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IMMUNIZATION OF YOUNG FOXES AGAINST RABIES: INTERACTION BETWEEN VACCINATION AND NATURAL INFECTION

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Résumé

IMMUNISATION ANTIRABIQUE CHEZ DES RENARDEAUX: INTERACTION ENTRE LA VACCINATION ET L'INFECTION NATURELLE. — Une expérience préliminaire de vaccination antirabique de renardeaux (*Vulpes vulpes L.*) à l'aide d'un vaccin inactivé administré par voie parentérale a été menée en Belgique en zone enzootique de rage (Province du Luxembourg). Parmi les 20 renardeaux capturés, 12 ont développé la rage durant leur période de captivité. La vaccination a induit une rapide et importante séroconversion chez la plupart des renardeaux sains mais n'a pu enrayer l'évolution de la maladie chez les renardeaux en période d'incubation. Chez ces derniers, la vaccination semble au contraire avoir accéléré l'évolution de la maladie.

Vaccination of foxes has received increased interest in recent years and several researchers have already proposed and studied this method. Several papers describe the use of live rabies vaccines given by the oral route either directly by instillation into the animal's mouth or indirectly by feeding them with vaccine-impregnated baits (Winkler and Baer, 1976; Blancou, 1979; Black and Lawson, 1980; Andral and Blancou, 1982; Steck *et al.*, 1982).

Other workers attempted to develop methods for vaccinating foxes in the field using an inactivated vaccine (Seidler *et al.*, 1982).

According to the recommendations of the Office International des Epizooties (OIE), the ultimate goal in the vaccination of foxes against rabies should be the use of an inactivated vaccine (Andral and Blancou, 1982).

This report describes a preliminary trial of vaccination with a killed vaccine in order to evaluate the possible consequences of vaccinating, in the field, already infected animals or animals infected a few days after vaccination.

Materials and Methods

Animals

Nineteen red fox cubs (*Vulpes vulpes L.*) belonging to four litters were captured from their dens between 7 and 21 May 1983 in the Province of Luxembourg (Belgium), using the method described by Seidler *et al.* (1982).

An isolated animal (number 12) was captured alone

and, as it was approximately of the same age as the animals of litter 2, it was joined to this group (table 1).

Vaccines

Inactived commercially available vaccine (Rabisin®, Mérieux) for domestic animals, conditioned in individual vials, was used (Précausta *et al.*, 1982).

This vaccine is produced from fixed rabies virus grown on NIL 2 (hamster embryo) cells, purified by filtration and inactivated with β-propiolactone.

Blood taking and vaccination

In each litter, half of the cubs were inoculated 2 or 3 days after capture with one dose of Rabisin® by the subcutaneous route. The others were kept as unvaccinated controls. They were bled from the jugular vein on days 0 (day of vaccination), 8, 15, 22, 29 and 36.

Serological analysis

Serum neutralizing rabies antibodies were assayed by a fluorescence-inhibition technique (RFFIT) (Smith *et al.*, 1973).

The titers obtained by this method are expressed in International Units (IU), as determined by comparison with a standard serum supplied by the World Health Organization, and are considered as protective when they exceed 0.5 IU/ml.

Rabies diagnosis

All the foxes that died after showing clinical signs of rabies and 4 out of the 8 healthy foxes (2 from each litter) that were euthanasied at the end of the experiment were examined for rabies. Brain tissue from each animal was tested for the presence of infectious rabies virus by the standard fluorescent rabies antibody (FRA) test (McQueen *et al.*, 1960). Those that were negative by this technique were tested by suckling mouse intracerebral inoculation (Koprowski, 1973).

Results

The characteristics, serological results and the length of survival of the rabid foxes vaccinated or not are given in table 1. The characteristics and serological results of the non rabid foxes are given in table 2. These animals were euthanasied at the end of the experiment.

Interaction between vaccination and natural infection in rabid foxes

All the contaminated animals (table 1), vaccinated or not, that were infected before vaccination (fox 12) or a few days thereafter (foxes 1 to 11) died of rabies after a more or less long evolution of the disease.

Table 1 shows that the foxes of litter 2, vaccinated or not, died very soon after capture after being contaminated by fox 12 that was already rabid at the time of capture.

In the older group (litter 1), it was observed that the three vaccinated animals (one of them did not elicit any rabies antibodies) (foxes 1, 2 and 3) died 3 to 15 days before the three foxes (4, 5, 6) kept as unvaccinated controls (table 1).

Serological data

— Rabid foxes

As confirmed by immunofluorescence, twelve out of twenty foxes died of rabies. The antibody titres of animals from litter 1 (foxes 1 to 6) and litter 2 (foxes 7 to 12) are given in table 1.

— Non rabid foxes

Table 2 illustrates the neutralizing antibody

Table 1. — Characteristics, serological results and length of survival for rabid foxes.

