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VACCINATION OF YOUNG FOXES (*VULPES VULPES, L.*) AGAINST RABIES: TRIALS WITH INACTIVATED VACCINE ADMINISTERED BY ORAL AND PARENTERAL ROUTES

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Résumé

VACCINATION ANTIRABIQUE DE RENARDEAUX (*VULPES VULPES, L.*) A L'AIDE D'UN VACCIN INACTIVÉ ADMINISTRÉ PAR VOIES ORALE ET PARENTÉRALE. — Un essai de vaccination antirabique à l'aide de virus inactivé a été pratiqué chez des renardeaux âgés de deux à trois mois. Trois différents procédés d'immunisation ont été testés: un premier groupe d'animaux fut vacciné par voie parentérale à l'aide d'une seule dose de vaccin (Rabisin®); les deux autres groupes ont été soumis à une ingestion quotidienne pendant dix jours du vaccin soit sous sa forme commerciale liquide (Rabisin®) soit présenté en comprimés gastro-résistants. Ces comprimés contenaient l'association du virus rabique inactivé à un virus potentiellement entéropathogène chez le renard (rotavirus bovin). Cette association virale a été réalisée pour voir si une séroconversion pouvait apparaître dans de telles conditions. L'immunisation conférée a été évaluée par examen sérologique et épreuve virulente. Une séroconversion fut observée dans deux groupes d'animaux à partir du 20e jour suivant la vaccination: les titres en anticorps sériques ont atteint des valeurs satisfaisantes chez les animaux vaccinés par voie parentérale; des valeurs plus faibles ont été enregistrées chez la plupart des renardeaux ayant ingéré le vaccin liquide. L'administration de tablettes gastrorésistantes contenant l'association virale n'a induit aucune séroconversion. De plus, aucune multiplication de la souche de rotavirus utilisée n'a pu être décelée. La vaccination par voie parentérale n'a conféré qu'une résistance partielle à l'épreuve virulente.

The red fox (*Vulpes vulpes, L.*) is the main vector of rabies in Belgium, as in most Western European countries. The reduction of its population either by shooting or by gassing the dens has not proved to be fully effective in controlling the disease. Furthermore, the threat to public health could become more serious since the suburban population of foxes appears to be increasing in these countries. Therefore, other methods such as vaccination of foxes need to be assessed. Several papers have described the use of modified-live rabies vaccines given by the oral route (Winkler and Baer, 1976; Dubreuil *et al.*, 1979; Blancou, 1979; Black and Lawson, 1980; Steck *et al.*, 1982; Haefliger *et al.*, 1982; Wachendoerfer *et al.*, 1984).

The safety and the innocuity of oral modified-live vaccines (MLV) tested in the fox is doubtful for non-target species susceptible to ingest the vaccine-containing baits. Vaccine-induced rabies in other wildliving mammals can have serious epidemiological consequences in the field. Rabies

Table 1. — Individual characteristics of the cubs and treatment applied to the five experimental groups formed.

Experimental group (vaccination procedure) litter animal (sexe)[a]	weight (kg) on day 0 (vaccination)	weight (kg) on day 30 (challenge)
Group A (unvaccinated)		
litter 1		
101 (F)	2.1	2.5
102 (M)	2.4	3.2
104 (M)	1.9	2.5
Group B (inactivated vaccine; oral route; 1 ml mixed with food, 10 daily ingestions)		
litter 2		
105 (M)	1.7	2.3
106 (M)	2.2	2.6
107 (F)	1.7	2.4
108 (M)	2.5	3.3
109 (M)	2.4	3.4
Group C (inactivated vaccine and live bovine rotavirus; oral route; 5 coated tablets mixed with food; 10 daily ingestions)		
litter 3		
110 (F)	3.2	3.4
111 (F)	3.1	3.5
112 (F)	3.0	3.5
113 (M)	3.2	3.4
114 (M)	3.2	3.4
litter 1		
103 (M)	2.3	3.0
Group D (inactivated vaccine; parenteral route (subcutaneous); one injection)		
litter 3		
115 (F)	2.6	3.5
116 (M)	1.6	2.3
117 (M)	2.0	3.0
litter 4		
118 (M)	1.9	2.6
119 (M)	2.5	3.3
Group E (live bovine rotavirus; oral route; one inoculation)		
litter 5		
120 (F)	2.2	2.5
121 (M)	2.2	2.5
122 (F)	2.4	3.2

