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EFFECT OF MATERNAL ANTIBODIES ON THE IMMUNE RESPONSE TO DIFFERENT CANINE PARVOVIRUS VACCINES AND ANTIBODY RESPONSE TO SELECTED VACCINES

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SUMMARY: Canine parvovirus (CPV) is the main cause of gastroenteritis and mortalities in young dogs worldwide. Despite vaccination, the outbreaks of canine parvovirus infection occur in many countries including Sri Lanka. Interference caused by maternally derived antibodies (MDA) is a main reason for vaccination failure. Present study assessed the level of CPV specific MDA in puppies prior to vaccination, the effect of MDA on the efficacy of different preparations of CPV vaccines and, evaluated the immunogenicity of selected CPV vaccines available commercially. Analysis of MDA in puppies born to vaccinated or unvaccinated mothers using commercially available point of care ELISA based test revealed the presence of protective levels of MDA titres at 8 weeks of age which can affect the immunogenicity of the vaccines containing inactivated virus or low viral titre (1000 HAU). The vaccines containing CCID 50 of $\geq 10^{3-5}$ were able to induce antibody titres higher than protective level. Analysis of CPV-2 specific antibody titres, two weeks after completing the primary CPV vaccination (16-18 weeks of age) revealed that the dogs who received the vaccines containing CPV-2 or CPV-2b strain with $>10^5$ CCID 50 induced significantly high levels of mean antibody titre (vaccine "B"- *p* value = 0.004; vaccine "D" - *p* value = 0.022; vaccine "F"- *p* value = 0.032) when compared to the vaccine containing 1000 HAU. In conclusion, it was evident that the interference of MDA on CPV vaccines could be circumvented by using a live attenuated CPV vaccine having a high viral dose of CPV. Low viral dose vaccines and inactivated vaccines are not suitable for primary vaccination as those could be interfered by maternal immunity

Key words: canine, parvovirus, vaccines, MDA

INTRODUCTION

Canine parvovirus (CPV) is endemic in many countries and despite extensive vaccination, disease outbreaks occur annually (Nandi *et al.*, 2013; Decaro *et al*., 2020; Gamage *et al*., 2020). The vaccination failure observed in such dogs could be due to several reasons, including the interference from maternally derived antibodies (MDA), lack of cross-protection by the vaccine against all variants of CPV-2, immaturity of the immune system of puppies, poor quality of the vaccine and inability to maintain the cold chain during vaccine storage and transport (Altman *et al*., 2017; Vila Nova *et al*., 2018).

The MDA, which provides immunity to puppies in the first few weeks of life, may neutralize the vaccine antigen and obstruct seroconversion even in the declining phase of MDA when antibody titres are lower than protective levels, but high enough to neutralize with the vaccine antigen (Tizard, 2004; Spibey *et al*., 2008) . The MDA titres as low as haemagglutination inhibition (HI) titres of 1:10 have been shown to interfere with the vaccine (Carmichael *et al*., 1981; Niewiesk, 2014). To overcome this

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problem, repeated vaccinations is recommended for puppies from 6-8 weeks of age to approximately 16-18 weeks of age (Day *et al*., 2016).

Although three variants of CPV-2, namely CPV-2a, CPV-2b and CPV-2c, are now distributed globally (Zhou *et al*., 2017) , most commercially available vaccines contain CPV-2 or CPV-2b as the vaccine virus (Pratelli *et al*., 2001a; Altman *et al*., 2017a). Some studies report that those vaccines provide cross-protection against all three variants, while others report lack of cross-protection for heterologous variants, particularly against CPV-2c (Hernández-Blanco and Catala-López, 2015). The level of immunity may vary with the type of vaccine and the immunity induced by inactivated vaccines may last only for several months whereas that of live attenuated vaccines last for several years (Pratelli *et al*., 2001) . Measurement of antibody titres through HI or virus neutralization tests is typically considered as the gold standard for assessing antibody titres (Mazar *et al*., 2009; Butler and Crawford, 2013). Antibody detection test kits specific for CPV and standardized against the gold standard test are available commercially (Day *et al*., 2016) .

The aims of this study were to assess the level of CPV specific MDA in puppies prior to vaccination, to determine the effect of MDA on the efficacy of different preparations of vaccines and to evaluate the immunogenicity of selected CPV vaccines available commercially in Sri Lanka.

MATERIALS AND METHODS

Ethical clearance for the study was obtained from the ethical approval committee, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, and consents were obtained from the owners before enrolling dogs into the study.

