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The administration of a single dose of a multivalent (DHPPiL4R) vaccine prevents clinical signs and mortality following virulent challenge with canine distemper virus, canine adenovirus or canine parvovirus

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ABSTRACT

Four challenge studies following vaccination of dogs with a multivalent vaccine containing canine parvovirus (CPV-2b), adenovirus (CAV-1/-2) and distemper (CDV) are described. Six week old puppies received a single vaccination while non-vaccinated control dogs received water. In each respective trial, groups of dogs were challenged 21 days after vaccination with heterologous viral isolates. Clinical observations, rectal temperature measurements, and blood and swab samples for analysis were collected throughout the study.

Dogs in all studies had normal temperatures and general health up to challenge. Clinical signs of infection and temperatures outside the normal range were observed in non-vaccinated dogs challenged with CDV, CPV, CAV-1 and CAV-2; vaccinated dogs remained clinically normal after challenge. All dogs were sero-negative prior to vaccination, non-vaccinated dogs remaining negative until challenge. Vaccinated dogs all sero-converted by 21 days after vaccination, with further increases seen after challenge. Non-vaccinated dogs sero-converted following challenge with CPV or CAV-2; no final blood samples were taken in the CDV and CAV-1 studies. Rectal swab analysis showed prevention of CPV shedding in vaccinated compared to non-vaccinated dogs, and nasal swab analysis following CAV-2 challenge showed longer duration and higher amount of viral shedding for non-vaccinated dogs.

In conclusion, we demonstrated that a single administration of a minimum titre, multivalent vaccine to dogs of six weeks of age is efficacious and prevents clinical signs and mortality caused by CAV-1 and CDV; prevents clinical signs and significantly reduces virus shedding caused by CAV-2; and prevents clinical signs, leucopoenia and viral excretion caused by CPV.

Introduction

Domestic dogs are at higher risk of contracting infectious diseases, and in particular viral infections, if they are not immune to the specific agent [1]. The widespread use of vaccines has led to reduced incidence of common infections; however, continuing evolution of vaccine technology results in products of varying efficacy [2] or range of protection. Vaccines have been grouped into core, non-core or non-recommended categories; with canine distemper, canine parvovirus and canine adenovirus considered core vaccine components recommended to be administered every three years [3]. These recommendations have been expanded and form the basis of the World Small Animal Veterinary Association (WSAVA) Guidelines for the Vaccination of Dogs and Cats [4].

Canine parvovirus (CPV) infection of dogs results in a contagious enteric disease leading to high rates of mortality or severe morbidity [5]. All naïve dogs are susceptible to infection with those under one year of age having the highest risk of developing severe disease. Since CPV2 was first identified [6], there have been multiple variations of virus strains which have increased in prevalence. Canine parvovirus type 2a [7], CPV-2b [8] and CPV-2c have been identified [9], and are becoming more frequently isolated [10,11]. Canine adenovirus type 2 (CAV-2) infection ranges from essentially non-apparent to a mild form of respiratory disease; however, the virus is considered to be one of the main causes of infectious

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tracheobronchitis in dogs [1]. The canine adenovirus type 1 (CAV-1) results in a more general infection, with predominant clinical signs being nausea, vomiting and diarrhoea [12]. Although CAV-2 was initially thought to be derived from CAV-1, later analysis [13] revealed they are genetically distinct. Despite this, dogs vaccinated with vaccines containing CAV-1 or CAV-2 were found to be cross protected [14,15]. As vaccines containing CAV-1 do have safety issues [12], current vaccines as recommended by the WSAVA [4] contain CAV-2. Canine distemper virus (CDV) is another highly contagious virus which results in acute to sub-acute systemic disease and a high mortality rate in dogs [16], although the host range [17] includes a variety of species. The introduction of efficacious vaccines has reduced the incidence of canine distemper disease in dogs although the possibility still exists that infection outbreaks can occur, even in vaccinated animals [16].

In this paper we describe the efficacy of a new multivalent canine vaccine containing the core viral components – CDV, CPV and CAV – in addition to canine para-influenza, rabies and four *Leptospira interrogans* and *kirschneri* serovar antigens. In four separate trials, dogs free of antibodies to the vaccine antigens received a single vaccination and then were challenged with different strains of each core viral antigen (distemper, adenovirus 1 and 2, and parvovirus). The impact of vaccination on clinical variables, serology and for some components (CPV and CAV-1) re-isolation of challenge virus was examined by comparing vaccinated dogs to non-vaccinated dogs in each study.

Materials and methods

This study reports the results of four separate trials with all studies designed to be compliant with the respective European Pharmacopeia monographs 01/2008:0448 (distemper), 01/2008: 1951 (adenovirus) and 01/2008:0964 (parvovirus). The studies were carried out in accordance with the Act on Animal Health and Animal Welfare of The Czech Republic, and had been approved by Bioveta a.s. and Zoetis ethical review committees.

