Veterinary Microbiology, 18 (1988) 103-108
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Use of Recombinant Vaccinia–Rabies Virus for Oral Vaccination of Fox Cubs (*Vulpes vulpes*, L) against Rabies

B.M. BROCHIER,[1] B. LANGUET[2], J. BLANCOU[3], M.P. KIENY[4], J.P. LECOCQ[4], F. COSTY[5], P. DESMETTRE[2] and P.-P. PASTORET[1]

[1]*Department of Virology and Immunology, Faculty of Veterinary Medicine, University of Liège 45, rue des vétérinaires, B-1070 Brussels (Belgium)*
[2]*Rhône-Mérieux, Laboratoire IFFA, 254, rue Marcel Mérieux, F-69007 Lyons (France)*
[3]*Centre National d'Etudes sur la Rage et la Pathologie des Animaux Sauvages, Ministère de l'Agriculture, BP 9, F-54200 Malzéville (France)*
[4]*Transgene, S.A., 11, rue de Molsheim, F-67082 Strasbourg (France)*
[5]*Department of Rabies, Institut Pasteur du Brabant, 28, rue du remorqueur, B-1040 Brussels (Belgium)*

(Accepted for publication 2 February 1988)

ABSTRACT

Brochier, B.M., Languet, B., Blancou, J., Kieny, M.P., Lecocq, J.P., Costy, F., Desmettre, P. and Pastoret, P.-P., 1988. Use of recombinant vaccinia-rabies virus for oral vaccination of fox cubs (*Vulpes vulpes*) against rabies. *Vet. Microbiol.*, 18: 103–108.

Thirteen fox cubs were orally administered $10^{7.2}$ plaque-forming units of live vaccinia-rabies glycoprotein recombinant virus. On Day 28 post-vaccination, all but 1 cub had produced rabies virus antibodies. Twelve animals were intramuscularly inoculated with $10^{3.2}$ mouse intracerebral LD_{50} of rabies virus suspension on Days 33 (5 foxes), 180 (4 foxes) or 360 (3 foxes) after vaccination. Eleven of them resisted rabies challenge. Unvaccinated foxes, either put in contact with 1 vaccinated animal or used as controls, died after challenge applied on Day 33. The absence of horizontal transmission of this vaccine strain and its innocuity to cubs were also demonstrated.

INTRODUCTION

Several wild species have replaced the dog as reservoirs and vectors of rabies: the red fox (*Vulpes vulpes*) in Europe and the red fox, the striped skunk (*Mephitis mephitis*) and the raccoon (*Procyon lotor*) in North America.

In Europe, the first attempts to control sylvatic rabies consisted of reducing the population of foxes. These measures have not proved to be fully effective in controlling the disease, therefore, other methods such as vaccination of foxes needed to be assessed (Baer, 1975). Oral administration is the only route ap-

0378-1135/88/$03.50

propriate for the vaccination of a large number of wild foxes (distribution of vaccine baits). Since 1978, several European countries have engaged, at different times, in large-scale field trials of fox vaccination using the Street Alabama Dufferin (SAD), standard or B 19 modified, live attenuated rabies virus (Steck et al., 1982; Schneider and Cox, 1983; Frisch et al., 1987; Artois et al., 1987, Brochier et al., 1987).

The encouraging results obtained from these vaccination campaigns attested to the feasibility and the efficacy of the method. However, the use of attenuated rabies virus remains controversial as far as innocuity is concerned.

A recombinant vaccinia virus expressing the immunising rabies G glycoprotein has been developed (Kieny et al., 1984). The potential of this live recombinant vaccinia virus, harbouring the rabies surface antigen (VVTGgRAB), to protect adult foxes against rabies has already been demonstrated (Blancou et al., 1986). The oral administration of VVTGgRAB to adult foxes, raccoons and skunks elicits high levels of rabies virus-neutralising antibodies and long-term protection against rabies. Vaccine bait ingestion or direct instillation into the mouth were shown to be similarly efficacious. Inoculation of the recombinant virus was totally innocuous in those species (Blancou et al., 1986; Rupprecht et al., 1986; Tolson et al., 1987).

In Europe, fox vaccination campaigns are performed in the field in May–June and/or September–October. During spring campaigns, fox cubs are potential targets for vaccination. The present paper deals with the use of this recombinant virus for the vaccination of fox cubs to test its efficacy, its innocuity and its absence of transmissibility in young foxes.

