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Efficacy of a baiting system for vaccinating foxes against rabies with vaccinia-rabies recombinant virus

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The efficacy of a vaccinia-rabies recombinant virus (10^8 TCID50) contained in a machine-made baiting system has been tested in 22 captive young foxes which were divided into three experimental groups of six and a control group of four foxes. Each fox in groups 1, 2 and 3 were fed one, two and three vaccine-baits, respectively, on successive days. The four unvaccinated foxes were housed separately. As shown by the incorporation of a tetracycline biomarker into their bones, all the baited foxes ingested at least one bait. Thirty days after baiting seroconversion to rabies was observed in 15 (83 per cent) of the foxes and seroconversion to vaccinia in 14 (78 per cent). Sixteen of the 18 (89 per cent) baited foxes resisted a rabies challenge 30 days after baiting. One cub was protected against rabies despite the absence of detectable anti-rabies antibody. The results demonstrate that the bait-sachet system permits a good release of the the virus suspension into the mouth.

SINCE 1978, several European countries have conducted large-scale field trials of oral vaccination of foxes (*Vulpes vulpes)* using the SAD, standard or B19 modified, attenuated strain of rabies virus (Steck and others 1982, Schneider and Cox 1988). However, the use of attenuated rabies virus remains controversial as far as its safety and stability are concerned, because these virus strains retain pathogenicity for laboratory and wild rodents and are heat-sensitive (Wandeler and others 1982, Leblois and Flamand 1988).

In order to improve the safety and stability of the vaccine used in the field, a recombinant vaccinia virus expressing the immunising G glycoprotein of rabies virus (VVTGgRAB strain 26D3 187XP) has been developed (Kiény and others 1984). The safety of VVTGgRAB for European target and non-target species has been tested experimentally and in the field (Brochier and others 1989, 1990).

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When they are fed 10^7 TCID50 of VVTGgRAB, 100 per cent of adult foxes are protected against a severe rabies challenge (Blancou and others 1986). When administered orally to 12 fox cubs, a dose of $10^{7.2}$ TCID50 of VVTGgRAB elicited significant levels of rabies virus antibody and protected 11 of them from rabies (Brochier and others 1988). The duration of immunity conferred by the VVTGgRAB (a minimum of 12 months in cubs and 18 months in adult animals) corresponds to the length of protection required for fox vaccination in the field (Brochier and others 1988, Schneider and Cox 1988, Blancou and others 1989).

It has been shown that vaccine bait systems can influence the contact of the virus with the oral mucosa and, if the system is inappropriate, lower the efficacy of the vaccine. In order to preserve the immunogenicity of the vaccine both the bait and the vaccine container must meet certain requirements (Wandeler and others 1988, Brochier and others 1990). The bait should be attractive to the target species, deliver the vaccine into the oral cavity, be hydrophilic and not inactivate the vaccine virus. The vaccine container should be easily ruptured not be swallowed or rejected intact and also not inactivate the vaccine. Finally, the ratio of the volume of bait to the volume of liquid vaccine should be as low as possible.

The field use of such a vaccine-bait system requires that the bait should be able to incorporate a biological marker, should be easily manufactured and resistant to heat, sunlight and biological breakdown; it should also be robust, so that it can be dropped from the air, and not need to be stored frozen.

In the present work, the authors have tested the efficacy of the VVTGgRAB fed to young foxes in a new machine-made bait.

Materials and methods

Animals

In May 1989, 22 wild foxes (*Vulpes vulpes)* aged between six and 12 weeks were trapped from seven dens situated in a rabies-free area of Belgium. After capture the foxes were weighed, sexed, marked and housed in individual wire cages. They were shown to be serologically negative for rabies virus and vaccinia virus antibodies.

All manipulations of the cubs were carried out under anaesthesia. Each animal was injected intramuscularly with Hypnorm (Janssen Pharmaceutica) 0·1 ml/kg, before weighing, bleeding or transport.

TABLE 1: Bait uptake, vaccinia and rabies-neutralising antibodies and protection from rabies challenge in foxes fed with one, two or three Rhone-Merieux baits containing 10^8 TCID50 of VVTGgRAB

