J. gen. Virol. (1989), **70**, 1601–1604. *Printed in Great Britain*

Key words: *fox/rabies/vaccinia virus*

Interaction between Rabies Infection and Oral Administration of Vaccinia–Rabies Recombinant Virus to Foxes (*Vulpes vulpes*)

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(*Accepted 3 March 1989*)

SUMMARY

We have investigated the influence of anti-rabies vaccination on the onset of the disease as well as the delay of death in foxes previously infected with rabies virus. A live vaccinia recombinant virus expressing the rabies virus glycoprotein (VVTGgRAB) was used as vaccine. Foxes were divided into six experimental groups of four animals. On day 0, each fox was experimentally infected with a rabies virus suspension. VVTGgRAB was administered by the oral route to each animal of three groups on day 0, 3 or 14. Foxes of other groups were used as unvaccinated controls or received, on day 0, the parental Copenhagen strain of vaccinia virus. The length of post-challenge survival and duration of clinical disease were recorded for each animal tested. All foxes, vaccinated or not, died from rabies as confirmed by fluorescent antibody tests. Animals vaccinated on days 0 and 3 died after a shorter period of incubation than unvaccinated controls. On the other hand, animals vaccinated on day 14 post-challenge died later than control animals. Foxes administered vaccinia virus died at the same time as unvaccinated controls. These results demonstrate that early and late death phenomena, as consequences of interactions between oral vaccination with VVTGgRAB and rabies infection, can occur in foxes.

Attempts to control rabies by vaccinating wild carnivores with attenuated strains of rabies virus seem promising (Steck *et al.*, 1982; Schneider & Cox, 1983; Pastoret *et al.*, 1987; Brochier *et al.*, 1988*a*). However, the use of conventional vaccines remains controversial as far as their innocuity and heat stability are concerned. In order to improve both the safety and stability of the vaccine used in the field, a recombinant vaccinia virus (VV) expressing the immunizing glycoprotein of rabies virus (VVTGgRAB) has been developed (Kieny *et al.*, 1984). The potential of this recombinant vaccinia–rabies virus to protect foxes (*Vulpes vulpes*) (adult and young), raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) against rabies has already been demonstrated. Oral, intradermal and subcutaneous administrations of VVTGgRAB to those target species elicit high levels of rabies virus-neutralizing antibodies and long-term protection against rabies (Blancou *et al.*, 1986; Rupprecht *et al.*, 1986; Tolson *et al.*, 1987; Brochier *et al.*, 1988*b*). VVTGgRAB oral administration was shown to be innocuous for target species (foxes, raccoons, striped skunks) as well as non-target animals (four laboratory, four domestic and 13 wild European species) (Wiktor *et al.*, 1985; Blancou *et al.*, 1986, 1988; Rupprecht *et al.*, 1986; Tolson *et al.*, 1987; Brochier *et al.*, 1988*c*; B. Brochier, unpublished results). Furthermore, as far as safety is concerned, the absence of horizontal transmission of VVTGgRAB has been confirmed in foxes, badgers (*Meles meles*), wild boars (*Sus scrofa*), cattle, dogs and ferrets (*Putorius furo*) (Brochier *et al.*, 1988*c*).

It is also of major importance to preclude some epizootic risks such as the emergence of asymptomatic carriers of rabies virus. This situation could occur in the field by vaccinating naturally infected animals during the incubation period. Among interactions between vaccination and infection, the early death phenomenon (i.e. reduction of the incubation period in an animal vaccinated after infection) has been experimentally observed in monkeys and mice (Sikes *et al.*, 1971; Blancou *et al.*, 1980; Bijlenga *et al.*, 1988). It appears that early death may also occur in humans who have been treated with vaccine, with or without accompanying serum therapy, after exposure to rabies virus. The humoral immune response (neutralizing antibodies) seems to play a role in the early death phenomenon (Blancou *et al.*, 1979*b*; Andral *et al.*, 1981; Prabhakar & Nathanson, 1981).

In a previous experiment on fox vaccination against rabies with an inactivated vaccine, an early death phenomenon was suspected but could not be demonstrated since several parameters (time interval between infection and vaccination, time of vaccination, route and dose of inoculation, site of biting, etc.) were not controlled (Thiriart *et al.*, 1985). The purpose of this study was to investigate in foxes the interaction between experimental rabies infection and post-exposure vaccination with VVTGgRAB.

Twenty-four adult foxes trapped in the wild were raised in the experimental centre of Atton (Nancy, France). After capture, they were sexed, marked, treated against parasites and vaccinated against distemper, Rubarth's disease and leptospirosis as previously described (Dubreuil *et al.*, 1979). They were shown to be serologically negative for rabies virus before being included in the study. In the first trial, 16 foxes were divided into four experimental groups of four animals (groups A, B, C and D). On day 0, each of them was challenged by inoculation in the right temporal muscle with 1 ml of a rabies virus suspension containing $10^{3\cdot6}$ mouse intracerebral (i.c.) LD_{50}. This virus challenge suspension consisted of a homogenate of salivary glands of foxes that had died from natural rabies (strain GS 7) (Blancou *et al.*, 1979*a*). A suspension of 10^8 $TCID_{50}$ of live modified vaccinia (Copenhagen strain)–rabies glycoprotein (ERA strain) recombinant virus (VVTGgRAB-26D3 187XP strain) was orally administered to each animal of groups A, B and C on day 0, 3 or 14 respectively. Foxes of group D were used as unvaccinated controls.

