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Source: Journal of Wildlife Diseases, 37(4) : 791-797

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-37.4.791>

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SHORT COMMUNICATIONS

Journal of Wildlife Diseases, 37(4), 2001, pp. 791–797
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Is it Possible to Orally Vaccinate Juvenile Red Foxes against Rabies in Spring Campaigns?

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ABSTRACT: The rabies antibody status of juvenile foxes (*Vulpes vulpes*) was evaluated in large-scale, long-term oral vaccination campaigns. Between 9% ($n = 659$) and 21% ($n = 42$) of the juvenile foxes examined in 1993–94 and 1997, respectively, showed rabies virus neutralizing antibody (nAb)-titers ≥ 0.5 IU/ml following bait distribution in spring. The presence of nAb may be due to either the passive transfer of maternal antibodies, or active immunization derived from spring vaccination campaigns. The latter alternative is supported by the finding of nAb throughout late spring and the summer months, and the finding of the tetracycline (TC) biomarker, used in the vaccine-baits, in 27% ($n = 43$) and 37% ($n = 155$) of juveniles in 1993–94 and 1997, respectively. It was not possible to distinguish nAb originating from passive immunity from that arising from active immunization. However, biological data on the whelping period of red foxes, on dynamics of maternal antibodies and the timing of oral vaccination, gave evidence that a superposition of these processes is likely. Evidence from these studies suggests that oral vaccination coinciding with the spring perinatal period may produce immunity in both parents and only in a certain percentage of the offspring simultaneously. This phenomenon should be useful in further enhancing the efficacy of oral vaccination in red foxes.

Key words: Immune response, juveniles, oral immunization, oral vaccination, rabies, red fox, *Vulpes vulpes*.

The successful establishment of oral rabies vaccination programs against fox rabies has been the basis for the considerable progress made towards rabies elimination in Europe (Stöhr and Meslin, 1996). Serological follow-up investigations have shown that a certain proportion of ju-

venile foxes (*Vulpes vulpes*) have rabies virus neutralizing antibodies (nAb) following vaccination campaigns (Vuillaume et al., 1998; Matouch et al., 1998). However, the origin of these nAb remains unknown. Are they the result of maternally transferred immunity or an induction of a specific immune response through active immunization by the oral route?

From experimental and epidemiological studies with dogs and mice, it is known that vaccination of dams against rabies results in a transfer of maternal antibodies (maAb) to the offspring (Winter, 1981; Xi-ang and Ertl, 1992). With respect to the red fox, it was for a long time assumed that cubs receive antibodies from their mother during pregnancy and lactation (Mayr et al., 1972; Vuillaume et al., 1998). Only recently, it could be experimentally shown that maternal immunity in fox cubs does occur (Müller et al., 1999; Cliquet et al., 2000). However, analysis of serological data from juvenile foxes (<1-yr-old) from areas vaccinated in successive years can provide some information on the occurrence of maternal antibodies (maAb) in the field. The objective of this study was to determine the prevalence of rabies nAb in juvenile foxes originating from large-scale and long-term vaccination areas in Germany after spring vaccination campaigns. In order to get information on the possible origin of nAb and to see whether young foxes can be orally vaccinated or not, subsequently, these results were in-

terpreted with data on the reproductive period of the red fox, the dynamics of maAb and the time of vaccination.

Two retrospective serological studies were carried out in two separate but topographically comparable areas of the Federal State of Brandenburg (Germany). The study areas have been continuously vaccinated twice a year since autumn 1991 using 18–20 baits/km² on average containing SAD P5/88 oral rabies vaccine and 250 mg of tetracycline (TC) as biomarker (Stöhr et al., 1994). The spring vaccination campaigns prior to sample collection took place in the second half of April.

In the first study (Study 1), 715 blood samples from juvenile foxes, shot between April and September of the years 1993 and 1994, were selected for serological testing from a wildlife serum bank. The animals originated from an area (4,500 km²) in northwestern Brandenburg (52.50–53.50°N, 11.50–13.00°E), and were identified as juveniles (3 to 12-mo-old) according to the secondary dentition. Data on TC in juveniles of this area originated from a central data bank of routine rabies and oral vaccination surveillance and were assayed separately during follow-up investigations of oral vaccination campaigns (Müller et al., 1994).

