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Long-term viremia and fecal shedding in pups after modified-live canine parvovirus vaccination

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ABSTRACT

Canine parvovirus (CPV) modified live virus vaccines are able to infect vaccinated dogs replicating in the bloodstream and enteric mucosa. However, the exact duration and extent of CPV vaccine-induced viremia and fecal shedding are not known. With the aim to fill this gap, 26 dogs were administered two commercial vaccines containing a CPV-2 or CPV-2b strain and monitored for 28 days after vaccination. By using real-time PCR, vaccine-induced viremia and shedding were found to be long lasting for both vaccinal strains. Vaccinal CPV-2b shedding was detected for a shorter period than CPV-2 (12 against 19 mean days) but with greater viral loads, whereas viremia occurred for a longer period (22 against 19 mean days) and with higher titers for CPV-2b. Seroconversion appeared as early as 7 and 14 days post-vaccination for CPV-2b and CPV-2 vaccines, respectively. With no vaccine there was any diagnostic interference using in-clinic or hemagglutination test, since positive results were obtained only by fecal real-time PCR testing. The present study adds new insights into the CPV vaccine persistence in the organism and possible interference with diagnostic tests.

1. Introduction

Along with canine coronavirus [1,2], canine parvovirus (CPV) is the main cause of acute hemorrhagic enteritis in young dogs, which may display very severe clinical signs such as leukopenia, fever, inappetence, hemorragic diarrhea, dehydration and death [3]. Currently, three different antigenic variants are known, namely CPV-2a, CPV-2b and CPV-2c, which are variously distributed worldwide [4–8]. The original type CPV-2, albeit no longer circulating in the field, is still contained in most CPV vaccines, whereas few commercially-available vaccines are prepared with CPV-2b [3]. Although CPV-2c is becoming the predominant strain in several countries [4–8], to date there are no licensed vaccines that contain the newest variant. Both CPV-2 and CPV-2b vaccinal strains cause viremia and are shed with the feces of immunized dogs, as also shown by their detection in specimens from dogs displaying acute gastroenteritis shortly after vaccination, alone or in addition to CPV field strains or other pathogens [9]. However, nothing is known about the real duration and extent of viremia and shedding of vaccinal CPVs.

The aim of the present paper is to report the results of virological investigations carried out on pups routinely undergoing CPV vaccination in order to assess the vaccine virus viremia and shedding with the feces.

2. Materials and methods

2.1. Vaccines

Two commercial modified live virus (MLV) vaccines were used in the study, Nobivac® PUPPY CP (Intervet Italia S.r.l., Milan, Italy) and Duramune® DAPPI + LC (Zoetis Italia S.r.l., Rome, Italy), containing $>10^7$ and $10^{4.7}$–$10^{6.5}$ tissue culture infectious doses of CPV-2 strain 154 and CPV-2b strain SAH, respectively.

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2.2. Vaccination protocol

A total of 26 pups belonging to two different breeding kennels were recruited in the study with the written consent of the breeders. The dogs, 16 males and 10 females, consisted of 13 American cocker spaniels and 13 Labrador retrievers that were randomly divided into two vaccine groups (on the basis of the vaccine administered) in order to include both breeds and genders in each group. At the age of 6 weeks, all pups were bled to collect sera that were submitted to hemagglutination inhibition (HI) in order to predict the most appropriate age of vaccination on the basis of their maternally-derived antibody (MDA) titers [10]. When MDA titers were below the levels interfering with CPV vaccination (<1:20), pups were administered subcutaneously one dose of CPV-2 or CPV-2b vaccine according to the vaccine group.

2.3. Sample collection

Vaccinated animals were monitored for a period of 28 days post-vaccination (dpv) in order to evaluate vaccinal strain viremia, shedding and seroconversion.

For virological investigations, EDTA–blood samples and fecal specimens were collected from vaccinated pups at dpv 3, 7, 10, 14, 17, 21, 24 and 28 by means of jugular venipuncture and anal swabs, respectively. Serological testing was carried out on serum samples taken once a week from each dog.

2.4. Virological investigations

2.4.1. In-clinic test

The in-clinic test was carried out with the commercial kit WITNESS Parvo test (Synbiotics Corporation, Pfizer), as previously described [11]. The fecal material was collected on the test kit swab following the manufacturer's instructions. The extraction buffer/conjugate was dispensed into the sample tube via the kit swab. Then, the sample swab was inserted into the tube containing the liquid and vortexed. The extracted fecal material/conjugate liquid was transferred to the WITNESS Parvo device using the swab pipette for the test kit as per manufacturer's instructions.

