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Review

Are licensed canine parvovirus (CPV2 and CPV2b) vaccines able to elicit protection against CPV2c subtype in puppies?: A systematic review of controlled clinical trials

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ABSTRACT

Severe gastroenteritis caused by canine parvovirus type 2 (CPV2) is a serious life-threatening disease in puppies less than 4-months of age. The emergence of new variants has provoked some concern about the cross-protection elicited by licensed canine parvovirus modified-live type 2 (CPV2) and type 2b (CPV2b) vaccines against the most recent subtype CPV2c. A systematic review was carried out to assess the efficacy of commercial vaccines.

We conducted a literature search of Pub Med/MEDLINE from January 1990 to May 2014. This was supplemented by hand-searching of related citations and searches in Google/Google Scholar.

Controlled clinical trials in which vaccinated puppies were challenged with CPV2c virus were evaluated. Reporting of outcome measures and results for vaccine efficacy were critically appraised through a variety of clinical signs, serological tests, virus shedding and the ability to overcome maternally derived antibodies (MDA) titres.

Six controlled clinical trials were included in the review. In most cases, the results of the selected studies reported benefits in terms of clinical signs, serological tests and virus shedding. However, MDA interference was not considered or evaluated in 5 of the selected trials. No accurate definitions of baseline healthy status and/or clinical outcomes were provided. Methods of randomization, allocation concealment and blinding were usually poorly reported.

As a result of the limited number of included studies matching the inclusion criteria, the small sample sizes, short follow-up and the methodological limitations observed, it was not possible to reach a final conclusion regarding the cross-protection of licensed CPV2 and CPV2b vaccines against the subtype 2c in puppies. Further and specifically designed trials are required in order to elucidate whether cross-protection is acquired from licensed CPV vaccines.

Contents

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1. Introduction

Canine parvovirus type 2 (CPV2) is a highly contagious pathogen for puppies up to 4 months (Appel et al., 1979; Jacob et al., 1980; Pollock and Coyne, 1993) and remains the most significant viral cause of severe gastroenteritis in young dogs (Wells and Hepper, 1999). Two diseases, enteritis (Appel et al., 1978; Woods et al., 1980) and myocarditis (Hayes et al., 1979), were initially associated with CPV2 infection. Although the cardiac disorder represented a life-threatening condition in 2-3-week-old seronegative puppies, it has not been reported in recent years. This is due to the fact that all bitches are now supposedly immune and transfer that immunity to their offspring through the colostrum conferring them protection during this critical period.

Maternally derived antibodies (MDA) confer immunity to dogs for the first few weeks of their lives and afterwards, they decline with a half-life of 9.7 days (Pollock and Carmichael, 1982). Accordingly, there is a period called "window of vulnerability" where MDA titres are too low to provide protection but high enough to prevent immunization with modified-live CPV vaccines. It has been determined that MDA with Hemaglutination Assay Inhibition (HAI) titres between 1:10 and 1:80 are able to interfere with an active immune response after vaccine administration, but such titres do not protect against CPV2 (Carmichael et al., 1983). Previous research revealed that puppies with HAI titres $\geq$ 1:80 were adequately protected against CPV2 intestinal replication (Pollock and Carmichael, 1982). Nevertheless, a recent paper has shown that pups with HAI MDA titres up to 1:160 were infected by CPV2b and excreted virus (Decaro et al., 2005; Elia et al., 2005). Protection from a minimum level of MDA titres has not been conclusively determined.

In the 1980s, two antigenic variants emerged, CPV2a and CPV2b, replacing the primary subtype (Parrish et al., 1991). Both strains differ from the original in five or six amino acid residues of VP2 capsid protein. In contrast, only two residues separate CPV2b from CPV2a. Furthermore, in 2000, the change of a single nucleotide (Glu426) resulted in a new strain, namely CPV2c. Its antigenic characteristics were suggested as affecting the diagnostic test and the predisposition of young dogs to infection when MDA are declined (Cavalli et al., 2008). As a consequence of the identification of this novel antigenic variant in several countries and regions where CPV-2c caused outbreaks of fatal gastroenteritis in pups and vaccinated adult dogs (Decaro et al., 2006, 2008; Sutton et al., 2013), concerns were raised regarding the ability of vaccines containing CPV2 or CPV2b to confer cross-protection against the last identified antigenic subtype CPV2c. The mismatch between the vaccine and an infecting strain may contribute to increasing the risk of an outbreak in the canine population. This variant CPV2c has been progressively distributed in Europe and other continents so it can be asserted that CPV2c is now the predominant subtype circulating in the different geographic regions (Decaro et al., 2007, 2011; Calderon et al., 2011; Perez et al., 2012; Vieira et al., 2008).

Factors that may affect the magnitude of the post-vaccination immune response to CPV vaccines are vaccine virus titration, degree of virus attenuation, antigenic properties of the vaccine strain, route of administration and breed. Vaccination by nasal route has proven to have a faster onset of action than other routes (Werneling et al., 2002; Martella et al., 2005). Furthermore, certain breeds, such as Rottweiler, Doberman pinchers, Labrador retrievers, Springer spaniels, Yorkshire terriers, American pit bull terriers and German shepherd dogs have been identified as having an increased risk of developing parvovirus enteritis (Houston et al., 1996). However, MDA interference in the vaccination course is by far the most influencing factor. It has been widely recognised as the first cause of vaccination failure (Pollock and Carmichael, 1982; Buonavoglia et al., 1985).