In a second study (Study 2), litters of fox cubs (<3-mo-old), with or without their respective vixens were investigated. These animals were collected by wildlife biologists within the course of a project on the ecology of the red fox in the county of Uckermark (1,000 km²) in northeastern Brandenburg (53.00–53.15°N and 13.30–14.00°E) during May and June 1997. A total of 49 foxes were collected from this area representing five complete litters, 21 cubs and five vixens, and 18 cubs from another seven litters. An additional, four single fox cubs were collected. Immediately after being killed, a bone sample from the lower jaw and blood were taken from the heart for the detection of TC and nAb, respectively.

After collection, all sera were centri-

fuged at 1,000 g for 10 min, aliquoted and stored at –30 °C. The sera were investigated in the Rapid Fluorescent Focus Inhibition Test (RFFIT) as described by Smith et al. (1973) with the modifications of that method as described by Cox and Schneider (1976). Prior to testing, sera were pre-diluted 1:2, heat inactivated for 30 minutes at 56 °C and centrifuged at 1,000 g for 10 min. A WHO standard (international standard immunoglobulin, 2nd human rabies immunoglobulin preparation, Potters Bar, UK), and sera from vaccinated and naive farm foxes served as controls (WHO, 1978). The nAb-titer was defined as the serum dilution showing a 50% inhibition (ND₅₀) of the virus control. For reasons of comparison the nAb-titres of the fox cubs were converted into international units (IU/ml) based on the WHO standard adjusted to 0.5 IU/ml. The presence of the biomarker was detected by demonstration of TC-induced fluorescence in the bone and dentine of teeth using a method described elsewhere (Linhardt and Kenelly, 1967; Johnston et al., 1987). Statistical analyses were conducted according to Sokal and Rohlf (1995).

Of the 715 sera available in study 1 for retrospective serological testing, 659 sera were analyzable by the RFFIT. The remainder was toxic or unsuitable. A total of 310 sera (47.1%, CI = 44.8%–49.2%) showed nAb of different titer-classes with 58 sera (9%) having nAb \geq 1:90 (\geq 0.5 IU/ml) (Fig. 1). Except for April, nAb-titers \geq 0.5 IU/ml were present in all samples between May to September in significant percentages ($P < 0.05$). Within the same time period of the years 1993 and 1994, a subset of 155 juvenile foxes were analyzed independently from the same area for the presence of the biomarker of which 58 (37.4%; CI = 29.8%–45.5%) showed TC-specific fluorescence.

Twelve of 43 fox cubs from Study 2 tested TC-positive (27.9%, CI = 15.3 – 43.7%), and of the 42 cub sera available 30 (71.4%, CI = 55.42 – 84.28) showed nAb, with nine (21%) having nAb \geq 1:90

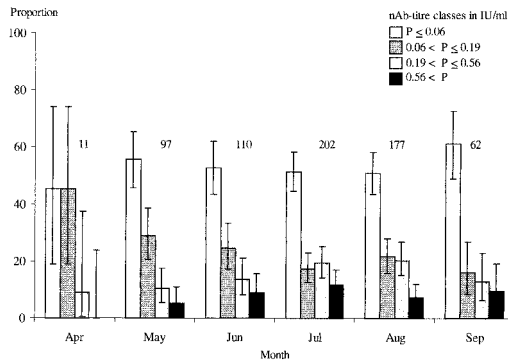


FIGURE 1. Monthly seroprevalence (95%-confidence intervals) of rabies neutralizing antibody (nAb) [classes of nAb-titers converted into International units (IU/ml) and sample sizes] in juvenile foxes of Study 1 (1993–94). Herein, 0.06 IU/ml = nAb-titre of 1:20; 0.18 IU/ml = nAb-titre of 1:60; 0.56 IU/ml = nAb-titre of 1:90.

(≥ 0.5 IU/ml). Although all combinations between TC and nAb were shown to occur, 47.6% of the cubs were nAb+/TC– (Table 1).

