1. Introduction

Parvoviral enteritis in dogs is a highly contagious disease that occurs worldwide but is still evolving (Shackelton et al., 2005; Truyen et al., 2000). Since the initial parvovirus enteritis pandemic following the emergence of canine parvovirus type 2 (CPV-2) (Hoelzer and Parrish, 2010), three variants, namely CPV-2a, 2b, and 2c completely replaced the original type 2 virus (Buonavoglia et al., 2000). Certain species of wildlife have not been spared during that pandemic and the following periods (Mech et al., 2008). Despite advances in biotechnology and recommended vaccination protocols by academia and professional veterinary associations, clinical manifestation of CPV infection remains a serious problem in recently sold pups and in breeding kennels.

Early studies following the emergence of CPV demonstrated significant interference by maternally derived antibody (MDA) to vaccination of puppies (Gooding and Robinson, 1982; Waner et al., 1996). Additionally, the recommendations of vaccine manufacturers have aided to advance the practice of accepting six weeks as the earliest age of vaccination against CPV. MDA accounts for the passive immunity that the pups acquire from the dam and is very useful at an early age, but makes implementation of standard vaccination schedules difficult (Chappuis, 1998). Concerns regarding the influence of MDA have also prompted general opposition by practitioners to earlier vaccination. Trials have reported the possibility to overcome residual MDA and to induce immunity post-vaccination (Chappuis, 1998) and serological response to be titre-related in puppies (Burtonboy et al., 1991), indicating that concerns regarding MDA are not necessarily applicable under all circumstances.

The age of first vaccination against CPV and MDA interference to CPV vaccination in pups have been...
discussed in numerous publications (Burtonboy et al., 1991; Carmichael et al., 1983; Chappuis, 1998; Day, 2007; Hoare et al., 1997; Iida et al., 1990; Moore, 1983; Pollock and Carmichael, 1982; Pratelli et al., 2000; Willem et al., 2001). It is known that colostral transfer accounts for approximately 90% of the MDA (Burtonboy et al., 1991; Pollock and Carmichael, 1982). MDA declines with a half-life of 9.7 days but this is variable (Pollock and Carmichael, 1982). In the absence of inhibitory MDA, pups and kittens are capable of mounting a protective immune response at a very young age (Chappuis, 1998; Day, 2007). The use of a similar high titre vaccine has been studied but with few cases at four weeks of age (Willem et al., 2001).

The magnitude of the MDA in the pup is of significance. Maternal antibodies with a haemagglutination inhibition (HI) titre of $\geq 1:20$ are able to interfere with an active immune response after vaccine administration, but such titres do not prevent infection with a virulent virus. In contrast, titres of $\geq 1:64$ are considered fully protective against both infection and disease. Such MDA titres, equivalent to 2–4 maternal antibody half-lives (about 2–5 weeks), may prevent successful immunisation and leave pups susceptible to infection (Pollock and Carmichael, 1982). This interference accounts for a window of susceptibility of the pups to CPV infections and failure of the vaccine to protect. The window of interference was reported to be between the ages of 40 and 69 days in one study (Iida et al., 1990).

The decline in MDA may also be influenced by whether the experimental group studied is subjected to exposure to virulent virus or not. One study demonstrated that MDA titres in pups declined more rapidly following challenge than before (Macartney et al., 1988), with authors postulating that this rapid decline of antibody was due to its sequestration by virus after the initial phase of viral replication in the lymphoid tissues. The more rapid decline of MDA, which could occur in endemically infected premises, may complicate immunisation programmes based on the isolation and segregation of puppies in anticipation of a predicted decline in MDA before vaccination (Macartney et al., 1988). It thus follows that it may be prudent to vaccinate pups in infected breeding kennels at four weeks of age, rather than the standard six weeks in order to shorten the window of susceptibility. Factors that may affect the magnitude of the post-vaccinal immune responses to CPV vaccines are the vaccine virus titre, the degree of virus attenuation, the antigenic properties of the vaccine strain and the route of administration (Martella et al., 2005).

