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Clinical efficacy studies of the vaccine against feline panleukopenia, calicivirus infection and viral rhinotracheitis Carnifel PCH in kittens

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ABSTRACT

Viral infections in cats can cause serious diseases and even death. Vaccines protect animals from diseases by inducing antibody production and cellular immune response. Primary and booster vaccinations are performed in accordance with the recommendations for the use of vaccines prescribed by the manufactures depending on the minimum duration of immunity. In case of feline panleukopenia, antibody titers correlate with the protection against infection, as for feline calicivirus infection and feline rhinotracheitis, there is no such correlation or it is less clear. Vaccination of cats against these diseases has been performed in the Russian Federation for many years, nevertheless, the feline panleukopenia virus (FPV), feline calicivirus (FCV) and feline herpesvirus (FHV) are still the main common cause of morbidity and mortality among cats. Virus-carrying cats play an important role in the transmission of respiratory viruses such as FHV and FCV in the feline population, and the long-term persistence of FPV in the body, stability in the environmental conditions and resistance to disinfecting agents can be a potential cause of the infection in susceptible kittens. Due to variety of antigenically different FCV strains, the use of the vaccines containing two or more viral strains may induce a broader heterologous protection. The purpose of this work was to evaluate the effectiveness of the vaccine against feline panleukopenia, feline calicivirus infection and feline viral rhinotracheitis developed at the Federal Center for Animal Health (Vladimir) subordinate to the Rosselkhoznadzor, containing 2 heterologous FCV strains (Pers strain genotype I and Fauna strain genotype II), FPV Sheba strain and FHV Lavr strain. The product was developed and tested for its quality in accordance with the requirements of the Russian Federation law. Clinical studies were conducted using 8–12 week-old kittens from different litters born from seronegative, non-vaccinated cats and kept in the household, in a veterinary hospital and animal shelters. The product has successfully passed comprehensive quality control and is registered in the territory of the Russian Federation.

Keywords: feline panleukopenia, feline calicivirus infection, feline viral rhinotracheitis, prevention, vaccine safety and efficacy

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Клинические исследования по оценке эффективности вакцины против панлейкопении, калицивироза и вирусного ринотрахеита кошек «Карнифел PCH» при иммунизации котят

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РЕЗЮМЕ

Вирусные инфекции у кошек приводят к серьезным заболеваниям, нередко становясь причиной летального исхода. Вакцины защищают животных от заболеваний, индуцируя образование антител и клеточный иммунный ответ. Первичная и бустерная вакцинация проводится в соответствии с рекомендациями по применению вакцинных препаратов, предоставленными организациями-разработчиками, исходя из минимальной продолжительности

иммунитета. При панлейкопении кошек титры антител коррелируют с защитой от инфекции, что касается калицивироза и ринотрахеита, то аналогичная взаимосвязь отсутствует или менее четкая. Вакцинация кошек против данных заболеваний доступна на территории Российской Федерации уже много лет, тем не менее вирус панлейкопении (FPV), калицивирус (FCV) и герпесвирус (FHV) продолжают оставаться основными распространенными причинами заболеваемости и смертности среди представителей семейства кошачьих. Кошки-вирусоносители играют важную роль в передаче таких респираторных вирусов, как FHV и FCV, в кошачьей популяции, а длительная персистенция FPV в организме, устойчивость в окружающей среде и к дезинфектантам приводит к заражению восприимчивых котят. Ввиду того, что существует множество обладающих антигенным разнообразием штаммов FCV, введение вакцин, содержащих два штамма вируса или более, будет приводить к более широкому спектру перекрестной защиты. Целью данной работы было оценить эффективность разработанной на базе подведомственного Россельхознадзору ФГБУ «Федеральный центр охраны здоровья животных» (г. Владимир) вакцины против панлейкопении, калицивироза и вирусного ринотрахеита кошек, состоящей из 2 гетерологичных штаммов FCV (штамм «Перс» генотип I и штамм «Фауна» генотип II), штамма «Шеба» FPV и штамма «Лавр» FHV. Разработку и контроль качества препарата осуществляли согласно требованиям законодательства Российской Федерации. Клинические исследования проводили с использованием котят 8–12-недельного возраста из разных пометов, рожденных от серонегативных, невакцинированных кошек и содержавшихся в домашних условиях, ветеринарном госпитале и приютах для животных. Препарат успешно прошел всесторонний контроль качества и зарегистрирован на территории Российской Федерации.

