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Canine parvovirus type 2 vaccine protects against virulent challenge with type 2c virus.

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Abstract

The ability of dogs vaccinated with a live attenuated CPV type 2 vaccine to resist challenge with a current CPV 2c isolate was investigated. Six SPF beagle dogs were given the minimum recommended course of vaccination, comprising a single inoculation of leptospirosis vaccine (Nobivac Lepto) at ten weeks of age followed two weeks later with a parvovirus vaccine in combination with distemper, adenovirus and parainfluenza virus (Nobivac DHPPi) and a repeat leptospirosis vaccine. Six control dogs were kept unvaccinated. All animals were challenged orally with a type 2c isolate of CPV and monitored for clinical signs , virus shedding, white blood cell fluctuations and serological responses. All vaccinated dogs were fully protected; showing no clinical signs nor shedding challenge virus in the faeces, in contrast to control animals which displayed all the typical signs of infection with pathogenic CPV and shed challenge virus in the faeces.

Keywords: Canine parvovirus, vaccine, protection

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Introduction

Canine parvovirus (CPV2) is a single stranded DNA virus which is responsible for an acute and sometimes fatal enteritis in dogs (Kelly, 1978; Appel et al., 1979). The virus, which first appeared in 1977 /1978, probably arose from a very closely related virus in cats, feline panleukopaenia virus (FPL) through a small number of mutations in the single capsid protein; a species jump which may have involved intermediate passage in other carnivores such as mink or raccoons (Truyen et al., 1996). As early as 1979 the first variants of CPV 2 appeared, termed CPV2a, and they were quickly followed by the appearance of CPV2b in 1984.(Parrish et al 1985, 1991). The original type 2 virus has now virtually disappeared from the field having been replaced by the 2a and 2b variants; although the relative proportions of these two types varies from country to country (Truyen et al., 1996; Chinchkar et al., 2006; Pereira et al., 2006).

The amino acid changes in the capsid protein (VP2) which characterise the shift from 2 to 2a and to 2b are very limited. Substitutions at positions 87 (Met to Leu), 300 (Gly to Ala), 305 (Tyr to Asp) and 555 (Val to Ile) occurred in the evolution of 2 to 2a and 426 (Asn to Asp) and 555 (Ile to Val) in the emergence of 2b from 2a (Parrish et al., 1991; Truyen et al., 1995). However as recent 2a strains lacking the Val to Ile substitution at position 555 have been reported (Wang et al., 2005; Martella et al., 2006), then a single amino acid change can differentiate the CPV2a and CPV2b VP2 sequences. More recently strains have emerged in Italy in which the amino acid at position 426 (Asn in 2a and Asp in 2b) has become a glutamic acid (Glu) residue (Buonavoglia et al., 2001; Martella et al., 2004). The fact that these Glu 426 variants,

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termed CPV2c viruses, are circulating and co-existing with other CPV types in Italy and other European countries (Decaro et al., 2006b:;C. Buonavoglia personal communication) and have also been isolated in countries as geographically diverse as Vietnam and Scotland (Nakamura et al., 2004; C. Buonavoglia personal communication) suggests that they have an advantage in at least a proportion of the dog population. The relatively rapid evolution of canine parvovirus has resulted in the loss and then re-gaining of the feline host range (Truyen et al., 1996), and this regained ability to replicate in cats may well account for the replacement of the original type 2 virus with the 2a, 2b and 2c variants.

In the late 1970's and early 1980's both live and inactivated FPL vaccines were used to protect dogs against CPV disease due to the shared antigens which stimulated cross protection, however the levels of protection they afforded was poor and duration of immunity was short. These vaccines were replaced by live attenuated CPV vaccines which provided excellent protection and longer duration of immunity. Currently the live attenuated vaccines are derived from either CPV2b isolates or the original type 2 virus. Since the type 2 virus has been entirely replaced in the field by 2a, 2b and now 2c viruses there has been concern over the level of protection afforded by attenuated type 2 vaccines (Pratelli et al., 2001). However, based on studies with available monoclonal antibodies each new antigenic variant has lost at least one neutralising epitope compared with the former variant (Strassheim et al., 1994, Pereira et al., 2006). Previously it has been demonstrated that the live attenuated CPV 2 vaccine is able to protect dogs against 2a and 2b field challenges (Greenwood et al., 1995) even though cross neutralisation studies conducted *in vitro* using sera raised against the various antigenic types do show marked differences (Pratelli et al., 2001).