a: M, male; F, female

has been reported in several species after vaccination with MLV (Esh *et al.*, 1982; Whetstone *et al.*, 1984).

The use of monoclonal antibodies confirms these vaccine-induced rabies cases; this technique has permitted the identification of vaccine-induced rabies in a fox vaccinated with the modified-live Flury LEP strain (Whetstone *et al.*, 1984). Moreover, the stability of a MLV contained in baits is required in order to keep the viral strain titre above the immunizing critical level.

According to the recommendations of the International Office of Epizooties (IOE), the ultimate goal for vaccination of foxes against rabies should be an inactivated vaccine (Andral and Blancou, 1982). Trials of vaccinating foxes using an inactivated vaccine parenterally administered were attempted in the field (Seidler *et al.*, 1982). Experimental studies have reported the failure to immunize or sufficiently immunize foxes either by oral vaccination with inactivated rabies virus contained in coated tablets (Blancou *et al.*, 1984) or by intestinal instillation (Lawson *et al.*, 1982). Nevertheless, seroconversion followed by protection against experimental rabies was obtained in cats orally vaccinated with inactivated virus (Metianu, 1981; Sureau *et al.*, 1981). The purpose of this paper was to study the immunizing capacity of an inactivated vaccine administered to young foxes.

The evolution of antibody titres and the length of survival post-challenge were evaluated after oral and parenteral vaccination. The oral vaccine was presented coated (gastro-resistant tablets) or uncoated (liquid form). The coated vaccine was associated with an enteropathogenic live virus in order to see if seroconversion can occur in these conditions.

A serological survey performed in order to determine the prevalence of enteric virus infections in foxes has reported that rotavirus infections are much more common and widespread in this species (Schwers *et al.*, 1984). Furthermore, multiplication and excretion of bovine rotavirus have been demonstrated in young dogs experimentally infected (Schwers *et al.*, 1983).

Therefore, assuming that the same is true for young foxes, bovine rotavirus was used as the vaccine-associated enteropathogenic virus.

Materials and Methods

Animals

Capture

In May 1984, twenty-two wild foxes (*Vulpes vulpes.* L.) aged between 2 and 3 months were captured from 5 dens situated in a rabies-free area of Belgium (Province

of Brabant Wallon). The method of capture is as described by Seidler and co-workers (1982).

Housing

The cubs were raised in captivity in the Institut Pasteur du Brabant (Brussels) for two months. The housing of the foxes consisted of individual (vaccination step) or familial mobile wire cages, daily cleaned and supplied with wood chips.

Diet

The diet consisted of reconstituted dog milk (Welpidog®) supplemented with minerals and vitamins (Pecutrine®). As the cubs grew up, milk was progressively substituted with water and meat consisting mainly of dead mammals (rats, mice, rabbits), birds (pigeons, chicken), fish and commercially available food for domestic carnivora.

Treatments

After capture, a parasitological examination by coprological analysis was applied and followed by a systematic treatment against coccidia (S-mez®), nematods and cestods (Lopatol®). All young foxes were weighed, sexed, marked with auricular buckles and then divided into five experimental groups (table 1). They were shown to be serologically negative for rabies antibodies before they were included in this study.

Handling

All manipulations of the live cubs were carried out under anaesthesia. Each animal was intramuscularly injected with Hypnorm® (Janssen Pharmaceutica, Beerse) before weighing, blood taking or transport.