Ключевые слова: панлейкопения кошек, калицивирусная инфекция кошек, вирусный ринотрахеит кошек, профилактика, безвредность и эффективность вакцины

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INTRODUCTION

Infections caused by the *Feline panleukopenia virus* (FPV), *Feline calicivirus* (FCV) and *Feline herpesvirus 1* (FHV), the main pathogens of cats, are widespread in all countries of the world and increasingly reported in Russia every year. Cats without specific immunity can become infected and even die. The viruses can be transmitted not only by diseased animals, but also by virus-carrying animals (possible healthy carriers) without apparent clinical signs, shedding the viruses into environment with feces, urine, and nasal discharge [1, 2].

Feline viral rhinotracheitis (*Feline herpesvirus 1*, FHV) is a contagious viral disease caused by FHV involving upper respiratory tract, causing conjunctivitis and keratitis. If the infections are complicated by secondary bacterial infections, the disease would be more severe and ultimately cause the death of the animal. About 80% of cats remain FHV-infected for the rest of their lives and the virus may become reactivated periodically, often due to stress or immunosuppression [2, 3].

Feline panleukopenia (*Feline panleukopenia virus*, FPV) is a highly contagious disease of viral etiology characterized by fever, acute hemorrhagic enteritis, leukopenia, dehydration and high mortality rates (from 25 to 100%) [1, 4, 5].

Feline calicivirus infection (*Feline calicivirus*, FCV) is a highly contagious viral disease characterized mainly by the involvement of the oral and upper respiratory mucosa. According to literature data, FCV was detected in 18–30% of cases suffering from upper respiratory diseases. Virus carriers are also common (up to 75%), especially among stray cats [2, 6, 7]. In addition to the common symptoms of oral ulcers, rhinitis and conjunctivitis, the disease signs may also include lameness, edema of the head and limbs, pneumonia, tongue and palate necrosis, gastrointestinal involvement. Various sources describe a systemic infection that causes death of up to 60% of diseased animals. FCV is characterized by a high genetic variability and antigenic diversity of strains, which significantly reduces the effectiveness of available vaccines [7, 8].

FPV, FHV and FCV are ubiquitous and infect domestic cats of all breeds and ages, as well as zoo and wild felines.

Live and inactivated mono- and combined vaccines are used to prevent viral diseases of cats. The vaccination effectiveness strongly relies on the presence of maternally derived antibodies (MDA), which usually persist in kittens up to 8–12 weeks of age, and sometimes even longer. There is a critical period for kitten vaccination, or «window of susceptibility», when MDA neutralize the vaccine virus, but do not protect against infection with field virulent viruses. The presence of MDA in high titers in kittens blocks the development of post-vaccination immunity and, as a result, affects the immunization outcomes [2, 9, 10, 11]. The level of MDAs will differ between litters and individual animals within litters, depending on the antibody levels in the colostrum of the queens and the amount of colostrum ingested. Therefore, it is common practice to perform the first core vaccination at 8–9 weeks of age (or earlier in higher risk or sheltered kittens), and to administer additional doses at 2–4-week intervals until the age of 12–16 weeks or older with the expectation that one of these vaccinations will occur after the blocking effect of the MDA has waned, and before exposure to virulent agents. Three international

expert groups: the Feline Vaccination Advisory Panel of the American Association of Feline Practitioners (AAFP), the Vaccination Guidelines Group of the World Small Animal Veterinary Association (WSAVA VGG) and the European Advisory Board on Cat Diseases (ABCD) provided veterinary practitioners with recommendations on the use of vaccines for cats. Despite some differences, all three groups recommend re-vaccinations for core vaccines at intervals of more than one year. The current recommendation for vaccination against FHV and FCV infection is primary vaccination with two injections at an interval of 3–4 weeks and revaccination after 1 year. Subsequent boosters should be administered every 3 years, except for higher-risk situations [4, 10, 11, 12, 13, 14, 15].

Unlike vaccines against feline panleukopenia, which provide long-term complete protection for cats, vaccines against feline viral rhinotracheitis and calicivirus infection significantly reduce the frequency of clinical cases, but do not confer full protection, and vaccine-induced immunity may decrease over time, requiring regular revaccination [16, 17, 18]. Therefore, along with the FPV antigen, FCV and FHV are considered the major components of the vaccine that all cats should receive regardless of age and gender [2, 4, 19].

