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Distemper vaccination of farmed fur animals in Finland

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Abstract

The most important farmed fur animal species in Finland are the American mink (*Mustela vison*), blue fox (*Alopex lagopus*), silver fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*); all are susceptible to canine distemper. The only distemper vaccines currently available are for mink, although they also have been used for fox and raccoon dogs in emergency situations. The efficacy in eliciting neutralizing antibodies and the safety of three mink-distemper vaccines were studied under field conditions with mink and silver fox. Two of the vaccines were also studied with raccoon dogs and blue fox. All three vaccines elicited a satisfactory antibody response in mink, whereas the response varied in the other species. No side effects were observed in any species tested. One of the vaccines was safe and immunogenic in all four species.

Keywords: Mink; Blue fox; Silver fox; Raccoon dog; Distemper; Vaccination

1. Introduction

The canine distemper virus (CDV) of canids and related species is a member of the family *Paramyxoviridae* and is classified in the genus *Morbillivirus* together with phocine distemper virus (PDV, recognized in seals and mink), measles virus (MV) of man, rinderpest virus (RPV, primarily of cattle and buffalo) and peste-des-petits-ruminants

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virus (PPRV, mainly of small ruminants) (Blixenkrone-Møller, 1993). Many different species of the order *Carnivora* are susceptible to canine distemper (CD), and the mortality varies greatly among species. In addition to the *Canidae*, species of the *Ailuridae*, *Felidae*, *Hyaenidae*, *Mustelidae*, *Procyonidae*, *Ursidae* and *Viverridae* are susceptible to CDV infection (Appel and Summers, 1995). Transmission may be either through contact, aerosol, or indirect; the virus may persist for several days in the environment (Gorham and Wilson, 1997; Pearson and Gorham, 1987).

In 1997, a total of 2 100 000 American mink (*Mustela vison*, later referred to as 'mink'), 2 300 000 blue fox (*Alopex lagopus*), 87 000 silver fox (*Vulpes vulpes*), 70 000 raccoon dog (*Nyctereutes procyonoides*), 130 000 hybrid fox (*Alopex* × *Vulpes*) and 17 000 ferret (*Mustela putorius*) pups/kits were produced in Finland (the Finnish Fur Breeders' Association). Vaccination against distemper is recommended for all fur animal species if distemper in fur animals occurs in Finland. Because no distemper vaccine registered for fox or raccoon dogs is currently available, vaccines registered for mink have been used for these species in emergency situations. The pups were vaccinated at 8–9 weeks of age if the dams are unvaccinated. If the dams have been vaccinated, the pups should not be vaccinated before 10–12 weeks of age due to the presence of maternal antibodies (recommendation by National Veterinary and Food Research Institute, Finland).

CD in dogs reappeared in Finland in 1990, during 1994–1995, an outbreak occurred in areas with high-density dog populations which also involved dogs vaccinated against the virus (Ek-Kommonen et al., 1997). Distemper in fur animals occurred in the early 1990s but not during the 1994–1995 outbreak in dogs. The last large outbreak of distemper in fur animals in Finland was recorded during 1985–1987 and originated from imported fox. Practically all fur animals were vaccinated during that outbreak, with apparently variable results among vaccine brands and animal species.

Although safe and efficient in dogs, modified live CD vaccines may be dangerous for a variety of wildlife and zoo carnivores (Appel and Summers, 1995; Halbrooks et al., 1981), depending (for example) on whether the vaccine strain is adapted to chicken eggs or canine-cell culture (Appel and Montali, 1994). The potential virulence of modified live CDV vaccines in different carnivores became apparent during the mid-1970s. Vaccine-induced distemper was reported in red panda (*Ailurus fulgens*), black-footed ferret (*Mustela nigripes*) and later in other species (Bush et al., 1976; Carpenter et al., 1976). Even the egg-adapted CDV vaccine may be fatal for some species (Appel and Summers, 1995; Saari et al., 1999).

