Persistence of Rabies Virus Antibodies in the Sera of Fox CubsVaccinated with the Vaccine Lysvulpen

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Received September 30, 2002
Accepted March 25, 2003

Abstract

Hostnik P., D. Barlič-Maganja, I. Toplak, J. Grom: *Persistence of Rabies Virus Antibodies in the Sera of Fox Cubs Vaccinated with the Vaccine Lysvulpen* . Acta Vet. Brno 2003, 72: 207-212.

Persistence of maternal antibodies transfer from rabies-immune vixens to their cubs was studied. Eight vixens (*Vulpes vulpes*) were vaccinated one month before pregnancy with Lysvulpen, vaccine for oral vaccination of foxes. Twenty-one cubs born in the first half of April were divided in two groups. One group (n = 18) of cubs was vaccinated and the control group (n = 3) was not vaccinated. The sera of adult foxes and their cubs were collected periodically and rabies neutralising antibody titre was measured by fluorescent antibody virus neutralisation (FAVN) test. Rabies neutralising antibodies were detected in all vaccinated vixens. The level of rabies neutralising antibody titre was not changed one year after oral vaccination. The geometric mean titre of rabies neutralising antibodies of fox cubs sampled in May was 1.31 IU/ml and has dropped successively to 0.54 IU/ml in June samples and to 0.18 IU/ml in July samples. We found that the persistence of rabies maternal antibodies in fox cubs was limited to two months after birth. Hence, the oral vaccination campaign in April or at the beginning of May is too early for southern part of Europe because juvenile foxes have still maternal antibodies that can prevent active immunisation. Moreover, they are too small to take the baits.

Maternal antibodies, lyssavirus, baits, flurescent antibody virus neutralization test, vaccination campaigns, timing

Rabies is a viral infection of the central nervous system, causing encephalitis and encephalomyelitis. The disease is fatal when clinical signs appear (Smith and Seidel 1993). Rabies virus is a member of the *Rhabdoviridae* family of the *Lyssavirus* genus, which can be divided into seven genotypes. In Europe, three different genotypes have been confirmed: genotype 1 – classical rabies virus; genotype 5 – European bat lyssavirus 1 (EBL 1) and genotype 6 – European bat lyssavirus 2 (EBL 2) (Gould et al. 1998). The red fox (*Vulpes vulpes*) is both the vector and reservoir of the disease and it is an important factor in the persistence of the disease. Rabies virus and other lyssaviruses are usually directly transmitted to domestic animals (Pastoret et al. 1985) and humans via bites or scratches, which provide direct access of the virus in saliva to exposed tissue and nerve endings.

In Europe, rabies in wildlife was first recorded in Poland and since 1939 has spread approximately 1400 km westwards (MacDonald 1988). The first outbreak of rabies in Slovenia was diagnosed in Prekmurje region in 1973 and spread only to the river Mura area and no further. In 1977, it has spread in the southwest direction, when it crossed the borders in the upper Sava valley region and in the Carinthian region. In 1980, the first cases were reported on the outskirts of Ljubljana, the capital of Slovenia and one year later it spread to the southern parts of the country. The disease reached its peak in years 1981 and 1982. Since then rabies has been present among wild animals and several cases in non-vaccinated domestic animals were recorded (Bidovec et al. 1993).

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During the period between 1995 and 2000, a total number of 11.425 animals were tested for rabies virus in Slovenia. Out of 1500 (13.12%) immunofluorescence (IF) rabies-positive samples 1415 (94.33%) were from wild animals and 85 (5.67%) were from domestic animals. The oral vaccination programme has been restarted in 1995 and the baits were laid all over the country. Baiting was performed in a row three times: in autumn (October) 1995 and in spring (April/May) and autumn (October) 1996. During the year 1997, vaccination was not performed. Vaccination was continued in spring and autumn of the years 1998, 1999, 2000 and 2001. The baits were dispersed by planes from height of 300 – 500 metres. The aeroplane pathways were 500 metres apart. The average bait discharge was 18-20 per km^2. The pilots used the GPS navigation system for orientation and the discharge was monitored by a computer (Hostnik and Bidovec 1999). The goal of oral vaccination was to eliminate rabies and to establish rabies-free areas. A rabies-free area is defined as an area of approximately 5.000 km^2 where after testing 8 foxes per 100 km^2 no cases of rabies are recorded during a two-year period (Blasco et al. 2001).

