SPECIFIC HUMORAL RESPONSE IN SILVER FOXES AFTER IMMUNISATION WITH VACCINES AGAINST CARNIVOROUS-ANIMAL PARVOVIRUSES

BARBARA MAJER-DZIEDZIC, ŁUKASZ JAROSZ, ANDRZEJ JAKUBCZAK, AND KRZYSZTOF KOSTRO

Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences, 20-612 Lublin, Poland

1Subdepartment of Veterinary Microbiology, Institute of Biological Bases of Animal Diseases, Faculty of Veterinary Medicine, University of Life Sciences, 20-033 Lublin, Poland

2Department of Biological Bases of Animal Production, Faculty of Veterinary Medicine, University of Life Sciences, 20-950 Lublin, Poland

bdziedzic@poczta.onet.eu

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Abstract

The research objective was to apply the haemagglutination-inhibition test to determine the antibody level in silver fox serum. The animals were immunised with inactivated vaccines against mink viral enteritis, as well as attenuated vaccines against parvovirosis of dogs. The tests involved 35 females aged 6-7 months. It was shown that none of the vaccines have a negative influence on fox reproduction, and all the vaccines are immunogenic and may be used in the prevention of parvoviral infections in farm foxes.

Key words: foxes, parvovirus, parvovirosis, vaccines, immunisation.

Parvoviruses of carnivorous animals were first recognised in cats in the 1930’s (19). Subsequent infections were observed in dogs (1, 4, 5), coyotes (6, 11, 18), and raccoons (12, 13). In foxes (Alopex lagopus), the disease was described by Phillips (16) in 1940 on the basis of clinical symptoms and histopathological examinations. It was not known until the 1980’s, when viral examinations confirmed the presence of parvovirus at an infected farm and among wild foxes (2, 13, 24-26). The isolated infectious agent blue fox parvovirus (BFPV) showed a significant genome homology (86%) to feline parvovirus (FPV), mink enteritis virus (MEV), and canine parvovirus (CPV – 2) (26). Based on the comparison between nucleotides and amino acid sequence of the capsid protein gene, the BFPV was classified in the group of feline parvoviruses, like viruses isolated from cats (FPV), raccoons (RPV), and minks (MEV). Blue fox (Alopex lagopus) isolates showed the presence of DNA sequences intermediary between FPV and CPV, although the corresponding amino acid sequences clearly grouped the virus as an FPV type (21). DNA obtained from red foxes (Vulpes vulpes) in Germany also showed the presence of intermediary sequences and the serologic overview confirmed the serodominance of parvovirus in foxes, which supports the assumption of interspecies virus transmission between farm and wild carnivores (21, 23).

Parvoviral infection in farm foxes is the disease with a frequent asymptomatic course. In females, it can cause reproductive disorders, manifested by lack or irregularity of oestrus, low effectiveness in covering and insemination, and death and resorption of embryos, as well as mumification of foetuses in the first 21 d of pregnancy. Abortions or stillbirths are also common. Offspring high-mortality rate in the first days of their life is noted, as well as scarce litters or no delivery, despite previous pregnancy symptoms. Low reproduction results in the economic inefficiency of breeding farms, since the number of the reared young is directly related to the number of skins for sale.

The BFPV reservoir comprises adult foxes that are shedding the virus without showing any signs of the disease. Under natural conditions the crucial role in virus transmission is played by the vertical path (ability to get through the placenta barrier) and infection takes place in the uterus. After crossing the placenta barrier the infectious agent permeates an embryo or foetus and replicates intensively, which leads to embryolysis or mumification.

Indirect methods proved their usefulness in the diagnosis of BFPV infections, as they enable the revealing of the increase of specific antibodies in blood serum. In the serologic diagnostics of BFPV infections both the haemagglutination inhibition test (HI) and
ELISA are the most useful. They are particularly effective in animal examinations on farms to identify and eliminate asymptomatic virus shedders (24-26, 28, 29).

The only effective method for the prevention of parvoviral infection in farm foxes is the specific immunisation of animals in the main herd before the covering period, by using attenuated or inactivated vaccines. Despite the lower immunogenic value of inactivated vaccines in comparison to the attenuated variety, the former are more widely applied in practice because of fewer side effects. In specific immunoprophylaxis against parvoviral infections in foxes, heterologic preparations are applied. The biologicals are intended to prevent parvoviral infections in minks and dogs, since, as yet, no specific vaccines have been made containing the immunogenic strain of BFPV (8).