In a preliminary trial, in 1992, mink, raccoon dogs and silver fox were vaccinated at 14 weeks of age and blue fox at 8 weeks of age with Distemink. All mink, raccoon dogs, blue fox and silver fox ($n = 10$ for each species) had positive antibody titres ($\geq$1/32) 2 months after Distemink (Distemink, United Vaccines, USA) vaccination. The antibody levels were promising but inconclusive, because no samples were taken before vaccination.

We ran these trials in 1993 and 1995 to evaluate the safety and immunogenicity of three distemper vaccines registered for mink in eliciting neutralizing antibodies in mink and silver fox under field conditions. Two of the vaccines also were studied with raccoon dogs and blue fox.

2. Materials and methods

2.1. Distemper vaccines

All three commercial vaccines contain freeze-dried modified live distemper virus and the vaccine strains are egg-adapted. Vaccine 1 (Distemink, United Vaccines, USA) is licensed for use in mink in Finland; vaccine 2 (Distem-R-TC, Schering Corporation, USA) has been licensed in mink in Finland, but is no longer in use. Vaccine 3 (intended for use in mink and ferret) has not been licensed in Finland and was used only for the purposes of this study.

2.2. Animals and farm

Healthy mink, raccoon dog, silver fox and blue fox young born within a time span of 2–3 weeks (hence, the limited sample sizes) were chosen from the population of the University of Kuopio Research Fur Farm, Finland. The young of each species were weaned at the age of 5–7 weeks, and placed in cages of the same shedhouse at the age of 10–12 weeks. A female and a male of the same litter usually were placed together. If there were more males than females, the extra males were kept alone. Animals on one side of the shedhouse were vaccinated with one vaccine and those on the opposite side with the other vaccine. All the animals at the farm were vaccinated annually against distemper, and the disease has never been detected at this farm. The farm was supervised by a veterinarian and the staff consisted of trained and highly experienced animal attendants. Ages vary within trials between species because all animals, all species were vaccinated simultaneously, and breeding seasons differ slightly.

2.3. Vaccination, blood sampling and safety monitoring

Vaccination and blood sampling were performed by the veterinarian. Vaccinations were given to healthy young ones. Each animal was vaccinated once subcutaneously in the neck, using the dose recommended for mink by the manufacturers (1 ml). Blood samples were collected from the cephalic vein of canid species with a vacuum-sampling device; for mink, a claw was cut and capillary blood collected openly. Adequate blood samples could not be drawn from all of the animals (especially from mink) at every sampling. Animals were monitored daily for changes in appetite, growth or any other signs of unthriftiness or clinical disturbances by the animal attendants. The staff had no knowledge about which animals received the vaccines used in these trials.

2.3.1. First trial

Two groups of each of the four species were vaccinated with either vaccines 1 or 2 (Table 1). Mink $(n = 20 + 20)$ were vaccinated at 12–14, raccoon dogs $(19 + 20)$ at 15–17, blue fox $(20 + 22)$ at 12–15 and silver fox $(20 + 20)$ at 14–16 weeks of age. Every other mink female and male of the same litter was placed on opposite sides of the shedhouse. Hence, every mink female and male of the same litter was vaccinated alternately with one vaccine or the other. With the other species every other litter

Table 1
Animals with detectable antibody responses after canine distemper vaccination (first trial, Finland, 1993)

Animal	*n*	Time since vaccination (months)[a]				
		0	1.5	2.5–4	8–10	11–12
Vaccine 1						
Mink	20	1/12	15/16	18/18	1/1	1/1
Raccoon dog	19	0/19	18/18	18/18	5/5	5/5
Blue fox	20	0/20	19/19	20/20	–	–
Silver fox	20	0/20	20/20	20/20	4/4	3/4
Vaccine 2						
Mink	20	0/12	13/14	19/19	3/3	3/3
Raccoon dog	20	0/18	20/20	20/20	6/6	6/6
Blue fox	22	0/21	19/22	16/17	5/5	1/1
Silver fox	20	1/20	19/20	18/20	6/7	6/7

[a] The values given in the table indicate the ''number with detectable antibodies/number sampled''.

according to age was placed on opposite sides of the shedhouse, the only exceptions being those litters with uneven numbers of females and males. Hence, the litters were vaccinated alternately with one vaccine or the other. The first blood samples were collected before vaccination, and subsequent sampling was performed 1.5 months and 2.5–4 months after vaccination. In addition, the animals selected for breeding (high indices for fertility and fur quality) were sampled twice more.