Rabies is now eliminated or nearly eliminated in many European countries due to using oral vaccination of foxes (World Health Organization, 2001). Experiences in many European countries such as Switzerland, France, Belgium and Germany have shown that a decrease of rabies incidence began very soon after oral vaccination. A residual focus has been often detected during the vaccination campaigns or after stopping of vaccination (Pastoret et al. 1985). Many authors have shown that the population of young foxes is very difficult to vaccinate during spring campaigns (Blasco et al. 2001) especially if baits are distributed during early spring (April). The fox cubs born in March or April cannot be vaccinated with oral vaccine baits, because at this age they are not able to consume solid food (Masson et al. 1999). The success of rabies eradication programme is dependent not only from sufficient bait density (World Health Organization, 2001) and period of vaccination but also from the location where the vaccination is performed. Slovenia is situated between the countries of southern part of the European Union area, where rabies has been eliminated by oral vaccination and south-east Europe where oral vaccination has not been performed systematically for a long time.

Complete rabies control programme cannot neglect the immune responses of fox cubs. Vixens possess an endotheliochorial placenta, whereby approximately 95% of maternal immunoglobulin transfer occurs via colostrum (Mohn et al. 1982). Depending on the immune status of vixens at parturition, significant immunological interference may occur in a young fox receiving colostrum (Brochier et al. 1985). Generally, fox cubs from unvaccinated vixens respond better to vaccination than cubs from rabies-vaccinated vixens. Maternal antibodies from vaccinated vixens prevent the production of antibodies in fox cubs, and even neutralizing antibodies decline below detectable levels (Müller et al. 2001b).

Post-vaccination immunity to rabies in foxes can be controlled *in vivo* by challenge test or by testing the presence of neutralizing antibodies in virus neutralization tests. Many authors studied the relations between antibody production and protection against rabies infection in many animal species and humans (Aubert 1993). The World Health Organization (WHO) recommends post-vaccination antibody titre 0.5 IU/ml as a limit of good protection for humans. The same titre value is used to evaluate the immune status of vaccinated foxes (Matouch et al. 1988). The resistance of the animal against rabies infection is dependent on neutralising antibody titre (Trimarchi et al. 1996). Rapid fluorescent focus inhibition test (RFFI) (Smith et al. 1973) was replaced by a fluorescent antibody virus neutralization (FAVN) test (Cliquet et al. 1998). Among animals immunised with rabies neutralising antibody titre higher than 0.5 IU/ml no rabies cases were observed (Aubert 1993).

In the present study the period of rabies maternal antibody titre detection was determined in healthy young foxes.

Materials and Methods

Experimental foxes

Eight vixens *(Vulpes vulpes)* were vaccinated approximately one month before pregnancy. They were 2-7-year-old. They were kept in individual cages and were fed and observed daily. Twenty-one fox cubs were born between April 1st and April 14th. Vixens were in good health condition following the vaccination and at the time of collection of serum samples. One bait of Lysvulpen (produced by Bioveta Ivanovice, Czech Republic) was placed in each cage and after one hour all the baits were taken. The residual envelopes of baits were collected. Each vaccine bait contained rabies virus, strain SAD-Bern at a titer of 107 TCID50/ml. One vixen and three of her cubs were used as negative non-vaccinated controls. Before blood samples were taken, the animals had been immobilised with an intramuscular injection of 0.2 ml/kg of Dormitor® (Orion, Helsinki, Finland). After blood sampling they were treated with 0.1 ml/kg of Antisedan® (Orion, Helsinki, Finland).

The serum samples were periodicaly collected from 9 fox mothers and 22 fox cubs. The blood of vixens were collected on the day of vaccination and later also from cubs in first weeks of May, June and July. After the collection all sera were centrifuged for 10 min at 1 000 *g*, aliquoted and stored at -20 ºC.