Canine parvovirus infection is the most common cause of enteritis in young dogs in animal rescue centres (Wells and Hepper, 1999). There is sometimes a perception among practitioners that only pups are susceptible to CPV-induced clinical disease. Outbreaks of CPV-associated enteritis and mortalities have been described in adult dogs (Decaro et al., 2008), but the incidence of true CPV-induced disease in older dogs is probably very low. Practitioners are often left confused by the multitude of published articles and comparative advertising material by the pharmaceutical industry. Many practitioners work in areas where CPV infection is rare, whereas others, despite consistent vaccinations, attend regularly to confirmed CPV cases. The former group practices in areas where there are few breeders and where vaccination compliance is high. These areas are often more affluent and practitioners are likely to perceive all vaccination programmes to be protective for puppies under all circumstances. Current recommended vaccination programmes are followed and early vaccination protocols are frowned upon. In addition, some owners and veterinarians erroneously hold the view that infectious diseases such as parvovirus infection can be controlled by frequent vaccination alone (Carmichael, 1999). In contrast, practitioners acting as consultants to large-scale breeders are well aware of the significance of CPV infections as a constant threat to susceptible dogs resulting in high morbidity and mortality.

It has been speculated that certain breeds are at higher risk for CPV infection than others. In one study Rottweilers, American Pit Bull Terriers, Doberman Pinschers and German shepherd dogs (GSDs) were at increased risk and Toy Poodles and Cocker Spaniels were at decreased risk for developing CPV-induced enteritis, compared with that for mixed-breed dogs (Houston et al., 1996). In another study Doberman Pinschers, Rottweilers and English Springer Spaniels had a significantly increased risk of CPV enteritis (Glickman et al., 1985). In another study evaluating the factors influencing the antibody response of dogs vaccinated against rabies, it was found that animal size, age, and breed strongly influenced results (Kennedy et al., 2007). The latter study also alluded to genetic susceptibility linked to haplotypes. In studies designed to determine vaccine efficacy, the choice of breed and size may therefore be of significance.

The aim of this study was to assess the efficacy of a high titre attenuated CPV vaccine in pups from four weeks of age under kennel conditions in South Africa. The primary objective was to record seroconversion rates achieved two weeks after vaccination in the presence of high maternally derived antibody levels.

2. Materials and methods

2.1. Experimental design

The study was designed to assess the ability of a high titre vaccine to stimulate seroconversion in puppies from 3 separate breeding establishments under natural husbandry conditions and with varying levels of maternal antibody.

2.2. Experimental animals

The study was conducted in three breeds, namely GSD, Rottweiler and Boerboel. Each breed belonged to a separate breeder. The number of bitches selected from each breeder included nine GSD bitches, four Rottweiler bitches and eight Boerboel bitches. A total of 52 GSD, 25 Rottweiler and 44 Boerboel pups were included of which 11, 4 and 18 were used as controls, respectively. All the pups were pedigree stock and registered with their respective breed associations.
The Boerboel breeder ran a medium-sized kennel with approximately 11 breeding bitches on site. No specific outbreaks of disease were encountered during the preceding three years. The GSD breeder managed a large breeding colony that experienced an outbreak of CPV infection 2 years before the trial despite an intensive vaccination programme. In excess of thirty breeding bitches were kept on the same premises resulting in large numbers of susceptible pups at any given time. Many adult working dogs left and entered the premises continuously. The Rottweiler breeder had a large breeding station with numerous breeds on the same premises. This breeder had sporadic mortality in neonates due to gastro-enteritis and septicaemia.

All breeding bitches were identified with either legible tattoo numbers or microchip transponders. Additional information such as treatments for illness, deworming, vaccine batch numbers, whelping dates, litter size, weight-gain and pup survival until 4 weeks of age were recorded. At 4 weeks of age, a transponder was inserted in all the pups.