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The aim of this study was to investigate the ability of a live attenuated type 2 vaccine (Nobivac- Intervet) to protect dogs from challenge with one of the most recent CPV variants, CPV2c.

Materials and Methods

Viruses & cell culture

Nobivac DHPPi vaccine (Intervet) containing canine parvovirus (CPV2 – strain 154), canine adenovirus (type 2), distemper virus, and parainfluenza virus, Nobivac Lepto (inactivated leptospirosis vaccine - Intervet), and Nobivac Pi (live parainfluenza virus only) were used.

A CPV2c pathogenic strain (kindly provided by C. Buonavoglia, Department of Animal Health and Well-being, Faculty of Veterinary Medicine of Bari, Italy) was used as challenge virus.

CPV2c and CPV2-154 were propagated and titrated in Crandell Rees feline kidney cells (CrFK); isolation of virus from rectal swabs was also performed in CrFK cells which were cultured essentially as described by Mochizuki et al (1993) using M6B8 medium (Intervet) supplemented with 5% foetal bovine serum containing penicillin and streptomycin.

Serology & immunofluoresence

Serum samples were assayed for antibodies to canine parvovirus using both haemaglutination inhibition (Churchill 1982) and serum neutralisation assays . The

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CPV2 and CPV2c viruses were used in the HAI test at a constant 4 HA units. In the serum neutralisation assays viruses were used at a titre of $10^{1.76}$ /well. Immunofluoresence was carried out as described previously (Vihinen-Ranta, 1998). Briefly, monolayers of CrFK cells were fixed ~72 hours post infection with methanol. The anti CPV monoclonal antibody A2F8 (Parrish et al., 1982) was used, followed by rabbit anti mouse FITC conjugate (SIGMA)

Efficacy Study

Twelve beagle dogs were obtained from unvaccinated unexposed bitches and therefore devoid of maternally derived antibodies against canine parvovirus. All the dogs were declared fit and healthy by veterinary inspection and shown to be sero negative with respect to CPV at the start of the experiment. The animals were divided into two groups, vaccinates and controls, with six animals in each group; each group was housed separately. The vaccinated group was given the minimum recommended course of vaccination which consisted of vaccination at 8 weeks of age with Nobivac Pi and Nobivac Lepto followed by a second vaccination at 10 weeks of age with Nobivac DHPPi and Lepto. The vaccinate group therefore only received a single vaccination with parvovirus vaccine. The control dogs received no vaccinations. Four weeks following vaccination both groups were challenged with the CPV2c parvovirus. Animals were deprived of food for 24 hours prior to, and for 12 hours following challenge; although water was available throughout. The challenge virus ($10^{5.0}$ $TCID_{50}$) was administered orally in a volume of 1.0ml. The dogs were bled pre-vaccination, pre-challenge and on selected days post challenge for measurement of serological responses and leucocyte/lymphocyte estimation. Animals were also swabbed at regular intervals for virus isolation and observed closely for clinical signs

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of disease including malaise, reduced appetite, poor general condition and blood in faeces from 2 days before until 14 days after challenge.

Statistical analyses.

A one way analysis of variance test was carried out using the Mini Tab™ statistics software package.

RESULTS

Clinical Observation

The clinical observations are set out in **TABLE 1**. The control animals started to show clinical signs from 4 days post challenge and by day 6 post challenge three of the control dogs showed severe clinical signs and were euthanased on welfare grounds. The remaining control animals exhibited less severe signs although oral electrolytes were needed to aid recovery. Nevertheless reduced appetite resulted in a marked check in their growth rate (results not shown). All the control animals exhibited a severe mucoid diarrhoea which was also haemorrhagic in the three dogs which required euthanasia, whereas the vaccinated group did not display any clinical signs of disease at any stage during the experiment. Rectal swabs taken post challenge were assayed for virus content by culture on CrFK cells (**TABLE 2**). Virus could be detected in swabs taken from all the control animals from day 3 to day 7 post challenge, whereas no evidence of viral excretion could be detected in any of the vaccinated dogs.

