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Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication?

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ABSTRACT

Despite extensive vaccination, canine parvovirus (CPV) remains a leading infectious cause of canine mortality, especially among juveniles. This review provides an update on CPV vaccine types and vaccination protocols. The design of CPV prevention strategies and vaccination programs with a goal of herd immunity has been hampered by deficiencies of studies that model companion animal viral infections and inform an understanding of the basic reproduction number. However, the most important issue in eradication of CPV disease is represented by immunisation failures including: i) the presence of interfering titres of maternally-derived antibodies; ii) the presence of non-responders; and iii) possible reversion to virulence. In contrast, the role of the CPV variants in immunisation failures is widely debated. Taking into account the reduced circulation of canine distemper virus and canine adenovirus type 1 in countries where extensive vaccination is carried out, more effort should be made to aim for CPV eradication, including antibody testing to determine the optimal time for vaccinations of pups and adults and homogeneous vaccine coverage of dog population.

1. Introduction

Canine parvovirus (CPV) has been known since the late 1970s and despite intensive vaccination, at least in developed countries, this virus still represents one of the main causes of acute gastroenteritis and death in juvenile pups (Decaro and Buonavoglia, 2012; Voorhees et al., 2020). In two independent studies aiming to assess the role of different pathogens in the occurrence of canine acute diarrhoea, only CPV and canine coronavirus (CCoV) were found to be significantly associated with enteric disease, although their prevalence in juvenile dogs was slightly different (Duijvestijn et al., 2016; Dowgier et al., 2017).

Prevention of CPV infection is based on the use of modified live virus (MLV) vaccines, which are able to stimulate both antibody- and cell-mediated immune responses, inducing a strong, long-lasting protection against subsequent challenge with virulent viruses (Day et al., 2016; Ford et al., 2017). However, not all vaccine administrations result in the development of active immunity against CPV, leading to immunisation failures that allow the vaccinated dogs to be exposed to CPV infection and disease (Decaro and Buonavoglia, 2012). Immunisation failures represent one of the main reasons for CPV continuous circulation throughout the world and may be due to different causes, including persistence of maternal immunity at the time of

vaccination, vaccination of non-responders and circulation of different antigenic variants of the virus. The scope of the present article is to provide an up-to-date review of the literature concerning CPV vaccines, vaccination protocols and causes of immunisation failures.

2. Canine parvovirus: one or more viruses?

According to the most recent classification, CPV is included in the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Protoparvovirus*, and it forms a unique species, *Carnivore protoparvovirus 1*, along with feline parvovirus and other parvoviruses of carnivores (Barrs, 2019).

CPV emerged as pathogen of dogs in the late 1970s, when it was responsible for a severe global panzootic in dogs of all ages, which at that time were naïve to the infection (Decaro and Buonavoglia, 2012). The original strain was named canine parvovirus type 2 (CPV-2) to distinguish it from the genetically and antigenically unrelated canine parvovirus type 1 (CPV-1 or canine minute virus), which has been reclassified as *Carnivore bocaparvovirus 1* (genus *Bocaparvovirus*), and is associated with neonatal mortality (Decaro et al., 2012). A few years after its emergence, CPV-2 gave origin to a first antigenic variant, named CPV-2a, which differs from the original type in 5–6 amino acid (aa) positions of the major capsid (VP2) protein. A second antigenic

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variant, CPV-2b, displayed a further mutation in the VP2 protein (mutation asparagine to aspartic acid at aa residue 426) (Parrish et al., 1985, 1991). In 2000, a third antigenic variant, CPV-2c, was detected, which displayed aa change asparagine/aspartic acid to glutamic acid at residue 426 of the VP2 protein (Buonavoglia et al., 2001). The three variants are variously distributed worldwide, while the old type CPV-2 is no longer circulating in the field and is present only in some vaccine formulations (Decaro and Buonavoglia, 2017). The presence of a single aa change among CPV-2a, -2b and -2c confers different antigenic properties, as evidenced by the different reactivity to specific monoclonal antibodies (Nakamura et al., 2004). However, the variants lack clear monophyletic segregation due to the accumulation of other point mutations in different parts of their genome and encoded proteins. Therefore, some parvirologists have suggested considering the three antigenic variants as belonging to a single “CPV-2a clade”, which by phylogenetic analysis forms a distinct branch from the old strain CPV-2 (Voorhees et al., 2019). Presently, there is no consensus in the scientific community, so that some parvirologists still refer to the three variants, whereas others recognise the existence of the single CPV-2a clade that encompasses all CPV variants and strains descendent from the initial CPV-2a global sweep. For the purposes of this review, the three-variant nomenclature will be kept, since this is most used in the current literature.