Sample and data collection

All puppies used in the study were aged 6-8 weeks and properly de-wormed. General clinical examination of each puppy was performed following the standard procedure. Age, gender, health status, vaccination status of the puppies and vaccination history of the mother were recorded. Blood smears were prepared using a blood drop obtained from the ear capillaries, stained with Leishman and examined for haemoparasites under x 100 objective of the microscope.

Evaluation of MDA titres of puppies born to vaccinated and unvaccinated mothers using a modified ELISA assay

Sixteen healthy puppies born to vaccinated mothers and eight healthy puppies born to unvaccinated mothers were used to evaluate CPV specific MDA titres. At the age of 8th week, 10 µl blood was collected from the ear pinnae into capillary tubes supplied with the commercial kit to test for Anti-CPV IgG antibodies using a point-of-care ELISA test according to the manufacturer's instructions (VacciCheck, Biogal, Israel). Briefly, the blood samples were diluted 1:10 in buffer and incubated with antigen spotted on the plastic comb for 5 minutes. After washing the combs to remove unbound antibodies, they were incubated with whole molecule goat anti-dog IgG alkaline phosphatase conjugate for 5 minutes. After two successive washing steps, bound antibodies were detected with a precipitating chromogen 5-bromo-4-chloro-3-indolyl phosphate and nitro-blue tetrazolium. Development of purple - grey colour was observed in different intensities depending on the antibody level in the test specimen.

Scoring was performed according to the guidelines of ImmunoComb Canine VacciCheck instruction manual. Positive reference value was calibrated to the S3 colour tone of the Comb Scale. Completely dried comb was aligned with the calibrated colour. The most closely matching tone of purple grey test result spot on the comb with the positive reference spot on the CombScale was selected and the CombScale value of that spot was recorded. If the colour tone

was equal or darker than the reference spot for S3, it was considered as a positive response (equivalent to HI ≥ 80). Colour tone matched with the S2 was considered as a week positive result and faint colour tone of S1 or less were considered as a negative response. ImmunoComb stained with blue appearance and comb showing spots without any colour change on spots after complete drying were assessed as invalid responses.

Determination of the effect of maternally derived antibodies (MDA) on the efficacy of different preparations of vaccines

Sixteen puppies with MDA titres S1-S3 of the CombScale (lower than protective level) were selected for the study. Puppies were assigned into four groups, and four different types of canine parvo vaccines were given to the puppies in each group.

Group 1: Multivalent vaccine containing inactivated canine parvovirus (1024 - 2048 HAU) with aluminium hydroxide adjuvant-unregistered vaccine with a user permit (Vaccine 1)

Group 2: Multivalent vaccine containing live attenuated parvovirus (10^2 - 10^3 HAU)-commercially available (Vaccine 2)

Group 3: Monovalent live attenuated canine parvovirus with a viral titre > 10^{3-5} CCID 50 – commercially available (Vaccine 3)

Group 4: Monovalent live attenuated canine parvovirus with a viral titre > $10^{5.5}$ CCID 50 – commercially available (Vaccine 4)

Two weeks after immunization, 10 µl blood were collected from each puppy to determine CPV specific antibody titres using the commercially available antibody detection kit (VacciCheck, Biogal, Israel) as described previously.

Determination of the efficacy of selected monovalent canine parvo vaccines

Six commercially available monovalent parvo vaccines were selected for the study. Dogs presented to the Veterinary Teaching Hospital for immunization (n = 82) were recruited for the study after obtaining the consent from the owner. The CPV vaccinations were done at 8th, 11th and 14th week of age, and CPV specific antibody titre was determined at the age of 16 weeks of each dog by using the commercial antibody detection kit. Antibody titres elicited by different vaccine preparations were compared using 't' test.

Table 1 Enrolment of dogs for the study for detecting efficacy of the selected vaccines

Vaccine	Specifications	Number of dogs inoculated with the vaccines
Vaccine A	Monovalent live attenuated canine parvovirus (type 2a) Viral dose- not indicated	12
Vaccine B	Monovalent live attenuated canine parvovirus (type 2) with a viral titre >$10^{5.5}$ CCID 50	28
Vaccine C	Monovalent live attenuated canine parvovirus with a viral titre > 10^{3-5} CCID 50	9
Vaccine D	Monovalent live attenuated canine parvovirus (type 2b) 10^7 CCID 50.	11
Vaccine E	Monovalent live attenuated parvo vaccine containing $10^{2.1} – 10^{3.6}$ HAU	11
Vaccine F	Monovalent live attenuated CPV-2 strain > 10^7 CCID 50	11

RESULTS

General clinical examination revealed all puppies selected for the study as being clinically healthy and the blood smears were negative for haemoparasites.