The mean white blood cell counts (mwcc) are shown in **TABLE 3**. Values were similar in both the vaccinates and control dogs prior to challenge, and in the vaccinated group the mwcc did not show a significant change after challenge (p=

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166 0.12). In the control group however there was a significant drop (p= 0.003) in the
167 mwcc post challenge to almost half the pre-challenge value.
168
169 **Serological responses**
170 In keeping with their SPF status and their derivation from unvaccinated mothers none
171 of the animals had any detectable antibodies to canine parvovirus prior to vaccination
172 (data not shown). At the time of challenge after the single parvovirus vaccination all
173 the vaccinated dogs had developed HAI antibody titres ranging from 1600 to 6400
174 (TABLE 4). There was no observable difference in HAI titre when the assay was
175 conducted with 2c or vaccine parvovirus antigens. The serological responses were
176 also measured in virus neutralisation assays against the challenge and vaccine viruses
177 (TABLE 4) and in these assays the vaccinates demonstrated a markedly higher
178 response to the type 2 strain compared to the 2c strain.
179 Following challenge the vaccinated animals did not show an anamnestic response to
180 CPV, in HAI or VN assays when either the CPV 2c antigen or the vaccine antigen
181 was used. The control animals remained seronegative up until the time of challenge,
182 however after challenge the control animals did mount an antibody response which
183 was noticeably higher in the recovered animals compared with the animals which
184 were subsequently euthanased.
185
186
187 **Discussion**
188
189 Canine parvovirus continues to be an important pathogen of dogs and is responsible
190 for serious occurrences of morbidity and mortality, despite the availability of safe and

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191 effective vaccines (Decaro et al., 2006a, 2006b). Since the replacement of the original
192 type 2 virus by the 2a , 2b variant and more recently the type 2c viruses (Parrish et al
193 1991, Martella et al 2004) there have been concerns expressed over the efficacy of
194 canine parvovirus vaccines which are based on the original type 2 strain (Martella et
195 al., 2005; Truyen, 2006).
196 Although it has previously been demonstrated that a type 2 vaccine is able to provide
197 protection against 2a and 2b field isolates (Greenwood et al 1995), the emergence of
198 the 2c variant naturally raises the question of whether the type 2 vaccines can provide
199 protection against this new variant also. We clearly demonstrate here that dogs
200 vaccinated with a single dose of one particular type 2 parvovirus vaccine are protected
201 from challenge with one of the type 2c field isolates; furthermore this isolate was able
202 to cause a severe enteritis in unvaccinated dogs. Analysis of the rectal swabs (**TABLE**
203 **2**) reveals that the vaccinated dogs were not only protected from clinical disease but
204 also that vaccination prevented shedding of challenge virus. This finding is in line
205 with the ability of this type 2 vaccine to prevent shedding of type 2a and type 2b virus
206 following challenge (Greenwood et al., 1995). In addition the duration of virus
207 shedding in the control animals was similar to that observed with other CPV strains
208 (Greenwood et al., unpublished observations). Leucopoenia is often a consequence of
209 CPV infection (Chalmers et al., 1999) and is therefore another criterion by which
210 infection and protection can be determined. The white cell counts (**TABLE 3)**
211 demonstrate that the type 2c virus causes a leucopoenia in the unvaccinated control
212 animals, whereas the vaccinated group remained normal. Interestingly a differential
213 white cell count did not show a specific drop in the lymphocytes normally associated
214 with CPV infection .
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There was no anamnestic response following challenge in the vaccinated dogs indicating that they had sterilising immunity to CPV. Moreover the HAI responses in the vaccinated group did not show a marked difference in titre whether the test was performed with the 2c antigen or the type 2 vaccine antigen. However the responses of the 3 control dogs which survived the challenge did show a difference in HAI when measured against the 2c antigen compared with the vaccine antigen. All the control animals were able to mount an immune response and it may be that differences in the serological responses observed in the control group may have been due in part to the different sampling intervals, in that the recovered dogs were sampled 7 days post challenge whereas the other control dogs were sampled at the point of euthanasia on day 6 post challenge.