A systematic review of controlled clinical trials was conducted to assess the cross-protection of licensed canine CPV2 and CPV2b vaccines against the variant CPV2c in puppies.

2. Methods, techniques

Overall, the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement was used as a guide for the reporting of this review (Moher et al., 2009).

2.1. Definitions and outcomes

The target population was less than 4-month-old puppies as CPV2 is the most significant cause of enteritis in dogs at this age. Licensed modified-live mono/multivalent vaccines containing CPV2/CPV2b fraction were selected as the interventions for this review. For the control group several options were eligible: a placebo-control (e.g. saline), the same vaccine with no CPV fraction or no vaccine.

A priori, CPV vaccine efficacy was defined on the basis of outcomes related to infectiousness and characteristic clinical signs of gastroenteritis (Nandi et al., 2010; Decaro and Buonavoglia, 2012; Stann et al., 1984):

- Clinical observations: reduction in dog morbidity due to clinical signs such as depression, vomiting, diarrhoea, anorexia, dehydration and fever and decrease in 4 to 16-weeks-old dog mortality (e.g. caused by severe gastroenteritis).
- No diagnosis of leucopenia: the virus is responsible for the destruction of young cells of the immune system and then knocking out the body's best defence mechanism. White blood cells counts (WBCC) was used as an indicator of developing leucopenia.
- Serological tests: resistance to infection with CPV2c subtype. There is a strong correlation between Hemaglutination inhibition (HI) or Serum neutralisation (SN) antibody titres and resistance to infection.
- Virus shedding: declining of virus excretion suggests reduction of infectiousness due to the potential impact of cross-protective vaccines on limiting onward transmission.
- Ability to overcome MDA in the primary course of vaccination. It has been determined that MDA titres represent the first reason for vaccination failure.

Table 1
Basic study and population characteristics.

Author (Publication year)		Wilson et al. (2013)	Siedek et al. (2011)	Larson and Schultz (2008)		Spibey et al. (2008)	Von Reitzenstein et al. (2012)	Glover et al. (2012)
Study design	Randomized or not	Randomized	Randomized	Non reported		Non reported	Randomized	Randomized
	Single/double blind	Non reported	Non reported	Single blind		Non reported	Non reported	Single blind
Modified Live Vaccine/s (MLV)	CPV stain	Multivalent with CPV2b fractionNon reported	Multivalent with CPV2 fraction	V1 Multivalent with CPV2b fraction	V2 Multivalent with CPV2 fraction	1 dose: 2 monovalent vaccines: 1 MLV with CPV2 component + 1 Leptospira vaccine 2 dose: Multivalent MLV with CPV2 fraction	Multivalent with CPV2 fraction	Multivalent with CPV2b fraction
	Level of CPV antigenic titres	$10^{4,7}$–$10^{5,5}$ $CCID_{50}$/ml	10^{7} $CCID_{50}$/ml	10^{7} $CCID_{50}$/ml	10^{7} $CCID_{50}$/ml	10^{7} $CCID_{50}$/ml	10^{7} $CCID_{50}$/ml	$10^{4,7}$–$10^{5,5}$ $CCID_{50}$/ml
	Dose no.	2	2	1	1	2	2	2
	Days within doses	21	21	Non applied	Non applied	21	21	28
Control		Sterile saline	Water for injection	Sterile saline		No vaccination	vaccine without ML- CPV2 fraction	Phosphate buffer saline
Duration(days)		56	56	49		63	70	98
Sample size		7	7	28		12	30	25
(no. i/no. c: n° puppies in vaccinated group/n° puppies in control group)		(5/2)	(5/2)	(9/9/10)		(6/6)	(20/10)	(20/5)
Breed		Beagles	Beagles	Beagles		Beagles	Beagles	–
Age (weeks)		8–9	8–9	12		8–10	6–8(iv)	6
Status	Description	Non reported	Clinically healthy	Non reported		Fit and healthy	Clinically healthy	Healthy
	Clinical check	Non reported	Non reported	Non reported		Veterinary examination	Non reported	Physical examination
Presence/absence MDA titres before intervention	Absence	Absence	Absence			Absence	Absence	Presence
Location	Non reported (i)	Non reported (i)	USA			USA	USA	USA
Funding	Non reported (ii)	Industry	Industry			Non reported (iii)	Non reported (iii)	Industry

(i) Europe pharmacopeia was fulfilled.
(ii) Industry reviewed and approved the study.
(iii) Study authors from pharmaceutical companies.
(iv) Controversial figures were described through the narrative: puppies were7–8 weeks old when the first dose was administrated and 9–10 weeks old for the second dose.

Table 2
Time of parameters measured

Author (Publication year)	Wilson et al. (2013)	Siedek et al. (2011)	Larson and Schultz (2008)	Spibey et al. (2008)	Von Reitzenstein et al. (2012)	Glover et al. (2012)
Clinical observations (times a day)	From the challenge day to the end of the study, daily (once)	From the challenge day, daily (once)	From the challenge day, daily (once)	2 days before challenge until 14 days after challenge (once)	2 days before challenge (once). The challenge day and post-challenge daily (twice)	2 days before challenge until 14 days after challenge (once)
WBC Pre-challenge (baseline: mean of values measured)	Three times	Three times	Non reported	Three times	Three times	Three times
Post-challenge	Five times	Five times		Seven times	Thirteen times	Ten times
Bloody samples	– Once before 1ªdose – Once the day 2ª dose before it – Once the challenge day before it – Once the last day of trial	– Once before 1ªdose – Once the day 2ª dose before it – Once the challenge day before it – Once the last day of trial	weekly	– 4 weeks after vaccination – 7 days post-challenge	– Once before 1ªdose – Once the day 2ª dose before it – Once the challenge day before it – Once the last day of trial	– Three times before 2 dose – Three times between 2 dose and challenge – The challenge day before challenge
Samples of faeces	– The challenge day – Daily post-challenge	– The challenge day – Daily post-challenge	– From day 4 to day 8 post-challenge	– From 2 days before challenge until 14 days after challenge	– The challenge day – Daily post-challenge	– From 2 days before challenge until 10 days post-challenge

(i) Post-challenge: period of 14 days after the challenge day.