Maternal derived canine parvovirus specific antibody levels of puppies at the age of 8 weeks

Of the eight puppies born to unvaccinated mothers, four puppies had CPV specific antibody titres higher than S3 of the CombScale (HI ≥ 80), while two puppies had antibody titres equal to S2 or S1 of the CombScale. The remaining two puppies did not have a detectable level of CPV specific antibody titres. Of the sixteen puppies born to vaccinated mothers, two puppies had titres ≥ S3, four had titres equal to S2, six had titres equal to S1 and four puppies did not have a detectable level of antibody titre.

Immunogenicity of four different commercially available CPV-2 vaccines in the presence of low levels of maternally derived antibodies.

The vaccine 1 (inactivated vaccine) and 2 (vaccine containing 10^2- 10^3 HAU units of parvovirus) were unable to elicit a protective level of antibody titres in the presence of low levels of MDA. The two live attenuated vaccines that contained 10^{3-5} and $10^{5.5}$ CCID 50 induced a good immune response (equal to S5 and S6 of the CombScale) in the presence of MDA (Figure 1)

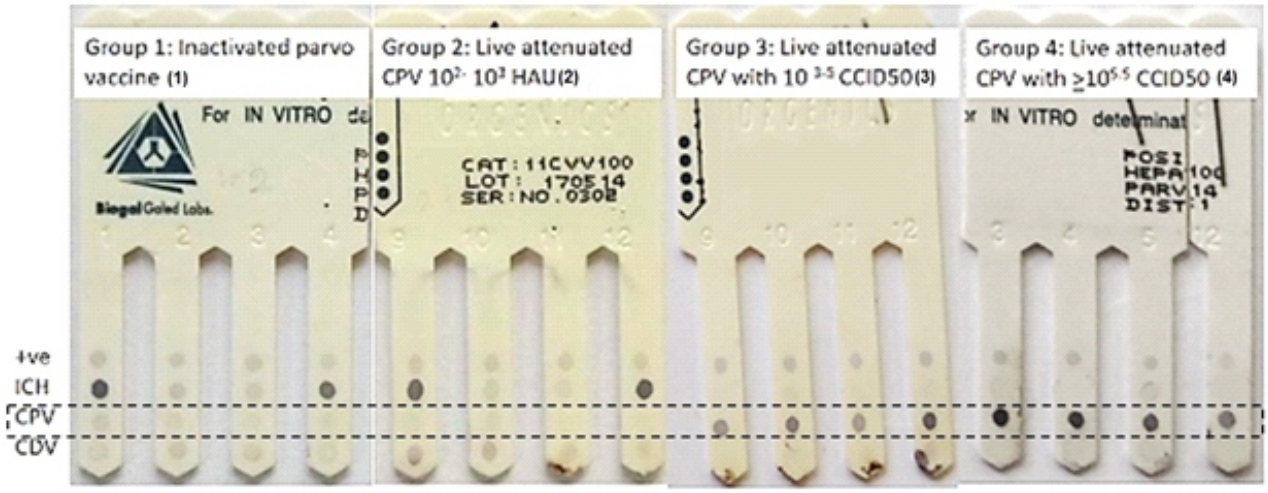

Figure 1: Canine parvovirus (CPV) specific antibody levels in puppies two weeks after vaccination with four different preparations of vaccines. ICH: Antibody specific for infectious canine hepatitis, CPV: Antibody specific for canine parvovirus, CDV: Antibody specific for canine distemper virus.

Efficacy of selected monovalent live attenuated canine parvovirus vaccines

The immunogenicity of six different live attenuated CPV vaccines commercially available in Sri Lanka was evaluated. The antibody levels were determined two weeks after the completion of the primary vaccination for CPV (around 16 weeks of age). Vaccines, B, C, E and F contained the CPV-2 strain, vaccine A contained CPV-2a strain and vaccine D contained CPV-2b strain. As shown in the Figure 2, two vaccines (B and F) containing CPV-2 and the vaccine (D) containing CPV-2b with CCID 50 > 1 x 10^6 produced CPV-2 specific antibody titres well above the protective level (> S5). One vaccine containing CPV-2 strain with 1 x 10^3 HAU (E) had failed to induce protective levels of antibody titres in 45% of dogs that received the vaccine. When compared to the vaccine E, a significantly high level of mean antibody titres were detected in dogs who received the vaccine B (p value = 0.004), vaccine D (p value = 0.022) and vaccine F (p value = 0.032). Even if the vaccine B had significantly high level of mean antibody titre, only 79% of the dogs that received the vaccine had the titres higher than the protective level.