These data indicate that whilst there may be antigenic differences between the type 2c virus and the precursor type 2 virus used in the vaccine these differences do not have a material significance in terms of protection from disease, i.e there is effective cross reactivity of the type 2 vaccine against the 2c virus.

Whilst the haemaglutination inhibition assay has been routinely used to assess protective serological responses in CPV studies, it may be argued that serum neutralisation would give a more accurate view of the protection afforded by a vaccine against any variant field strains. Not surprisingly in all the vaccinated dogs the neutralisation titres are higher when measured against the vaccine strain compared with the 2c challenge virus. However after challenge the neutralisation titres against 2c or the vaccine did not increase indicating that as shown with the HAI responses the animals had sterilising immunity. Therefore it is interesting to note that antibody titres

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241 in these dogs were as high as in the recovered control dogs. These and other data
242 support the view that despite the minor differences between the original type 2 virus
243 and the 2a, 2b and now 2c variants, dogs vaccinated with this type 2 vaccine will
244 mount a robust immune response to CPV and are fully protected against challenge
245 from any of the current CPV types.
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TABLE 1 Clinical observations of dogs challenged with CPV Glu-426

Animal Number	Group	Clinical Observation (days post challenge)														
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
5256		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5260		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9815		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9819	**Vaccinate**	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9823		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9829		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5254		N	N	N	N	M, RA, BF	M, RA BF	E	-------	------	------	------	------	------	------	------
5258		N	N	N	N	M, RA, BF	M, RA, BF	E	-------	------	------	------	------	------	------	------
9813	**Control**	N	N	N	N	M, RA, BF	M, RA, BF	M, RA, BF	PC, RA	RA	N	N	N	N	N	N
9817		N	N	N	N	M, RA, BF	M, RA, BF	E	-------	------	------	------	------	------	------	------
9821		N	N	N	N	M, RA, BF	M, RA, BF	M, RA, BF	PC, RA	PC, RA	RA	N	N	N	N	N
9827		N	N	N	N	M, RA, BF	M, RA, BF	M, RA, BF	PC, RA	RA	N	N	N	N	N	N

N= Normal, M= Malaise, RA= Reduced appetite, BF= Blood in faeces, PC= Poor condition

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TABLE 2 Post challenge viral excretion

Group/ Animal Number		CPV titre (Days post challenge) 0	3	4	5	6	7
Control	5254	0	3.30	6.70	6.30	5.45 (euthanased)	-
	5258	0	4.45	6.20	7.45	7.10 (euthanased)	-
	9813	0	3.45	5.54	7.20	6.20	5.01
	9817	0	4.30	7.10	6.45	3.30 (euthanased)	-
	9821	0	3.95	5.70	5.85	5.85	6.30
	9827	0	<1.45	4.20	7.95	6.30	6.70
Vaccinate	5256	0	0	0	0	0	0
	5260	0	0	0	0	0	0
	9815	0	0	0	0	0	0
	9819	0	0	0	0	0	0
	9823	0	0	0	0	0	0
	9829	0	0	0	0	0	0

Titres are given in $TCID_{50}$/ml

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TABLE 3 White Blood Cell Counts