Viruses

Rabies vaccine

Inactivated vaccine, kindly provided by Dr. Vet. G. Chappuis (Rhône Mérieux, France) was produced with fixed rabies virus (strain GS 57 WISTAR) on cultures of NIL 2 cells, inactivated with β propiolactone and absorbed onto aluminium hydroxyde (Précausta *et al.*, 1982).

The batch employed had a titre of minimum 10^7 LD 50 mouse per ml dose. The vaccine was presented in a commercially available liquid form (Rabisin®) either by oral or parenteral route. On the other hand, the rabies vaccine associated to live bovine rotavirus was given orally in coated tablets (5 tablets = 1 ml dose). The rabies virus contained in these tablets was not adsorbed onto aluminium hydroxyde (absence of adjuvant).

Live bovine rotavirus

Rotavirus (strain S 14), isolated from stools of diarrhoeic calves was cultured on MA 104 cells (Dagenais *et al.*, 1981). The viral suspension used for inoculating the control foxes (Group E, table 1) had a titre of 5×10^7 PFU (Plaque Forming Unit) per ml.

Challenge virus

The virus challenge suspension consisted of a homogenate of salivary glands of foxes dead of natural rabies (lot GS 6). The titre was determined by intracerebral (IC) inoculaton of mice.

The inoculum consisted of one millilter with a titre of $10^{3.6}$ (Groups A, B, D) or $10^{3.7}$ (Groups C, E) mouse IC LD 50.

Vaccination

The 22 animals were divided into five experimental groups and every cub in a group was treated by one of the following procedures (table 1):

Group A (3 foxes):
unvaccinated controls.

Group B (5 foxes):
day 0 to day 10, daily ingestion of 1 ml Rabisin® mixed with the food.

Group C (6 foxes):
day 0 to day 10, daily ingestion of five gastroresistant tablets containing Rabisin® associated to live bovine rotavirus. The tablets were mixed with the food.

Group D (5 foxes):
day 0, parenteral administration (subcutaneous injection) of 1 ml of Rabisin®.

Group E (3 foxes):
day 0, inoculation by the oral route with 0.5 ml of a live bovine rotavirus suspension.

Sampling

Plasma was obtained by centrifugation of 5 ml of blood collected from the jugular vein of all foxes before vaccination (day 0) and at different times thereafter (days 4, 8, 12, 16, 20, 24 and 28).

Faeces were collected in the cages on days 0, 4, 8, 12, 16, 20, 24 and 28.

Challenge

For this experimental step, all foxes were transfered to the Centre National d'Études sur la Rage of Malzéville (Nancy, France).

The rabies challenge was made by inoculation in the right temporal muscle with 1 ml of the viral dilution on day 30 for groups A, B, D and on day 42 for groups C, E.

Serological analysis

Serum neutralizing antibodies were determined by a technique of inhibition of fluorescence (RFFIT) (Smith *et al.*, 1973). The titres obtained by this method are expressed in International Units (IU) as determined by comparison with a standard serum furnished by the World Health Organization and are considered as protective when exceeding 0.5 IU/ml.

On day 28 post-vaccination, the serum titres were also determined using the technique of seroneutralization on mice recommended by the WHO (Kaplan and Koprowski, 1973). The titres of the sera are expressed in IU determined by comparison with a standard serum (titre: 65 IU/ml) furnished by the Institut Pasteur in Paris. The sera collected on days 0 and 28 were tested for the presence of antirotavirus antibodies using the counterimmunoelectroosmophoresis (CIEOP) technique described by Middleton *et al.* (1976).

Rotavirus excretion

The presence of rotavirus in stools was tested by CIEOP following the method described by Middleton *et al.* (1976).