MATERIALS AND METHODS

Animals

In May 1986, 17 wild foxes (*Vulpes vulpes*) aged between 6 and 12 weeks were captured from 8 dens situated in a rabies-free area of Belgium. After capture, all of these young foxes were weighed, sexed and marked with auricular buckles. They were shown to be serologically negative for rabies virus antibodies before they were included in this study.

All manipulations of live cubs were carried out under anaesthesia. Each animal was intramuscularly injected with Hypnorm^{R} (Janssen Pharmaceutica, Beerse), 0.1 ml kg^{-1}, before weighing, bleeding or transport.

Viruses

A live-modified vaccinia (Copenhagen ts strain)–rabies glycoprotein (ERA strain) recombinant virus (VVTGgRAB-26D3 strain) (Kieny et al., 1984)

was used as vaccine and contained $10^{7.2}$ plaque-forming units (p.f.u.) per 1-ml dose.

The virus challenge suspension consisted of an homogenate of salivary glands of foxes which had died of natural rabies (Blancou et al., 1979). The titre was determined by intracerebral (I.C.) inoculation of mice. Foxes were intramuscularly inoculated with 1 ml of virus suspension containing $10^{3.2}$ mouse I.C. LD_{50} ml^{-1}.

Experimental protocol

The cubs were divided into 5 experimental groups (see Table 1). On Day 0, each cub of Groups 1 (4 foxes), 2 (4 foxes) and 3 (4 foxes), and 1 cub of Group 4 (3 foxes) received the 26D3 strain of VVTGgRAB. Vaccine was administered by direct application into the mouth via a needleless syringe (volume 1 ml). Two unvaccinated cubs (Group 5) were separately housed and used as controls. In Group 4, the vaccinated cub was held in contact with 2 unvaccinated animals.

Serum was obtained by centrifugation of 5 ml of blood collected from the jugular vein of all foxes before vaccination (Day −3), on Day 14 and before challenge (Days 28, 180 or 360). It was stored at −20°C until testing.

Foxes of Groups 1, 4 and 5 were challenged by inoculation in the right temporal muscle with 1 ml of the viral dilution on Day 33. Foxes of Groups 2 and 3 were similarly challenged on Days 180 and 360, respectively.

Serological analysis

On Days −3, 14, 28 (all groups), 180 (Group 2) and 360 (Group 3), rabies virus-neutralising antibodies were determined by a fluorescence inhibition technique (RFFIT). Antibody titres are expressed in I.U. (International Units) per millilitre as determined by comparison with a standard serum (titre: 4.4 I.U. ml^{-1}) (Smith et al., 1973). The arbitrary defined level of 0.5 I.U. ml^{-1} in humans is considered indicative of successful rabies immunization.

On Days −3, 28 (Groups 1, 4 and 5), 180 (Group 2) and 360 (Group 3), vaccinia virus-neutralising antibodies were determined by a technique of seroneutralisation on Vero cells. Titres are expressed as the logarithm of the dilution neutralising 30 $TCID_{50}$ of vaccinia virus.

Rabies diagnosis

The rabies virus was sought in the dead foxes' brains by the analytical techniques recommended by the World Health Organisation: immunofluorescence and I.C. inoculation of mice (Kaplan and Koprowski, 1973).

TABLE 1

Vaccinia and rabies virus-neutralising antibodies and protection from rabies challenge in fox cubs vaccinated with $10^{7.2}$ p.f.u. of VVTGgRAB by the oral route

Animal		Sex	Treatment[a]	Rabies antibody titres (I.U. ml^{-1}) on day					Vaccinia antibody titres[b] on day				Resistance to challenge applied on day[c]		
Group	No.			−3	14	28	180	360	−3	28	180	360	33	180	360
1	152	M	Vaccinated	<0.46	4.45	9.95	–	–	0.47	0.82	–	–	S	–	–
	153	F		<0.46	3.07	4.92	–	–	0.70	0.82	–	–	S	–	–
	154	M		<0.46	7.85	20.90	–	–	<0.35	1.28	–	–	S	–	–
	155	M		<0.46	1.57	9.95	–	–	<0.35	0.82	–	–	S	–	–
2	156	M	Vaccinated	<0.46	1.37	3.06	0.73	–	<0.35	–	<0.35	–	–	S	–
	157	F		<0.46	3.06	5.25	3.17	–	<0.35	–	<0.35	–	–	S	–
	158	F		<0.46	5.25	4.45	5.33	–	<0.35	–	<0.35	–	–	S	–
	159	M		<0.46	3.06	5.25	6.07	–	<0.35	–	<0.35	–	–	S	–
3	160	M	Vaccinated	<0.46	1.52	4.45	–	7.62	<0.35	–	–	<0.35	–	–	ND
	161	F		<0.46	1.82	2.81	–	3.81	<0.35	–	–	<0.35	–	–	S
	175	F		<0.46	<0.46	2.81	–	5.09	<0.35	–	–	<0.35	–	–	S
	176	M		<0.46	<0.46	<0.46	–	<0.46	<0.35	–	–	<0.35	–	–	D(11)
4	172	F	Vaccinated	<0.46	<0.46	0.89	–	–	0.58	<0.35	–	–	S	–	–
	173	M	Unvaccinated	<0.46	<0.46	<0.46	–	–	<0.35	0.47	–	–	D(26)	–	–
	174	F	Unvaccinated	<0.46	<0.46	<0.46	–	–	0.58	0.58	–	–	D(25)	–	–
5	150	F	Unvaccinated	<0.46	<0.46	<0.46	–	–	<0.35	<0.35	–	–	D(23)	–	–
	151	F	Unvaccinated	<0.46	<0.46	<0.46	–	–	0.58	0.47	–	–	D(25)	–	–