The majority of dogs who received the vaccines D (91%) and F (82%) also had antibody titres above the protective level. One puppy was unable to seroconvert even after receiving three doses of high viral titre vaccine (Vaccine B).

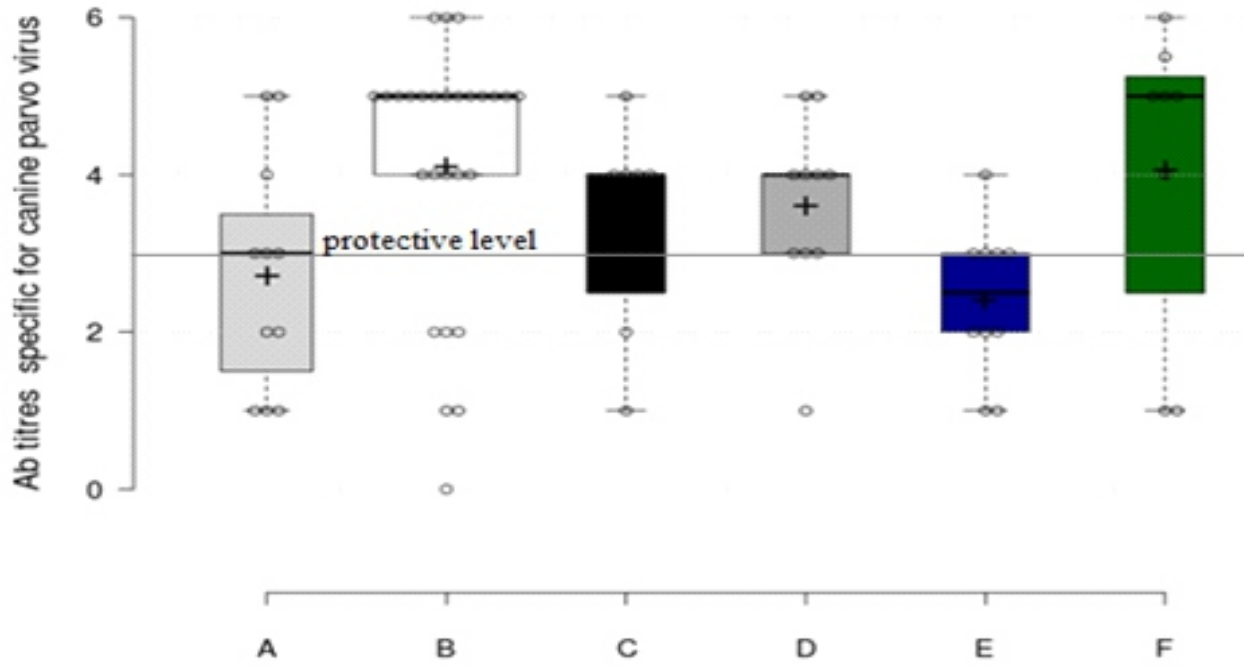

Figure 2: Canine parvovirus specific antibody titres in dogs vaccinated with six different commercially available canine parvovirus vaccines. Protective level is above 3.Vaccine A: Live attenuated parvo vaccine (CPV-2a strain); Vaccine B: Monovalent live attenuated canine parvovirus strain with a viral titre $\geq 10^{5.5}$ CCID 50; Vaccine C: Monovalent live attenuated canine parvovirus, with a viral titre $\geq 10^{3-5}$ CCID 50; Vaccine D: Monovalent live attenuated canine parvovirus (type 2b) 10^{7} CCID 50, Vaccine E: Monovalent live attenuated parvovirus vaccine containing $10^{2.1} - 10^{3.6}$ HAU; Vaccine F: Monovalent live attenuated CPV-2 strain > 10^{7} CCID 50 /dose); (CCID 50: cell culture infective dose 50 %, HAU: Haem agglutinating units)

DISCUSSION

Failures observed in canine parvovirus vaccination could be due to various reasons of which the interference caused by MDA is one of the main reasons (Tizard, 2004; Decaro *et al*., 2020). Though half-life of MDA specific for CPV is about 9-13 days, MDA titres specific for CPV have been shown to persist in puppies up to 13 weeks of age (Pollock and Carmichael, 1982; Decaro *et al*., 2004). Our results revealed that 66 % of puppies had detectable levels of MDA titres (S1-S3 of the CombSclae) at the 8th week of age and 25 % of them had the antibody titres equal or more than protective level. Of those puppies who had high MDA titres, four were born to unvaccinated mothers, which may have been exposed to field strain of the parvovirus.