3. Canine parvovirus vaccination

Vaccination is the most effective measure to control the spread of the infection in dogs and to prevent the development of clinical CPV infection. Therefore, CPV vaccines are considered core vaccines by professional associations with international outreach, such as the Vaccination Guidelines Group of World Small Animal Veterinary Association (WSAVA)¹ and the American Animal Hospital Association (AAHA)². A core vaccine is a vaccine that all dogs should receive, regardless of circumstances or geographical location, since it protects animals from severe, life-threatening diseases that have global distribution (Day et al., 2016). However, despite the intensive vaccination programs that are adopted worldwide, CPV infection represents one of the most frequent infectious disease and cause of death in juvenile dogs even in developed countries (Decaro and Buonavoglia, 2012). For example, based on data from a recent nationwide survey of veterinary hospitals in Australia, the estimated incidence of CPV infection among owned and shelter-housed dogs is 4.12 cases per 1000 dogs (Kelman et al., 2019b).

3.1. CPV vaccine types

Very few inactivated CPV vaccines are available on the market, since these formulations have low immunogenicity, and therefore require repeated administration during the primary course and annual boosters. Their use is suggested only in exotic animals and pregnant bitches, for which most MLV vaccines are not yet registered (Day et al., 2016). In a recent study (Altman et al., 2017), inactivated vaccines were more frequently associated with immunisation failures than MLV vaccines in pups of less than twelve weeks of age, likely as a consequence of a better ability of replicating MLV to override residual maternally-derived antibodies (MDA).

In contrast, MLV vaccines are widely used, since they induce a strong, long-lasting (usually life-long) immunity by replicating within the host, without producing significant tissue damage or clinical signs.

¹ WSAVA Vaccination Guidelines. <https://www.wsava.org/Global-Guidelines/Vaccination-Guidelines> (Accessed 6 May 2020)

² 2017 AAHA Canine Vaccination Guidelines. <https://www.aaha.org/aaha-guidelines/vaccination-canine-configuration/vaccination-canine/> (Accessed 6 May 2020)

To date, in most countries there are only two CPV types that are included in the CPV MLV vaccine formulations, the original CPV-2 strain and its variant CPV-2b. Both MLV CPV vaccine strains are able to cause viraemia and replicate in the intestinal mucosa, albeit at lower titres than field strains, being shed in the faeces of vaccinated dogs for at least 3–4 weeks post-vaccination (Decaro et al., 2014; Freisl et al., 2017). Their ability to replicate in the intestinal mucosa has been demonstrated even in dogs with protective circulating antibody titres, which shed low DNA titres of vaccine virus in their faeces (Freisl et al., 2017).

CPV MLV vaccines are not recommended by the WSAVA vaccination guidelines group for use in wildlife, in pups less than 4–6 weeks of age, or during pregnancy, due to possible vaccine-associated adverse effects (Day et al., 2016). However, some recent MLV vaccines have been demonstrated to be innocuous for the both foetuses and for 4-week-old pups, and are registered for administration during pregnancy and in young pups (N. Decaro, personal observation).

CPV MLV vaccines are characterised by early onset of immunity (OOI) and long duration of immunity (DOI). Some studies have demonstrated that dogs administered MLV vaccines were protected against challenge with virulent CPV as early as 3 days post-vaccination (Schultz and Larson, 1996; N. Decaro, unpublished data). DOI after natural CPV infection is considered life-long, but there is evidence of a long-term DOI even after CPV vaccination (Day et al., 2016). Dogs vaccinated against CPV, canine distemper virus (CDV) and canine adenovirus (CAV) were protected from disease and/or infection after challenge 9 years from vaccination (Schultz et al., 2010). Most CPV licensed vaccines are registered for 3-year-interval boosters, but even those claiming a required revaccination interval of 1 or 2 years can be administered at 3-year intervals (Day et al., 2016; Ford et al., 2017).

All CPV vaccines available on the market are registered for administration through the parenteral route. However, some experimental and/or commercial vaccines have been proposed for intranasal or oral administration to better overcome MDA interference (Buonavoglia et al., 1995; Martella et al., 2005; Cavalli et al., 2020).