Vaccines against feline calicivirus infection do not provide complete protection due to considerable antigenic variability amongst FCV strains [8, 20], this means poor efficacy of the vaccines and inability to completely prevent infection with field virulent strains and further transmission of the virus among cats [7, 8]. For several decades, commercial vaccines for cats have been based on FCV F9 or 255 vaccine strains or a combination of two G1 and 431 vaccine strains, however, some publications state that due to frequent FCV mutations, these vaccines are not always effective [16, 17, 21]. In addition, vaccines against feline calicivirus infection or viral rhinotracheitis do not prevent infection, but rather reduce the severity of clinical signs and sometimes viral shedding [6, 9, 10, 21, 22, 23, 24]. Although commercial combined vaccines against feline panleukopenia, viral rhinotracheitis and calicivirus infection are used worldwide, significantly reducing both morbidity and mortality rates, nevertheless, these viral diseases are still common among cats in various countries, including the territory of the Russian Federation. The development of the vaccines against these infectious feline diseases requires taking into account the wide variety of FCV genotypes, as well as the genetic and antigenic variability of the strains. That is why it is urgently needed to update the strain composition of core vaccines against feline panleukopenia, viral rhinotracheitis and calicivirus infection.

Thus, the specific protection against FPV, FCV, FHV, and prevention of the diseases caused by these viruses among felines are of paramount importance to ensure the favourable animal health situation in the country.

Based on the above, the Federal Centre for Animal Health, subordinate to the Rosselkhoznadzor, was tasked to develop and register a safe and effective vaccine for cats against feline panleukopenia, calicivirus infection and viral rhinotracheitis in the Russian Federation.

MATERIALS AND METHODS

Carnifel PCH vaccine against feline panleukopenia, calicivirus infection and viral rhinotracheitis was developed and tested for quality in accordance with the requirements of Federal Law No. 61-FZ "On circulation of medicines" and Order No. 101 of the Ministry of Agriculture of the Russian Federation "On approval of the Rules for preclinical studies, clinical studies and bioequivalence studies of veterinary medicinal products".

Vaccine. The active ingredients of the Carnifel PCH vaccine include inactivated FPV (Sheba strain), FCV (Pers strain genotype I and Fauna strain genotype II) and FHV (Lavr strain). Aluminum hydroxide is used as an adsorbent. All components of the vaccine undergo comprehensive input quality control, including control of sterility and innocuity in Crandell-Rees Feline Kidney Cells (CRFK) using three consecutive passages.

Animals. Clinical studies were conducted using 8–12-week-old kittens (n = 37) from different litters born from seronegative, non-vaccinated cats and kept in a household, in a veterinary hospital and animal shelters.

Animal handling complied with the ethical standards adopted by the European Convention ETS No. 123 and approved by the Bioethics Commission of the Federal Centre for Animal Health.

Serological tests. Kitten sera obtained before vaccination and at 7, 14, 21, 28, 35, 42 days post vaccination (dpv) were tested. Then sera were collected every month for a year, and tested for antibodies to FPV by haemagglutination inhibition test (HI test), and to FCV and FHV by virus neutralization test (VNT). Before tests sera were inactivated by heating at 56 °C for 30 minutes.

Haemagglutination inhibition test (HI test). For the purposes of the test 25 µL of heat-inactivated serum was subjected to 2-fold serial dilutions started at 1:10 with phosphate buffered saline solution (pH 6.8) in a 96-well U-bottom microplate. Then an equal volume of FPV containing 8 haemagglutination units was added to the diluted sera. Following one-hour incubation, 0.8% porcine erythrocytes were added to each plate well and incubated overnight at 4 °C. The reaction was interpreted after the red blood cells completely settled in the control wells (in the form of a button). The reaction result was considered positive if the tested serum contained FPV-specific antibodies at a titer of ≥ 1:40 (HI titer of ≥ 5.3 $\log_2$) and higher. The antibody titer was expressed as the highest serum dilution causing complete inhibition of hemagglutination.

