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POST VACCINAL ANTIBODY TITRES SPECIFIC FOR CANINE DISTEMPER VIRUS AND CANINE ADENOVIRUS IN DOGS VACCINATED WITH SELECTED COMMERCIAL VACCINES: A PRELIMINARY STUDY

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SUMMARY: Vaccination of dogs against canine distemper virus (CDV) and canine adenovirus (CAV), are recommended to prevent severe disease outbreaks caused by those viruses. Antibody (Ab) titre testing has proven to be an effective method of monitoring immunity and determining whether re-vaccination is necessary. Post-vaccinal immunoglobulin (IgG) titres specific for CDV and CAV with three selected commercial multivalent vaccines (A, B, and C) were evaluated in this study. When antibody titres of a total of 40 client-owned dogs (16 vaccinated with vaccine A, and 12 each with vaccine B and C) were tested three weeks after the primary vaccination using VacciCheck in-clinic test kit, only 33 yielded valid responses. Pair-wise comparison using the Kruskal-Walli's test showed that vaccine C induced a significantly higher CDV-specific Ab titres ($p = 0.001$) than vaccine A or B. Both vaccines A and C induced significantly high levels of CAV-specific Ab titres ($p = 0.001$) when compared to vaccine B. Vaccine B containing the Rockborn strain of CDV and Manhattan strain of CAV induced a very poor immune response against both viruses. Since many factors can affect the immune response elicited by a vaccine, selecting a reliable vaccine product is important to ensure a good immune response. The vaccine C produced by a leading manufacturer containing the viral titres of $1x10^4$ TCID50 and $1x10^6$ TCID50 or CDV and CAV, respectively was effective in inducing a protective humoral immune response in all dogs tested in this study.

KEYWORDS: canine, CDV, CAV, vaccine, immunity

INTRODUCTION

Vaccination is an important component of preventive care for dogs. As a result of improving veterinary diagnostic facilities, the prevalence of previously uncommon diseases affecting dogs has been identified and vaccines have been introduced to prevent those infections as well (Coyne *et al*., 2001). When the increased occurrence of vaccine-induced immune-mediated diseases in dogs was brought to notice, widespread discussions on the effective use of vaccines in companion animals were initiated (Cole 1998). It results in categorizing canine vaccines as core, non-core, or not recommended (Schultz 1998). As per the vaccination guideline group (VGG) of the World Small Animal Veterinary Association (WSAVA), the vaccination of dogs throughout the world against canine distemper virus (CDV), canine adenovirus (CAV) and canine parvovirus type -2 (CPV-2) are essential. Hence, those vaccines are defined as canine core vaccines (Day *et al*., 2016). To overcome the interferences caused by maternally derived antibodies (MDA), those guidelines recommend the repeated vaccination of young dogs in two-to-four-week intervals starting from six to eight weeks of age up to 16 weeks of age. In the absence of MDA, a single dose of modified live viral vaccine (MLV) can induce protective immune response.

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Until recently, re-vaccination of dogs for CPV-2, CDV, and CAV was done annually as the early work on duration of immunity (Baker, 1960) has indicated that some puppies were unable to maintain a protective level of immunity after one year of vaccination. Within the last two decades, vaccination technologies have been improved greatly, and so has the efficacy of vaccines. Serological studies conducted by many research groups in recent times have confirmed the persistence of vaccine-induced antibodies specific to those diseases for many years following the administration of the MLV (Böhm *et al*., 2004; Mouzin *et al*., 2004; Schultz *et al*., 2010). Therefore, WSAVA guidelines also recommend triennial re-vaccination of adult dogs or performing serological testing at triennial intervals to avoid unnecessary vaccination (Day *et al*., 2016). However, it is important to note the wide variation in the immune response induced by different vaccines according to the vaccine type (i. e. live, killed, subunit, or recombinant), viral dose, viral strain, and potency of the strain. In addition to those vaccine-related factors, animal, administration, or storage-related factors could also interfere with the development of a protective immune response (Tizard, 2017). The internal factors of animals that affect the development of immunity are difficult to control, but other factors which reduce the immune response could be easily manipulated (Baxter, 2007). The selection of an appropriate vaccine is important to induce a long-lasting protective immune response (Cunha et al., 2020). The immune responses of dogs following vaccination are usually evaluated by serum antibody titres. Nevertheless, protection against infectious agents involves both cellular and humoral immunity. Several studies have indicated that the serum antibody titres are correlated well with protection against CDV, CAV, and CPV-2 (Schultz et al., 2010). Therefore, the post-vaccinal antibody (Ab) titres could be used to predict the protection against those diseases.

Given the background, the objective of this study was to compare the vaccine-induced Ab responses specific for CDV and CAV in dogs vaccinated with three different commercial vaccines available in Sri Lanka.