To evaluate vaccine efficacy regarding resistance to infection, the study groups needed to be challenged using the virus subtype CPV2c. Efficacy was assessed by monitoring the pups for various clinical signs, laboratory testing of antibody titres and detection of CPV in the faeces.

2.2. Data sources and search

A systematic search in Pub Med/MEDLINE was conducted for published articles on CPV vaccine (between January 1st 1990 and May 7th 2014). The search strategy consisted of combinations of free texts and medical subject heading (MeSH) terms distributed into three groups: "parvovirus", "dogs/puppies" and "vaccination/vaccine". Specifically, we used the following search terms: ("parvovirus"[MeSH Terms] OR "parvovirus"[All Fields]) AND ("dogs"[MeSH Terms] OR "dogs"[All Fields] OR "dog"[All Fields] OR "canine"[All Fields] OR "puppy"[All Fields] OR "puppies"[All Fields]) AND ("vaccines"[MeSH Terms] OR "vaccines"[All Fields] OR "vaccine"[All Fields] OR "vaccination"[MeSH Terms] OR "vaccination"[All Fields]) AND ("1990/01/01"[PDAT]: "2014/05/07"[PDAT]).

This primary search was supplemented by complementary hand-searching of reference lists of eligible studies and review articles and searches on Google/Google scholar (last search in August 2014).

2.3. Study selection process and data extraction

Titles and abstracts from the literature search were screened and relevant full-text articles were also tested on inclusion criteria.

Published controlled clinical trials evaluating modified-live (ML) CPV2/CPV2b vaccine efficacy against CPV2c were exclusively selected. In CPV infection, attenuated CPV vaccines offer a longer duration of immunity than killed vaccines so only live vaccines were worth including. Additionally, as commercial ML CPV vaccines have only CPV2 or CPV2b parvovirus fractions available, cross-protection against CPV2c was determined.

CPV vaccination of dogs is usually performed by administrating multivalent vaccines. However, monovalent vaccines are strongly recommended for the initial dose. Therefore, the collected papers referred to both polyvalent and/or monovalent vaccines. Finally, only controlled clinical trials where an intranasal and/or oral challenge dose was administered were eligible. Thus, the route of administration reproduced the common ways of animals of contracting infection. Each dose should contain the CPV2c variant.

For each paper included, a reviewer (BHB) extracted the characteristics of eligible studies (e.g. study design, author and publication year; features of study population: breed, age and health status), parameters of intervention, outcome measures and results of interest (see Tables 1–6).

2.4. Analysis

A descriptive analysis of the characteristics and results of the selected studies using evidence tables was performed. The heterogeneity among studies did not make it possible to carry out a quantitative synthesis of the results using a meta-analysis.

3. Results

3.1. Literature search

The Pub Med/MEDLINE search resulted in a total of 245 references, 59 of which were deemed potentially relevant (Fig. 1). After full-text level screening, 4 studies met the inclusion criteria and from a complementary hand-searching, 2 more studies were added. Finally, a total of 6 trials were definitely included (Wilson et al., 2013; Siedek et al., 2011; Larson and Schultz, 2008; Spibey et al., 2008; Von Reitzenstein et al., 2012; Glover et al., 2012).

3.2. Trial and population characteristics

The main study characteristics are described in Table 1.

Table 3
No. of puppies with clinical signs and leucopenia (White blood cells counts -WBCC) in control (c) and vaccinated (v) group.

Outcomes (Outcomes defined or not and n° affected animals/ group total)	Wilson et al. (2013)	Siedek et al. (2011)	Larson and Schultz (2008)	Spibey et al. (2008)	Von Reitzenstein et al. (2012) (i)	Glover et al. (2012)
Fever	Non defined	>39.4°C	Non defined	Non reported	>103.4F or 1 degree more referred to temperature baseline C:6/10 V:0/19	>103.4F or 1 degree more referred to temperature baseline C:3/5 V:1/20
Diarrhoea	Non defined C:2/2 V:0/5	Non defined C:2/2 V:0/5	Non defined C:5/10 no described signs V1:0/9	Non defined C:6/6 V:0/6	Non defined C:10/10 V:4/19	Non defined C:4/5 V:5/20
Bloody diarrhoea	Non reported	Non reported	V2:0/9	Non defined C:3/6 V:0/6	Non defined C:8/10 V:0/19	Non defined C:4/5 V:5/20
Vomit	Non defined C:2/2 V:0/5	Non defined C:2/2 V:0/5		Non reported	Non defined C:9/10 V:2/19	Non reported
Depression	Non defined C:2/2 V:0/5	Non reported		Non reported	Non reported	Non reported
Lethargy	Non reported	Non reported		Non reported	Non defined C:2/10 V:0/19	Non reported
Dehydration	Non defined C:1/2 V:0/5	Non reported		Non reported	Non defined C:2/10 V:0/19	Non reported
Anorexia	Non defined C:2/2 V:0/5	Non reported		Non defined C:6/6 V:0/6	Non defined C:7/10 V:0/19	Non reported
Leucopenia (n° puppies)	Defined C:2/2 V:0/5	Defined C:1/2 V:0/5	Non reported	Defined C:1/6 V:0/6	Defined C:10/10 V:1/19	Defined C:5/5 V:3/20
Death	Euthanized C:2/2 V:0/5	Euthanized C:2/2 V:0/5	Euthanized C:5/10 V1:0/9 V2:0/9	Euthanized C:3/6 V:0/6	Euthanized C:10/10 V:0/19	Died C:1/5 V:0/20