Table 2. — Post-vaccination serological data and

Group	Fox No.	RFFIT[a] day 0	4	8	12	16	20	24	28
Group A									
	101	<0.16	<0.07	0.09	<0.32	<0.32	<0.32	<0.32	<0.32
	102	<0.16	<0.07	<0.33	<0.32	<0.32	<0.32	<0.32	<0.32
	104	<0.16	<0.07	<0.07	<0.32	<0.32	<0.32	<0.32	<0.32
Group B									
	105	<0.16	<0.07	<0.22	<0.22	<0.22	0.41	0.48	0.45
	106	<0.16	<0.09	<0.22	<0.22	0.30	*0.53*	*0.55*	0.43
	107	<0.16	<0.07	<0.16	<0.22	*1.62*	*0.61*	0.41	0.27
	108	<0.16	<0.07	<0.15	<0.22	0.32	*0.58*	*0.79*	0.48
	109	<0.16	<0.15	<0.25	<0.31	<0.32	0.40	*0.53*	<0.31
Group C									
	110	<0.16	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24
	111	<0.16	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24
	112	<0.16	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24
	113	<0.16	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
	114	<0.16	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
	103	<0.16	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
Group D									
	115	<0.16	<0.31	<0.31	<0.31	0.41	*0.60*	*0.77*	*0.80*
	116	<0.16	<0.31	<0.31	0.49	*0.63*	*2.64*	*4.22*	*2.64*
	117	<0.16	<0.36	<0.36	<0.36	*0.50*	*0.74*	*1.26*	*2.88*
	118	<0.16	<0.36	<0.36	<0.36	0.36	*0.91*	*0.99*	*3.11*
	119	<0.16	<0.36	<0.36	<0.36	0.48	*1.06*	*1.39*	*2.11*
Group E									
	120	<0.16	<0.32	<0.32	<0.32	<0.32	<0.32	<0.32	<0.32
	121	<0.16	<0.32	<0.32	<0.32	<0.32	<0.32	<0.32	<0.32
	122	<0.16	<0.32	<0.32	<0.32	<0.32	<0.32	<0.32	<0.32

a: rabies antibody titres (IU); EU: animal euthanasied on day 90 post-challenge; ND: not done

Rabies diagnosis

The rabies virus was sought in the dead foxes brain by the analytical techniques recommended by the WHO: immunofluorescence, IC inoculation of mice (Kaplan and Koprowski, 1973).

Results

The evolution of rabies antibody titres as evaluated by the RFFIT technique is given in table 2. Antibody titres exceeding 0.5 IU/ml appeared in two out of the five groups from approximately day 20 post-vaccination: all the cubs in group D and 4 out of the 5 cubs in group B developed neutralizing antibodies. Titres recorded in group D were significantly higher than those obtained in group B.

The foxes that received gastroresistant tablets (group C) failed to elicit any demonstrable antibody.

The technique of seroneutralization on mice, performed on day 28 post-vaccination detected a seroconversion only in the parenterally vaccinated foxes (group D) (table 2).

The absence of anti-rotavirus antibody was noticed in all the foxes before vaccination (day 0). On day 28, none of the cubs inoculated with bovine rotavirus (groups C and E) presented seroconversion.

However, an asymptomatic excretion of rotavirus was observed in four cubs (group C): no. 111

deaths post-challenge of the foxes in groups A, B, C, D and E.

Group	Fox No.	Seroneutralisation on mice[a] day 28	Seroneutralisation on mice[a] day 120	Death in days post-challenge	Rabies diagnosis
Group A					
	101	0	...	24	+
	102	0	...	20	+
	104	0	...	19	+
Group B					
	105	0	...	21	+
	106	0	...	21	+
	107	0	...	21	+
	108	0	...	18	+
	109	0	...	21	+
Group C					
	110	0	...	20	+
	111	0	...	16	+
	112	0	...	17	+
	113	0	...	22	+
	114	0	...	17	+
	103	0	...	17	+
Group D					
	115	0.03	0.33	EU	—
	116	0.33	...	60	ND
	117	0.33	0.09	EU	—
	118	0.09	...	90	+
	119	0.13	0.03	EU	—
Group E					
	120	0	...	18	+
	121	0	...	18	+
	122	0	...	17	+

on day 0, no. 112 on days 0 and 16, no. 113 on days 4 and 20, no. 114 on days 0 and 4.