With respect to juvenile foxes, it is supposed that oral vaccination is ineffective, and that during dispersal non-immunized foxes are responsible for the spread and persistence of the disease because they are not protected (Breitenmoser et al., 1995; Müller, 1997). Interestingly, in our two studies 47% and 71% of the juvenile foxes showed nAb following normal spring vaccination campaigns (Table 1, Fig. 1). However, when compared to the arbitrarily defined threshold of 0.5 IU/ml (WHO, 1978) which equals a nAb-titer of $\geq 1:90$ in our study, the percentage of juveniles considered immune in Study 1 and 2 is estimated as 9% and 21%, respectively. This is in accordance with assumptions of Matouch et al. (1998) and Vuillaume et al. (1998). Sera with nAb-titers between $\geq 1:20 < 1:90$ (< 0.5 IU/ml) may be due to low level rabies nAb, but may also arise from unspecific serum factors, or other virus-toxic effects that result from the often poor serum quality which may also mimic low level nAb-titers, but are difficult to differentiate.

Rabies specific antibodies in fox cubs

can derive from various sources. One major source of nAb in fox cubs could be decreasing levels of maAb with increasing age (Müller et al., 2000). Following rabies vaccination of female dogs, maAb were transferred to puppies transplacentally and via colostrum. They could be detected in decreasing concentrations, on average, up to 6–7 weeks post partum (Winters, 1981; Aghomo et al., 1990). In contrast to dogs, most maAb in foxes have disappeared in RFFIT after 23 days (Müller et al., 2000). Considering this fact and a maximal duration of the reproductive period of nearly two months (Lloyd and Englund, 1973; Goretzki and Paustian, 1982) complete disappearance of maAb would have been expected in the fox cubs investigated by May (Fig. 1). A possible explanation for the occurrence of nAb in June–September is that a certain but unknown percentage of fox cubs may have had contact with vaccine baits after the spring vaccination campaigns resulting in an active immunization. This might only apply to fox cubs that were born very early during the reproductive season. However, cubs having maAb show a partially impaired immune response to active immunization which outlasts the time during which maternal antibodies are present at detectable levels. In contrast, cubs born of naive vixens develop a protective immunity at a relatively early age post partum (5 wks) (Müller et al., 1999). Other possible sources like immuno-stimulation via the gastro-intestinal route from ingested rabies virus infected material (Ramsden and Johnston, 1975; Lawson et al., 1987) or naturally occurring rabies virus nAb can be excluded as no rabies cases have been diagnosed from these areas since 1992.

The detection of the biomarker can provide further information on the source of nAb. Although, 37% and 28% of the juveniles in Study 1 and 2 tested TC-positive, respectively, it is possible that these animals may have acquired TC (i) transplacentally, (ii) via colostrum or (iii) by bait up-take. The possibility of a transplacental

TABLE 1. Results of TC-biomarker and serological testing for rabies in litter cubs with and without the respective vixen from Study 2 (1997) in northeastern Brandenburg (Germany).

Status	N°	Vixen			nAb-GMT			nAb-range			n ≥			
		Date	nAb-titre	TC	n	Titer	IU/ml	Titer	IU/ml	0.5 IU/ ml	nAb+/TC+	nAb+/TC-	nAb-/TC+	nAb-/TC-
Litters with respective vixens	1	05-11	1:149	+	6*	1:20	0.12	1:(13-43)	0.08-0.27	—	—	1	—	4
	2	05-20	>1:810	+	6	1:92	0.57	1:(42-511)	0.26-3.19	2	—	6	—	—
	3	05-25	1:66	+	5	1:34	0.21	1:(10-85)	0.06-0.53	1	—	4	—	1
	4	05-26	n.d.	+	3	1:18	0.11	1:(8-81)	0.05-0.51	1	—	1	—	2
Litter without vixens	5	06-12	>1:810	+	1	1:102	0.63	1:102	0.64	1	—	1	—	—
	6	05-13	—	—	3	1:96	0.6	1:(48-139)	0.30-0.87	2	3	—	—	—
	7	05-19	—	—	2	1:23	0.14	1:(19-27)	0.12-0.17	—	1	—	1	—
	8	05-19	—	—	2	1:16	0.1	1:(8-30)	0.05-0.19	—	1	—	1	—
	9	05-26	—	—	3	1:32	0.2	1:(14-112)	0.09-0.70	1	2	—	—	1
	10	05-30	—	—	2	1:28	0.17	1:(22-33)	0.14-0.21	—	—	2	—	—
Singles	11	06-05	—	—	2	1:40	0.25	1:(35-45)	0.22-0.28	—	1	1	—	—
	12	06-10	—	—	4	1:84	0.52	1:(21-810)	0.13-5.06	1	—	4	—	—
	13	05-19	—	—	1	1:25	0.15	—	—	—	1	—	—	—
	14	05-28	—	—	1	1:10	0.06	—	—	—	—	—	—	1
Total	15	05-31	—	—	1	1:16	0.1	—	—	—	—	—	—	1
	16	06-12	—	—	1	1:34	0.21	—	—	—	1	—	—	—
Total					43					9	10	20	2	10