2.3.2. Second trial

Two groups of mink and silver fox were vaccinated with either vaccines 1 or 3 (Table 2). Mink $(20 + 20)$ were vaccinated at 12–13 weeks of age and silver fox $(20 + 20)$ at 12–15 weeks of age. Every other litter of both species was placed on opposite sides of the shedhouse, the only exceptions being litters with uneven numbers of females and males. Therefore, the litters were vaccinated alternately with one vaccine or

Table 2
Animals with detectable serum antibody responses after canine distemper vaccination, $n = 20$ in each group (second trial, Finland, 1995)

Animal	Time since vaccination (months)[a]						
	0	1	2	3	4	5	6
Vaccine 1							
Mink	0/17	20/20	18/18	17/19	20/20	1/1	1/1
Silver fox	0/20	17/19	17/19	17/19	16/18	9/10	9/10
Vaccine 3							
Mink	0/18	14/16	13/13	18/20	18/19	8/8	7/7
Silver fox	1/20	9/20	9/20	9/20	12/20	4/13	4/13

[a] The values given in the table indicate the ''number with detectable antibodies/number sampled''.

the other. Animals were sampled at the time of vaccination and then four times (once per month) until pelting in December. In addition, those animals with high indices for fertility and fur quality that were selected for breeding were sampled twice more.

2.4. Antibody determination

Neutralizing antibodies were measured with a modification of the microneutralization test described by Appel and Robson (1973). Briefly, the inactivated serum samples were diluted 4-fold (1/8, 1/32, 1/128 and 1/512) and mixed with an equal volume of medium containing 100 $TCID_{50}$/ml of the Onderstepoort strain of CDV and incubated at 37°C for 1 h. The mixture then was inoculated into vero cells and incubated again at 37°C for 1 h; after this incubation, maintenance medium was added to the wells. A standard virus titration and a positive in-house control serum (1/128) were included in each test series. The test was read microscopically after 6 days, and serum samples with titres <1/8 were classified as undetectable for virus-neutralizing antibodies and those $\geq$1/8 as detectable.

2.5. Statistical evaluation

A value of 1 was used for titres <1/8 to simplify the statistical analysis. The mean titres and standard deviations (S.D.) were calculated from $\log_{10}$-transformed reciprocal values. Statistical tests were applied to the transformed values. The antibody levels between the groups of vaccinates stratified on sampling time, species and trial were compared with Student's two-sample *t*-test (two-tailed). Statistical significance was inferred with $p < 0.05$. The software package used was Statistix for Windows (Analytical Software, USA).

3. Results

3.1. First trial

No changes in appetite, growth or any other signs of unthriftiness or clinical disturbances were reported in any of the four species during the trial.

No detectable antibodies were present in the samples taken before the vaccination, except for low levels of antibodies (1/8 in both) in one 13-week-old mink and one 16-week-old silver fox, both of which seroconverted 1.5 months after vaccination (Table 1).

Antibody levels in mink vaccinated with either vaccines 1 or 2 did not differ significantly 1.5 months after vaccination (Fig. 1), whereas the antibody levels in mink given vaccine 1 were significantly higher ($p = 0.008$) 4 months after vaccination than in those receiving vaccine 2.

Antibody levels in raccoon dogs vaccinated with vaccine 1 were significantly higher than in those receiving vaccine 2, both at 1.5 and 3 months after vaccination ($p = 0.002$ in both). The antibody levels in raccoon dogs were extremely high compared with the other fur animals. Ten and 12 months after vaccination, the animals given