As shown by immunofluorescence, 18 out of the 22 foxes died after rabies challenge. The length of post-challenge survival is given in table 2. In group D, one fox died from rabies on day 90 post-challenge.

Discussion

As shown by the RFFIT technique, the parenterally vaccinated foxes (group D) raised antirabies antibodies from approximately the 20th day post-vaccination. On the challenge day, the recorded titres are elevated but only partially protective since one cub of this group (no. 118) died of rabies three months post-challenge. One cub of this group (no. 117) died accidentally and the others were euthanazied three months post-challenge. Therefore, the real persistence of protection in unknown; however, this is not the purpose of this study.

The young age of the foxes and the administration route (subcutaneous) could be related to this partial protection; similar trials should be attempted using adult foxes and/or other parenteral routes (intramuscular, intradermal).

A lower but significant seroconversion was detected in most of the cubs of the group B (uncoated vaccine, repeated administration by the oral route). Nevertheless, these antibody titres are not protective since all the animals of this group died at the same time as those kept as unvaccinated controls (approximately the 20th day post-challenge). In this group, the specific antibodies

appeared from the 20th day post-vaccination but the titres decreased rapidly. The antigenic processing could have occurred through natural lesions encountered in the oro-pharyngeal mucosa. Previously, it has been observed that inactivated rabies vaccine, exploded in foxes'mouths, induces a rise in serum-neutralizing antibodies accompanied by protection (Baer, 1975).

In fact, the vaccine was used in this case in a form completely unprotected against digestive processing. In this context, oral vaccines containing mechanical agents susceptible to induce artificial lesions of the oral mucosa should be tested.

The absence of seroconversion characterized the group C (gastro-resistant tablets containing the viral association; repeated administration by the oral route). As observed in the control group E (rotavirus inoculation), the associated bovine rotavirus did not multiply in the gut mucosa of the 6 treated foxes; therefore an eventual helping effect of rotavirus infection could not occur. Moreover, the absence of seroconversion could partially be explained by the absence of adjuvant in the tablets.

Previous experiments have already shown that foxes are more easily immunized in the buccal cavity than in the stomach or intestines (Baer, 1975).

Other biological agents such as coccidia and hematophage nematods are more susceptible to induce injuries in the gut mucosa of foxes. Moreover, these natural parasitical infestations are widespread and have more clinical consequences in cubs (haemorrhagic diarrhœa).

Therefore, a similar trial of oral vaccination could be carried out in cubs untreated with anthelmintics and coccidiostatic compounds. This was not the case in the present experiment. Among the enteropathogenic viruses, the coronavirus represents a candidate but the incidence of this infection is low in the fox population and this virus is less resistant than rotavirus (Barrat *et al.*, 1984).

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Summary

Foxes aged between two and three months were vaccinated with an inactivated rabies vaccine. Three immunization procedures were attempted: a first group of animals was parenterally injected while two other groups daily ingested during ten days either an uncoated vaccine (commercially available liquid form: Rabisin®) or a coated vaccine (gastro-resistant tablets) associated with a potential enteropathogenic virus (bovine rotavirus). The latter viral association was used in order to see if seroconversion can occur in these conditions. Rabies antibody titres and the length of survival postchallenge were recorded in each treated fox. Seroconversion was observed from approximately day 20 post-vaccination in two groups of cubs: satisfactory antibody titres were found in parenterally vaccinated foxes while lower titres characterized most of the cubs which ingested the uncoated liquid vaccine. Gastro-resistant tablets containing the viral association did not induce seroconversion. It was shown that bovine rotavirus did not multiply in the gut mucosa of young foxes, on the contrary to young dogs. Protection against experimental rabies was partially obtained in parenterally vaccinated foxes.

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