MATERIALS AND METHODS

Ethical clearance for the study was obtained from the ethical approval committee of the Faculty of Veterinary Medicine and Animal Science at the University of Peradeniya.

Samples to evaluate Ab responses specific for CDV and CAV after primary vaccination

Client-owned dogs (n=40) between 18-22 weeks of age presented to the Veterinary Teaching Hospital at the University of Peradeniya in 2017 for immunization were conveniently selected for the study with the consent of the owner. They had been vaccinated with three different types of multivalent vaccines containing modified live attenuated CDV, modified live attenuated CAV type 2, and killed bacterin of *Leptospira (*2 or 4 serovar*)*. Of these dogs, 16 had been vaccinated with vaccine A, 12 with vaccine B, and 12 with vaccine C.

Vaccine A: multivalent vaccine containing live attenuated CDV, live attenuated CAV type 2 (in freeze dried form) and killed bacterin of *Leptospira* (liquid). The viral doses or the strains were not indicated.

Vaccine B: multivalent vaccines with lyophilized fraction containing attenuated live CDV, Rockborn strain and attenuated live CAV type 2 Manhattan strain. The viral dose was not indicated. Liquid fraction contains inactivated *Leptospira*.

Vaccine C: multivalent vaccine containing live attenuated strains of CDV ($1x10^{4.0}$ $TCID_{50}$) and CAV type 2 ($1x10^{6.0}$ $TCID_{50}$) (freeze-dried) and killed *Leptospira* bacterin.

Collection and processing of blood samples

Approximately 10 µl of whole blood was collected from the ear pinna of test dogs three weeks after the last CDV, CAV-2 and leptospira combined vaccine using the pipette and tips provided with the commercial kit. Ab titres specific for CDV and CAV viruses were assessed using the VacciCheck (Biogal, Israel) test kit. Briefly, the collected blood samples (10 µl) were immediately deposited in wells in row A of the kit containing a diluent (1:10). Then, the comb was inserted into the well and incubated for five minutes to facilitate the binding of Abs. The comb was transferred to wells in row B containing a washing solution and washed for two minutes to remove unbound antibodies. Then, the comb was incubated in well C containing anti-dog IgG b, for five minutes. After two consecutive washing steps in wells D and E, the comb was dipped for five minutes in well F containing the chromogen, 5-bromo-4-chloro 3- indolyl phosphates and nitro-blue tetrazolium. Upon colour development, the comb was incubated back in row E for colour

fixation. The results were read after drying the comb for five minutes.
The Ab titres were scored as described in the manual using the Comb Scale: S0 - no immunity; S1–2 - insufficient immunity; S3 positive; and S5-6 - strong positive.

Statistical analysis

Statistical analysis was performed using R software (version 4.0.3) (https://www.R-project.org/). Shapiro-Wilk test was used to determine the normality of Ab titres in 33 dogs for the data analysis. The vaccinated groups were compared using Kruskal-Wallis H test and Wilcoxon test was used for pairwise comparison of the Ab titres induced by different vaccines. Boxplots were created to visualise the differences in Ab responses induced by different vaccine preparations using the package "ggpubr". The *p* values less than 0.05 were considered as statistically significant.

RESULTS

Seven of 40 dogs (three given vaccine B and four given vaccine C) were removed from the study due to inappropriate colour development in the VacciCheck kit. All 33 dogs used in the study had been repeatedly vaccinated with vaccines A, B, or C between 10-18 weeks of age and Ab titres were determined 3 weeks after the last CDV and CAV vaccination. Demographic data, the type of vaccine given and the Ab responses for CD and CAV are summarized in Table 1.

Table 1: Summary of demographic data of dogs, characteristics of the vaccine and antibody responses of dogs for different vaccines

	Vaccine A	**Vaccine B**	**Vaccine C**
Type of vaccine	Multivalent live attenuated CDV, CAV- 2 & killed Leptospira	Multivalent live attenuated CDV, CAV-2 & killed Leptospira	Multivalent live attenuated CDV, CAV-2 killed Leptospira
Vaccines strains	Not indicated	CDV, Rockborn strain CAV-2 Manhattan strain	Not indicated
Viral Dose	Not indicated	Not indicated	CDV: $1x10^{4.}TCID_{50}$) CAV-2:$1x10^{6}$ $TCID^{50}$)
No. dogs vaccinated	16	12	12
Male to female ratio	0.78:1	1:1	1:1
Breeds	German shepherd 5 Labrador retriever 6 Rottweiler 3 Golden retriever 1 Cros bred 1	German shepherd 4 Labrador retriever 2 Rottweiler 3 Golden retriever 1 Dalmatian 2	German shepherd 4 Labrador retriever 4 Rottweiler 4
No. omitted due to inappropriate results	-	3 (Rottweilers)	4 (1 Rottweiler & 3 Labradors)
Protective Ab titers			
- CDV	5/16 (31.25%)	1/9 (11.1%)	8/8 (100%)
- CAV-2	15/16 (93.7%)	0/9 (0%)	8/8 (100%)

Canine distemper virus specific IgG antibody titres induced by three commercial vaccines.