It has been indicated that the MDA titres >1:20 (equal to S1) is capable of neutralizing modified live attenuated-CPV vaccines and prevent the establishment of protective immunity (Buonavoglia *et al*., 1992). However, another study reported that interference could occur by only 1:80 MDA titres (Waner *et al*., 1996) . We used four vaccines containing different viral doses of killed or live attenuated CPV-2 to vaccinate puppies with MDA titres ≤ 1:80 (S3). Our results showed that the puppies with low levels of MDA titres were able to seroconvert

when modified live virus (MLV) vaccines with ≥ 10^5 CCID 50 were given. Two vaccines with 1000 HAU were incapable of eliciting protective level of immune response in the presence of low level of MDA. As it is difficult to compare the HAU with CCID 50, we are unable to state whether the viral dose of those two vaccines are lower or equal to the vaccines which elicited good protection. One of these two vaccines which were incapable of eliciting protective level of antibody titres contained inactivated virus. It is important to note that the use of inactivated vaccines are recommended only in exotic animals and pregnant bitches and not for routine vaccination (Day *et al.*, 2016). A study conducted by Altman and colleagues (2017) has shown that the inactivated vaccines are more frequently associated with immunisation failures than MLV vaccines in puppies. Prevention of CPV infection is based on the use of MLV vaccines, which has the ability to stimulate both antibody and cell-mediated immune responses (Parrish *et al*., 2015; Ford *et al.*, 2017).

In order to overcome the influence of MDA, the repeated vaccinations starting from 6-8 weeks up to 16-20 weeks are recommended by the professional associations such as Vaccination Guidelines Group of World Small Animal Veterinary Association (Day *et al*., 2016). In this study we evaluated the immunogenicity of six commercially available MLV vaccines after repeated vaccination at 8^{th}, 11^{th} and 14^{th} weeks. Our results showed significant variations in the antibody titres induced by different preparation of vaccines. Importantly one vaccine containing $10^{2.1} – 10^{3.6}$ HAU was incapable to elicit antibody titres above protective level even after repeated vaccination. However, the presence of CPV antibodies, irrespective of titre, in a vaccinated dog over the age of 20 weeks is considered to be protected from the disease (Day *et al*., 2016; Decaro *et al*., 2020). The MLV vaccines induce both humoral and cell mediated immune responses and the serological tests can only measure the humoral responses. To ensure the efficacy of the vaccine, it is necessary to expose the vaccinated dogs to the field strain of the virus. We did not attempt to expose the dogs to the filed strain of the virus as the study was conducted using client owned dogs. However, two dogs who received the vaccine "A" and three dogs who received the vaccine "D" developed parvo viral infection (confirmed by PCR) during the study period (Gamage *et al.*, 2020). A study conducted in Australia has shown that 3.3 % of dogs infected with CPV were adult dogs who had completed the primary vaccinations appropriately. This finding indicates an apparent immunisation failure which could be related to the host or vaccine (Ling *et al*., 2012). Certain dogs are unable to mount an immune response due to inherited or acquired conditions. In our study also one puppy was unable to seroconvert even after repeated vaccination.

In this study, we followed the vaccination schedule recommended to use in Sri Lanka (Silva, 2016). However, according to recent publications, the last CPV vaccine of the primary vaccination should be given after 16 weeks of age (Day *et. al*., 2016).

CONCLUSION

In conclusion, it was evident that the interference of MDA on CPV vaccines could be circumvented by using a MLV-CPV vaccine having a high titre. Therefore, vaccines with high viral dose are recommended for primary vaccination to ensure required protection for puppies. Low viral dose vaccines and inactivated vaccines given to young puppies as a primary vaccination resulted in low immunity due to interference by maternal immunity. Repeated vaccination up to the age of 16-18 weeks is a good practice as recommended by the Vaccination Guidelines Group of World Small Animal Veterinary Association, to overcome the maternal antibody interference, since MDA titre greatly differ between individuals. It would be a good practice to measure antibody level before commencing primary vaccination.

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