2.3. Housing and nutrition

The experimental animals were housed at the respective breeders in kennels with outside runs and fed a standard commercial pelleted ration.

2.4. Blood collection

Blood was collected for serology by jugular or cephalic venipuncture using a sterile syringe and needle. Blood was centrifuged and the serum transferred to glass serum tubes. Serum samples were kept frozen until the end of the trial and tested simultaneously. Serum was collected from all puppies at 4, 6, 9 and 12 weeks, and where possible at 16 weeks.

2.5. Vaccines and vaccinations

The early vaccine administered was a non-adjuvanted, modified-live vaccine containing parvovirus strain Cornell 780916-115 strain with a viral titre of 10^7.8 TCID\textsubscript{50} (PRIMODOG Merial, Lyon, France, for puppies as determined on canine cell line). Inoculations were performed at the breeding establishments or at one designated private veterinary hospital. The vaccines used and the vaccination schedules are set out in Table 1.

Control dogs included puppies from the respective litters. They did not receive the initial vaccination at 4 weeks of age but were vaccinated from 6 weeks. One pup did not receive any vaccination and acted as a sentinel. All the dams in the study were vaccinated with EURICAN DHPPi2-LR\textsuperscript{®}, Merial, France, within the preceding 6 months before parturition but not later than 30 days before the expected date of parturition.

2.6. Serology

Serum antibodies were detected by means of the haemagglutination-inhibition (HI) technique (Carmichael \textit{et al.}, 1980), with minor modifications. Sera were diluted 1:2 in phosphate buffered saline (pH 7.4) and heat inactivated at 56 °C for 30 min. Duplicate serial twofold dilutions of the sera were made in a barbital-acetate buffer (pH 6.2). To the first dilution series, 8 HA units of a field strain of CPV-2 isolated in South Africa was added, while the second set of serum dilutions contained buffer and therefore served as a control for non-specific inhibition. After the addition of the antigen, the plates were held at room temperature for 90 min. This was followed by the addition of 1% porcine red blood cells (prepared in barbital-acetate buffer). Plates were placed at 4 °C overnight and the titre was read as the highest dilution at which 50% inhibition occurred. A positive control serum with a known antibody titre was included with each batch of tests. Seroconversion was confirmed when antibody titres showed a numerical increase of fourfold or more. Puppies with HI titres of 1:64–1:80 can be infected by parvovirus (Chappuis, 1998). For the purpose of this study an HI titre of ≥128 was regarded as protective.

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2.7. Analysis of the results

Statistical analyses were done using SIGMASTAT\textsuperscript{®} and SIGMAPLOT\textsuperscript{®} software. The level of significance was set at \( P \leq 0.05 \). Seroconversion rates were compared using a standard t-test or the Mann–Whitney Rank Sum Test.

3. Results

The seroconversion rate after the 4-week vaccination in the Rottweiler group was 80% (17/21) and was statistically significant when compared to the control group \(( P = 0.013, \text{ Mann–Whitney Rank Sum Test})\). Only 4 puppies were non-responders, but by the second vaccination all pups had seroconverted. None of the vaccinates ever had a titre less than 128. In the control group no pups seroconverted at six weeks (Fig. 1).

The seroconversion rate after the 4-week vaccination in the Boerboel group was 62% (16/26) and was statistically significant when compared to the control group \(( P = <0.001)\). Ten puppies were non-responders, but by the second vaccination two more seroconverted. By the third vaccination only three puppies had not responded, but all pups in the group had seroconverted by the fourth vaccination. Three of the pups had a titre of less than 128 for a maximum period of 3 weeks between the ages of 9–12 weeks. In the control group, 1/18 seroconverted unexpectedly (Fig. 2).