3.2. CPV vaccination protocols

Vaccination is a “customised” action, which should take into account a series of individual factors, including age, breed, lifestyle of the dog, disease prevalence in a particular geographic region, etc. Therefore, no standard vaccination policy will cover all possible situations. Nevertheless, there are some general protocols that are recommended for use of canine core vaccines that include MLV CPV, CDV and CAV. In client-owned pups, the minimum age to begin the CPV primary vaccination protocol is 6–8 weeks, although some vaccines are licensed for use in 4-week-old pups (Day et al., 2016; Ford et al., 2017). After the vaccine is given, a 2–4-week interval revaccination protocol is suggested until the age of 16 weeks or even older. In fact, there is some evidence that some pups can still not be immunised at 16 weeks, and that a last vaccination at 20 weeks may be helpful in situations with high incidence of CPV. In dogs older than 16 weeks, which should no longer have interfering MDA titres, a single CPV MLV vaccine dose is acceptable (Day et al., 2016; Ford et al., 2017), although administration of two doses 2–4 weeks apart is also considered (Ford et al., 2017). Dogs should receive a booster within 1 year of the primary vaccination course given as a pup (Ford et al., 2017) or at any time point between 26 and 52 weeks of age (Day et al., 2016), with subsequent revaccinations being given at intervals of 3 years or longer. This vaccination protocol should be followed even if some MLV vaccines against CPV are still licensed for 1-year or 2-year boosters and/or claim to induce protection with a primary vaccination series completed at 10- or 12-weeks of age. However, in some countries practitioners may be legally required to strictly comply with the vaccine registration data and administer 1-year or 2-year boosters.

More intensive vaccination programs are recommended in the shelter environment, where the virus burden could be high, dogs of

unknown vaccination and hygiene status are housed, and the population turnover is high, so that the risk of exposure to CPV is very high (Day et al., 2016; Ford et al., 2017).

An alternative to revaccination is to perform antibody testing of dogs that completed the primary vaccination series using external laboratory or in-clinic tests. The gold standard for detection of CPV antibodies is haemagglutination inhibition (HI), which can only be performed in highly specialised laboratories, requiring fresh swine erythrocytes and trained personnel. The CPV HI antibody titre is expressed as the reciprocal of highest serum dilution still inhibiting the haemagglutination activity of a known amount of virus (Decaro et al., 2005). A more reliable assay for detection of CPV protective antibodies is virus neutralisation (VN), which requires laboratory expertise in cell culture and virus isolation. The VN titre is the reciprocal of the highest serum dilution that completely neutralizes the virus (Cavalli et al., 2008). In-clinic assays are usually dot-ELISA assays licensed to determine the titre of antibodies against canine core vaccines (CAV-1, CPV and CDV). The concentration of CPV antibodies in serum samples is defined by the colour intensity of the dots, which corresponds to the antibody level in the test specimen. Results are scored using positive reference dots and a scale (Killey et al., 2018; P. Dall'Ara, manuscript in preparation).

The presence of CPV antibodies, regardless of titre, in an actively immunized dog over the age of 20 weeks is correlated with protection (Day et al., 2016; Decaro et al., 2020). Antibody testing could be performed at least 1 month after the final primary series vaccination administered at 16 weeks or older and repeated every 3 years in the case of a positive result.

3.3. Herd immunity and vaccination coverage

“Herd immunity” to a pathogen refers to the indirect protection of susceptible members of a population by immune members, due to a lower risk of exposure to an infected individual, and is also influenced by the basic reproduction number (R_0) of the pathogen (Horzinek, 2006). R_0 is defined as the number of new infections generated by the first infectious individual in a wholly susceptible population (Metcalf et al., 2015).

Few studies have modelled R_0 for companion animal viral infections, although such an approach would be useful for CPV to better inform prevention strategies. Since R_0 is not a fixed parameter and is sensitive to multiple variable host factors such as demography, husbandry and genetics, modelling of R_0 for CPV would need to be done in different settings (Woolhouse et al., 2016).