The research objective was to determine the serum level of specific haemagglutination antibodies in silver foxes after immunisation with inactivated vaccines against mink viral enteritis, as well as with attenuated vaccine against dog parvovirosis.

Material and Methods

Animals. The research was carried out on a farm where the main animal herd consisted of 250 females of silver foxes and 50 females of blue foxes. The farm sanitary conditions were assessed as good. The foxes were fed conventionally, complying with the standards recommended for this animal species, including mineral-vitamin supplements and free access to water. The study was performed in the preparatory period for reproduction at the turn of November and December. Only female silver foxes were selected for experimental groups as they had not been vaccinated against parvovirosis, and their sera did not reveal any specific haemagglutination antibodies against parvoviruses.

Vaccines. Two commercial vaccines, as well as a vaccine prepared by the authors, were used in the experiment. The first commercial preparation was a monovalent vaccine Biovac (United Vaccines, USA) containing inactivated strains of MEV, types 1 and 2. The second preparation was polyvalent vaccine Febrivac 3-Plus (Nordvacc, Denmark) containing inactivated MEV, toxoid type C of Clostridium botulinum, and inactivated precipitate of Pseudomonas aeruginosa serotypes 5, 6, and 7/8. The authors’ own vaccine was prepared on the basis of attenuated parvovirus strain isolated from dogs with clinical parvoviriosis. This strain was passaged 100 times in a cell culture of CCC clone 81 (clone 81 of transformed feline kidney cells) and five times cloned by the plaques method. The virus titre in the vaccine was as high as 10^6.5 CCID50 ml⁻¹ (9).

Vaccination. Thirty-five females of silver foxes, aged 6-7 months were involved in the experiment. The animals were divided into three experimental groups (I, II, and III). Group I consisted of 14 females, which further was divided into two subgroups (Ia and Ib) of seven females each. All these foxes were given subcutaneously the commercial vaccine Biovac in the amount of 1 ml (subgroup Ia) and 2 ml (subgroup Ib) following revaccination with the same dose after 28 d. Fourteen females in the group II were divided in the same way into two subgroups (IIa and IIb) and they received twice subcutaneously Febrivac 3-Plus in the dose of 1 ml (subgroup IIa) and 2 ml (subgroup IIb), at the same interval as the animals in group I. The seven females in group III were given 2 ml of the vaccine prepared by the authors, in the same manner as the animals in groups I and II. The above-mentioned dose of the vaccine in group III had been determined in previous experiments, in which it was proved that giving it twice in the amount of 2 ml resulted in the highest level of serum antibodies determined by the HI and seroneutralisation tests (SN) (10). Control group involved seven randomly-selected females of the same age and breed. They were not vaccinated, and the level of specific antibodies was determined at the start and the end of the experiment.

The haemagglutination-inhibition test (HI). The level of specific antibodies in the immunised foxes was determined by means of an HI test carried out according to the β-method, applying double solutions of sera mixed with 4 HAU of the virus. Natural haemagglutinins were removed by the thermal inactivation of the sera (56°C for 30 min) followed by saturation with pig erythrocytes. In addition, nonspecific agglutinins were detected by carrying out the first serum dilutions twice, which made it possible to replace the antigen with the buffer used in the research (10). The level of specific haemagglutination-inhibiting antibodies in animals’ sera was determined before the vaccination (day 0) and then after giving the first (7th, 14th, 21st, and 28th d) and second dose (35th and 42nd d) of the tested vaccines.

Statistical analysis. The obtained results were statistically analysed by calculating the means, as well as the standard deviation. Additionally, the significance of differences between the groups was verified by the Duncan test. The calculations were made using the statistical package SAS.

Results

In the examined blood serum samples taken before the vaccination, no specific antibodies determined by HI test were found neither in all the experimental groups nor in control animals. The antibodies were not detected in the animals from the control group at the end of the experiment. HI antibodies appeared in the immunised foxes on the 7th d after vaccination (Fig. 4). The kinetics of immunological response to the first vaccination with Biovac and Febrivac 3-Plus was identical after both doses (1 ml and 2 ml); however, there were significant differences in the antibody titres strictly depending on the applied dose (Figs 1, 2). Significantly-different antibody levels were observed on the 14th d.
**Fig. 1.** Level of haemagglutination inhibiting antibodies titre (HI) in the serum of silver foxes immunized with Biovac vaccine (x ± SD)

\[ y = -63,543x + 833,07 \]
\[ R^2 = 0,1157 \]

