Trials in Vaccinology 4 (2015) 14–18

Contents lists available at ScienceDirect

Trials in Vaccinology

journal homepage: www.elsevier.com/locate/trivac

The efficacy of a multivalent calicivirus, herpesvirus and parvovirus vaccine and a rabies vaccine is not affected when administered in combination

Stephen Wilson [a,*], Vickie King [b], Gordon Sture [b]

[a] Zoetis, Veterinary Medicine Research and Development, 1930 Zaventem, Belgium
[b] Zoetis, Veterinary Medicine Research and Development, Kalamazoo, USA

ARTICLE INFO

Article history:
Received 29 September 2014
Revised 11 December 2014
Accepted 24 January 2015
Available online 25 February 2015

Keywords:
Feline
Calicivirus
Herpesvirus
Panleucopaenia virus
Rabies

ABSTRACT

The results of a serological study examining the antibody responses generated in cats following administration of a trivalent feline vaccine (feline calicivirus [FCV], feline herpesvirus [FHV] and feline panleucopaenia virus [FPV]; Versifel CVR) in combination with an inactivated rabies vaccine, in compliance with European Pharmacopoeia requirements to support new product registrations, are presented.

Nine week old cats were allocated to one of three groups, 10 cats per group. Group 1 received the CVR vaccine on days 0 and 21, group 2 received the rabies vaccine on day 21 and group 3 received the CVR vaccine on day 0 and the CVR vaccine reconstituted with the rabies vaccine (i.e., administered simultaneously) on day 21. Blood samples were collected from each animal, on days 0, 21, 28, 35, 42 and 49; and antibody titres determined using haemagglutination inhibition assay or virus neutralisation test.

FHV and FPV antibody responses in the group 3 combination administration were considered non-inferior to the responses in the group 1 CVR-only at all time points (days 28, 35, 42 and 49). However for FCV, group 3 was considered non-inferior to the responses in group 1 on days 28, 35 and 42; but not on day 49. For rabies, group 3 was only considered non-inferior to the responses in group 2 on day 49; an apparent inferiority was observed on days 28, 35 and 42. However, in all cases cats that received the combination administration seroconverted with antibody titres at a magnitude shown in other studies to be protective against virulent challenge, and also at a titre deemed adequate by the European Pharmacopeia monograph 04/2013:0451 (Rabies vaccine [inactivated] for Veterinary use).

In conclusion, the data show that combining these two separate vaccines in one administration has limited impact on their ability to generate serological responses, and that these responses are still of a magnitude previously demonstrated to be protective.

1. Introduction

The severity of feline calicivirus (FCV), feline herpesvirus (FHV) and feline panleucopaenia (FPV) infection is such that vaccines to these agents are considered core feline vaccine requirements [1], and are often produced as combination products.

Feline calicivirus is a highly infectious pathogen of cats, with a widespread distribution [2,3]. Studies have shown the prevalence is broadly related to the number of cats in a specific location; with, for example, privately owned animals present in small numbers [4] having a lower prevalence than those kept in a cat shelter. FCV has significant genetic diversity with the potential to produce multiple strains, and therefore the range of clinical signs following infection can be broad [5]. The presence of antibodies, either maternally-derived or induced through active vaccination, has been demonstrated to play a role in reducing or eliminating the clinical signs of FCV challenge [6], but does not prevent infection [7].

Feline herpesvirus infection results in feline viral rhinotracheitis. The virus replicates in the mucosal tissues of the conjunctiva and upper respiratory tract and can result in a latent state through infection of neuronal tissue [8]. The presence of latent carrier cats can lead to the infection of others through reactivation of the virus and resultant shedding into the environment [9]. Lesions are a common result of mucosal infection with epithelial necrosis and immune cell infiltration [10]; but other clinical signs can be observed.

* Corresponding author.
E-mail address: stephen.wilson@zoetis.com (S. Wilson).

http://dx.doi.org/10.1016/j.trivac.2015.01.001
1879-4378/

Feline panleucopaenia virus is defined as the prototypical carnivore parvovirus [11], infection resulting in systemic disease through cellular depletion within lymphoid tissues thus causing immune suppression of the animal. The bone marrow is also impacted with virus replicating in early progenitor cell lines thereby affecting virtually all of the myeloid cell populations. Infection of kittens while in the uterus or in the immediate period after birth can affect the central nervous system leading to ataxia and tremors in the neonatal animals. Significant mortality resulting from FPV infection is found in younger cats, although the virus affects all age groups [12].

Rabies infection of cats has significant mortality unless rapidly detected or prevented through vaccination, with virus neutralising antibodies being particularly important in protection [13]. Although rabies is endemic across the world certain areas are designated as free of the virus; e.g. countries such as the UK, Japan, Australia and other islands [14]. The disease is spread from infected animals by saliva through cuts or scratches to the skin, and has a variable incubation period depending on virus dose and location of the entry into the host [15]. The rabies virus replicates in either striated muscle or connective tissue at the point of entry, and from there to peripheral nerves or directly into nervous tissue. The virus travels to the salivary glands, at which point the animal is infectious but prior to onset of any clinical signs [16].