Of the three vaccines tested, only five (31.25%) out of the 16 dogs vaccinated with vaccine A had developed Ab titres (mean 1.9 ± 1.6) above the protective level (≥ 3 of the CombScale) for CDV while all eight dogs (100 %) vaccinated with vaccine C developed Ab titres higher than the protective level for CDV (4.75 ± 1). In comparison, vaccine B elicited a very poor immune response for CDV (0.78 ± 1.3). Only one out of the nine dogs (11%) vaccinated with this vaccine was able to induce a titre higher than the protective level. Vaccine C had developed a significantly high level of Ab titres than vaccine A (*p* value = 0.001) and vaccine B (*p* value = 0.001) (Figure 1).

Canine adenovirus specific IgG antibody titres induced by different commercial vaccines.

Twenty three out of 33 dogs developed titres higher than the protective level for CAV. Those 23 included, 15 animals vaccinated with vaccine A (4.7 ±0.89), and all eight dogs vaccinated with vaccine C (4.75 ± 0.7). None of the dogs vaccinated with vaccine B (0.22 ± 0.44) was able to induce protective titre levels. A statistically significant difference was observed between the Ab titres induced by the three vaccine products (Figure 2). Ab response specific for adenovirus, induced by vaccine A and vaccine C were almost equal and both vaccine "A" (*p* value £0.001) and vaccine C (*p* value £0.001) had induced significantly high Ab response than Vaccine "B".

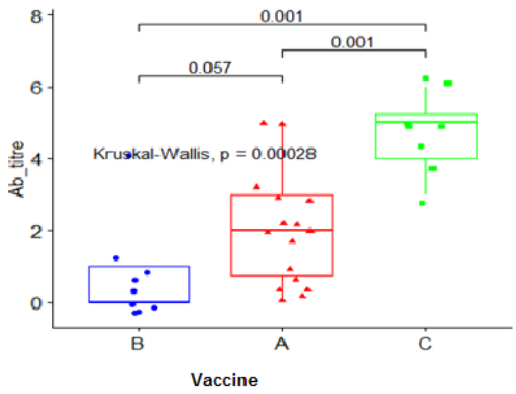

Figure 1: Comparative analysis of Ab titres specific for canine distemper virus induced by three different commercial vaccines.

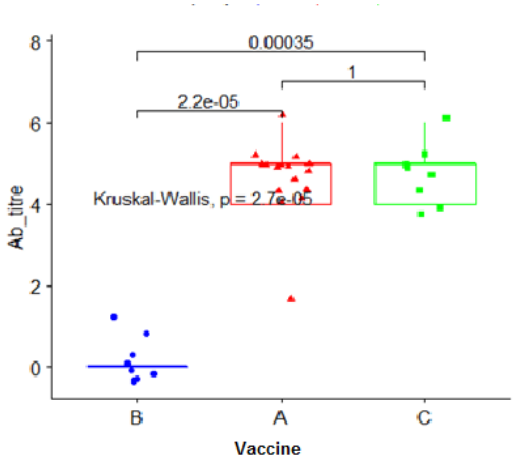

Figure 2: Ab titres specific for canine adenovirus induced by three different commercial vaccines.

DISCUSSION

Results of this study revealed that vaccine C from a leading manufacturer had induced protective antibody titres specific for CAV and CDV in all vaccinated dogs. When the Ab titres produced by vaccine C were compared with the other two vaccines, the former induced a significantly high level of Ab titres against both viruses. All 15 dogs vaccinated with vaccine A had induced protective levels of Ab titres against CAV but only one-third had developed protective Ab titres for CDV. Vaccine B containing the Rockborn strain of CDV and Manhattan strain of CAV-2 yielded very low IgG titres against both viruses following primary vaccination.

Reasons for the failure of vaccine A to induce a protective immune response against CDV and vaccine C to induce protective titres against both viruses in the majority of dogs remain unclear. It has been reported previously when the modified live CDV is combined with CAV-2, the risk of immune suppression increases in puppies (Dodds, 2001). All three vaccines evaluated in this study were combined vaccines and hence, it could not be considered the main reason for the poor immune response observed in groups A and B. As the viral dose of vaccine B was not indicated, it is impossible to compare it with other vaccine products to confirm that the viral dose used in the vaccine is adequate. Information on the strains used in vaccines A and C was also not indicated in the product description. Demeter et al. (2010) reported that although vaccine C claimed to contain the Synder Hill strain belonging to the America-1 group, it showed higher genetic similarity to a wild-type strain from the America-2 group. This controversy may have prompted the exclusion of the virus strain name in

the product description of vaccine C. However, it is crucial to indicate the name of the vaccine strain in the product description as it would help to select the appropriate vaccine and to understand any post-vaccinal complications that may occur.