Animals

In the studies determining efficacy to CDV, CAV-1 and CPV, seven dogs aged 6 weeks old, were enrolled into each study with five dogs vaccinated and two dogs receiving water for injection acting as controls. In the CAV-2 study, 20 dogs were enrolled with 10 dogs vaccinated and 10 dogs receiving water for injection as controls. All dogs were confirmed to be free of antibodies (methods described in laboratory analysis) against the respective challenge virus.

Vaccine

An experimental vaccine batch was produced which contained live CDV, CPiV, CAV-2, CPV-2b (DHPPi); inactivated *L. interrogans* sv Canicola, Icterohaemorrhagiae and Bratislava, and *L. kirschneri* sv Grippotyphosa, and rabies virus (L4R). The DHPPi component was freeze-dried while the L4R component was a liquid containing adjuvant (aluminium hydroxide). The control product was sterile water. Administration (1 ml) was by the subcutaneous route behind the left shoulder blade on day 0 using standard aseptic technique. Where appropriate vaccine antigens were formulated at minimum titre and maximum passage.

Challenge

The challenge isolates were different strains to the vaccine antigens and had been used previously in validation studies. CDV isolate Snyder Hill was obtained from the American Type Culture Collection; CAV-1 isolate Mirandola and the CAV-2 isolate Manhattan were obtained from the Animal and Plant Health Inspection Services at the Centre for Veterinary Biologics; and CPV 2b isolate 212/98 was obtained from the University of Bari, Italy. For the CDV (10^{-1} dilution of virus provided which was of unknown titre) and CAV-1 ($10^{5.8}$ $TCID_{50}$/mL) studies 1 mL of challenge material was administered by the intravenous route; for CPV ($10^{6.8}$ $TCID_{50}$/mL) a 2 mL dose was administered with 1 mL orally and 1 mL intranasally (0.5 mL per nostril); for CAV-2 ($10^{5.3}$ $TCID_{50}$/mL), 1 mL of challenge material was administered intranasally.

Observations and samples

Rectal temperatures (°C) of all animals were recorded on days-2, -1, 0 (prior to and 4 h after vaccination) and then daily thereafter for a period of seven days. Further measurements were recorded on day 21 (prior to and 4 h after challenge administration), then daily until the end of the study.

Clinical observations were performed once daily from day-2 until the end of the study. Clinical observations on days 0 and 21 were performed prior to vaccine and challenge strain administration respectively, with additional observations approximately 4 h after challenge strain administration. Observations performed following challenge administration assessed developing clinical disease. However, for animal welfare reasons specific clinical endpoints were defined for each challenge whereby animals would be euthanased prior to reaching end-stage clinical disease.

Blood samples were collected into plain blood tubes from each animal prior to test material (vaccine or control) administration on day 0, prior to challenge administration on day 21, and at the end of the study on day 35 for CAV-2 and CPV or day 42 for CDV and CAV-1. For the CPV study further blood samples (0.5–1 ml) for white blood cell (WBC) counts were collected from each animal 4, 2 and 0 days before and then 3, 5, 7, 10, 12 and 14 days post-challenge administration.

To determine virus shedding in the CPV study, faecal swabs were collected into sterile tubes on day 21 before CPV-2b challenge administration (before challenge) and then 3, 5, 7, 10, 12 and 14 days after CPV-2b challenge. In the CAV-2 study, two nasal swab samples (one from each nostril) were collected into sterile tubes from each animal prior to challenge administration on day 21 and then daily from day 23 (2nd day after challenge) until day 31 (10th day after challenge).

Laboratory analysis

Serum samples from the respective studies were examined for the presence of antibodies to CDV, CPV-2, CPV-2b, CAV-1 and CAV-2 by serum-neutralisation test. Briefly, duplicate two-fold dilutions of test and control sera in MEM cultivation medium were prepared in micro titre plates; approximately 100 $TCID_{50}$ of the respective virus was added followed by incubation at 37 °C for 1 h in 5% CO_2. Susceptible cells (VERO – CDV; MDCK – CAV; CRFK – CPV) were added as appropriate; with a further incubation at 37 °C for 3–7 days in 5% CO_2. The end point was assessed as the serum dilution where more than 50% of the characteristic cytopathic effect was attenuated. For the CPV analysis the haemagglutination assay was used to definitively confirm virus presence and visualisation; the end point was assessed as the serum dilution where inhibition of haemagglutination was observed.

For the CPV study, whole blood samples were analysed for leukocyte counts by staining cells with Tűrk's solution and counting them in a Bűrkerusing standard counting chamber. Faecal samples were examined for virus presence and titre by re-isolation on a