C: control V: vaccinated.
(i) A duplicated sample number in the challenge day so from 20 vaccinated dogs only 19 were observed post-challenge.
Non reported- parameter non measured.
Non defined-parameter measured without precise definition (subjectively).
Leucopenia definition: Reduction ≥50% of counts compared to pre-challenge baseline

Table 4
Serological tests.

Author (Publication year)	Wilson et al. (2013)	Siedek et al. (2011)	Larson and Schultz (2008)	Spibey et al. (2008)	Von Reitzenstein et al. (2012)	Glover et al. (2012)
CPV fraction of the vaccine	CPV2b strain SAH	CPV2 strain NL-35-D	V1: CPV2 strain 154 V2: CPV2b	CPV2 strain 154	CPV2 strain NL-35-D	CPV2b strain SAH
HAI: CPV antigen as a reference	CPV2	CPV2	CPV2	CPV2c CPV2	–	–
HAI: expression of results	Reciprocal of the highest dilution to show HAI	Reciprocal of the highest dilution to show HAI	Reciprocal of the highest dilution to show HAI	Non reported	–	–
SN: CPV antigen as a reference	CPV2c	CPV2c CPV2	–	CPV2c CPV2	CPV2	CPV2b
SN: Expression of results	Spearman-Kaeber formula	Spearman-Kaeber formula	–	Non reported	Spearman-Kaeber formula	Reed and Muench method

CPV vaccines were examined in 6 controlled clinical trials which enroled a total of 109 puppies (sample size ranged from 7 to 30 animals in each study). In these studies, the mean age of puppies was 8.5 weeks and the mean duration of trials was 65.3 days (range: 49–98).

Methods of randomization, allocation concealment and blinding were usually poorly reported.

Although two vaccinations were administrated in almost all clinical trials (except in one study (Larson and Schultz, 2008)), one dose was reported satisfactory to elicit immunity. Five studies selected MDA free beagle pups. Animal status at the beginning

Table 5
Serological assay in control (c) and vaccinated (v) group. Geometric mean titre -GMT (minimum-maximum.)

Author (Publication year)		Wilson et al. (2013)	Siedek et al. (2011)	Larson and Schultz (2008)	Spibey et al. (2008)	Von Reitzenstein et al. (2012)	Glover et al. (2012)
HAI test (against CPV2 except (c))	Baseline	<10	<10	<20	–	–	–
	Day of 2 dose before admon	C:<10 V:768 (640-1280)	C:20-40 V:1664 (640-5120)	–	–	–	–
	Challenge day, before admon	C:<10 V:704 (320-1280)	C:20-40 V:704 (320-1280)	C:<20 V1:3982 (2560-10240) V2:7111 (2560-10240)	C:<10 (c) C:<10 V:3200 (1600-6400) (c)V:2133 (1600-3200)	–	–
	Post-challenge	V:576 (320-640)	V:768 (640-1280)	C:16384 (10240-20480) V1:2702 (1280-5120) V2:6826 (2560-20480)	C:2560 (c)C:10240 V:2560 (c)V:2560	–	–
SN test (against CPV2c except (a) (b))	Baseline	<10	–	–	–	1 (a)C:1 (a)V:3990.8 (2048-5793)	$4 \leq \times \geq 64$
	Day of 2 dose before admon	C:<10 V:1280 (640-2560)	–	–	–		
	Challenge day, before admon	C:<10 V:1152 (640-1280)	C:<10 V:448 (320-640) (a)V:6144 (5120-10240)	–	C:<3 (a)C:<3 V:21597 (10624-36781) (a)V:294973 (147123-370328)	(a)C:1 (a)V:2243.7 (431-4871)	**(b)** V:>15000
	Post-challenge	V:1024 (640-1280)	–	–	C:35739 (13141-55109) V:22833 (7298-46341) (a)C:24770 (11585-46341) (a)V:174761 (65536-339959)	(a)C:1 (a)V:3723.5 (1448-5793)	

C: control group.
V: vaccinated group.
(a) SN against CPV2.
(b) SN against CPV2b.
(c) HAI against CPV2c

Table 6
Virus shedding.