Serological surveillance has provided some insights into herd immunity to CPV among different dog populations. Among owned-dog populations (e.g. household pets, dogs in breeding kennels) within the last twenty years, in which most dogs tested have typically been vaccinated, high CPV seroprevalences have been reported in most studies, ranging from 86 to 98.5 % (Tward and Dodds, 2000; Bohm et al., 2004; Mitchell et al., 2012; Riedl et al., 2015; Killey et al., 2018; Rota et al., 2019). By contrast, among shelter-housed dogs, which are usually unvaccinated stray dogs or variably vaccinated guardian-surrendered dogs, lower seroprevalences of 67–84% have been reported (Lechner et al., 2010; Litster et al., 2012; Spindel et al., 2018). The minimum level of vaccine coverage required to prevent disease outbreaks among owned dog populations is lower than that for shelter-housed dogs because of a lower risk of exposure and transmission. A minimum vaccination coverage of 70–75% has been suggested previously to be adequate to prevent disease outbreaks in owned dog populations (Horzinek, 2006; Day et al., 2016; Riedl et al., 2015). Such estimates may have partially been based on two CPV seroprevalence studies of owned dogs in the UK and US in the 1990s where CPV seroprevalence was 70 % and 73 %, respectively (Tennant et al., 1991; McCaw et al., 1998). However, both of these studies were small and seroprevalence was based on a threshold CPV titre (1:80). If all positive antibody titres

had been considered protective, in accordance with current WSAVA guidelines (Day, et al., 2016), the actual seroprevalence of those dog populations would have been higher.

In the absence of interfering titres of MDA, attenuated CPV vaccines are strongly immunizing, as demonstrated in a field study of dogs entering two shelters that were vaccinated with 1 or 2 doses of a MLV vaccine; protective antibody titres were present in 98 and 100 % of dogs, respectively, within 2 weeks of vaccination (Litster et al., 2012). However, when vaccination coverage is not uniform there may be pockets of highly susceptible individuals within an otherwise immune population. Geographic “hot-spots” of antibody-negative dogs were identified in one study, which analysed census data and zip code of origin in unvaccinated dogs presenting to shelters in the US. Some of these hot-spots were regions with low access to veterinary resources (Spindel et al., 2018). Similarly, Kelman et al. (2019a) found a strong correlation between case numbers of CPV infection and occurrence in regions of socioeconomic disadvantage.

4. Immunisation failures

In an epidemiological survey conducted in Australia, 3.3 % of dogs infected with CPV were adults that had received a complete primary vaccination course as a pup, indicating apparent immunisation failure (Ling et al., 2012). Immunisation failures can be vaccine-related or host-related. Causes of vaccine-related failures include vaccine storage or administration errors, non-compliance with vaccine schedules, and failures in vaccine immunogenicity (Decaro et al., 2008a; Altman et al., 2017). In a 2017 survey of Australian veterinarians' vaccination protocols, nearly half of respondents did not comply with the recommended guideline to finish primary vaccination at or after 16 weeks of age (Kelman et al., 2020).

Host-related factors are associated with age, genetic factors, and impaired health, nutrition or immune status (Wiedermann et al., 2016). Primary immunisation failure, occurring in 2–10 % of vaccinated healthy humans, is due to failure to develop detectable antibodies, while secondary immunisation failure is associated with loss of previously acquired protection faster than expected (waning immunity) (Wiedermann et al., 2016). Age-related immunosenescence, a well-documented phenomenon in humans, is associated with both primary and secondary immunisation failure (Grubeck-Loebenstein, 2010). A low CPV seroprevalence of 81 % detected among sick dogs admitted to an intensive care unit may, in part, have been due to secondary immunisation failure (Mahon et al., 2017). The main causes of immunisation failures are summarised in Fig. 1.

4.1. Role of maternal immunity

One of the main causes of CPV immunisation failures is the presence of interfering titres of MDA. In dogs, due to the low permeability of the canine placenta to immunoglobulins, only 5–10 % of MDA are transferred during pregnancy. Most MDA, mainly represented by immunoglobulin G antibodies, are transferred from the bitch to the offspring through ingested colostrum, reaching the small intestine, and transported across the intestinal epithelium into the neonatal circulation (Winters, 1981). Passive transfer of CPV MDA through milk has been also demonstrated up to at least 38 days after parturition, such that this continued lactogenic immunity may contribute further to protection from CPV infection (Decaro et al., 2004).

