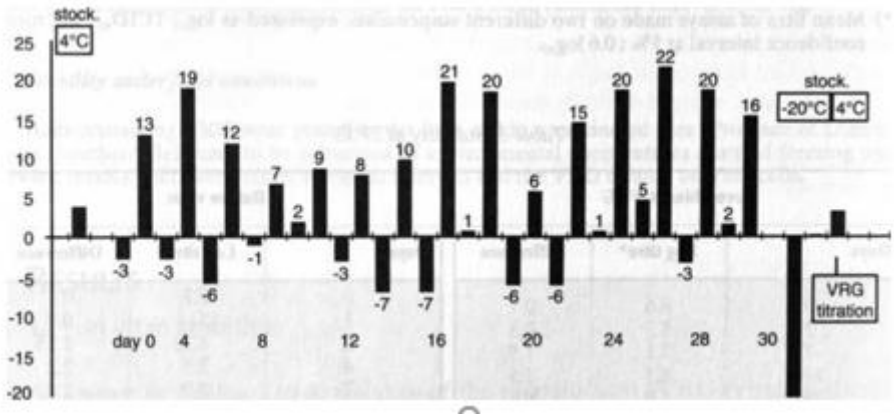

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Table 5. *Titres of samples of Raboral (VRG) after field exposure.*

Dilution (log10)	**Day0 (9.395)**		**Day 4 (13.3.95**		**Day 8 (17.3.95)**		**Day 14 (23.3.95)**		**Day 20 (29.3.95)**		**Day 30 (8.4.95)**	
Samples	**N°1**	**N°2**	**N°1**	**N°2**	**N°1**	**N°2**	**N°1**	**N°2**	**N°1**	**N°2**	**N°1**	**N°2**
4	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5
8	2	4	2	2	1	3	1	1	1	1	3	2
9	0	0	0	0	0	0	0	0	0	0	0	0
Titres (log10)	7.93	8.48	7.93	7.93	7.73	8.27	7.73	7.73	7.73	7.73	8.27	7.93
Means	8.21		7.93		8.00		7.73		7.73		8.10	

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