Author (Publication year)	Wilson et al. (2013)	Siedek et al. (2011)	Larson and Schultz (2008)	Spibey et al. (2008)	Von Reitzenstein et al. (2012)	Glover et al. (2012)
Virus shedding (no. of affected puppies/ group total)	C:2/2 V:0/5	C:2/2 V:(i)	C:10/10 V1:0/9 V2:2/9	C:6/6 V:0/6	C:10/10 V:0/19	C:5/5 V:1/20
Duration (days)	Non reported	C:2–3	C:2–5 V2:1–2	C:5	Non reported	C:4–8 V:1
Measure virus excretion	Increase of baseline (<1.5 log $_{10}$ $TCID_{50}$/ml)	Marked increase of reciprocal of the highest dilution to show HAI	presence	presence	$\geq 10^{3.3}$ $TCID_{50}$/gr	>1.8 $\log_{10}$ FAID (*)/ml

C: control group.
V: vaccinated group.
(*) FAID: 50% Fluorescent Antibody Infectious Dose.
(i) The result reported in the vaccinated group is ≤160.

was: not mentioned (Wilson et al., 2013; Larson and Schultz, 2008), only reported (Siedek et al., 2011; Von Reitzenstein et al., 2012) or clinically checked (Spibey et al., 2008; Glover et al., 2012) but in no trial was the status defined.

3.3. Clinical observations

The measures and results of clinical manifestations are presented in Tables 2 and 3.

In 4 of the clinical trials, the vaccinated dogs appeared to be largely protected against key signs such as diarrhoea/bloody diarrhoea and vomiting, and severe disease leading to euthanasia/death which occurred in 50–100% controls (details Table 3).

However, results were subjectively reported and most of clinical parameters were not defined.

3.4. White blood cells counts (WBCC)

The total number of leucocytes (10^9/l) was compared between the pre-challenge and post-challenge stage in 5 studies (see Table 3). Leucopenia, in the post-challenge stage, was established as a decrease of 50% or more in the number of leucocytes from the

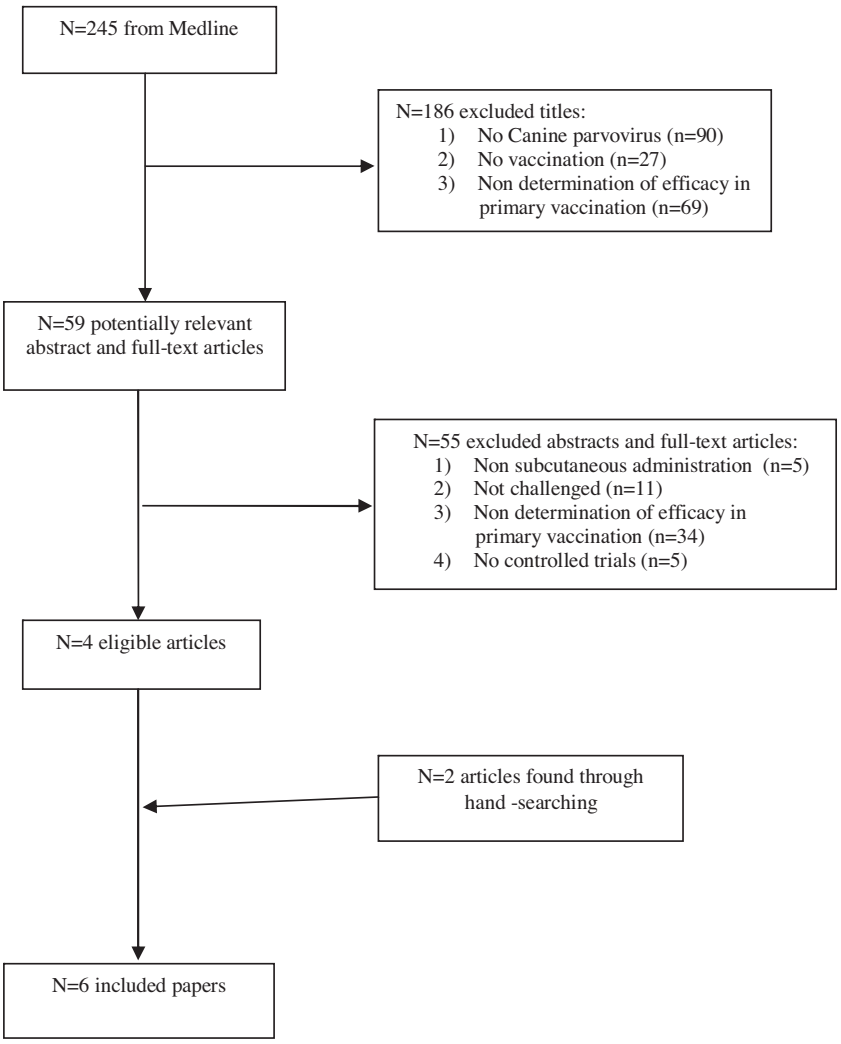

Fig. 1. Flowchart for studies selection.

pre-challenge baseline. The baseline was the mean of three measures recorded before the challenge administration (pre-challenge). Leucopenia was markedly manifested the control group in two studies of larger sample size (Von Reitzenstein et al., 2012; Glover et al., 2012).

3.5. Serological assays

The characteristics of serological tests are described in the Table 4. Although results were obtained and expressed by different methods, both HAI assays and SN tests showed an increase of antibody titres in vaccinated dogs after vaccination. Studies reported that results of HAI were independent of the antigen employed. On the other hand, a serum neutralisation (SN) assay carried out with various antigens revealed divergent results, with CPV2 antigen showing higher values. In the Spibey clinical trial, the Geometric Mean Titre (GMT) of 2560 was obtained against CPV2 and CPV2c by the HAI test. However, the SN test revealed a higher GMT range against CPV2 (65536-339959) than against CPV2c (7298-46341).