Virus neutralization test (VNT). To determine the level of neutralizing FCV and FHV antibodies, monolayers of CRFK cells were used. The antibody titer was determined by serial dilution of serum sample, which was then added to the standard amount of the virus: 50 µL of diluted serum and 50 µL of an infectious culture medium containing 100 $TCID_{50}$ of the selected virus strain, mixed and incubated for 2 hours at 37 °C and 5% CO_2. Then, the antibody-virus mixture was inoculated into CRFK monolayer cells in 96-well microplates with CellBIND-treated surface. Each serum dilution was added to 4 wells. The cultures were incubated for 5 days at 37 °C and 5% CO_2. The reaction was interpreted using a microscope. The neutralization titer was expressed as the reciprocal of the highest dilution at which cell infection was blocked (VNT).

Statistical analysis of the results. Microsoft Excel software statistical methods were used to process the obtained data. The mean group titers and standard deviation were determined. The specific antibody titer was calculated using Kaerber formula and expressed as $\log_2$.

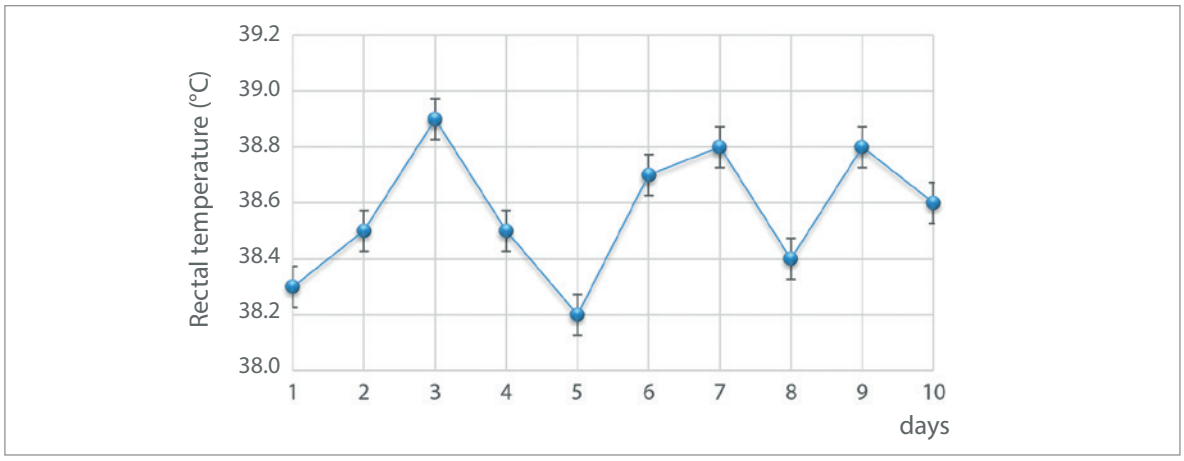

Fig. 1. Body temperature of animals (kittens)

RESULTS AND DISCUSSION

For clinical studies to evaluate the effectiveness of vaccination against FPV, FCV and FHV infections with Carnifel PCH, outbred 8–12 week-old kittens from different litters (n = 37) were used. The kittens were vaccinated subcutaneously twice at a 21 day interval at a dose of 1.0 cm^3. The animals were monitored and their body temperature was measured during 10 days post vaccination.

Figure 1 shows the dynamics of the average body temperature in animals after vaccination. It was established that during 10 days after the first immunization, the body temperature of the kittens remained within the normal range; no depression and loss of appetite were observed.

Within the observation period, it was found that during 21 days post first immunization and after booster vaccination, kittens remained healthy, no changes in behavior and clinical symptoms of FPV, FCV and FHV infection were noted, which suggests the safety of the vaccine used.

Testing of sera collected from kittens before vaccination showed that the animals were seronegative to FCV and FHV (VNT results); FPV specific antibodies (HI test results) were determined at a titer of ≤ 1:20 (4.3 log_2).

Figure 2 shows the dynamics of the humoral immune response development in kittens to the vaccination with Carnifel PCH. It was established that the immune system of animals actively reacted to the antigens included in the vaccine, the concentration of FCV, FHV and FPV antibodies increased gradually over time. The level of FPV antibodies was above the threshold value ≥ 1:40 by 14 dpv; the maximum titers (1:640–1:1,280) were recorded at 42 dpv and persisted throughout the entire study period.