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**Table 1**

<table>
<thead>
<tr>
<th>Age at vaccination</th>
<th>Vaccine used</th>
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<tr>
<td>4 weeks</td>
<td>PRIMODOG\textsuperscript{®} (Merial, France) monovalent canine parvovirus vaccine</td>
</tr>
<tr>
<td>6 weeks</td>
<td>EURICAN\textsuperscript{®} DA2PPi2 (Merial, France)</td>
</tr>
<tr>
<td>9 weeks</td>
<td>EURICAN\textsuperscript{®} DA2PPi2 (Merial, France)</td>
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<tr>
<td>12 weeks</td>
<td>EURICAN\textsuperscript{®} DA2PPi2 + LR (Merial, France)</td>
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The seroconversion rate after the 4-week vaccination in the GSD group was 90% (35/39) and was statistically significant when compared to the control group ($P = <0.001$). Four puppies did not seroconvert. These 4 pups remained non-responders after the second and third vaccinations and their 16 weeks titres were not determined. None of the pups ever had a titre of less than 128. In the control group, 1/11 seroconverted uneventfully (Fig. 3).

The combined results for all the puppies showed that 80% (69/86) of the pups in the experimental groups seroconverted after the first vaccination at four weeks. In the control group 2 of 27 pups seroconverted uneventfully. Three of the pups in the experimental group had a titre less than 128 for a maximum period of 3 weeks between the ages of 9–12 weeks.

4. Discussion

The efficacy of a high titred CPV-2 vaccine was studied by monitoring antibody titres following a series of vaccinations. It is evident from the CPV titres at 4 weeks of age prior to vaccination that transfer of colostral...
antibodies was satisfactory in all the pups. The MDA titres ranged from 64 to 2048 with an average of 256. Despite high levels of MDA at 4 weeks, 80% of pups that received the 4-week vaccination seroconverted. In contrast, only 2 pups that did not receive the 4-week vaccination did not seroconvert at six weeks. These control pups showed a sharper increase in titres than the 4-week vaccinates as measured at nine weeks of age. Their titres ranged from 256 to 8192 but generally averaged 2048 and above and remained protected. These results are in contrast to another study where a similar vaccine was used at 4–8 weeks of age but good seroconversion rates were only achieved in the group with MDA titres below 128 (Willem et al., 2001). All the pups in the experiment maintained high serum CPV titres until the last bleed. This suggested that under the particular circumstances specific to these dogs, vaccination beyond 12 weeks would have been of little or no value.

The Boerboel group performed markedly poorer than the Rottweiler and GSD groups. This breed is a larger breed than the Rottweiler and GSD breeds, and it remains speculative whether size influenced the seroconversion rates as reported for rabies vaccination (Kennedy et al., 2007).

During the trial two pups were hospitalised with signs of lethargy, depression, dehydration, gastroenteritis, vomiting and died despite intensive therapy. Electron microscopic examination of the faeces did not reveal the presence of enteric viruses. *Toxocara* spp. worm eggs and coccidial oocysts were found in abundance with the aid of faecal floatations in these pups and its littermates. One pup in the GSD group that did not receive the 4-week vaccination, presented with severe haemorrhagic gastro-enteritis at 9 weeks of age end died after 2 days of treatment in hospital. Histological examination of sections of the small intestines was suggestive of CPV but viral isolation was not performed.

It is not clear why some pups in the control groups seroconverted between the ages of 4–6 weeks without having been vaccinated. Although histopathological examination in one animal suggested that wild type CPV virus was active during the trial, confirmation by means of electron microscopy or viral isolation was not attempted.

It must be borne in mind that some vaccine efficacy studies are performed under conditions similar to field conditions and not under gnotobiotic conditions. Given the worldwide distribution and the endemic nature of CPV infection in many areas, it may be difficult to assess whether the immunity was boosted by natural exposure during the test period in field trials, thus leading to the erroneous conclusion that the primary vaccination is the sole source of the antibody status of the experimental animal in question. This was illustrated in a study (Bohm et al., 2004) where natural CPV boosting was strongly suspected. The dogs had significantly higher titres three years after their primary vaccination than two weeks after it and three unvaccinated dogs had acquired protective antibody levels uneventfully.