In addition, no differences were appreciated (see Table 5):

- Using a vaccine with CPV2 parvovirus fraction versus CPV2b parvovirus component.
- Between vaccines with high CPV2 antigenic titres or lower parvovirus titres.

MDA interference was not considered or evaluated in 5 trials. In the only trial where population with MDA titres was selected for the study (Glover et al., 2012), serological information was poorly described. Furthermore, the last value of antibody response was calculated on the challenge day (before administration) so the efficacy of the vaccine in dogs with MDA titres was not proved.

3.6. Virus shedding

All the studies reported a CPV excretion rise after challenge (see Table 6). Criteria of infection involved different ranges from the sole presence of virus until a significant increase of virus excretion.

No identification of the CPV variant was made in any study.

Complementary data were the prolonged shedding of CPV. Virus in faeces was observed more than 3 days only in the control groups of three papers (Larson and Schultz, 2008; Spibey et al., 2008; Glover et al., 2012).

4. Discussion

This analysis reviewed characteristics and reporting of methods and results of 6 published controlled clinical trials that examined the effects of licensed canine CPV2 and CPV2b vaccines against the variant CPV2c in puppies. In general, the results of controlled trials reported benefits in terms of reductions of clinical signs, serological tests and virus shedding in most cases.

Regarding the above, no vaccinated animals displayed clinical signs in the majority of studies. However, in two trials (Von Reitzenstein et al., 2012; Glover et al., 2012), with much larger samples of vaccinated population, leucopenia and more than one clinical sign were reported in the vaccinated group.

With regard to serological tests, the following advantages and disadvantages of each test should be considered:

- Determination of HAI titres is currently the most widely used procedure for evaluating the antibody status of dogs but it is no longer recommended against the antigenic variants (Cavalli et al., 2008).
- The SN test is a more sensitive assay but it provides diverse results depending on the antigen used as reference.

Regarding virus excretion, hemaglutination and virus isolation assays used for detecting CPV shedding are less sensitive than real-time PCR. These techniques provide negative results even at moderate titres of virus (Elia et al., 2005). This is due to sequestration of viral particles by antibodies, especially during late infection (Desario et al., 2005; Decaro et al., 2010, 2013).

Despite MDA being considered the main reason of vaccination failure, five trials selected free MDA puppies to evaluate initial vaccination efficacy. As was expected from free MDA puppies, they developed high antibodies titres with only one dose. Therefore, the following question arises to challenge external validity: if it is suggested in the vaccination schedule of less 4-month-old puppies that have MDA titres (they need two doses to complete vaccination), why were these trials carried out in a free MDA population?

In this review, the ability to overcome MDA in the primary vaccination course could not be evaluated. In this respect, the European Medicines Agency (EMA) requires: "if the indication or specific claims for the vaccine are related to efficacy in the presence of maternal antibodies against the vaccine agent(s), the trial protocol shall include animals with titres of these antibodies normally occurring in the field". (EMA, 1999).

In terms of reporting of methods, randomization, allocation concealment and blinding (e.g. only 2 studies were reported as blinded studies) this was usually poorly reported.

Our results highlight the importance of improving study designs to include adequate size sample and follow-up so as to accomplish reliable information and clinical impact. For example, in this review the total number of control puppies represented less than 50% of vaccinated animals (only 35 dogs). In the majority of included trials, the challenge was administrated close to the time estimated to acquired immunity after vaccination. Short-term studies did not permit knowing if the acquired antibody level would decrease in the time period before infection.

On comparing human and veterinary vaccine evaluation methods, the following assertions were extracted (Knight-Jones et al., 2013):

- "Despite the large number of veterinary vaccines in use, the literature on their evaluation is small. This is exacerbated by a failure to publish findings by trial lists and/or vaccine manufacturers leading to potential bias (e.g. selective reporting and publication bias).
- Field studies play a very limited role in veterinary vaccine authorization and are typically used to evaluate safety rather than efficacy.
- The evaluation of veterinary vaccines relies heavily on challenge studies. Sometimes, the controlled conditions of a challenge study do not reflect the suboptimal application of vaccines in the field.
- Owing to concerns about animal welfare, cost and laboratory pathogen escape, the number of animals used for challenge evaluation is generally small and the length of follow-up limited. Consequently, results can be statistically uncertain."
- In this regard, the findings of our analysis suggest an urgent need to improve the reporting quality of trials in this field. Poor reporting can introduce biased estimates of an intervention's effects and thus impact on animal care and veterinary decision making. Medical journals regularly publish new evidence as regards some aspects of poor reporting of methods and results of clinical trials (Hopewell et al., 2010). Improving the completeness of reporting research will not only maximise transparency and replicability, but also may help to reduce a waste in science (Glasziou et al., 2014).

This systematic review has several limitations that should be considered. One of these was the systematic search of published articles mainly from a sole database: PubMed/MEDLINE. Although this is the most important bibliometric source of peer-reviewed articles, the existence of non-indexed studies in the database (for example, non-English speaking journals) may have involved the loss of some locally published studies despite complementary searches made. In addition, there may be other studies which may not have been identified (unpublished trials).

5. Conclusion

Based on the available evidence and considering the small sample sizes of included trials, short follow-up and methodological limitations shown, it is not possible to reach any final conclusion regarding the cross-protection of licensed CPV2 and CPV2b vaccines against the subtype 2c in puppies. Further and specifically designed trials are needed (including the evaluation of vaccine efficacy in the presence of MDA) to elucidate whether CPV2c cross-protection is acquired from licensed CPV vaccines.