MDA titres decline exponentially over time, with CPV-specific MDA half-lives in serum ranging from 8.3–13.5 days (Parrish et al., 1982; Pollock and Carmichael, 1982; Mila et al., 2014), although they can persist for 13–15 weeks (Pollock and Carmichael, 1982; Buonavoglia et al., 1985). Maternal immunity represents the first defence of pups against infectious diseases. A correlation between passive immune transfer, in terms of the titre of CPV MDA absorbed, and duration of protection against parvovirus infection in weaning pups was observed

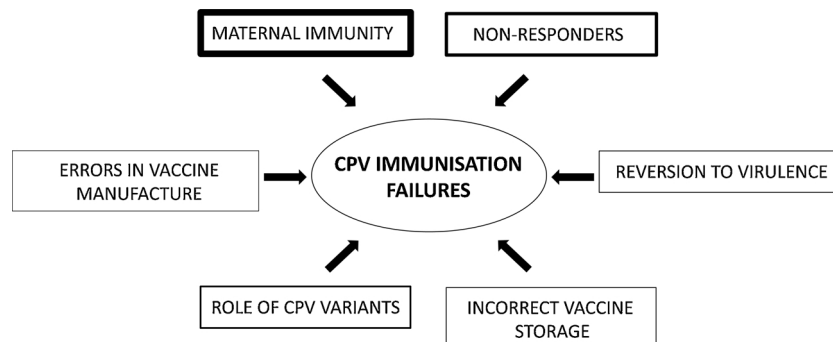


Fig. 1. Schematic representation of causes of immunisation failures after canine parvovirus vaccination. The thickness of the border of each box is proportional to the impact of the relative factor.

(Mila et al., 2014). In another study, pups with high CPV-specific MDA titres were protected against virulent challenge, while pups with intermediate and low or absent CPV-specific MDA titres developed mild and severe disease, respectively (Decaro et al., 2005).

MDA are able to block active immunisation after administration of CPV vaccines (Pollock and Carmichael, 1982; Buonavoglia et al., 1985; Chappuis, 1998). Vaccination of pups with interfering MDA titres (HI titres > 1:20) may result in lack of seroconversion due to neutralisation of vaccine viral antigen by maternal antibodies. Since only HI titres \geq 1:80 are considered protective against infection by field strains, there is a period, known as the “window of susceptibility” or “immunity gap”, usually lasting 2–3 weeks, during which the MDA titre falls below that required for protection but is able to neutralise vaccine virus. During this period pups can be infected and sometimes develop disease (Pollock and Carmichael, 1982). Indeed, more recent studies have demonstrated that the CPV variants are able to induce active infection (and disease) even in the presence of MDA titres previously considered protective, i.e., 1:80–1:160 (Decaro et al., 2005). Similarly, some vaccines are claimed to confer protection in pups with MDA titres previously reported as interfering (N. Decaro, personal observation).

Different strategies have been proposed to overcome MDA interference, including i) determination of MDA titres; ii) use of high-titre vaccines, and iii) alternative routes of vaccine administration. MDA titration at 4–6 weeks of age through the HI test, which represents the reference standard for detection or determination of antibodies titres against CPV, can be useful to predict the best time to vaccinate pups, taking into account the curve of MDA decline, which is based on the MDA half-life. For instance, if a pup displays an HI antibody titre of 1:640 at 4 weeks of age, considering an MDA median half-life of 10 days, the optimal period for vaccination could be estimated approximately at 10–11 weeks, when MDA will presumably drop below titres of 1:20–1:40. Although this approach can be cumbersome, requiring serum collection and delivery to a specialised laboratory, future validation of in-clinic tests, currently registered for assessment of post-vaccinal antibody response, for MDA titre determination may facilitate routine use of this strategy (P. Dall’Ara, manuscript in preparation).

Administering CPV vaccines via alternative routes to the parental one may help limiting vaccine virus neutralisation by MDA. Experimental vaccines, based on MLV CPV-2b, have been administered intranasally and are proven to partially overcome MDA interference (Buonavoglia et al., 1995; Martella et al., 2005). More recently, the oral administration of a commercially available CPV monovalent vaccine was also proven to be effective in overcoming the MDA interference (Cavalli et al., 2020). However, no commercially available CPV vaccine is currently registered for oral administration, so that administration of CPV vaccines via alternative routes to the parental is considered off-label. In addition, according to the WSAVA vaccination guidelines, these alternative routes of CPV vaccination are not as effective as parental (subcutaneous or intramuscular) vaccination (Day et al., 2016)

Another strategy to overcome the MDA interference is the use of

high-titre vaccines (Burtonboy et al., 1991; Buonavoglia et al., 1992; Hoare et al., 1997; De Cramer et al., 2011). These vaccines, also commercially available, have the advantage of containing viral titres 2–3 logs higher than those of traditional vaccines, such that in the presence of intermediate MDA titres not all viral particles are neutralised and are able to infect the vaccinated dog inducing an active immune response (Truyen, 2006).