Group	Fox	Vaccine baiting	Bait* uptake	Rabies antibodies†			Vaccinia antibodies‡			Response to rabies challenge	Rabies diagnosis	Tetracycline detection
				Day 0	Day 15	Day 30	Day 0	Day 15	Day 30			
1	1	1 bait on day 0	+	<0·55	2·55	3·87	0·08	0·43	0·40	R	–	+
	2		+	<0·55	11·47	25·79	0·07	0·49	0·72	R	–	+
	3		–	<0·55	<0·26	<0·55	0·10	0·06	0·04	D (16)	+	+
	4		+	<0·55	14·29	6·67	0·07	0·40	0·39	R	–	+
	5		+	<0·55	0·47	4·10	0·05	0·03	0·06	R	–	+
	6		+	<0·55	2·37	14·96	0·03	0·26	0·22	R	–	+
2	7	1 bait on day 0	–+	<0·55	>32	>32	0·16	0·45	0·32	R	–	+
	8	1 bait on day 1	–+	<0·55	>32	>32	0·07	0·38	0·58	R	–	+
	9		++	<0·55	7·39	>32	0·04	0·27	0·35	R	–	+
	10		++	<0·55	>32	>32	0·11	0·63	0·81	R	–	+
	11		++	<0·55	25·4	>32	0·05	0·56	0·90	R	–	+
	12		++	<0·55	0·57	0·93	0·05	0·06	0·04	R	–	+
3	13	1 bait on day 0	+–+	<0·55	>32	>32	0·06	0·60	0·68	R	–	+
	14	1 bait on day 1	–++	<0·55	>32	>32	0·05	0·51	0·79	R	–	+
	15	1 bait on day 2	+++	<0·55	>0·26	>32	0·06	0·55	0·68	R	–	+
	16		+++	<0·55	>32	30·78	0·05	0·38	0·50	R	–	+
	17		–??	<0·55	<0·26	1·03	0·05	0·02	0·02	D (18)	+	+
	18		+++	<0·55	11·35	24·02	0·02	0·57	0·61	R	–	+
4	19	Unvaccinated		<0·55	<0·55	<0·55	0·05	0·08	0·04	D (19)	+	–
	20	controls		<0·55	<0·55	<0·55	0·05	0·04	0·03	D (16)	+	–
	21			<0·55	<0·55	<0·55	0·04	0·04	0·07	D (16)	+	–
	22			<0·55	<0·55	<0·55	0·06	0·08	0·05	D (18)	+	–

* Negative results = vaccine sachet rejected intact, † Results are expressed in iu/ml serum, ‡ Results are expressed as the optical density for a 1/160 diluted serum, R Survived, D Died (day of death after challenge)

Viruses

A live-modified vaccinia (Copenhagen strain) — rabies glycoprotein (ERA strain) recombinant virus (VVTGgRAB, strain 26D3 187XP) (Kiény and others 1984) was used as vaccine and contained 10^8 TCID50 2·5 ml dose.

The virus challenge suspension consisted of an homogenate of salivary glands of foxes which had died from natural rabies (strain GS8) (Blancou and others 1979). The titre was determined by intracerebral inoculation of mice.

Baiting system

The vaccine baiting system consisted of a bait made of a mixture of plant and animal proteins and fish oil aggregated by a synthetic polymer and containing tetracycline (150 mg/bait) as a biomarker. A sealed plastic sachet containing one dose of 2·5 ml of liquid vaccine (> 10^8 TCID50/dose) was introduced into the bait which was stitched closed.

Each complete bait weighed about 35 g, was stable from –20°C to +45°C and was strong enough to be dropped from an aeroplane.

Experimental protocol

The 22 foxes were divided into three experimental groups of six, and a control group of four foxes. The foxes of group 1 received one bait, the foxes of group 2 received two and the foxes of group 3 received three baits fed on successive days. The four control foxes were housed separately and not vaccinated (Table 1).

Serum was obtained from all the foxes just before they were given the baits, 15 days later, and 30 days later, just before they were challenged with rabies. The samples were stored at –20°C until they were tested. All the foxes were challenged by intramuscular inoculation with 1 ml of a virus suspension containing $2·3 \times 10^4$ mouse LD50 of strain GS8 natural rabies. The survivors were destroyed 60 days after challenge.

Control of bait uptake

The ingestion of bait and the perforation of the vaccine sachets by the foxes were monitored one or two days after baiting. Any rejected plastic sachet was removed from the cage. The ingestion of the bait was confirmed by the detection of tetracycline in bone. A jaw bone was removed and stored at –20°C. Thin transverse sections (400 µm) were cut with a diamond saw (Isomet-Bueler) and examined directly by ultraviolet fluorescence microscopy (Capt 1981).

Serological analysis

Rabies virus-neutralising antibodies were determined in the serum samples by a fluorescence inhibition technique. The antibody titres are expressed in International Units (iu)/ml as determined by comparison with a standard serum (with a titre of 4·4 iu/ml) (Smith and others 1974). The arbitrary level of 1 iu/ml was considered indicative of successful rabies immunisation.

Vaccinia virus-neutralising antibodies were determined by an ELISA technique. The results are expressed as the optical density of a 1/160 dilution of serum and an optical density of 0·2 was considered indicative of vaccinia virus seroconversion.

