Short communication

Oral administration of modified live canine parvovirus type 2b induces systemic immune response

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Highlights:

- Interfering levels of maternally derived antibodies (MDA) can hamper the immune response against canine parvovirus type 2 (CPV-2) vaccination.
- Fourteen pups with MDA titres ranging from 1:20 to 1:160 were orally administered a commercial modified live CPV-2b vaccine.
- All pups but one seroconverted after a single vaccine administration.
- The remaining pup raised an immune response after administration of a second vaccine dose.
- Oral administration of CPV-2 vaccines can help overcome MDA interference.
Abstract

Different strategies have been proposed to overcome maternally derived antibodies (MDA) interference with canine parvovirus type 2 (CPV-2) immunisation, including intranasal vaccination, which presents some practical limitations. In the present study, the results of the oral administration of a commercial CPV-2b modified live virus (MLV) vaccine in pups with MDA are reported. The CPV-2b vaccine was orally administered to 14 6-week-old pups with a bait. Blood samples and rectal swabs were collected at different days post-vaccination (dpv) to determine CPV-2 antibody titres and DNA loads. Thirteen pups were positive to serological and virological tests after the first vaccination and one pup became positive after the second vaccine administration. The findings of this study suggest that systemic immunity against CPV-2 may be achieved by the use of an MLV CPV-2b vaccine administered orally even in the presence of MDA titres that usually interfere with vaccination.

Key words: pups; canine parvovirus; maternally derived antibodies; oral vaccination.

1. Introduction

Canine parvovirus type 2 (CPV-2) is the causative agent of a severe, highly contagious gastroenteric disease in pups [1]. Since its first emergence in the mid-1970s, the original type CPV-2 has evolved giving rise to new antigenic variants designated CPV-2a, CPV-2b and CPV-2c, which have completely replaced the original virus and are now distributed worldwide [2], [3].
Typically, CPV-2 infects 4-to-12 week-old pups especially during the decline of maternally derived antibodies (MDA) [2]. Modified live virus (MLV) vaccines are prepared by using either the original type CPV-2 or its variant CPV-2b. Despite the high efficacy of vaccines, outbreaks of infection continue to arise in vaccinated dogs, likely as a consequence of the MDA interference, which prevents an active immune response by vaccinated pups. Thus, in order to avoid or reduce this interference, vaccines should be administered to pups only after waning of MDA [2]. Different strategies have been proposed to overcome the MDA interference, including the administration of high-titre vaccines [4] or intranasal vaccine administration [5].

In a previous study [5], CPV-2b MLV vaccine administered intranasally induced high antibody titres even in the presence of high MDA titres. The intranasal vaccination can significantly reduce the MDA interference, resulting particularly effective in infected kennels. However, this procedure is not easy to perform, requires an adequate containment of the animal and, consequently, it can cause stress and/or the accidental loss of part of the vaccine dose during the administration.

In order to overcome the limitation of intranasal vaccination, we selected an alternative approach based on the oral administration of a MLV vaccine. Therefore, in the present study, the results of the oral administration of a commercial modified live CPV-2b vaccine in pups with MDA are reported.

2. Materials and Methods

2.1 Dogs

The study was approved by the Ethics Committee of the Department of Veterinary Medicine of University of Bari (Protocol number 756 – III/13).

Fourteen privately owned healthy pups presented to the Department Veterinary Teaching Hospital for preventive medicine examination and veterinary counseling were included in the study. All owners were informed of the nature of the study and gave their written consent (Client Informed Consent Form for clinical trials). The study was conducted on one litter comprising 11 pups (A1 to
A11) and on 3 other pups (C1, C2, C3) belonging to three different owners. Litter A consisted of 11 English short haired pointers (6 males and 5 females), whose mother had received last vaccination against CPV-2 18 months before parturition, while puppies C1 (male), C2 and C3 (females) were mongrel dogs. All pups at the start of the study were 40 days old.