Serum neutralising antibody titre determination

All sera were heat inactivated (for 30 min at 56 °C) and analysed for the presence of rabies virus antibodies with fluorescent antibody virus neutralisation (FAVN) test. The method was described by Cliquet and coworkers (1998). Briefly, the serial three-fold dilutions of serum samples were prepared in duplicate in minimum essential medium (MEM, Gibco, Paisley, UK) and were placed on a 96-well microplate. A challenge virus strain (CVS, obtained from OIE/WHO, Nuncy, France) in titre 30-200 $TCID_{50}$/0.1 ml was added in each well. After incubation for 60 min at 37 °C BHK cells were added. The cells were fixed after 48 h of incubation with cold acetone (stored at -20 °C) and stained with anti-rabies fluorescent conjugate (Sanofi Diagnostic Pasteur, Marnes-la-Coquette, France). The highest neutralising antibody titre of the serum samples was considered the dilution, which completely blocked CVS propagation. The OIE reference serum with known neutralising antibody titre (0.5 IU/ml), negative serum controls, virus and cell controls were also included in the tests. The neutralising antibody titres obtained in sera were transformed into International Units/ml (IU/ml). Geometric mean titre (GMT) was determined by programme Excel.

Results

All vaccinated vixens had neutralising antibodies whereas unvaccinated animals were negative in FAVN test. All animals survived the application of Dormitor and Antisedan and no other patho-clinical symptoms were evident. The first serum samples from young foxes could be obtained at the age of 25 days. Rabies neutralising antibody titres higher than 0.5 IU/ml were found in 17 out of 18 fox cubs sampled in May 9th when fox cubs were 25 to 39 days of age. In three out of 18 fox cubs sampled in June 5th (when aged 52-66 days) the antibody titre was higher than 0.5 IU/ml, the others had lower antibody titre than 0.5 IU/ml. On sampling day July 2nd (when aged 79-93 days) all tested fox cubs had lower antibody titre than 0.5 IU/ml. All control, non-vaccinated cubs tested negative for rabies neutralising antibodies.

The level of rabies neutralising antibody titre measured in eight vaccinated foxes has not changed one year after oral vaccination by Lysvulpen (not shown).

Rabies antibody titres in vixens and their cubs expressed in international units (IU/ml) are listed in Table 1.

Table 1
Geometric mean titres (GMT) and rabies antibody titres range in 8 vaccinated foxes and their 18 cubs, and in 1 non-vaccinated fox and her 4 cubs during sampling period

		Geometric mean titres (GMT) and (rabies antibody titres range in IU/ml)		
		Date of sampling		
	No. of foxes	May, 09	June, 05	July, 02
Vixens (vaccinated)	8	13.4 (4.2-24.8)	12.4 (3.8-32)	12.5 (4.9-32)
Cubs	18	1.31 (0.2-8.5)	0.54 (0.2-2.6)	0.18 (0.03-0.4)
Vixens (control)	1	< 0.1	< 0.1	< 0.1
Cubs (control)	4	< 0.1	< 0.1	< 0.1

Discussion

The programme of oral immunisation of foxes against rabies is an effective method for control and eradication of rabies in wildlife. The use of attenuated rabies virus strains in different vaccines has led to the elimination of sylvatic rabies from large areas in several European countries (Blasco et al. 2001). The World Health Organization (2001) strategies for rabies control has established vaccination, and requires spring and autumn campaigns every year until no cases of rabies has been detected for over two years in the vaccination area.

In Slovenia two oral vaccination campaigns of wildlife have been carried out yearly. The vaccinations are carried out in spring (April) and autumn (October) when temperatures are relatively low and the stability of vaccines can be ensured. Spring vaccination period is critical for the immunisation of young foxes. Only between 9% to 21% of the juvenile foxes showed rabies virus neutralising antibodies (Müller et al. 2001a). Observed seroconversion rates in fox cubs population (20-24%) were significantly lower than in adult foxes (78-79%) when vaccination campaign was carried out in April or first part of May (Bruyere et al. 2000). However, the origin of neutralising antibodies in juvenile foxes remains unknown. They can be a result of maternal transferred immunity or an induction of a specific immune response through active immunisation by the oral route. There is experimental evidence that the presence of maternal antibodies results in the inhibition of a specific immune response following active rabies immunisation (Blasco et al. 2001; Müller et al. 2001b). It means that a large number of young foxes could stay unprotected against rabies infection during summer and early autumn. 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). Early baiting can induce the origin of a residual rabies focus within the vaccination area (Müller et al. 2001b).

