Paper

Vaccination of dogs with Duramune DAPPi+LC protects against pathogenic canine parvovirus type 2c challenge

S. Wilson, C. Stirling, S. Borowski, A. Thomas, V. King, J. Salt

In this study, we determined whether vaccination with Duramune DAPPi+LC containing canine parvovirus (CPV) type 2b protects against challenge with virulent CPV antigenic type 2c. Seven healthy dogs, seronegative for CPV2, were enrolled into two treatment groups; five were vaccinated twice, 21 days apart, with minimum titre vaccine, and two were given saline. Dogs were challenged with CPV 2c three weeks later. Clinical observations, body weight and rectal temperature measurements, blood samples for serology and white blood cell counts and faecal samples for virus excretion were collected. Control dogs remained seronegative until challenge; vaccinated dogs seroconverted and were positive for antibodies to CPV2 from day 21. Four days after challenge, clinical signs associated with parvovirus infection (vomiting, paroxysmal shivering, depression, loose stools) were observed in the control dogs. Both animals were withdrawn from the study for welfare reasons one day later. On day 47, leucopenia was observed in controls, with white blood cell counts less than 50 per cent prechallenge values. No specific clinical sign of parvovirus infection were observed in the vaccinated dogs, nor was (detectable) challenge virus shed in faeces suggesting that antibodies generated contributed sterilising immunity. We conclude that vaccination of dogs with Duramune DAPPi+LC protects against challenge with a virulent field strain of CPV 2c.

Introduction

Canine parvovirus (CPV) infection of dogs results in a highly contagious enteric disease which can lead to high rates of mortality or severe morbidity (Larson and Schultz 2007). Symptoms of CPV infection vary and can range from mild to severe haemorrhagic enteritis, fever, vomiting and often death in severe cases. All naive dogs are susceptible to infection, and those under one year of age have the highest risk of developing severe disease. Those naive dogs older than one year are also considered highly susceptible, but have a tendency to shed virus in faeces and develop a milder form of the disease and have lower incidence of mortality. Due to the risk of virus shedding and the resultant risk of contact infection, CPV is routinely considered as a core vaccination (American Animal Hospital Association Canine Vaccination Task and others 2011).

Since CPV2 was first identified (Appel and others 1979), there have been multiple iterations which have increased in prevalence. CPV type 2 was replaced by type 2a (Truyen and others 1996), and a further variant was then later identified (Parrish and others 1991). These CPV2 types differ by a single amino acid residue at position 426 of the VP2 capsid protein. More recently, a third variant has been identified (Buonavoglia and others 2001) called CPV 2c. The CPV type 2c which is becoming more commonly isolated, has been identified (Decaro and others 2011, Touihri and others 2009) in a number of countries within Europe, and exhibits similar clinical manifestations to type 2a and 2b; ranging from mild to severe disease and often death. As the CPV2 variants differ by only a number of amino acids, there has been debate that vaccines containing a single variant may offer cross-protection against the full range. Indeed, vaccination of dogs with vaccines containing CPV2 strains have been shown to protect against virulent CPV2c challenge (Spibey and others 2008) or virulent CPV2b and CPV2c challenge (Siedek and others 2011).

As the CPV 2c prevalence is increasing, we wanted to determine whether vaccination with the Duramune DAPPi+LC vaccine, containing the CPV 2b strain SAH, protects against a challenge with virulent CPV antigenic type 2c in male and female dogs of eight to nine weeks of age at time of first vaccination.

Materials and methods

The study was randomised and controlled, and run in accordance with the European Pharmacopoeia monograph 01/2008:0964—canine parvovirosis vaccine (live) for immunogenicity at a contract research organisation. The study was reviewed and approved by Zoetis and the organisation's ethical review board, and complied with all appropriate national regulations and guidelines.

Seven pure-bred, specified, pathogen-free beagles were obtained from a commercial breeder; they were confirmed seronegative for CPV prior to initiation of the trial, and were a minimum of eight to nine weeks old at first vaccination. The dogs received two vaccinations with either Duramune DAPPi+LC (Zoetis) (n=5) or sterile saline

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(n=2), three weeks apart. The two treatment groups were housed separately to minimise potential contamination from shed vaccine virus.

The vaccine used was a commercial batch, but to obtain minimum titre CPV, the freeze-dried fraction containing CPV antigen was diluted in sterile water. The vaccinated dogs, therefore, received a 1 mL dose of diluted freeze-dried fraction, and a 1 mL dose of the liquid fraction on each vaccination day.

Three weeks after the second vaccination, all dogs were challenged by the oral route with 10^5 $TCID_{50}$ per dose of CPV type 2c (strain 7/97, second passage, University of Leipzig, Germany). Animals deemed unfit to continue the study upon veterinary examination and fulfilment of clinical endpoints (Jones and others 2002 were withdrawn from the study and euthanased with an overdose of sodium pentobarbital (Euthadorm; CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany).