Animals	Vaccination on day 0	Serological results (IU/ml) Day 0	Day 8	Day 15	Day 22	Day 28	Day 36	Day of death
Litter 1 (3 months-old)								
Fox 1 (M)	Yes	< 0.5[a]	ND[b]	5	13.94	†[c]	†	28
Fox 2 (F)	Yes	< 0.5	ND	4.63	2.39	< 0.5	†	29
Fox 3 (M)	Yes	< 0.5	ND	< 0.5	< 0.5	< 0.5	†	29
Fox 4 (F)	No	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	40
Fox 5 (M)	No	< 0.5	ND	< 0.5	< 0.5	< 0.5	†	33
Fox 6 (M)	No	ND	ND	< 0.5	< 0.5	< 0.5	< 0.5	44
Litter 2 (5 weeks old)								
Fox 7 (M)	Yes	< 0.5	< 0.5	< 0.5	†	†	†	18
Fox 8 (F)	Yes	< 0.5	ND	< 0.5	†	†	†	16
Fox 9 (M)	Yes	< 0.5	ND	< 0.5	†	†	†	16
Fox 10 (M)	No	ND	1.05	1.35	†	†	†	17
Fox 11 (M)	No	< 0.5	< 0.5	< 0.5	†	†	†	15
Fox 12 (M)	Yes	< 0.5	†	†	†	†	†	3

a : titres inferior to 0.5 are considered as negative. b : ND : not done. c : † animal dead.

titres of this group. Three out of four foxes developed neutralizing antibodies; the last animal failed to elicit any demonstrable antibodies over four weeks after vaccination.

Discussion and Conclusions

The 20 young foxes used in this study were captured in an enzootic area.

As shown by immunofluorescence, 12 out of the 20 foxes (litters 1 and 2) died of rabies.

Rabies seems to occur in very young animals much more often than commonly admitted (Artois and Aubert, personal communication). But as the animals die in the den, this phenomenon remains hidden most of the time.

Half of the rabid foxes were vaccinated either during the incubation period or perhaps a very few days before infection. If vaccination of foxes against rabies were applied in the field to control rabies, such a condition would be largely encountered. In spite of vaccination, all the rabid foxes died. The vaccine used was therefore neither curative nor protective during the first days post vaccination. Some of the vaccinated foxes died even before the unvaccinated control animals (table 1). Early death (Blancou *et al.*, 1980; Porterfield, 1981; Prabhakar and Nathanson, 1981) can occur in foxes as well as in other species. Therefore, there does not seem to be any hazard arising from the use of inactivated vaccines given by the oral route. Since such vaccines do not protect rabid foxes from death, the risk of creating asymptomatic carriers can be discarded.

Serological data on rabid foxes is given in table 1. These results are striking because none of the five unvaccinated rabid foxes (except fox 10 that elicited a response) produced antibodies against rabies. Infected animals would in fact be expected to elicit antibodies against natural rabies virus in the final stages of the disease. Only two of the six vaccinated rabid animals responded to vaccination but they showed very high antibody titres.

Serological data obtained from the four healthy foxes (table 2) show that 3 out of 4 vaccinated foxes developed rabies neutralizing antibodies. The titres recorded were high. A rapid seroconversion occurred since titres exceeding 0.5 IU/ml appeared on day 8 for foxes 14 and 16, and on day 15 for fox 19.

In conclusion, these results seem to be encouraging but further experiments are needed to confirm these preliminary observations on seroconversion in foxes parenterally vaccinated with an inactivated vaccine. If the serological results are confirmed and if the protection confered by such a vaccine turns out to be of the same length as in other species (3 years), vaccination of foxes would be worth being applied in the field since the protection confered could last during all the animal's life expectancy (less than three years) (Anderson, 1982).

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Table 2. — Characteristics and serological results for non-rabid foxes.

Animals	Vaccination (on day 0)	Serological results (IU/ml) Day 0	Day 8	Day 15	Day 22	Day 29	Day 36
Litter 3 (2.5 months old)							
Fox 13 (M)	No	< 0.5[a]	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fox 14 (F)	Yes	< 0.5	0.85	0.94	0.6	0.95	0.97
Fox 15 (F)	No	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fox 16 (M)	Yes	< 0.5	6.91	2.12	4.77	3.49	4.49
Litter 4 (2.5 months old)							
Fox 17 (F)	No	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fox 18 (F)	No	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fox 19 (F)	Yes	< 0.5	< 0.5	< 0.9	< 0.7	2.31	7.04
Fox 20 (M)	Yes	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

a: titres inferior to 0.5 are considered as negative.

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Summary

A preliminary experiment of vaccination against rabies with a parenterally administered killed vaccine was performed in Belgium with young foxes (*Vulpes vulpes L.*) captured in an enzootic area (Province de Luxembourg). Out of 20 young foxes, 12 developed spontaneous rabies. Vaccination induced a rapid and important seroconversion in most of the non rabid animals but failed to stop the evolution of the disease in young foxes incubating rabies. On the contrary, vaccination seemed to accelerate the evolution.

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