After booster vaccination, at 35 dpv all kittens had high titers of specific antibodies to FCV, FHV and FPV. For example the mean group titer of virus neutralizing antibodies to FHV (Lavr strain) was 6.3 log_2, to FCV (Fauna strain) – 6.5 log_2, to FCV (Pers strain) – 7.2 log_2; the mean group titer of specific antibodies to FPV (Sheba strain) was at the level of 10.3 log_2 (by HI test).

The mean group titers determined at 7, 14, and 21 dpv significantly differed from the same value at 35 dpv ($p \geq 0.1$). At the same time, the mean titers detected at 35 and 42 dpv were statistically identical ($p \geq 0.05$). Biologically this meant that the period up to 35 dpv corresponded to the active phase of the humoral immunity development, the period after 35 dpv corresponded to the stabilization phase. Based on the data obtained, it was concluded that after double vaccination with Carnifel PCH, a strong humoral immune response in kittens develops by 35 dpv, i. e. 14 days post booster vaccination.

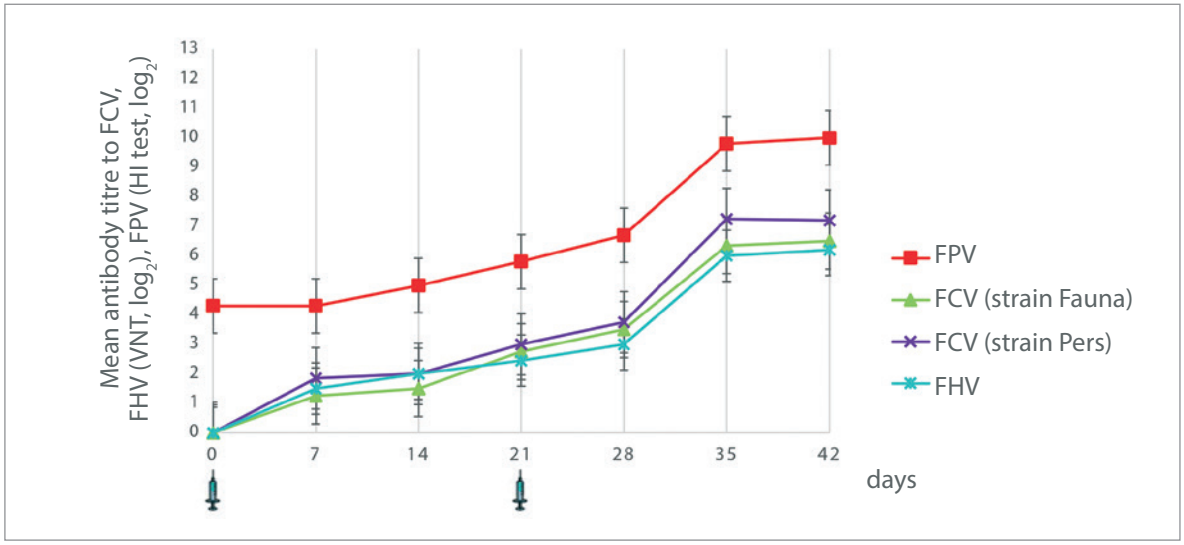

Fig. 2. Development of humoral immune response in kittens following vaccination with Carnifel PCH