In the second trial, eight foxes were divided into two experimental groups of four animals (groups E and F). On day 0, each fox was challenged by inoculation in the right temporal muscle with 1 ml of the virus suspension containing $10^{2\cdot8}$ mouse i.c. LD_{50}. On day 0, $10^{7\cdot3}$ $TCID_{50}$ of the parental Copenhagen strain of VV was administered by the oral route to each animal of group E; foxes of group F were used as uninoculated controls. In both trials, VVTGgRAB and VV were administered by direct application into the mouth via a needle-less syringe (vol. 1 ml). The duration of clinical disease and the day of death post-challenge were recorded for each animal tested. Since the delays of death do not follow a Gaussian distribution (Blancou *et al.*, 1979*b*), these data were analysed according to the non-parametric Mann-Whitney test. The results were compared according to the one-failed-critical values given by Zar (1974). All foxes succumbed to rabies virus challenge. Rabies was confirmed by the analytical techniques recommended by the World Health Organization: fluorescent antibody tests and i.c. inoculation of mice (Koprowski, 1973).

Table 1 gives the length of post-challenge survival and duration of clinical disease of each fox included in trial no. 1. The delays of death of groups A and B, taken as a whole, were statistically shorter (mean values 17·25 days) than that of unvaccinated control foxes (group D) (mean value 19·25 days). The significance level was 5%. Although animals of group C died later (mean value 22·25 days) than those of group D, the significance level of this difference was only 10%. Table 1 also gives the length of post-challenge survival and duration of clinical disease of each fox included in trial no. 2. Animals of groups E and F, challenged with $10^{2\cdot8}$ LD_{50} (mouse i.c.), similarly died on days 17 to 19 post-challenge (mean values 18·25 days). As shown in Table 1, no difference in the duration and pattern of clinical disease could be observed between the six experimental groups (mean values 2·75 days in groups A, B, C and D; 1 and 1·25 days in groups E and F respectively).

These results demonstrate that the early death phenomenon, as a consequence of interaction

Table 1. *Effect of oral inoculation of foxes with VVTGgRAB (groups A, B and C) or VV (group E) on the same day or several days after rabies challenge*

Trial	Group	Fox no.	Rabies challenge (mouse i.c. LD_{50}; day 0)	Day of vaccination	Day of death post-challenge		Duration of clinical disease (days)	
1	A	655	$10^{3\cdot6}$	0	15	$\bar{x}=17\cdot25$	3	$\bar{x}=2\cdot75$
		656		0	17		2	
		657		0	18		2	
		658		0	19		4	
	B	664	$10^{3\cdot6}$	3	15	$\bar{x}=17\cdot25$	2	$\bar{x}=2\cdot75$
		665		3	17		4	
		666		3	18		3	
		667		3	19		2	
	C	168	$10^{3\cdot6}$	14	20	$\bar{x}=22\cdot25$	2	$\bar{x}=2\cdot75$
		169		14	22		3	
		170		14	23		3	
		171		14	24		3	
	D	162	$10^{3\cdot6}$	Unvaccinated controls	17	$\bar{x}=19\cdot25$	2	$\bar{x}=2\cdot75$
		163			20		2	
		177			20		2	
		178			20		5	
2	E	TVP1	$10^{2\cdot8}$	0	19	$\bar{x}=18\cdot25$	1	$\bar{x}=1$
		TVP2		0	19		2	
		TVP3		0	18		0	
		TVP4		0	17		1	
	F	700	$10^{2\cdot8}$	Unvaccinated controls	19	$\bar{x}=18\cdot25$	1	$\bar{x}=1\cdot25$
		718			19		2	
		720			18		1	
		777			17		1	

between vaccination and rabies infection, can occur in foxes as well as in mice and monkeys as previously demonstrated by other workers. It may explain the previous observations of early death occurring in naturally infected foxes vaccinated by the parenteral route with an inactivated vaccine (Thiriart *et al.*, 1985). Moreover, a late death phenomenon as previously observed in mice (Bijlenga *et al.*, 1988) appears in foxes when vaccinated a few days before clinical disease. Such variations in the incubation period may occur in the field during fox vaccination campaigns.

The absence of protection is observed in all foxes included in this study. Both death phenomena are thus likely to impede the emergence of survivors of rabies infection and subsequently lead to asymptomatic carriers of rabies virus. Therefore, there do not seem to be peculiar epidemiological risks in similar natural conditions. Nevertheless, other effects could occur when vaccinating at other time intervals after or before natural infection. In this regard, field conditions need to be considered: foxes are liable to ingest one or several vaccine doses at different times before or after natural contamination; furthermore, the time intervals between successive ingestions can vary widely. Post-exposure protection with two successive intramuscular administrations of VVTGgRAB has already been conferred to hamsters (Wiktor *et al.*, 1985). It could be suggested that curative vaccination of foxes incubating rabies would be a possible event in the wild. Further investigations including other time intervals between vaccination and natural infection need to be done.

It is also interesting to note that the rabies virus glycoprotein (the only rabies virus component of VVTGgRAB) administered by the oral route can induce the early death phenomenon. This effect could not be induced in animals inoculated with the parental strain of VV. It does not mean that the other rabies virus antigens do not participate in the phenomenon, but rather the glycoprotein may account for the effect. This result confirms that the early death reaction to vaccine in post-exposure treatment is likely to be caused by neutralizing antibodies (induced by the glycoprotein) as has been demonstrated in other animal species (Blancou *et al.*, 1979*b*). Previous studies have also demonstrated that neutralizing antibodies and interferon are

involved in the post-exposure protection. The immune response induced by oral administration of VVTGgRAB (i.e. kinetics and levels of interferon and neutralizing antibodies) needs to be evaluated and compared to the response induced by attenuated strains of rabies virus.

The authors thank J. Bailly and A. Dardaine for maintenance of captive animals and l'Association des Déterreurs (France) for fox captures. They thank also M. Muys for the assistance in preparing this manuscript. This work was supported by a grant from the Ministry of Région wallonne for Agriculture and Environment.

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(Received 26 October 1988)