Blood samples were collected for serology on day 0 and 21 prior to vaccination, day 42 prior to challenge, and at the end of the study on day 56. Additional blood samples for white blood cell counts were collected on days 40, 41, 42, 45, 46, 47, 49, 52 and 56. Clinical observations, rectal temperature measurements and faecal samples collection were performed daily from the day of challenge until the end of the study on all dogs. White blood cell analysis was performed at the contract research organisation while serology and virus isolation from faecal material was performed at the University of Leipzig, Germany, using published methods. Serum was examined by haemagglutination inhibition (HAI) assay to CPV2 strain 265 (Parrish and others 1991), or serum neutralisation (SNT) assay (Siedek and others 2011) to CPV 2c strain 7/97. Briefly, for the HAI test, sera was titrated in doubling dilutions from 1 : 10 in barbital-borate albumin buffer (pH 6.2), inactivated at 56°C for 30 minutes, and preadsorbed to pig erythrocytes. The prepared sera was subsequently incubated with approximately eight haemagglutination (HA) units of CPV, and incubated again for one hour at room temperature. A suspension of pig erythrocytes (0.5 per cent in buffer solution) was added and incubated for another two hours at 4°C or overnight. The test plates were then examined for haemagglutination. Results were reported as the reciprocal of the highest dilution to show HAI. Sera were considered to be positive at an HAI titre of > 10. For the SNT assay, twofold dilutions of the sera were incubated with the virus in duplicates and incubated for one hour at 37°C. The virus-serum suspension was then transferred to susceptible Crandell Rees feline kidney (CRFK) cells and further incubated for 3–4 days. Cells were fixed with acetone and stained with a pool of parvovirus-specific monoclonal antibodies, and FITC-conjugated with antimouse IgG. Neutralising antibody titres were calculated according to Spearman and Kaerber. The serum dilution was only considered positive when not a single nucleus was stained. Virus isolation from faecal material was performed as previously described (Strassheim and others 1994). Briefly, faecal material was 1 : 10 suspended in phosphate buffered saline and extracted by filtration. The aqueous phases were titrated in triplicates on CRFK cells, on two different plates. After four days incubation at 37°C the plates were stained by indirect immunofluorescence as described for the neutralisation test (Siedek and others 2011). Virus titres were calculated by the method of Spearman and Kaerber and expressed as $TCID_{50}$/mL.

Results

Analysis of body weights showed all animals gained weight during the course of the study, with no obvious impact of vaccination or challenge on any of the animals. Vaccination and challenge also had minimal impact on rectal temperature measurements, with only one of the non-vaccinated control dogs having a slightly elevated temperature recorded after challenge. All other dogs had temperatures within the normal physiological range.

Clinical examination of dogs revealed no specific findings in any animal of either group during acclimatisation, or from day 0 to 45, three days after challenge. From four days after challenge (day 46) with the CPV 2c strain, clinical signs typically associated with parvovirus infection, such as vomiting, paroxysmal shivering and loose faeces, were observed in control dogs. Additionally, reduced feed intake, depression and pale pink mucous membranes were seen in both control dogs, while unsteady gait, possible dehydration and gaunt appearance was seen in one of the two control dogs. One day later (day 47), both control animals were withdrawn from the study for welfare reasons. In the vaccinated dogs no animals exhibited any specific clinical signs from the period following challenge until study completion.

TABLE 1: White blood cell counts, total number of leukocytes (10^9/L)

	Control dogs		Vaccinated dogs				
Day of study	1	2	1	2	3	4	5
40	13.3	12.6	12.4	11.4	23.2	10.0	10.2
41	12.9	10.7	10.3	10.5	15.0	9.0	9.1
42	9.3	9.7	8.3	9.1	10.3	8.7	8.4
Baseline mean*	11.83	11.00	10.33	10.33	16.17	9.23	9.23
45	13.4	12.5	10.7	10.5	13.8	11.0	9.6
46	11.3†	7.9†					
47	4.8	4.5	10.8	9.4	9.9	10.1	10.0
49			9.6	11.3	12.2	10.4	9.1
52			10.1	10.3	10.5	9.6	9.4
56			9.3	11.2	10.4	9.6	9.2

*Baseline mean is the average of days 40, 41 and 42 values.
†Additional sampling due to observed clinical findings.

White blood cells counts (Table 1) stayed relatively stable in all dogs until day 45; in the control dogs, the counts declined sharply on days 46 and 47 to a value of 41 per cent of the prechallenge mean for both dogs. By contrast, no decline indicative of developing leucopenia was observed in any of the five vaccinated dogs. One vaccinated dog did have high white blood cell counts, albeit not consistently so prior to challenge, giving a higher mean baseline value. Although the percentage decrease after challenge was approximately 38 per cent, which is not considered leucopenic by the European Pharmacopeia, it is considered unlikely to be related to CPV due to the absence of detectable virus from faecal samples after challenge (data not shown).