4.2. Non-responders

Humoral immunity is the primary mechanism of protective immunity against CPV. The duration of immunity depends on the persistence of memory B and T-cells and long-lived plasma-cells, or “memory effector B cells”, which synthesise CPV-specific antibodies for years subsequent to the initial challenge or vaccination (Schultz, 2006). In veterinary medicine, primary vaccine failures that are considered to be genetic non-responders include dogs that repeatedly fail to develop detectable antibodies after both the primary vaccination course (finishing at 16 weeks of age or older) and subsequent revaccination (Kennedy et al., 2007b; Day et al., 2016).

A strong genetic factor associated with primary immunisation failure in humans is the major histocompatibility complex, encoded for by the human leukocyte antigen (HLA) gene complex. Certain HLA type II haplotypes are risk factors for primary immunisation failure to specific vaccines such as hepatitis B or influenza (Gelder et al., 2002). Some canine breeds have been reported to be at higher risk of primary immunisation failure to CPV vaccines, including Rottweilers and Doberman pinschers (Houston et al., 1996). Dog leukocyte antigen (DLA) type II haplotype diversity in dogs varies widely between but not within breeds and is restricted in Rottweiler as compared to other breeds (Kennedy et al., 2007a). These dogs are assumed to have a higher risk for canine parvovirus and should be excluded from breeding. However, direct associations between primary vaccine failure and DLA haplotype have not been investigated in dogs. While the incidence of genetic non-responders among CPV-vaccinated dogs has been estimated at one in 1000 dogs, (Day et al., 2016) epidemiological data are lacking, and prospective studies to evaluate the prevalence of primary vaccine failures are needed.

4.3. Reversion to virulence

Reversion of CPV MLV vaccine strains to virulent virus is theoretically possible, but has not been confirmed as a cause of immunisation failure. There is also the chance that the vaccine itself can cause disease after administration. An investigation of 29 dogs that developed severe gastroenteritis shortly after CPV vaccination used a minor groove binder probe quantitative PCR assay to differentiate between vaccine and field strains of CPV (Decaro et al., 2007b). Disease was confirmed to be caused by field strains of CPV in over half of the samples (18 of 29), which contained either field strains alone ($n = 15$) or together

with the vaccine strain ($n = 3$). In 11 samples in which vaccine virus was detected but not a field strain, reversion to virulence of CPV MLV vaccine strains was considered unlikely, since co-infections with other pathogens including CCoV, CDV and *Cystoisospora canis* were detected. In three pups in which only the CPV MLV vaccine strains were detected, quantities of vaccine virus in faeces were lower than those associated with disease from CPV field strains, ranging from 1.03×10^3 to 1.78×10^5 DNA copies/mg, thus suggesting that the vaccine virus did not have an etiological role in the onset of diarrhoea (Decaro et al., 2007b).

4.4. Errors in vaccine manufacture and storage

Although so far errors in manufacturing have not been documented for CPV vaccines, theoretically vaccine design and manufacture may affect the immunogenicity of a particular batch of vaccine. Tempesta et al. (1998) reported that an experimental CPV vaccine strain displayed a shorter VP1/VP2 gene, thus leading to the hypothesis that defective particles (non-replicating virus) may hamper the development of a strong immune response. However, large manufacturers marketing their vaccines at the international level are subjected to strict control quality and potency tests that reduce the chance to licence low-quality products (Day et al., 2016). Taking into account that vaccines must be stored at optimal temperatures of 2–8 °C, incorrect storage and transportation, i.e., interruption of the cold chain, may potentially inactivate MLV vaccines, especially in warm climate regions (Day et al., 2016). In some countries, vaccines are transported long distances from the point of manufacture or importation to the veterinary practice, which may hamper the continuation of the cold chain. However, incorrect storage and transportation are unlikely to affect the viability of CPV, due to its environmental resilience, in contrast to CDV, for which the interruption of the cold chain has been involved in apparent vaccination failures (van de Bildt et al., 2002).