2.2 Vaccine

Canigen® Puppy 2b vaccine (Virbac srl, Milan, Italy), containing a MLV CPV-2b strain (CPV 39) and having a titre of $10^{5.6} - 10^{7.5}$ Tissue Culture Infectious Doses (TCID$_{50}$/dose, was used during the study (Lot n. 72ZT; expiry date: May 2020).

2.3 Experimental procedure

The pups were orally vaccinated using a bait containing the vaccine dose. Each bait consisted of a friable biscuit filled, immediately prior to use, with one dose of vaccine (1 ml) and freely taken by pups. The chewing of the bait resulted in a persistence of the vaccine in the oral cavity for 1-2 min. Clinical examinations were performed daily to evaluate the general conditions of the pups.

Blood samples were collected at 0 (T0), 15 (T15) and 30 (T30) dpv to determine individual CPV-2 antibody titres. Rectal swabs were collected at 0 (T0), 4 (T4), 8 (T8) and 15 (T15) dpv for virological testing (vaccinal virus shedding).

At T15, in the absence of seroconversion (increase of antibody titres), pups were vaccinated again, using the same protocol (route of vaccination, sampling, serological and virological investigations).

In particular, the following sampling was scheduled: T19 and T23 (for virological testing), T30 (for serological and virological testing).

2.4 Serological testing

Antibody titers against CPV-2 were evaluated by haemagglutination inhibition (HI) test, which was performed in V-shaped 96-well microtitre plates, at +4°C, using 0.1% pig erythrocytes and 10 haemagglutinating (HA) units of CPV-2b strain 29/97 [6].

Serial twofold dilutions of each serum, starting from 1:10 to 1:10240, were prepared in phosphate buffered saline (PBS, pH 7.2). An equal volume (25 µl) of virus (10 HA units) was added to the
wells containing the dilutions of the sera. Thereafter, the plates were incubated at room temperature for 60 min, then refrigerated at +4°C for 30 min before adding to each well 50 µl of the cold erythrocyte suspension. HI titres were read after 12 hours at +4°C and expressed as the reciprocal of the highest serum dilution that completely inhibit the HA activity.

2.5 Virological testing

Faecal samples were homogenised in PBS (10% w/v) and subsequently clarified by centrifugation at 8,000 rpm for 5 min. Viral DNA was extracted from the supernatants of faecal 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 [7].

CPV-2 DNA was detected by real-time polymerase chain reaction (PCR) using a conventional TaqMan probe [7], whereas virus characterisation was obtained by a panel of minor groove binder (MGB) probe assays able to predict the viral type [8] and to discriminate between vaccine and field strains of CPV-2 [9], [10].

2.6 Statistical analysis

Normality of distribution of continuous variables was assessed by Shapiro-Wilk test. Antibody titers at T0 and T15 were summarized as median and interquartile range and compared to each other by the non-parametrical Wilcoxon signed rank test with continuity correction. Viral DNA copies at T0, T4, T8, T15, T19, T23 and T30 were summarized as median and interquartile range and subjected to the non-parametrical Friedman test. Pairwise comparisons using Wilcoxon signed rank test with Bonferroni post-hoc test were performed to evaluate significant differences in viral DNA copies between T0, T4, T8, T15, T19, T23 and T30. Statistical analyses were performed by using the freely available online tool EZR [11]. A p-value<0.05 was considered for statistical significance.

3. Results

3.1 Vaccine administration
Thirteen puppies chewed and then ingested the bait containing the vaccine dose, freely and without any difficulty. Only pup C2 showed an initial distrust for the bait and expelled it twice from the oral cavity, before ingestion. However, the bait was better tolerated by this pup at the second vaccine administration. None of the pups showed local or general reactions following oral vaccination.

### 3.2 Serological testing

The results of serological tests are shown in Table 1. The sera taken at T0 showed HI MDA titres ranging from 1:20 to 1:160. All pups orally vaccinated, except pup C2, seroconverted after the first vaccination, displaying at T15 HI titres ranging from 1:1280 to 1:10240. In the comparison between antibody titers detected at T0 and T15 a statistically significant difference (p-value = 0.001293) was observed.