**Fig. 2.** Level of haemagglutination inhibiting antibodies titre (HI) in the serum of silver foxes immunized with Febrivac 3-Plus (x ± SD)

\[ y = -130,74x + 1081,6 \]
\[ R^2 = 0,2731 \]

\[ y = -76,343x + 904,53 \]
\[ R^2 = 0,0749 \]
Fig. 3. Level of haemagglutination inhibiting antibodies titre [HI] in the serum of silver foxes immunized with the attenuated vaccine (x±SD)

Fig. 4. Comparative results of mean titre values of haemagglutination inhibition antibodies [HI] in the serum of silver foxes immunized with different vaccines against parvovirosis (x±SD)
In the case of Biovac vaccine applied in the dose of 1 ml, the antibody titre was 864, but for 2 ml dose 1,280. After vaccination with Febrivac 3-Plus using 1 ml and 2 ml doses, the antibody level on the 14th d was 608 and 1,280, respectively. After vaccination with a 1 ml dose the highest mean antibody titre was recorded on the 21st d, and on the 14th d for 2 ml dose. On the 28th d, the antibody level dropped significantly and reached similar values, irrespective of the applied doses of Biovac and Febrivac 3-Plus (Figs 1, 2). The second dose application of Biovac of 1 ml and 2 ml significantly declined the trend in haemagglutination inhibiting-antibody titres in relation to the first dose (Fig. 1). However, the booster application of Febrivac 3-Plus in the dose of 1 ml increased antibody titres, whereas the amount of 2 ml showed falling trend towards antibody levels compared to their content after a single vaccine application in the same doses (Fig. 2).

Immunisation of foxes with the vaccine made by the authors in the amount of 2 ml resulted in a rising trend of HI antibody titres and this tendency held also after revaccination. The antibody level, which declined on the 28th d of the experiment, increased after the revaccination, and showed similar values on the 42nd d after the immunisation (Figs 3, 4).

**Discussion**

The lack of commercial biologicals for the immunoprophylaxis of parvoviral infection in farm foxes requires the use of vaccines containing parvoviruses isolated from other carnivores. The possibility of using heterologic vaccines is additionally supported by the fact of a close genetic and antigenic relationship among parvoviruses in different carnivorous species (7, 14, 15, 20, 21, 26, 27). Therefore, it is justifiable to use for fox immunisation already developed vaccines against parvoviriosis of the similar animal species. The results of the experiments performed by Majer-Dziedzic et al. (10) proved that the attenuated vaccine against the dog parvovirus induced a good humoral response, as measured by the level of HI and SN antibodies, in both silver and arctic foxes. The vaccine efficacy was assessed on a large number of foxes. Forty female blue foxes before their covering periods, and 280 male blue and 61 silver foxes from different farms, were immunised. The obtained HI antibody titres were of a high protective value and ranged from 80 to 640. The vaccination of female foxes before the covering period had a beneficial influence on both the reproduction process and offspring health conditions in the first period of rearing. It led to a satisfactory level in the young foxes rearing rate. Many authors (3, 9, 10, 19, 20, 27, 29) claimed that the determination of specific level of antibodies using the HAI test is a reliable, convenient, quick, and highly recommended method of immunological response monitoring as well as an assessment of the protective value of the vaccines used against parvovirosis in dogs, cats, minks, raccoons, and foxes. They also admit that antibody titre of 80 and higher protect the animals against illness (3, 9). If this threshold value is assumed to be protective, then even a single vaccination of foxes with the Biovac and Febrivac 3-Plus vaccines in the doses of 1 ml or 2 ml, as well as with the authors’ vaccine applied in the amount of 2 ml, is an effective preventive measure against parvovirus infection in this animal species.

The authors’ research has proved that an application of Biovac in the second dose of 1 ml or 2 ml, as well as of Febrivac 3-Plus in 2 ml dose resulted in the declining trend line. Presumably, a single application of 2 ml of the vaccines, which can be regarded as an effective immunogenic dose for farm foxes, would be a less time-consuming and less expensive method in immunoprophylaxis of parvoviral infections in foxes. Booster dose application has a partial inhibiting influence on the secondary immunological response. It is possible that the reason for the weaker response is the partial inactivation of vaccine virus by the antibodies. A further increase in antibodies level after the second 1 ml dose of Febrivac 3-Plus confirms that a weaker immunological response to its single application in an inappropriate amount can be compensated by a booster dose.

In conclusion, the initial results of the study determine the proper dose of the vaccines to be used in farm foxes, as well as the optimum immunisation scheme. Moreover, the results prove that the applied vaccines do not have negative influence on reproduction in foxes and show their good immunogenic properties. Therefore, they can be used in the immunoprophylaxis of parvoviral infections in this animal species.

**References**

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