Rabies diagnosis

The rabies virus was demonstrated in the dead foxes' brains by the analytical techniques recommended by the World Health

Organization, immunofluorescence and the intracerebral inoculation of mice (Koprowski 1974).

Results

The results of the vaccine-bait ingestion are given in Table 1. The baits were affected in three different ways: 28 of the 36 (77·7 per cent) had been ingested and the vaccine sachet was found perforated and empty; six (16·6 per cent) had been ingested but the vaccine sachet was rejected intact; two (5·5 per cent) had been ingested but the vaccine sachet was not found. The incorporation of tetracycline in bone showed that all the baited foxes had consumed at least one bait.

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

Fifteen and 30 days after baiting, rabies virus antibody titres exceeding 1 iu/ml appeared in 14 (77 per cent) and 15 (83 per cent) of the foxes, respectively. At the same times vaccinia virus antibody titres with an optical density exceeding 0·2 appeared in 14 (77 per cent) of the animals. Foxes 3, 12 and 17 failed to develop any significant virus specific antibody to either rabies or vaccinia virus.

Sixteen (89 per cent) of the baited foxes resisted a rabies challenge applied 30 days after baiting. Two of the baited foxes (3 and 17) and all the control foxes failed to produce any virus specific antibody and died from rabies, as confirmed by immunofluorescence in brain smears and the intracerebral inoculation of mice.

Fox 12 was protected against rabies despite the absence of significant anti-rabies antibodies.

Discussion

The bait was taken voluntarily by all the foxes in less than 48 hours, confirming earlier observations (Brochier and others 1990). The serological results and the responses to a severe rabies challenge demonstrate that the bait-sachet system permitted a good release of the virus suspension into the mouth and its contact with the buccal mucous membranes, where the recombinant virus multiplies (Thomas and others 1990). Significant or high levels of rabies and vaccinia virus neutralising antibodies were observed 15 days after baiting in 77 per cent of the treated animals. This result demonstrates a rapid immune response and a good correlation between rabies and vaccinia seroconversions. Moreover, 16 (89 per cent) of the baited foxes were protected against an experimental rabies challenge infection 30 days after baiting. One of the two treated foxes which succumbed to rabies (fox 3) had rejected the unperforated sachet. Unfortunately, the failure of immunisation recorded in fox 17 could not be explained because two of the three vaccine sachets could not be found in its cage; it is possible that the fox swallowed a sachet without perforating it. As has been observed in previous experiments (Brochier and others 1988) one of the vaccinated animals resisted the challenge despite the absence of any significant specific antibody against rabies virus.

The VVTGgRAB is effective, safe and heat-stable, and is an excellent candidate for the oral vaccination of foxes in the field. A large-scale field trial using this system started in Belgium in November 1989. Twenty-five thousand similar baits each containing 10^8 TCID50 of VVTGgRAB were were dropped by helicopter in a 2200 km^2 area in south Belgium. This campaign will be repeated in March and November 1990 and the efficacy of the vaccine-bait and its distribution will be evaluated.

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Bovine Abstracts

Immunity of bolus-treated calves to lungworm

THE development of immunity to *Dictyocaulus viviparus* by grazing calves which had been treated with an oxfendazole pulse release bolus was compared with the level of immunity developed by untreated calves, by challenging them with the parasite. Even though signs of parasitic bronchitis appeared only in the untreated grazing calves, the calves treated with the bolus had developed an adequate although lower level of immunity, which protected them from disease after a substantial challenge infection. There was evidence that the level of immunity developed by the bolus-treated calves was related to their initial levels of infection. The results, therefore, suggest that the pulse release bolus should control parasitic bronchitis, provided that the initial levels of infestation of pasture with larvae are sufficient to provoke the development of immunity.

EYSKER, M., BOERSEMA, J. H. & KOOYMAN, F. N. J. (1990) *Research in Veterinary Science* **48**, 301

Distribution of *Taenia saginata* cysts in calves

THE distribution of *Taenia saginata* cysts was recorded in 23 calves which had been infected with between 11,000 and 12,000 eggs, either 10 to 12 weeks or 52 weeks previously. The heart, liver, kidneys, lungs, oesophagus, diaphragm, the muscles of the head and tongue and the muscles of one half of the body were cut into slices less than 0·5 cm thick. The calves harboured between two and 2569 viable cysts. A median value of 15·7 per cent of the cysts were found in the heart and 6·5 per cent in the masseter muscles, the organs usually thought to be the predilection sites for the parasite. A probability model was derived from the results to estimate the sensitivity of conventional meat inspection at different levels of infection; the probability of detecting an animal with 10 cysts was estimated to lie between 20 and 60 per cent.

KYVSGAARD, N. C., ILSOE, B., HENRIKSEN, S. A. & NANSEN, P. (1990) *Research in Veterinary Science* **49**, 29