All dogs were seronegative (titres <10) to CPV2 (HAI test) and CPV2c (serum neutralisation test) on day 0, the controls also remaining seronegative on days 21 and 42 prior to challenge (Tables 2 and 3). In vaccinated dogs, titres rose to 640–1280 (HAI tests)

TABLE 2: Serological analysis, haemagglutination inhibition test; CPV2 (265)

	Controls		Vaccinates				
Day of study	A	B	C	D	E	F	G
0	<10	<10	<10	<10	<10	<10	<10
21	<10	<10	1280	640	640	640	640
42	<10	<10	640	640	320	1280	640
47	40*	80*	NA	NA	NA	NA	NA
56			640	640	320	640	640

Vaccinated dogs were administered a vaccine containing CPV2b on days 0 and 21, and all dogs were challenged orally with a CPV2c strain (10^5 $TCID_{50}$/dose) on day 42
*Animal euthanased for welfare reasons. NA=samples only collected at euthanasia of controls
CPV, Canine parvovirus

TABLE 3: Serological analysis, serum neutralisation test; CPV 2c (7/97)

	Controls		Vaccinates				
Day of study	A	B	C	D	E	F	G
0	<10	<10	<10	<10	<10	<10	<10
21	<10	<10	2560	640	640	1280	1280
42	<10	<10	1280	1280	640	1280	1280
47	20*	40*	NA	NA	NA	NA	NA
56			1280	1280	640	640	1280

Vaccinated dogs were administered a vaccine containing CPV2b on days 0 and 21, and all dogs were challenged orally with a CPV2c strain (10^5 $TCID_{50}$/dose) on day 42
*Animal euthanased for welfare reasons. NA=samples only collected at euthanasia of controls
CPV, Canine parvovirus

and 640–2560 (serum neutralisation test) by 21 days after first vaccination and remained relatively stable thereafter, even following challenge (Tables 2 and 3). In control dogs, only low titres (20–80) were seen when euthanased on day 47 (five days after challenge). Faecal samples (data not shown) were analysed for titration of virus excretion, and prior to challenge on day 42, titres were <1.5 $\log_{10}$ $TCID_{50}$/mL in all animals of both groups. After challenge of control group animals with CPV antigenic type 2c, titres increased to 5.8 and 6.2 $\log_{10}$ $TCID_{50}$/mL on day 46. By contrast, titres remained <1.5 $\log_{10}$ $TCID_{50}$/mL in all vaccinated animals during the entire postchallenge period (ie, days 43–56) indicating absence of any detectable virus shedding after vaccination with Duramune DAPPi+LC.

Discussion

Despite the ready availability of safe and efficacious vaccines to CPV, this virus continues to be a very important pathogen in dogs. The advent of new CPV variants, type 2a and 2b (Parrish 1991, Truyen and others 1996), and more recently type 2c (Buonavoglia and others 2001), has obviously raised concerns about the continued ability of older vaccines based around the original CPV2 strain to protect dogs from infection. The present study investigated whether administration of Duramune DAPPi+LC containing CPV 2b at minimum titre to dogs aged eight weeks old would protect against challenge with a virulent CPV 2c field strain, this challenge protection is a claim already held for the CPV 2a and CPV 2b types by this vaccine. As shown, we were able to successfully demonstrate protection from the vaccine against clinical disease, leucopenia and virus shedding following a highly virulent challenge. Both control animals had clinical signs of enteric parvovirus infection, were found to shed virus after challenge, and had to be withdrawn from the study for welfare reasons five days after challenge.

Researchers have questioned the need for new vaccines (Truyen 2006) to combat the rise of different CPV types, from the original CPV2 to CPV 2a and CPV 2b to the more recent CPV 2c, given older vaccines have tended to use antigens derived from CPV2. Although the CPV types can be differentiated by a change in one amino acid, there is clearly high variability between them. Cavalli and others (Cavalli and others 2008) examined CPV2, CPV 2a, CPV 2b and CPV 2c types using SNT and HAI analytical tools. Their study identified clear antigenic differences between the CPV2 and variants as might be expected with the CPV 2c found to have a unique antigenic profile, and poorly recognised by sera from animals vaccinated with the other CPV types. Our current study demonstrates alongside previous investigations (Siedek and others 2011, Spibey and others 2008) based on type 2 vaccines that vaccination of dogs with a CPV type 2b-based vaccine also offers considerable protection against the new CPV 2c variant. The two serological assays we have used, examining antibody development against CPV2 (HAI) and CPV 2c (SNT), show vaccinated dogs generate high antibody titres to both virus types by three weeks after primary vaccination, demonstrating data contrary to that of Cavalli and others (2008) with CPV2 vaccinated dogs generating highly reactive antibodies to CPV 2c virus. The lack of anamnestic responses in vaccinated dogs following challenge seen in the current study is comparable with the situation seen by Spibey and others (Spibey and others 2008), and is indicative of sterilising immunity. Using both SNT and HAI assays in our study, peak antibody responses were observed at day 21 or day 42, prior to challenge, with no further increases observed after this. Both control dogs were essentially seronegative up to challenge, and mounted limited immune responses thereafter; this is explained by the rapid decrease in white blood cell counts observed at the same time, a common consequence of CPV infection (Chalmers and others 1999). Both controls were euthanased prior to study completion due to reaching defined welfare endpoints, so full analysis was not possible.

The results obtained here would indicate that although there are clear antigenic differences between the CPV variants, there is still considerable homology also with which a vaccine can prove efficacious and protect against infection.

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