Dog ID/Group	Days Prior to Challenge								Days post challenge													
	5		3		0		mean		1		2		3		4		5		7		9	
Control	twc	ly	twc	ly	twc	ly	twc	ly	twc	ly	tec	ly	twc	ly	twc	ly	twc	ly	twc	ly	twc	ly
5254	15.30	7.04	15.60	8.27	11.70	4.91	***14.20***	6.74	13.60	5.98	13.10	4.19	18.00	2.52	8.93	2.59	8.25	1.73	Euthanased			
9813	14.90	8.2	16.90	8.28	11.80	4.48	***14.53***	6.99	15.00	6.15	13.90	4.73	14.60	2.48	10.20	4.69	12.70	2.67	9.87	4.84	8.77	6.67
9817	13.00	7.15	16.30	9.94	12.00	5.52	***13.77***	7.54	12.40	5.83	11.30	3.96	10.20	1.94	8.84	1.86	1.56	0.66	Euthanased			
9821	12.20	5.73	12.30	5.66	9.14	3.93	***11.21***	5.11	10.30	5.05	16.00	6.24	11.70	1.17	8.68	2.86	8.03	1.98	3.18	3.02	6.33	2.66
5258	12.50	5.75	14.30	6.44	13.00	5.59	***13.27***	5.93	15.90	7.47	13.60	6.26	17.10	2.22	7.55	1.06	7.79	3.82	Euthanased			
9827	15.20	6.99	15.10	8	11.10	5.66	***13.80***	6.88	16.30	6.68	14.10	6.63	13.60	5.71	13.10	2.1	7.55	1.06	9.87	2.86	8.63	6.3
mean	**13.85**	**6.81**	**15.08**	**7.77**	**11.46**	**5.0**	**13.46**	**6.53**	**13.92**	**6.19**	**13.67**	**5.34**	**14.20**	**2.67**	**9.55**	**2.53**	**7.65**	**1.99**	**7.64**	**3.57**	**7.91**	**5.18**
Vaccinate																						
9815	11.10	5	12.00	6.24	8.85	3.19	***10.65***	4.81	13.30	6.25	12.90	5.29	13.80	7.59	13.90	5.14	13.70	4.8	9.86	4.63	9.56	4.4
9819	18.00	7.74	14.10	4.79	9.59	2.78	***13.90***	5.10	15.90	4.61	13.20	6.2	12.50	5.63	12.00	4.8	11.70	4.68	11.10	5.11	11.00	4.84
9823	15.30	6.89	14.60	7.74	10.70	3.32	***13.53***	5.98	13.80	5.66	12.30	4.55	15.20	6.84	13.40	7.91	15.10	5.74	13.90	6.81	12.90	7.35
5256	14.10	4.65	13.00	4.94	10.60	3.82	***12.57***	4.47	16.40	6.56	13.90	4.87	13.80	5.11	12.20	5.37	14.60	4.23	12.00	5.64	11.70	4.1
5260	17.50	5.95	14.40	3.02	10.20	3.88	***14.03***	4.28	11.90	4.17	11.60	4.99	12.40	5.33	8.92	3.75	12.10	4.24	11.90	3.81	10.90	4.58
9829	15.00	5.4	14.40	4.9	10.40	2.5	***13.27***	4.27	13.90	3.38	12.80	5.12	10.80	3.89	13.80	6.35	13.70	3.7	11.10	2.44	13.20	4.22
mean	**15.17**	**5.94**	**13.75**	5.27	**10.06**	**3.25**	**12.99**	**4.82**	**14.20**	**5.11**	**12.78**	**5.17**	**13.08**	**5.73**	**12.37**	**5.55**	**13.48**	**4.57**	**11.64**	**4.74**	**11.54**	**4.92**

twc= total white cell count; ly= lymphocyte count
Counts are given in 10^9 cells/litre

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TABLE 4 Serum neutralisation and HAI responses

Group	Animal ID	Post Vaccination				Post challenge*			
		HAI		VN		HAI		VN	
		2c	Vaccine	2c	Vaccine	2c	Vaccine	2c	Vaccine
Control	5254	<10	<10	<3	<3	†1280	†320	†2896	†2656
	9813	<10	<10	<3	<3	10240	2560	38968	16384
	9817	<10	<10	<3	<3	†5120	†640	†2896	†2656
	9821	<10	<10	<3	<3	10240	2560	13141	11585
	5258	<10	<10	<3	<3	†5120	†640	†2299	†4598
	9827	<10	<10	<3	<3	10240	2560	55109	46341
Vaccine	9815	1600	3200	18390	>370328	2560	2560	7298	105130
	9819	1600	6400	36781	>370328	2560	2560	23170	339959
	9823	3200	1600	12634	339959	2560	2560	14218	~210261
	5256	1600	3200	10624	147123	2560	2560	9195	65536
	5260	3200	1600	32768	339959	2560	2560	46341	~262144
	9829	1600	3200	18390	202141	2560	2560	36781	65536
+ve control		800	1600	2896	13141	1280	2560	2896	13141

* Samples taken 7 days post challenge

† Samples taken at time of euthanasia

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