4.5. Role of the CPV variants

The ability of old-type (CPV-2 based) vaccines to protect against the CPV variants is a topic of debate among parvovirologists. There is concern that the antigenic differences from the currently circulating field strains may decrease the effectiveness of CPV-2 based vaccines (Greenwood et al., 1995; Yule et al., 1997; Pratelli et al., 2001). *In-vitro* cross-neutralisation studies have demonstrated that there is a one-way cross-reactivity between the CPV variants and the old type CPV-2 (Pratelli et al., 2001). Animals vaccinated with CPV-2 displayed significant lower virus neutralising (VN) antibody titres against CPV-2a, CPV-2b and CPV-2c with respect to the homologous virus (Cavalli et al., 2008). In a Korean study, the poor cross-reactivity between CPV-2 and the CPV variants was evident not only by VN that is able to detect protective antibodies, but also by HI, which also detects non-neutralising antibodies (Kang et al., 2008).

Accordingly, there are an increasing number of case reports describing the occurrence of parvoviral disease, mainly caused by CPV-2c, in dogs “regularly” vaccinated with CPV-2. One outbreak occurred in a breeding kennel in Italy, affecting 11 dogs aged between 6 months and 2.5 years and leading to the death of a 20-month-old Bernese mountain dog pregnant bitch (Decaro et al., 2008a). In that kennel, all adult dogs had not only completed their primary course of vaccinations as pups, but they had also received annual boosters. Similarly, a CPV clinical case occurred in a 12-year-old crossbreed bitch, which had received annual vaccinations using a CPV-2 vaccine (Decaro et al., 2009). Subsequent studies also reported the occurrence of parvoviral disease in vaccinated dogs, but in most cases the vaccines administered were unknown and the infected dogs had not completed a primary vaccination course as pups. In addition, it could not be ruled out that the few adult animals that had received multiple boosters were non-responders (Calderon et al., 2009; Mittal et al., 2014; Woolford et al., 2017).

In a comprehensive study aiming to assess the distribution of the

three CPV variants in Italy in a 24-year period, about a third (32.5 %) of CPV-infected dogs had been vaccinated, raising doubts regarding the ability of the vaccine to confer protective herd immunity (Battilani et al., 2019). However, in this study although dogs were categorised as “completely” or “incompletely” vaccinated, these terms were not defined, and vaccination protocols were unknown. Since the median age of affected dogs was 3 months, immunisation failure in many of these cases may have been due to MDA interference. Similarly, the immunisation failures reported in one Australian study were likely caused not by genetic variation of CPV field viruses but by MDA interference in the response of pups to vaccination (Meers et al., 2007).

Despite the number of reports, sometimes anecdotal, concerning the putative lack of efficacy of traditional vaccines against the CPV variants currently circulating in the field, several studies have demonstrated that currently available vaccines, including those prepared with CPV-2, confer a good degree of protection against CPV-2a, CPV-2b and CPV-2c (Brunet et al., 2007; Larson and Schultz, 2008; Spibey et al., 2008; Siedek et al., 2011; von Reitzenstein et al., 2012; Wilson et al., 2013; Glover et al., 2015). Most of these studies have been recently reviewed by Hernández-Blanco and Catala-López (2015), showing that CPV-2 vaccines were generally beneficial in terms of significant reduction of clinical signs and virus shedding caused by subsequent challenge with field isolates. However, in one of these studies, which was the largest study in terms of the number of vaccinates ($n = 20$), leukopenia, diarrhoea and vomiting occurred in some vaccinated pups, although vaccination was carried out in the absence of MDA interference since all dogs were specific-pathogen free (von Reitzenstein et al., 2012). In the same study, the CPV-2 vaccine was able to prevent faecal shedding of the challenge virus (CPV-2c), although viral shedding was evaluated by virus isolation, which has been proven to be poorly sensitive (Desario et al., 2005).

A recent study by Altman et al. (2017) found no strict correlation between immunisation failures and the antigenic CPV type contained in the vaccines, whereas the main risk factor identified was early termination of the primary vaccination course schedule. Accordingly, both WSAVA and AAHA claim that all CPV MLV vaccines are expected to provide protection from disease caused by any CPV field variant currently recognised (Day et al., 2016; Ford et al., 2017). In conclusion, there is no definitive evidence for an unequivocal role of the CPV variants in vaccination failures.