2.4.2. Hemagglutination (HA)

Two-fold dilutions of the supernatant of each fecal homogenate were made in PBS (pH 7.2) starting from a 1:2 dilution [12]. Tests were carried out in 96-well V-plates (50 μl of sample dilution per well); equal amounts of a suspension containing 0.8% pig erythrocytes and 1% fetal calf serum (FCS) were added to each dilution. Results were read after 4 h at +4 °C and expressed as the reciprocal of the highest sample dilutions able to produce HA.

2.4.3. Real-time PCR assays for CPV detection, quantification and characterization

Specimens were homogenized (10%, w/v) in Dulbecco's modified Eagle's medium (DMEM) and subsequently clarified by centrifuging at 2500 × *g* for 10 min. Viral DNA was extracted from the supernatants of fecal homogenates by boiling for 10 min and chilling on ice. To reduce residual inhibitors of DNA polymerase activity to ineffective concentrations, the DNA extract was diluted 1:10 in distilled water [13].

Detection and quantification of CPV DNA was obtained by real-time PCR using a conventional TaqMan probe [13]. Briefly, the 25-μl reaction contained 12.5 μl of master mix (Bio-Rad Laboratories S.r.l., Milan, Italy), 600 nM of primers (5′-AAACAGGAATTAACTATACTAATATATTTA-3′) and CPV-Rev (5′-AAATTTGACCATTTGGATAAACT-3′), 200 nM of probe CPV-Pb (5′-TGGTCCTTTAACTGCATTAAATAATGTACC-3′) and 10 μl of standard or template DNA. For the standard-curve construction, ten-fold dilutions of a plasmid containing the nearly full-length CPV genome (kindly supplied by C.R. Parrish, Cornell University, Ithaca, NY, USA) were processed. All standard dilutions and unknown samples were tested in duplicate. The following thermal protocol was used: activation of iTaq DNA polymerase at 95 °C for 10 min and 40 cycles consisting of denaturation at 95 °C for 15 s, primer annealing at 52 °C for 30 s and extension at 60 °C for 1 min.

A panel of minor groove binder (MGB) probe assays able to predict the viral type [14,15] and to discriminate between vaccine and field strains of CPV [16,17] was used to confirm the viral strain detected in vaccinated dogs.

2.5. Serological investigations

Sera of dogs collected at dpv 0, 7, 14, 21 and 28 were submitted to an HI test using a standardized protocol [10]. The tests were performed at +4 °C in 96-well V-plates, using 10 hemagglutinating units of CPV-2 antigen and 0.8% pig erythrocytes. Two-fold dilutions in PBS of each serum sample, starting from 1:10, were tested. The HI titer was indicated as the highest serum dilution completely inhibiting viral hemagglutination.

2.6. Statistical analysis

The data were analyzed using the Prism 6 software (version 6.0d). All hypothesis tests were conducted at the 0.05 level of significance (two-sides). The areas under curve (AUC) for the viremia and fecal shedding of the vaccine viruses were calculated for each group and the statistical significance was evaluated using the Wilcoxon–Mann–Whitney test. Prior to analysis the AUC values were logarithmically transformed. HI antibody titers were also analyzed by the Wilcoxon test.

3. Results

3.1. CPV post-vaccinal viremia

By real-time PCR, dogs immunized with the CPV-2 strain displayed viremia for 19 mean days, from dpv 3 to 21, with mean viral titers peaking at dpv 10 (1.12×10^6 DNA copies 10 μl^{-1} of template). In dogs vaccinated with CPV-2b, viremia started at dpv 3 and stopped at dpv 24 (22 mean days), with maximal titers of 6.39×10^7 DNA copies 10 μl^{-1} of template being observed at dpv 7 (Fig. 1A).

Minor groove binder (MGB) probe assays developed to predict the antigenic type and discriminate between filed and vaccine strains [14–17] confirmed the positivity for the vaccine strain (CPV-2 or CPV-2b) according to the vaccine group.

The mean values for the area under the curve of real time PCR results from EDTA–blood for the observation period were 4.55×10^6 and 2.37×10^8 for CPV-2 and CPV-2b vaccinated dogs, respectively ($P < 0.001$).

3.2. CPV post-vaccinal shedding

None fecal swab collected after vaccination tested positive by either in-clinic testing or HA. By real-time PCR, CPV-2 immunized pups shed the virus with the same pattern observed for viremia (19 mean days), although viral DNA loads detected in the feces were slightly lower than those observed in the blood. The highest DNA loads (mean of 3.80×10^5 DNA copies 10 μl^{-1} of template) were shed at dpv 7. Vaccinal CPV-2b shedding occurred for a shorter period (12 mean days, from dpv 3 to 14) but with greater viral load, which peaked at dpv 10 (mean titer of 1.12×10^6 DNA copies 10 μl^{-1} of template) (Fig. 1B). The vaccine strain shed through the

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