Conflict of interest

We declare that we have no conflicts of interest.

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None.

Appendix.

Immunological terms

Cross-protection: broadly include (A) protection of vaccinated animals by reducing viral shedding and /or morbidity and mortality but not necessarily preventing infection and (B) offering this protection against different subtypes and strains of the same subtype.

Challenge: exposure of the immune system to pathogenic organisms or antigens. Thus, the pre-challenge is the period before administrating challenge.

Vaccine efficacy: percentage reduction in disease incidence in a vaccinated group compared to an unvaccinated group under optimal conditions.

Controlled trial: experimental study in which animals are allocated to a vaccinated or control group using methods that are random or not.

References

Appel, M.J.G., Cooper, B.J., Greisen, H., Carmichael, L.E., 1978. Canine viral enteritis. J. Am. Vet. Med. Assoc. 173, 1516–1518.

Appel, M.J.G., Scott, W.F., Carmichael, L.E., 1979. Isolation and immunization studies of canine parvo-like virus from dogs with haemorrhagic enteritis. Vet. Rec. 105, 156–159.

Buonavoglia, C., Nardo, P., de Reitano, M., Orfei, Z., 1985. Persistence of maternal antibody to canine parvovirus in puppies, and interference with vaccination. Clin. Vet. 108, 19–23.

Calderon, M.G., Romanutti, C., D'Antuono, A., Keller, L., Mattion, N., Torre, J.L., 2011. Evolution of canine parvovirus in Argentina between years 2003 and 2010: CPV 2c has become the predominant variant affecting the domestic dog population. Virus Res. 157, 106–110.

Carmichael, L.E., Joubert, J.C., Pollock, R.V.H., 1983. A modified live canine parvovirus vaccine. II. Immune response. Cornell Vet. 73, 13–29.

Cavalli, A., Martella, V., Desario, C., Camero, M., Bellacicco, A.L., De Palo, P., Decaro, N., Elia, G., Buonavoglia, C., 2008. Evaluation of the antigenic relationships among canine parvovirus type 2 variants. Clin. Vaccine Immunol. 15, 534–539.

Decaro, N., Buonavoglia, C., 2012. Canine parvovirus–a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet. Microbiol. 155, 1–12.

Decaro, N., Campolo, M., Desario, C., Elia, G., Martella, V., Lorusso, E., Buonavoglia, C., 2005. Maternally-derived antibodies in pups and protection from canine parvovirus infection. Biologicals 33, 259–265.

Decaro, N., Martella, V., Desario, C., Bellacicco, A.L., Camero, M., Manna, L., d'Aloja, D., Buonavoglia, C., 2006. First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. J Vet. Med. B Infect. Dis. Vet. Public Health 53, 468–472.

Decaro, N., Desario, C., Addie, D.D., Martella, V., Vieira, M.J., Elia, G., Zicola, A., Davis, C., Thompson, G., Thiry, E., Truyen, U., Buonavoglia, C., 2007. Molecular epidemiology of canine parvovirus, Europe. Emerg. Infect. Dis. 13, 1222–1224.

Decaro, N., Desario, C., Elia, G., Martella, V., Mari, V., Lavazza, A., Nardi, M., Buonavoglia, C., 2008. Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. New Microbiol. 31, 125–130.

Decaro, N., Desario, C., Beall, M.J., Cavalli, A., Campolo, M., Dimarco, A.A., Amorisco, F., Colaianni, M.L., Buonavoglia, C., 2010. Detection of canine parvovirus type 2c by a commercially available in-house rapid test. Vet. J. 184, 373–375.

Decaro, N., Desario, C., Billi, M., Mari, V., Elia, G., Cavalli, A., Martella, V., Buonavoglia, C., 2011. Western European epidemiological survey for parvovirus and coronavirus infections in dogs. Vet. J. 187, 195–199.

Decaro, N., Desario, C., Billi, M., Lorusso, E., Colaianni, M.L., Colao, V., Elia, G., Ventrella, G., Kusi, I., Bo, I., Buonavoglia, C., 2013. Evaluation of an in-clinic assay for the diagnosis of canine parvovirus. Vet. J. 198, 504–507.

Desario, C., Decaro, N., Campolo, M., Cavalli, A., Cirone, F., Elia, G., Martella, V., Lorusso, E., Camero, M., Buonavoglia, C., 2005. Canine parvovirus infection: which diagnostic test for virus. J. Virol. Methods 126, 179–185.

Elia, G., Cavalli, A., Cirone, F., Lorusso, E., Camero, M., Buonavoglia, D., Tempesta, M., 2005. Antibody levels and protection to canine parvovirus type 2. J. Vet. Med. B. Infect. Dis. Vet. Public Health 52, 320–322.

EMA. Note for guidance: field trials with veterinary vaccines. London: EMEA; 1999. Available from:http://www.ema.europa.eu/docs/en_GB/document_librar/Scientific_guideline/2009/10/WC500004598.pdf.

Glasziou, P., Altman, D.G., Bossuyt, P., Boutron, I., Clarke, M., Julious, S., Michie, S., Moher, D., Wager, E., 2014. Reducing waste from incomplete or unusable reports of biomedical research. Lancet 383, 267–276.