5. Challenges to eradication

Fig. 2 reports schematically the main factors affecting eradication of CPV disease. In countries where dogs’ vaccination programmes are extensively carried out, CDV and CADV-1 circulation has been

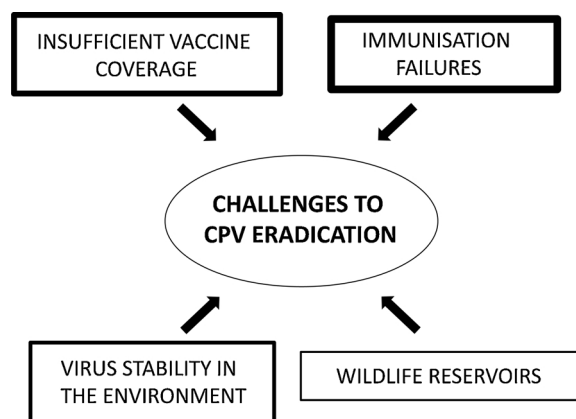


Fig. 2. Schematic representation of challenges to eradication of canine parvovirus disease. The thickness of the border of each box is proportional to the impact of the relative factor.

considerably reduced, so that outbreaks caused by these two viruses are now only sporadically reported (Decaro et al., 2007a; MacLachlan and Dubovi, 2017; Mitchell et al., 2017). One almost insurmountable challenge to canine distemper and infectious canine hepatitis eradication is the existence of wildlife reservoirs of infection in wild carnivores (Decaro et al., 2008b; Beineke et al., 2015). Importantly, CDV and CaDV-1 outbreaks in wildlife seem to represent spillover events of wide virus circulation in domestic dogs (Martella et al., 2009; Di Sabatino et al., 2015, 2016; Dowgier et al., 2018), although spillback events from wildlife reservoir hosts to domestic animals occur (Kapil and Yeary, 2011). In addition, autochthonous circulation of CaDV-1 in European red foxes was demonstrated, which may represent a potential threat to domestic dogs (Balboni et al., 2013; Verin et al., 2019). Analogously, a diverse range of wildlife in the order *Carnivora* can be infected by CPV, in which subclinical infection appears to be common, or may even be the norm (Allison et al., 2014). Thus, there appear to be significant wildlife reservoirs of CPV, and viral transmission between domestic dogs and wildlife is frequent and bidirectional (Allison et al., 2014). One recent study analysed the geospatial distribution of wild canids in Australia and CPV cases in owned dogs, and found that postcodes in which CPV cases were reported were significantly correlated with the presence of wild canids (Van Arkel et al., 2019).

Vaccination against CPV, CDV and CaDV-1 is not performed extensively in many areas of the world, especially in developing countries, such that these areas can represent pockets of infections that can spillover into countries where virus circulation has been reduced (Martella et al., 2006; Decaro et al., 2008b, 2010). This, however, does not explain why canine distemper and infectious hepatitis are well controlled by vaccination, while canine parvovirus still represents a great threat to the canine population. An explanation could be that both maternal immunity towards CPV and window of susceptibility are longer-lasting as compared to the situation for CDV and CaDV-1 (Griot et al., 2004; Day et al., 2016; N. Decaro, personal observation). In addition, in contrast to CDV and, to a lesser extent, CaDV-1, where survival persistence outside the host is relatively short-lived, CPV is resilient, environmentally persistent and able to exist outside the host in favourable environments for 12 months or longer (Greene and Decaro, 2012). CPV is also resistant to commonly used disinfectants (e.g. quaternary ammonium compounds), although a 0.75 % sodium hypochlorite solution displayed a good efficacy against the virus (Cavalli et al., 2018). These factors present another major challenge to halting transmission of CPV, which is mainly indirect through fomites (Greene and Decaro, 2012). The relative impact of environmental reservoirs of CPV on disease transmission is incompletely understood. However, studies that found an association between occurrence of CPV and periods of lower rainfall, suggested that extended dry periods may contribute to environmental persistence and increased risk of exposure (Kelman et al., 2019a; Rika-Heke et al., 2015).

6. Conclusions

Despite intensive vaccination (at least in developed countries), CPV infection remains a leading cause of death from infectious disease amongst domestic dogs worldwide and at the moment we are far from disease eradication. Immunisation failures are uncommon and are mostly the result of interference from MDA in pups under the age of 16 weeks. More research is required to determine the prevalence and genetic causes of non-responders. The most effective tools available for disease prevention are antibody testing to determine the optimal time for vaccination of both pups and adults, and homogeneous coverage of dog populations by vaccination with CPV MLV vaccines in accordance with current global guidelines (Day et al., 2016). Antibody testing to determine the optimal time for vaccinations of pups and adults and homogeneous coverage of dog population are the next logical steps towards achieving disease eradication or at least an improvement of the current situation.

Declaration of Competing Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetmic.2020.108760>.

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