The first oral vaccination of pup C2 did not result in seroconversion. This pup showed at T0 and T15 HI MDA titres of 1:160 and 1:40, respectively. According to the protocol described above, pup C2 was vaccinated a second time at T15, using the same procedure as for the first vaccination. At T30 (15 days after the second vaccination), the pup seroconverted, showing an HI titre of 1:1280.

Therefore, the vaccine was able to induce active seroconversion in all pups having HI MDA titers ranging from 1:20 to 1:80, as well as in 1 (A9) out of 2 pups displaying an HI titre of 1:160 at the time of the vaccination.

### 3.3 Virological testing

The results of virological tests are shown in Table 2. All rectal swabs taken at T0 were negative for CPV-2 by the TaqMan real-time PCR assay [7].

At T4, 6 puppies (A3, A4, A9, A10, A11 and C3) were positive for CPV-2. By using MGB probes able to discriminate between the three CPV-2 variants [10] and between vaccine and field CPV-2b [9], the virus in the swabs was characterized as CPV-2b vaccine strain CPV 39, with viral loads ranging from $3.1 \times 10^2$ to $4.75 \times 10^3$ DNA copy numbers $10^{-1} \mu l$ of template.
At T8, additional 7 pups (A1, A2, A5, A6, A7, A8 and C1) tested positive for the vaccine strain. At T15, the rectal swabs of all pups but C2 tested positive for CPV-2b strain CPV-39. The copy numbers ranged from $3.89 \times 10^3$ to $1.57 \times 10^7$ DNA copies $10^{-1} \mu l$ of template at T8 and from $6.13 \times 10^3$ to $9.06 \times 10^4$ DNA copies $10^{-1} \mu l$ of template at T15.

At T19, the vaccine strain was detected in the swabs of all dogs, including the C2 pup and the copy numbers $10^{-1} \mu l$ of template ranged from $3.8 \times 10^2$ to $5.29 \times 10^3$. The viral shedding was detected in rectal swabs of pup C2 up to T30 while other pups tested negative at T23 and T30.

Therefore, the vaccine virus was detected at T4 (6 pups), T8 (7 pups) and T19 (pup C2, fours days after the second vaccination) and lasted for 11 days (A1, A2, A5, A6, A7, A8, C1, C2) or 15 days (A3, A4, A9, A10, A11, C3).

Overall, the comparison between viral DNA copies at T0, T4, T8, T15, T19, T23 and T30 showed statistically significant differences ($p$-value = 0.000000000677). The pairwise comparisons revealed significant differences in viral DNA copies between T4 and T19 ($p$-value = 0.0051), T8 and T19 ($p$-value = 0.0051), T8 and T23 ($p$-value = 0.0487) T8 and T30 ($p$-value = 0.0077), T15 and T19 ($p$-value = 0.0051) and T15 and T30 ($p$-value = 0.0051).

4. Discussion

One of the primary obstacles in immunisation of dogs against CPV-2 is the persistence of high levels of MDA in pups which may interfere with the development of the vaccine-induced immunity. The administration of high-titre vaccines [4] and the intranasal vaccination [5] are currently the only strategies proposed to overcome the MDA interference. Compared to the parenteral inoculation, the intranasal administration of the vaccine has the advantage of stimulating a better local immunity together with a faster humoral response [2], [5]. However, this route of administration has many practical limitations with regards to the forced containment of the animals and the possible loss of part of the vaccine dose during the inoculation into the nostrils. For these reasons, the intranasal
vaccination may not be easy and may cause stress, suffering and/or alteration of the animal welfare.

Based on the above, the need for alternative vaccination routes against CPV-2 is evident.

In the present study, oral administration of a commercial MLV CPV-2b in pups induced a systemic immune response (in terms of HI antibodies) already at the first vaccine administration. All pups with HI MDA titres up to 1:80 and 1 out of 2 pups with MDA titres of 1:160 seroconverted after the first vaccination, showing that oral administration of the vaccine is able to overcome MDA titres that interfere with vaccination using the parental route [2]. Only pup C2, which displayed a MDA titre of 1:160 and had some problems in ingesting the bait, failed to seroconvert after the first vaccination but responded to the second vaccination. In 6-week-old pups, such MDA levels were not so common in the past, but nowadays repeated vaccination of dams and or continuous virus circulation are leading to a longer-term persistence of MDA especially in breeding kennels [2, personal observation].