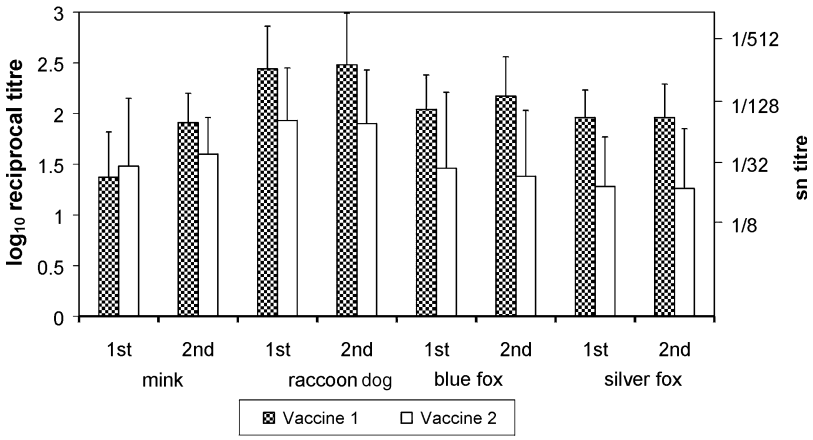

Fig. 1. First trial of distemper vaccination in Finland (1993); mean titres with S.D. achieved with the two vaccines in different species (approximately 20 animals per vaccine group per species).

vaccine 2 ($n = 6$) had antibody titres $\geq$1/8 and those receiving vaccine 1 ($n = 5$) had titres $\geq$1/512.

Antibody levels in blue fox vaccinated with vaccine 1 were significantly higher than in those receiving vaccine 2 both 1.5 and 2.5 months after vaccination ($p = 0.004$ and 0.0001, respectively). Eight months after vaccination, all five animals remaining in the vaccine 2 group had titres $\geq$1/8.

Antibody levels in silver fox vaccinated with vaccine 1 were significantly higher than in those receiving vaccine 2, both 1.5 and 3 months after vaccination ($p < 0.0001$ and $p = 0.0001$, respectively).

3.2. Second trial

No changes in appetite, growth or any other signs of unthriftiness or clinical disturbances were reported in either species during the trial.

There were no detectable antibodies in the samples taken before vaccination, except for low levels of antibodies (1/8) in one 14-week-old silver fox, which seroconverted 2 months after vaccination (Table 2). Antibody levels in mink receiving either vaccine did not differ significantly 1, 2 or 3 months after vaccination (Fig. 2). Four months after vaccination, the titres were significantly higher in the vaccine-1 group ($p = 0.02$). All eight minks sampled 6 months after vaccination had titres $\geq$1/128.

Antibody levels in silver fox receiving vaccine 1 were significantly higher than those of vaccine 3 vaccinates 1, 2, 3 and 4 months after vaccination ($p = 0.005$, 0.008, 0.003 and 0.008, respectively). Eight animals receiving vaccine 3 showed no detectable antibody levels at any of the samples taken 1–4 months after vaccination. Three months after vaccination, 2 out of 19 (10%) and 11 out of 20 (55%) silver fox vaccinated with vaccines 1 and 3, respectively, showed no detectable antibodies. The two silver fox belonged to different litters and the 11 silver fox to three different litters.

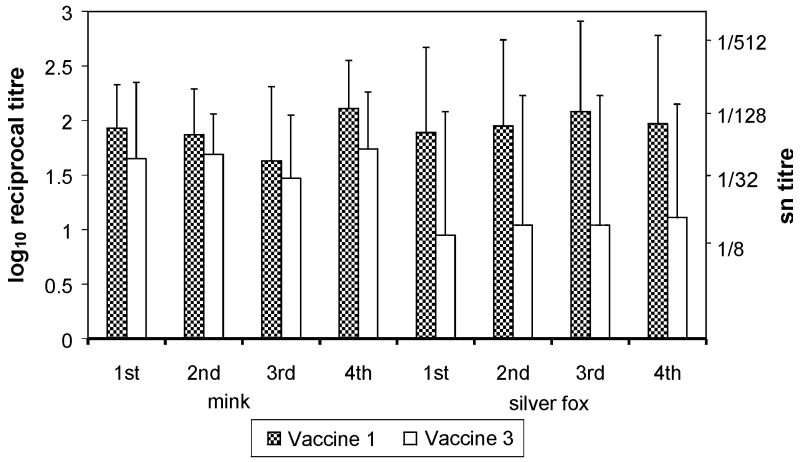

Fig. 2. Second trial of distemper vaccination in Finland (1995); mean titres with S.D. achieved with the two vaccines in mink and silver fox (approximately 20 animals per vaccine group per species).