This study revealed the shortcomings of a field trial. Trials conducted in commercial breeding stations and large breeding concerns have continuous movement of both animals and people on their premises. These cannot be considered closed isolated kennels. The implication is that the introduction of problems like verminosis, coccidiosis, CPV and other causes of gastro-enteritis cannot be adequately controlled. Notwithstanding these constraints, a field trial should be considered valuable as it mimics real life conditions under which the researched product will be used in practice. A field trial of this nature may be equated with post marketing surveillance with the difference that there is more reliable data collection and feedback.

Companion animals visiting private veterinary clinics or hospitals may be healthy at the time of presentation (as is the case with admissions for routine surgery), or be
compromised by injury, disease or immunosuppressive therapy. Any private clinic or hospital is considered a high-risk environment for nosocomial infections. This is due to constant movement of animals and sharing of entrances in spite of disinfection efforts and isolation of infectious patients in separate wards. The “effective quarantine” of such animals in the standard private clinics may be questioned. Many of these animals hospitalised have either an incomplete or unknown vaccination history, placing them at risk for infectious disease. Vaccination at the time of hospitalisation of dogs with incomplete or unknown vaccination histories therefore seems reasonable.

It has been documented that compromised immune function in dogs affects their response to vaccinations (Strasser et al., 2003). This does not, however, imply that the newly hospitalised patient may not be vaccinated to reduce risk. Vaccination pre- and post-surgery neither induces severe immuno-suppression, nor potentiates the severity of concurrent disease, and does not cause inapparent infections to become clinically apparent (Miyamoto et al., 1995). They also recorded comparatively good production of serum antibody titres, including antibody against CPV. Human transplant patients that are severely immuno-suppressed were still able to mount protective antibody levels against influenza virus albeit lower than normal controls (Cavdar et al., 2003). Similarly corticosteroid-treated mice could mount protective immune responses following vaccination against normally fatal Aspergillus fumigatus infections (Ito and Lyons, 2002).

With regards to the risk period between vaccination and exposure to virulent virus, it has been shown for canine distemper that dogs given a single vaccination only hours before being placed in a contaminated environment did benefit (Larson and Schultz, 2006). The value of immediate vaccination of possibly unvaccinated dogs in a high risk environment is also supported by another study (Carmichael et al., 1983) where parenteral vaccination of sero-negative dogs resulted in HI antibody titres as early as post-vaccination day 2. Dogs mounted a significant immune response in as little as seven days post vaccination (Miyamoto et al., 1995) and maximal titres occurred within 1 week in a recent trial performed by Minke et al. (unpublished results).

The utilization of a modified-live virus (MLV) CPV vaccine with a titre of $10^7$ TCID$_{50}$/dose was effective even when HI titres of pups were $\geq 1:80$ (Buonavoglia et al., 1992). They speculated that high titre MLV vaccines might be superior to others. In independent studies, other researchers concluded that seroconversion rates attained in their studies despite high MDA were dose related (Burtonboy et al., 1991; Hoare et al., 1997; Martella et al., 2005). Nevertheless, the titre in a vaccine may be meaningless if the minimum protective dose is not known (Appel, 1999), suggesting that over-attenuated virus may have a high titre in tissue culture but may not be adequately immunogenic. The use of an MLV CPV-2b vaccine with a relatively low virus titre administered parenterally proved to be highly effective in overcoming the obstacle of MDA (Pratelli et al., 2000).

The findings of our study showed that the use of a high titre CPV vaccine in pups at four weeks of age under field conditions in South Africa, and in the presence of high MDA, resulted in significant seroconversion rates of 80% in puppies with quantitative values higher or equal to the known minimum protective antibody levels against CPV. Earlier vaccination than the accepted norm did indeed bridge the window of susceptibility in most vaccines. This makes the use of such a vaccine very suitable in breeding kennels. Reduction, but not complete elimination of CPV-induced disease in large breeding kennels or in highly contaminated environments is a realistic expectation using this approach.

Conflict of interest

It is declared that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vetmic.2010.11.004.

References