The successful oral vaccination was also confirmed by the results obtained from the virological tests. In fact, all the pups shed the virus for 11-15 days after the vaccination, albeit with different trends and peaks of viral titres. This is in agreement with a previous study showing that dogs parenterally administered a MLV CPV-2b strain shed the vaccine virus in their faeces for 12 mean days [12]. In contrast, Freisl and colleagues [13] observed an MLV CPV-2 shedding up to 28 days in 20% of the vaccinated dogs, but they used a CPV-2 (original type) vaccine, which was found to be shed for a longer period with respect to MLV CPV-2b [12]. In addition, in the Freisl’s study dogs shed the virus discontinuously and at very low titres [13].

The main drawback of the present study is that only the immunogenicity of the vaccine administration (in terms of antibody response) was evaluated, whereas the efficacy evaluation would have required the challenge of the vaccinated dogs 22-24 days after vaccination, along with the recruitment of a control group consisting of unvaccinated dogs subjected to the challenge with the pathogenic virus. Albeit strictly required by the European Pharmacopeia, in the present study the challenge test was not performed to avoid suffering of the infected pups (control group) and
because this was conceived as clinical study not subjected to authorization of the Ministry of Health but only to the advise of the Ethics Committee. Indeed, it is recognized that seroconversion for CPV-2 not only represents a marker of vaccine immunogenicity but also it can be correlated with clinical protection [12], [3], [14]. This approach is consistent with the principles of the 3Rs (Refinement, Reduction and Replacement) and avoids efficacy studies based on infectious challenges in vivo that result in the death or euthanasia of the infected animals.

In conclusion, the present study demonstrates that the oral administration of a MLV CPV-2 vaccine is not only immunogenic, but also easy and it do not cause any stress to the animals that ingested the bait spontaneously and without containment. However, the failure of the first vaccination of pup C2 suggests that the palatability of the bait could be improved, especially when it is intended for pups accustomed to a milk diet.

Moreover, further studies are required to establish the ability of oral administration of MLV CPV-2 to induce immune response in pups, even in the presence of higher (≥1:160) interfering MDA titres. Although the application of the 3R principles is acceptable under a scientific point of view, the eventual registration of a commercial oral vaccine will inevitably require oral challenge studies according to the European Pharmacopeia.

5. References


Table 1. Haemagglutination inhibition antibody titers in pups orally vaccinated with a commercial modified live CPV-2b vaccine.\(^a\)

<table>
<thead>
<tr>
<th>Dog</th>
<th>T0</th>
<th>T15</th>
<th>T30</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>80</td>
<td>10240</td>
<td>ND</td>
</tr>
<tr>
<td>A2</td>
<td>80</td>
<td>10240</td>
<td>ND</td>
</tr>
<tr>
<td>A3</td>
<td>80</td>
<td>10240</td>
<td>ND</td>
</tr>
<tr>
<td>A4</td>
<td>80</td>
<td>1280</td>
<td>ND</td>
</tr>
<tr>
<td>A5</td>
<td>40</td>
<td>5120</td>
<td>ND</td>
</tr>
<tr>
<td>A6</td>
<td>40</td>
<td>10240</td>
<td>ND</td>
</tr>
<tr>
<td>A7</td>
<td>80</td>
<td>10240</td>
<td>ND</td>
</tr>
<tr>
<td>A8</td>
<td>80</td>
<td>10240</td>
<td>ND</td>
</tr>
<tr>
<td>A9</td>
<td>160</td>
<td>1280</td>
<td>ND</td>
</tr>
<tr>
<td>A10</td>
<td>80</td>
<td>5120</td>
<td>ND</td>
</tr>
<tr>
<td>A11</td>
<td>80</td>
<td>2560</td>
<td>ND</td>
</tr>
<tr>
<td>C1</td>
<td>20</td>
<td>5120</td>
<td>ND</td>
</tr>
<tr>
<td>C2</td>
<td>160</td>
<td>40</td>
<td>1280</td>
</tr>
<tr>
<td>C3</td>
<td>20</td>
<td>10240</td>
<td>ND</td>
</tr>
</tbody>
</table>