4. Discussion

Vaccines 1 and 2 had been used occasionally to treat the other species in emergency situations with usually satisfactory but undocumented results, but a methodical evaluation of the safety and efficacy was considered indispensable.

The safety of the vaccines was an overriding concern — especially, because high mortality in grey fox (*Urocyon cinereoargenteus*) associated with vaccination with a canine-cell-adapted CDV vaccine has been reported. However, this vaccine was well tolerated by red fox (*V. vulpes*) (Halbrooks et al., 1981). Both species have been vaccinated without side effects with the egg-adapted CDV vaccine (Appel and Summers, 1995). All three vaccines used in the present study are of the egg-adapted type, and no adverse or side effects were observed in any of the species. There are also no reports of unfavourable effects of the emergency use of these vaccines in the field.

Capability of an animal to produce neutralizing antibodies and strong early cell-mediated response both correlate with survival after infection (Appel, 1969; Appel et al., 1982). Neutralizing antibodies are considered to correlate directly with the protection *in vivo* (Murphy et al., 1999) and are the ''gold standard''. In the present study, the production of detectable levels ($\geq$1/8) of neutralizing antibodies after vaccination was taken to indicate the presence of an active immune response with involvement of immune memory. A level of 1/8 was chosen for the first dilution, because dilutions <1/8 in some samples cause nonspecific cell death in the virus-neutralizing test due to serum toxicity. The low levels of neutralizing antibodies detected in 0-samples of three animals are presumably of maternal origin. In general, the amounts of maternal antibodies decline to insignificant levels by about 10–12 weeks, but may in extreme cases persist for as long as 16–20 weeks (Tizard, 1997). The levels of these antibodies were apparently insignificant, because all three animals seroconverted after vaccination. The three vaccines induced moderate or high antibody levels in mink as expected. Vaccine 1 was superior to the other

two vaccines in this species only at the last sampling of all of the vaccinates (4 months after vaccination). However, the antibody levels induced by vaccine 1 in raccoon dogs, silver fox and blue fox were significantly higher than those induced by vaccine 2 and those induced by vaccine 3 in silver fox at each sampling. Interestingly to us, the antibody levels elicited in raccoon dogs were very high compared with those elicited by the same vaccine in other species. With this species, antibody titres against parvovirus are also clearly higher than in other fur animal species (Neuvonen et al., 1982). Egg-adapted vaccine strains are less immunogenic than canine-cell-adapted strains in the immunization of dogs (Appel and Summers, 1995), but the egg-adapted CD vaccines also differ (Rikula et al., 2000). The immunogenicity of a vaccine also depends on virus attenuation, passage level and method of production. The superiority of vaccine 1 is probably due both to the original virus strain and to the presence of an optimized passage level. Vaccine 2 also induced detectable levels of antibodies in most animals.

The proportion of animals with detectable antibody levels in a group is an important measure of a vaccine's performance, due to the protective effect of herd immunity. This applies especially to conditions under which large numbers of animals are kept in close confinement. In the case of measles (another morbillivirus infection), coverage of 91–95% is required to provide herd immunity — even though vaccines with high impact are available (Nokes and Anderson, 1988; Woolhouse and Bundy, 1997). If a large proportion of a group of immunocompetent animals has not produced neutralizing antibodies within 4–6 weeks after vaccination, it is questionable whether the vaccine is immunogenic enough. In this respect, both vaccines 1 and 2 performed well with the species tested — whereas vaccine 3 raised the proportion of immunized silver fox only to an insufficient 45–60%.

5. Conclusion

This is the first published report on the safety and immunogenicity of commercial mink-distemper vaccines used under field conditions in blue fox, silver fox and raccoon dogs. All three commercial distemper vaccines registered for mink were immunogenic and safe in this species and also can be used safely to immunize the other species against distemper. However, the vaccines differed in their immunogenicity in the other species. The best results consistently were obtained with vaccine 1.

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