A recent study conducted in the USA reported that the three CDV vaccine strains, Onderstepoort, Snyder Hill, and Rockborn, could induce a protective immune response and that the Rockborn strain is the most immunogenic (Anis *et al*., 2018). Therefore, further studies are warranted to understand the reasons for the low IgG titres in the local dogs vaccinated with vaccine B in this study. According to an initial report, the Rockborn strain retains virulence even after the 36th passage on primary cells but further passaging to reduce immunogenicity needs to be done cautiously as the immunogenicity of the virus could be lost after the 70th passage (Rockborn, 1960). However, it is impossible to comment on whether vaccine B lost its immunogenicity due to excessive attenuation during increased passaging as the number of passages was not indicated in the product description. Nonetheless, it is important to note that dogs in Great Britain had developed encephalitis after vaccination with the CDV vaccines containing the Rockborn strain, and consequently, vaccines containing this strain were withdrawn from the market (Bestetti et al., 1978; Cornwell et al., 1988; Hader and Osterhaus, 1997). It must be noted that a dog vaccinated with vaccine B in the present study later developed neurological signs similar to distemper. Anis *et al*., (2018) reported the existence of antigenic differences in the H glycoprotein among different CDV strains. Therefore, breakthrough infections reported in vaccinated dogs could be due to the retaining virulence of the vaccine strain or due to the lack of cross-protection provided by the vaccine as a result of antigenic variation between the vaccine strain and the circulating field strain of the virus.

Martella *et al*., (2011) sequenced the H gene of the Rockborn stain and reported that it differs from commonly used CDV vaccine strains but closely resembles a CDV strain detected from a Lesser Panda in China. Thus, it is important to investigate whether the Ab induced by CDV vaccine strains in commercial vaccines provides cross-protection against the distemper strains circulating in the country.

Failures observed in primary vaccination could also be due to the presence of high MDA titres, which can neutralize the vaccine antigens (Nandi *et al.*, 2013; Killey *et al*., 2018). The interference of MDA on vaccinal antigens could be reduced effectively by using an MLV with a high antigenic titre (Silva, 2016). Therefore, vaccines with high antigen titers are recommended for primary vaccination to confer adequate protection for puppies (Gamage *et al.*, 2020). It is also important to note that serum antibodies are only a part of the immune response, and most MLV vaccines induce protection via a cell-mediated immune (CMI) response as well (Tizard, 2017). Dogs may have solid resistance to the virus even if they do not have protective levels of Ab titres due to the presence of memory T cells generated from CMI (Mitchell, *et al*., 2012).

Vaccination aims to protect individuals from diseases and maintain a high proportion of the immune population to prevent rapid transmission of the disease. In areas with high risks of infectious diseases, the threshold level of immune individuals or herd immunity must be maintained to protect the entire population from those diseases. Certainly, a significantly high proportion of dogs in Sri Lanka are not vaccinated against distemper and ICH, but the chances are high for those dogs to get exposed to wild strains of these viruses and elicit a natural immune response or boost the vaccinal immunity. Therefore, it would be imperative to do a cross-sectional seroprevalence study to determine the level of herd immunity and factors associated with Ab titres for these diseases.

We evaluated the serum IgG titres specific for CDV and CAV using the VacciCheck kit, which is an enzyme-linked immunosorbent assay-based semi-quantitative test designed for use in veterinary clinics. Veterinary practices throughout the world are now using this or similar kits to monitor serum IgG titres specific for canine core vaccines for the decision-making process on re-vaccination as recommended by the vaccination guidelines of WSAVA (Day *et al*., 2016; Besten, 2018; Killey *et al*., 2018; Meazzi, *et al*., 2022). The test is suitable for veterinary clinics due to its rapidity, simplicity, and reliability. The only limitation in its use in veterinary practices in developing countries like Sri Lanka could be the high cost of the kit.

CONCLUSION

Of the three commercially available vaccines tested in the study, vaccine B containing the Rockborn strain of CDV and Manhattan strain of CAV induced a very poor immune response against both viruses.

It warrants further studies to identify the reasons for this vaccination failure. The vaccine C produced by a leading manufacturer containing the viral titres of $1x10^4$ TCID50 and $1x10^6$ TCID50 or CDV and CAV, respectively was effective in inducing a protective humoral immune response in all dogs. The absence of necessary details of the vaccine on the leaflets provided, hampered comparing three vaccines. Selecting a reliable vaccine product is important to ensure a good immune response.

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