In this study we examined whether the combination of a multivalent feline vaccine (Versifel CVR) with a feline rabies vaccine (Vanguard R) impacted their respective efficacy as measured by serological response when administered to minimum age kittens. The serological responses of cats administered the combination were compared to those of cats administered the two vaccines when given alone.

2. Materials and methods

There were three treatment groups (group 1 – Versifel CVR, group 2 – rabies vaccine (Vanguard R) and group 3 – Versifel CVR + Vanguard vaccine). Group 1 containing ten animals received the CVR vaccine on days 0 and day 21, group 2 containing ten animals received the rabies vaccine on day 21 and group 3 containing ten animals received the CVR vaccine on day 0 and the CVR vaccine reconstituted in the rabies vaccine (i.e. administered simultaneously) on day 21. On day −3, 30 animals were selected for inclusion in the study. Animals were randomised to Treatment Groups as per a randomised complete block design with blocking based on age by a Zoetis Biometrician. The animal age, the vaccine batches used and the study design was selected using the existing guidelines for the concurrent administration of immunological veterinary products (EMEA/CVMP.550/02-FINAL). Ten animals per treatment group allowed testing for non-inferiority using an equivalence limit difference of 2.0 on the logarithm_2 scale assuming a common standard deviation of 1.71 on the logarithm_2 scale, 80% power, and a 0.05 level of significance using a 1-sided test. The values were based on historical values from other Zoetis studies.

The study was run at a Contract Research Organisation (CRO), and the design was approved by both the Zoetis and CRO institutional ethical review committees, and fulfilled all local and national animal welfare and care regulations.

2.1. Animals

Thirty (30) specified pathogen free cats aged 59–64 days old at the time of first vaccination were enrolled into three groups. Each animal had a subcutaneously implanted microchip; the last five digits of the microchip number were used to identify each animal for the study. Blood samples collected three weeks prior to study start showed all cats to be negative to the vaccinal antigens. Further samples collected on day 0, prior to vaccination, showed that all of the 30 animals were sero-negative to FHV and rabies; 29 animals were sero-negative to FPV) (the seropositive cat was in group 1 – Versifel CVR); 25 animals were sero-negative to FCV) (the seropositive animals comprised two animals from group 1 – Versifel CVR, one animal from group 2 – rabies and two group 3 – Versifel CVR + rabies animals). For logistical reasons the pre-screening samples collected on day 0 were not analysed until the completion of the study, but due to the limited sample volumes obtained during the course of the study the decision was taken to not exclude these animals from subsequent analysis.

To avoid the potential risk of viral shedding from animals vaccinated with the live vaccine (groups 1 and 3) to animals vaccinated with the killed vaccine (group 2) animals from group 2 were housed in a separate building. Cats had *ad libitum* access to water and commercial cat food during the course of the study.

2.2. Vaccine

A commercial batch of Versifel CVR (Zoetis) vaccine against feline calicivirus (strain F9 $10^{6.8}$ $CCID_{50}$), feline panleucopaenia virus (Snow Leopard strain $10^{4.6}$ $CCID_{50}$) and feline herpes virus (strain FVRm $10^{6.3}$ $CCID_{50}$), and a minimum titre inactivated monovalent rabies vaccine (Vanguard R, Zoetis; Vnukovo-32 strain 2.0 IU/mL), were used as per the design described above. The Versifel CVR vaccine is lyophilised and requires reconstitution in either sterile water (group 1 – Versifel CVR) or with the Vanguard R vaccine (group 3 – Versifel CVR + Rabies) which is supplied as a liquid formulation. For each vaccination a 1 mL dose was administered to cats by the subcutaneous route. On day 0 all treatments were administered into the left side of the neck and on day 21 into the right side of the neck.

2.3. Observations and samples

A Veterinary examination was performed for each animal on days −7 and −3. Only animals assessed as being healthy on day −3 were enrolled. General health observations were carried out daily by a qualified technician from day −7 to day 49 inclusive.

Blood samples were collected from the jugular vein of each animal into 5 mL whole blood tubes containing no anti-coagulant. Samples were collected pre-vaccination on days 0 and 21 and on days 28, 35, 42 and 49. The tubes containing the whole blood were allowed to coagulate for a minimum of one hour at room temperature and then placed in a centrifuge and spun at 3000 rpm for fifteen minutes at 4 °C. The resulting serum was removed and divided between two serum tubes to obtain approximately equal aliquots.

2.4. Laboratory analysis

The serum was analysed at a commercial diagnostic laboratory using proprietary assays. Haemagglutination Inhibition (HAI) was used to detect the presence of antibodies to FPV, Virus Neutralisation Test (VNT) was used to detect the presence of antibodies to FHV and FCV, and Fluorescent Antibody Neutralisation Test (FAVN) was used to detect the presence of antibodies to rabies. Briefly, for the HAI assay (FPV) blood samples were centrifuged, sera removed and inactivated by heating for 30 min at 56 °C. The appropriate FPV dilution was added to micro-titre plates and twofold dilutions made in PBS containing 1% BSA, positive and negative controls are added to appropriate wells. Twofold dilutions of control and test sera are made across the plate, and the plates are incubated at room temperature for one hour. A 1% suspension of porcine red blood cells are added to all wells and incubated. The titre is