transfer can be excluded because breeding vixens can only get TC during vaccination campaigns in spring and autumn, that means outside the gestation period unless it is from environmental sources. Furthermore, the durability of the TC-labeling is limited by the bone calcification process. This makes it very unlikely that TC once deposited in adults, can be reactivated during pregnancy in a high enough concentration and transferred to mark fetal tissues (Frost, 1968). Although TC is known to cause considerable residues in the milk of dams (Dinsmore et al., 1996), a transfer of TC via colostrum in this case does not seem very plausible. Of the litters examined in Study 2 (Table 1) with a complete data-set, all cubs tested TC-negative, but all vixens tested TC-positive indicating bait-uptake once in their life. An up-take of old buried baits can also result in TC-labeling of juveniles, but in this case seroconversion is not to be expected due to a decline in vaccine potency. The high proportion (47%) of nAb+/TC- animals in Study 2 (Table 1) is probably a result of the presence of maAb. However, other possibilities cannot be ruled out, e.g., mechanical transport of liquid vaccine by adults to the cubs through perinatal care (grooming, suckling and regurgitative feeding) (Rupprecht et al., 1988).

In central Europe, more than 80% of the vixens have given birth by the end of March (Fig. 2). Considering this fact, a certain proportion of the offspring might, in theory, have the chance to consume baits or to have contact with the vaccine depending on their date of birth and timing of the vaccination campaigns. This is supported by experimental studies showing that fox cubs aged 3 weeks were already able to consume solid food offered (Englund, 1969; Kolb and Hewson, 1980). In the study areas, spring vaccination campaigns took place in the second half of April when most of the cubs were already at least 4-wk-old (Fig. 2).

Our field data verified experimental results obtained on the occurrence of maAb

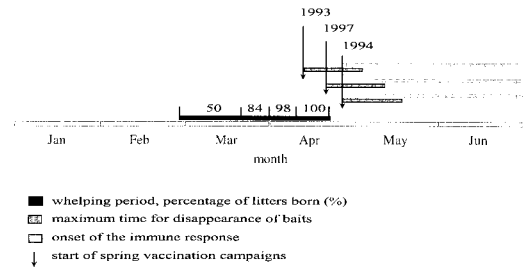


FIGURE 2. Correlation between time of vaccination in spring and the reproductive period of the red fox in the study areas as described by Lloyd and Englund (1973) and Goretzki and Paustian (1982). Spring vaccination campaigns started on April 16, 26 and 19 for Study 1 (1993–94) and Study 2 (1997), respectively.

in fox cubs. There is evidence that nAb in fox cubs from normal spring vaccination campaigns are attributed to two sources, that is a transfer of maAb, and an active immunization response due to contact with the vaccine. Because there is experimental evidence that the presence of maAb results in the inhibition of a specific immune response following active rabies immunization (Xiang and Ertl, 1992; Müller et al., 1999) a superposition of these processes during spring vaccination campaigns is most likely. Whereas, the source of nAb in juvenile foxes shot in April and May cannot be precisely determined, fox cubs having nAb $\geq 1:90$ (≥ 0.5 IU/ml) shot between June and September probably have derived immunity from active immunization. Considering that the timing of vaccination is correlated with spring whelping activity, this has far reaching consequences on the effectiveness of alternative vaccination strategies, e.g., den baiting, double vaccination or additional vaccination during early summer. Therefore, if also young foxes are to be vaccinated in spring vaccination campaigns, baits should not be distributed before the end of May (Vos et al., 2000).

The authors thank D. H. Johnston, and R. Zanoni, their critical comments and suggestions to the manuscript. This study was funded by the Ministry of Nutrition, Agriculture and Forestry of the Federal

State Brandenburg and the German Federal Foundation Environment.

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Received for Publication 5 May 2000.