Median 80 7680 ND

IR [50;80] [3200; 10240] ND

\(^a\) Antibody titres are expressed as the reciprocal of the highest serum dilution able to cause haemagglutination inhibition. T, day post-vaccination; ND, not determined; IR, interquartile range.

Table 2. Real-time PCR results for CPV DNA in rectal swabs of pups orally vaccinated with a commercial modified live CPV-2b vaccine.\(^a\)

<table>
<thead>
<tr>
<th>Dog</th>
<th>T0(^b)</th>
<th>T4</th>
<th>T8</th>
<th>T15</th>
<th>T19</th>
<th>T23</th>
<th>T30</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Neg</td>
<td>Neg</td>
<td>1.82×10(^6)</td>
<td>1.23×10(^4)</td>
<td>1.57×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>A2</td>
<td>Neg</td>
<td>Neg</td>
<td>2.95×10(^4)</td>
<td>6.84×10(^3)</td>
<td>4.37×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>A3</td>
<td>Neg</td>
<td></td>
<td>5.70×10(^2)</td>
<td>8.28×10(^3)</td>
<td>1.84×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>A4</td>
<td>Neg</td>
<td></td>
<td>3.70×10(^2)</td>
<td>1.52×10(^4)</td>
<td>3.31×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>A5</td>
<td>Neg</td>
<td></td>
<td>6.64×10(^5)</td>
<td>5.36×10(^4)</td>
<td>3.51×10(^3)</td>
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<td>Neg</td>
</tr>
<tr>
<td>A6</td>
<td>Neg</td>
<td></td>
<td>8.63×10(^5)</td>
<td>4.20×10(^4)</td>
<td>3.40×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>A7</td>
<td>Neg</td>
<td></td>
<td>2.69×10(^6)</td>
<td>1.20×10(^4)</td>
<td>3.30×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>A8</td>
<td>Neg</td>
<td></td>
<td>1.40×10(^6)</td>
<td>6.13×10(^3)</td>
<td>1.69×10(^3)</td>
<td>Neg</td>
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<tr>
<td>A9</td>
<td>Neg</td>
<td></td>
<td>3.70×10(^2)</td>
<td>6.71×10(^3)</td>
<td>5.29×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>A10</td>
<td>Neg</td>
<td></td>
<td>3.60×10(^2)</td>
<td>7.82×10(^3)</td>
<td>3.11×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>A11</td>
<td>Neg</td>
<td></td>
<td>4.75×10(^3)</td>
<td>9.06×10(^4)</td>
<td>4.66×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>C1</td>
<td>Neg</td>
<td></td>
<td>3.89×10(^3)</td>
<td>2.56×10(^4)</td>
<td>1.70×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>C2</td>
<td>Neg</td>
<td></td>
<td>4.75×10(^3)</td>
<td>3.80×10(^2)</td>
<td>3.29×10(^5)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>C3</td>
<td>3.10×10(^2)</td>
<td>4.86×10(^4)</td>
<td>8.63×10(^3)</td>
<td>1.57×10(^3)</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
</tr>
</tbody>
</table>

Median ND ND 2.57×10\(^5\) 1.03×10\(^4\) 3.20×10\(^3\) ND ND

IR ND ND [4.72×10\(^5\);1.58×10\(^6\)] [7.08×10\(^5\);2.30×10\(^4\)] [1.69×10\(^5\);3.48×10\(^3\)] ND ND

\(^a\) Results are expressed as CPV-2 DNA copy numbers 10 µl\(^-1\) of template.

T, day post-vaccination; Neg, negative; ND, not determined; IR, interquartile range