[a]Day of vaccination = Day 0. [b]Results are expressed in logarithms of the dilution neutralising 30 $TCID_{50}$ of vaccinia virus. [c]S = survived, D = died, ND = accidentally died on Day 361; day of death after challenge is in parentheses.

RESULTS

The evolution of rabies and vaccinia virus-neutralising antibody titres and the length of post-challenge survival are given in Table 1.

On Day 28 post-vaccination, rabies virus antibody titres exceeding 0.5 I.U. ml^{-1} appeared in all but 1 cub vaccinated with VVTGgRAB (Groups 1, 2 and 3, fox No. 172 in Group 4). One cub in Group 3 (No. 176) failed to elicit any demonstrable antibody. A rabies seroconversion was recorded from the 14th day post-vaccination in 11 out of 13 vaccinated cubs. On Days 180 and 360 post-vaccination, cubs in Groups 2 and 3 (except No. 176) still possessed antibodies.

A vaccinia seroconversion was detected on Day 28 only in one cub (No. 154). This animal had developed the highest titre of rabies virus antibodies.

Eleven out of 12 animals receiving VVTGgRAB resisted challenge applied on Days 33 (Group 1 and fox No. 172), 180 (Group 2) and 360 (foxes Nos. 161 and 175 in Group 3). Fox No. 160 accidentally died on Day 361.

The unvaccinated cubs, held in contact with a vaccinated animal (foxes Nos. 173 and 174) did not elicit rabies or vaccinia virus-neutralising antibodies nor did the control animals (Group 5). All these unvaccinated animals and only the vaccinated cub No. 176 succumbed to rabies challenge. Rabies virus was confirmed by immunofluorescence in brain smears and I.C. inoculation of mice.

No clinical signs or lesions were recorded in any of the vaccinated foxes during 1, 6 or 12 months after vaccination.

DISCUSSION

Strain 26D3 of VVTGgRAB ($10^{7.2}$ p.f.u.), administered by the oral route to fox cubs, induces significant levels of rabies virus antibody and protects 92% of them (11/12) from rabies. Only 1 fox did not produce any rabies antibody nor resisted challenge. The duration of immunity conferred by this recombinant virus (a minimum of 12 months) corresponds to the length of protection required for fox vaccination in the field (Schneider and Cox, 1983). As shown by the absence of seroconversion in unvaccinated cubs maintained in close contact with a vaccinated animal, the excretion and transmission of immunising titres of VVTGgRAB vaccine could not be observed. This absence of direct or indirect horizontal transmission together with the absence of residual pathogenicity for a long-term period (a maximum of 12 months of observation) are 2 important factors indicative of vaccine safety. Despite the limited number of test animals, the efficacy and the innocuity of this VVTGgRAB vaccine can be established in young foxes as it has been in adults (Blancou et al., 1986).

The effect of maternal antibodies (cubs born from vaccinated vixen) on active immunisation of the young animal with the VVTGgRAB virus is now under study.

ACKNOWLEDGEMENTS

We express our gratitude to the following people who have made the work possible or have been involved in the study: D. Ducarme, Minister of Région Wallonne for Agriculture and Environment and Dr. R. Hens, President of the Société Vétérinaire pour la Protection Animale (S.V.P.A.) for financial support; E. Lejeune, D. Peharpre, J. Ambert, J. Bailley, A. Dardaine and F. Patron for laboratory analysis and maintenance of foxes.

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