The specific rabies neutralising antibody titre higher than 0.5 IU/ml protects the foxes against reinfection (Müller et al. 2001b). The results of a challenge test showed that foxes vaccinated by Lysvulpen vaccine have been protected for three years after vaccination (Matouch et al. 1988). It has been reported about different success of vaccination campaign against rabies. The level of protection depends also on the season and ratio of young foxes in the population. The optimal time for vaccination and the system of baits delivery has been discussed (Vos et al. 2000). Baits distribution by hunters is difficult to organize over several years. Many countries distribute baits by plane. The bait density also differs from country to country, ranging from 13 to 25 baits per km^2. Müller (1997) recommended 30 baits per km^2 in areas with a high population density of foxes. The efficiency of vaccination programme is evaluated by the detection of biomarker in mandibular bone and by detection of specific antibodies in blood samples of hunted foxes (Bruyere et al. 2000). Rabies has been eradicated in areas where over 75% of fox population was immunized by oral vaccination during several years (Müller 1998).

Comparing spring and autumn vaccination campaign high differences in sero-conversion rate were observed between populations of young and adult foxes. In southern part of Europe the foxes are being born from the end of March to the end of April. During the spring vaccination campaigns (the end of April or first week of May) the fox cubs are too small to eat the baits. At the same time maternal antibodies can prevent active immunization. The interference between maternal antibodies and active immunization has been described by many authors (Brochier et al. 1988; Vuillaume et al. 1998). Bruyere and coworkers (2000) using SAG2 vaccine suggested that spring vaccination campaign could be carried out during late May. The SAG2 vaccine is more stable at higher temperatures than the vaccine containing SAD strain of rabies virus (Flamand et al.1993).

In our study it has been shown that the duration of maternal antibodies in young foxes from previously vaccinated vixens was limited to two month after birth. Our findings

studying kinetics of maternal antibody persistence was in close agreement with the results of Gangadhar and Raghavan (1996). During the spring oral vaccination campaign young foxes have rabies maternal antibodies which can cause the inhibition of immune response.

The differences of vaccination efficacy between adult foxes and fox cubs were reported by Bruyere et al. (2000). When vaccination was carried out in the same area at the end of April or at the end of June, 85% of adult foxes had antibodies at both terms of vaccination. The large differences in sero-conversion were observed in young foxes. In the case when vaccination was performed at the end of April only 24% of fox cubs sero-converted. However, 75% of fox cubs developed antibodies if vaccination was done in June. Our experiment on fox cubs shows that maternal antibodies persist in young foxes until June. Müller et al. (2001b) reported that maternal antibodies interference could be the reason for low sero-conversion in young foxes. The baits should not be distributed before the end of May if we would like also to protect young foxes. The immunisation of fox cubs is more successful if vaccine baits are distributed around fox dens entrances Vuillaume et al. 1998). This way of bait distribution is expensive and more difficult to put into practice.

In conclusion we can say that the oral vaccination campaign of foxes in April or at the beginning of May is too early for this part of Europe because juvenile foxes have still maternal antibodies and are too small to take the baits.

Persistence protilátek proti vzteklině v séru liščat vakcinovaných vakcinou Lysvulpen

V práci jsem sledovali persistenci mateřských protilátek proti vzteklině u mláďat imunizovaných lišek. Osm lišek (*Vulpes vulpes*) jsme vakcinovali měsíc před zabřeznutím vakcínou Lysvulpen pro orální vakcinaci lišek. Mláďata narozená v první polovině dubna (n= 21) jsme rozdělili do dvou skupin; první z nich (n = 18) byla vakcinována a druhá (n = 3) byla nevakcinována, ponechána jako kontrola. Séra matek a liščat byla byly pravidelně odebírána a titr virus neutralizačních protilátek byl stanovován pomocí testu fluorescenční neutralizace protilátek (FAVN). Protilátky byly nalezeny u všech vakcinovaných lišek a jejich hladina se nezměnila během jednoho roku po orální vakcinaci. Geometrický průměrný titr protilátek u mláďat, stanovený po odběru v květnu byl 1,31 IU/l, do června klesl na 0,54 IU/l, a do července dokonce na 0,18 IU/l. Prokázali jsme, že persistence mateřských protilátek byly u liščat omezena na dva měsíce po narození. Provádět vakcinační kampaně v zemích jižní Evropy v dubnu a na počátku května proto není vhodné; v té době jsou liščata chráněna kolostrálními protilátkami, které mohou bránit aktivní imunizaci. Mimo to jsou návnady pro mladá liščata příliš velké.

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