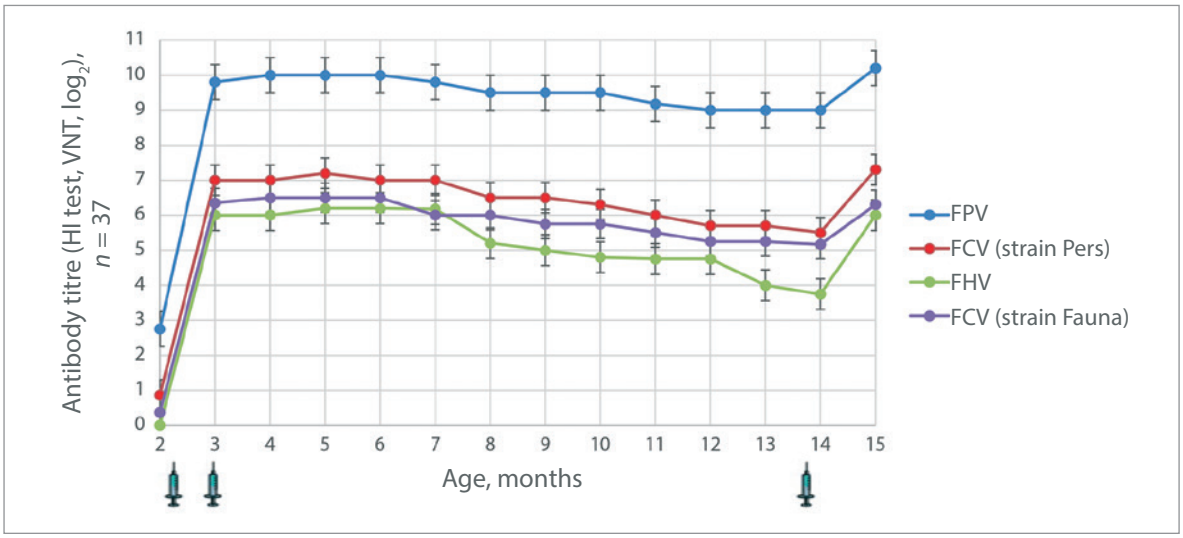

Fig. 3. Duration of immunity in kittens following vaccination with Carnifel PCH

To evaluate the strength of post-vaccination immunity against FPV, FCV and FHV, blood samples were collected from kittens every month during a year. Tests of collected sera showed that during 12 months the mean titer of FHV specific antibodies was in the range from 4.0 to 6.5 $\log_2$, to FCV (Pers strain) – from 5.5 to 7.0 $\log_2$, to FCV (Fauna strain) – from 5.0 to 6.0 $\log_2$ (by VNT), to FPV – from 9.0 to 10.0 $\log_2$ (by HI test).

Figure 3 demonstrates that kittens showed a slight decrease in the level of specific antibodies, which by 14 months of age was the following (mean group titer): to FHV – 3.8 $\log_2$, to FCV (Pers strain) – 5.5 $\log_2$, to FCV (Fauna strain) – 5.2 $\log_2$, to FPV – 9.0 $\log_2$ (by HI test). The kittens were revaccinated in a year with Carnifel PCH once subcutaneously at a dose of 1.0 cm^3, and in 30 days more blood samples were collected for testing. The results showed that the mean group titer of FHV specific antibodies increased by 2.3 $\log_2$, to FCV (Pers strain) – by 1.6 $\log_2$, to FCV (Fauna strain) – by 1.1 $\log_2$ (by VNT), to FPV – by 1.2 $\log_2$ (by HI test).

Thus, based on the data obtained, it was found that Carnifel PCH induced seroconversion after booster subcutaneous vaccination at a dose of 1.0 cm^3 with 21 day interval between the doses; the duration of immunity was at least 12 months.

Most cat vaccination guidelines recommend using a basic vaccination scheme: primary vaccination and subsequent revaccination in a year [4, 10, 11, 14, 25]. The same vaccination scheme of cats was used in our study, which proved its effectiveness for Carnifel PCH vaccination. The vaccine induced strong immunity and specific antibodies to FPV, FHV and FCV at high titers after revaccination.

CONCLUSION

Based on the conducted studies, it was concluded that the vaccine causes the development of an immune response in cats against FPV, FCV and FHV 14 days post double vaccination with a 21-day interval between the doses. The duration of the immunity against these diseases is at least 12 months. When studying the dynamics of FPV, FCV and FHV post-vaccination immunity strength in kittens vaccinated with Carnifel PCH vaccine, its specific effectiveness was proven. The results of serology by HI test and VNT demonstrated strong immune response.

During the tests, it was shown that the developed vaccine has a good tolerability in kittens at 8–12 weeks of age. Double vaccination of animals with a 21-day interval between the doses at a dose of 1.0 cm^3 induces antibodies to FCV, FHV and FPV in high titers. It was found that Carnifel PCH vaccine against FPV, FCV and FHV is safe, non-reactogenic and potent and can be recommended for cats to prevent these infections.

The risk of cat infection with infectious diseases is high at any age, so it is important to understand the need for immunization, which is a tool to keep the diseases under control. Vaccination of even a single animal significantly contributes to the prevention of infectious disease spread in the feline population. The higher the percentage of vaccinated animals in a population, the lower the risk of epizootics.

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