Glover, S., Anderson, C., Piontkowski, M., 2012. Canine Parvovirus (CPV) Type 2b Vaccine Protects puppies with maternal antibodies to CPV when challenged with virulent CPV-2c Virus. Int. J. Appl. Res. Vet. Med. 10, 217–224.

Hayes, M.A., Russel, R.G., Mueller, R.W., Lewis, R.J., 1979. Myocarditis in young dogs associated with a parvovirus-like agent. Can. Vet. J. 20, 126–132.

Hopewell, S., Dutton, S., Yu, L.M., Chan, A.W., Altman, D.G., 2010. The quality of reports of randomised trials in 2000 and 2006: comparative study of articles indexed in PubMed. B. M. J. 340, c723.

Houston, D.M., Ribble, C.S., Head, L.L., 1996. Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982–1991). J. Am. Vet. Med. Assoc. 208, 542–546.

Jacob, R.M., Weiser, M.G., Hall, R.L., Kowalski, J.J., 1980. Clinic pathogenic features of canine parvoviral enteritis. J. Am. Vet. Med. Assoc. 16, 809–813.

Knight-Jones, T.J., Edmond, K., Gubbins, S., Paton, D.J., 2013. Veterinary and human vaccine evaluation methods. Proc. Biol. Sci. 281, 2839.

Larson, L.J., Schultz, R.D., 2008. Do two current canine parvovirus type 2 and 2b vaccines provide protection against the new type 2c variant? Vet. Ther. 9, 94–101.

Martella, V., Cavalli, A., Decaro, N., Elia, G., Desario, C., Campolo, M., Bozzo, G., Tarsitano, E., Buonavoglia, C., 2005. Immunogenicity of an intranasally administered modified live canine parvovirus type 2b vaccine in pups with maternally derived antibodies. Clin. Diagn. Lab. Immunol. 12, 1243–1245.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., 2009. PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann. Int. Med. 151, 264–269.

Nandi, S., Chidri, S., Kumar, M., Chauhan, R.S., 2010. Occurrence of canine parvovirus type 2c in the dogs with haemorrhagic enteritis in India. Res. Vet. Sci. 88, 169–171.

Parrish, C.R., Aquadro, C.F., Strassheim, M.L., Evermann, J.F., Sgro, J.Y., Mohammed, H. O., 1991. Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. J. Virol. 65, 6544–6552.

Perez, R., Bianchi, P., Calleros, L., Francia, L., Hernandez, M., Maya, L., Panzera, Y., Sosa, K., Zoller, S., 2012. Recent spreading of a divergent canine parvovirus type 2a (CPV-2a) strain in a CPV-2c homogenous population. Vet. Microbiol. 155, 214–219.

Pollock, R.V., Carmichael, L.E., 1982. Maternally derived immunity to canine parvovirus infection: transfer, decline, and interference with vaccination. J. Am. Vet. Med. Assoc. 180, 37–42.

Pollock, R.V., Coyne, M.J., 1993. Canine parvovirus. Vet. Clin. North Am. Small Anim. Pract. 23, 555–568.

Siedek, E.M., Schmidt, H., Sture, G.H., Raue, R., 2011. Vaccination with canine parvovirus type 2 (CPV-2) protects against challenge with virulent CPV-2b and CPV-2c. Berl. Munch. Tierarztl. Wochenschr. 124, 58–64.

Spibey, N., Greenwood, N.M., Sutton, D., Chalmers, W.S., Tarpey, I., 2008. Canine parvovirus type 2 vaccine protects against virulent challenge with type 2c virus. Vet. Microbiol. 128, 48–55.

Stann, S.E., DiGiacomo, R.F., Giddens, W.E., Evermann, J.F., 1984. Clinical and pathological features of parvoviral diarrhoea in dogs. J. Am. Vet. Med. Assoc. 185, 651.

Sutton, D., Vinberg, C., Gustafsson, A., Pearce, J., Greenwood, N., 2013. Canine parvovirus type 2c identified from an outbreak of severe gastroenteritis in a litter in Sweden. Acta Vet Scand. 55, 64.

Vieira, M.J., Silva, E., Oliveira, J., Vieira, A.L., Decaro, N., Desario, C., Muller, A., Carvalheira, J., Buonavoglia, C., Thompson, G., 2008. Canine parvovirus 2c infection in central Portugal. J. Vet. Diagn. Invest. 20, 488–491.

Von Reitzenstein, M., Ludlow, D., Marcos, S., Kopta, L., Sandbulte, J., Mischnick, C., Slade, D., Hawkins, K.F., King, V., Inskeep, G., Sture, G., 2012. Cross protection of vanguard 5L4-CV vaccine against virulent canine parvovirus-2c circulating in the USA. Int. J. Appl. Res. Vet. Med. 10, 187–197.

Wells, D.L., Hepper, P.G., 1999. Prevalence of disease in dogs purchased from an animal rescue shelter. Vet. Rec. 144, 35–38.

Werneling, D.P., Miller, J., Rudy, A.C., 2002. Systemic intranasal drug delivery: concepts and application of intranasal delivery. Drug Deliv. 2, 1–8.

Wilson, S., Stirling, C., Borowski, S., Thomas, A., King, V., Salt, J., 2013. Vaccination of dogs with Duramune DAPPi+LC protects against pathogenic canine parvovirus type 2c challenge. Vet. Rec. 172, 662.

Woods, C.B., Pollock, R.V.H., Carmichael, L.E., 1980. Canine parvoviral enteritis. J. Am. Anim. Hosp. Assoc. 16, 171–179.