Canine Parvovirus in South Africa - selected topics

The virus

Canine parvovirus is a tiny single stranded DNA virus. Its capsid consists of 2 proteins, one of which is used to bind to host cells. It binds to host transferrin receptors which are usually used to transport iron into cells. The exact structure of this capsid protein determines which species the particular parvo strain is able to infect.

Practically, the most important structural property of paroviruses is that they are not enveloped. Envelopes are formed when a virus hijacks some host cell membrane or endoplasmic reticulum. Enveloped viruses are better at evading the immune system, but are also much more susceptible to environmental damage. The fact that parvoviruses are not enveloped means that they are extremely tough, surviving exposure to many routine disinfectants and surviving for months - years in soil or on fomites.

Disinfectants that do work:

- Quaternary ammonium compounds like F10 and Vetguard
- Bleach diluted 1:30 with water

Both need a contact time of AT LEAST 10 MINUTES

Huge numbers of virus particles are shed, mainly in faeces, for 7-14 days after the onset of clinical signs, contaminating the environment. This means that CPV is ubiquitous in any environment frequented by dogs. For example, CPV can remain viable on a dog’s coat for weeks and if you want to remove it from a lawn, you have to lift up the turf as well as underlying soil and replace the whole lot. Thus most clinical cases are infected by invisible faecal residues left in the environment and distributed further by shoes, clothing, feeding bowls and other animals.

The related feline panleukopenia virus (FPV) has been around for > 100 years and is antigenically very stable. CPV-2 is thought to have evolved from FPV in the early 1970s and first caused severe disease outbreaks between the late 70s and early 80s. A mutation in one of the two capsid proteins allowed the new parovirus to infect dogs and is also one that remains labile. Thus CPV-2 mutated again and was rapidly replaced by CPV 2a. CPV 2 is now only found in vaccines. CPV2b emerged by 1984 and in some areas has become the dominant strain. In 2001, CPV 2c was reported from several sites (Italy, Vietnam) and appears to be spreading slowly across the globe. The capsid protein mutations also determine the hosts the virus can infect: Both FPV and CPV2 only rarely infect members of the opposite domestic species and hardly ever cause clinical signs. The capsid protein mutations that changed the virus to CPV 2a, 2b and 2c also conferred the ability to infect cats and cause clinical signs indistinguishable from feline panleukopenia in some cases.

There is only 1 published study of CPV strains in South Africa. In 1998, CPV 2b represented 67% and CPV 2a 33% of South African and Namibian isolates. This experiment was repeated in South Africa and Nigeria last year and has been submitted
for publication. Approximately 120 virus isolates were tested and no CPV 2c was found. Thus CPV2c has not been isolated on the African continent yet (Moritz van Vuuren, personal communication July 2011). As dogs and cats regularly travel across continents and as CPV is so difficult to kill, it is likely to only be a matter of time before this strain appears in South Africa too.

**Pathophysiology (the minimum you need to understand the Dx)**

Paroviruses target rapidly dividing cells. In pups, these are typically the small intestinal (crypt)cells, the bone marrow and lymphoid organs explaining the typical clinical signs we’re all familiar with: foul smelling haemorrhagic or just mucoid diarrhoea +/- vomiting; leucopenia.

Signs do not appear to be significantly determined by the strain. They are influenced by AGE at infection: Paroviruses infect rapidly dividing cells. In utero and in very young (< 8w old) pups, the myocardium is growing rapidly and can become infected resulting in myocarditis and presenting as sudden death in pups < 12 w old (most commonly) or congestive heart failure (very rare). On PM, the myocardium is initially infiltrated with lymphocytes. Areas of inflammation are then replaced with fibrosis resulting in characteristic tiger striping. Myocarditis is very rarely seen these days because CPV is so ubiquitous and persistent in the environment and vaccination is common - which means that it’s very rare for a bitch to be infected during pregnancy or pups not to receive any CPV antibodies in the colostrum.

Severity of clinical signs may also be affected by concurrent problems eg intestinal worm burdens, coccidiosis, giardiasis; by management issues eg food change; concurrent disease eg distemper infection, demodeciosis, ehrlichiosis; host genetics; host age (older dogs typically have much milder signs unless Immunosuppressed. Some dogs (many adult dogs) are asymptomatically infected.

**Diagnosis**

The benchtop snap / rapid immunomigration tests are very specific (ie are unlikely to call anything that isn’t CPV positive) but will definitely miss cases. The reasons are

- no test is perfect
- CPV is only shed in faeces for a maximum of 2 weeks
- For the later part of that period, the virus will be coated with antibodies, ‘hiding’ it from the test reaction. ‘Uncoated’ virus will be present in faeces of the ill dog for 1-7d after the onset of clinical signs.
- There is no one benchtop test that shows clear superiority over others - at least as far as I could tell
- False positive reactions are possible if the dog was vaccinated in the last 2 weeks

| If your benchtop test is negative but you still think it’s parvo you have 2 options |
|----------------------------------------|------------------------------------------|
| **PCR**                                 | **Electronmicroscopy**                    |
| Most sensitive: may detect CPV in clinically healthy dogs | More sensitive than benchtop
You can look for concurrent GI viruses (distemper, rota, corona) at the same time
You can’t ID strains / distinguish CPV 1 |
| Can identify CPV strain                |                                         |
| Can differentiate vaccine and wild virus |                                         |
| Can differentiate CPV1 and CPV2       |                                         |
Faecal inhibitors can be a problem
Most susceptible to technical error

Treatment - why do we use what? (for details, see reference texts)
- Fluid: rehydration, maintain blood pressure, replace blood lost, maintain oncotic pressure. Calculate required amounts and use multiple measures to assess adequacy of hydration eg skin fold, pulse quality, pulse rate
  o Potential problems:
    § overhydration of very small animal
    § hypoalbuminaemia resulting in oedema / anasarca
- Glucose: hypoglycaemia common in young animals that are severely ill
- Potassium: lost in vomit, results in weakness and ileus (which means pup continues to vomit for entirely preventable reasons). Monitor and supplement.
- Antibiotics: Pups are leukopenic and have lost their SI lining - so all those gram -ve and anaerobic bacteria in the GIT have free access to the circulation. Antibiotics are used to prevent bacterial sepsis in animals that are neutropenic and / or have haematemesis or melena. Pups’ immune system is not functional, so you’re looking for CIDAL antibiotics that cover all 4 quadrants. Traditionally, amoxicillin or amoxy/clav with an aminoglycosides is used. The latter is obviously only started once the pup is properly hydrated and out of shock.
- Feeding: Research has shown that early feeding shortens hospitalization time. Enterocytes are entirely dependent on luminal nutrients.
- Treat concurrent infections (eg worms, coccidian, Giardia): to prevent further damage to the SI, further protein loss
- Gastroprotectants: consider especially if vomiting blood and to guard against oesophagitis
- IFN-omega (Virbac): Has been shown in a field trial to improve survival (4.4 times less likely to die), but is not licensed in SA, is very expensive, and also needs to be shipped in controlled conditions, so it is not practical for the majority of our clinical cases in SA.

Prognosis - can we predict who’s gonna die?
- Hypothermia (< 37°C) and poor peripheral pulses are associated with a poor prognosis and were a better predictor of mortality than acute phase proteins
- Lymphocyte count > 1x10^9 /l after 24 and 48 hrs was relatively good at pinpointing puppies that would survive
- People have looked at T4 and cortisol levels but these are both expensive and don’t separate survivors and pups that will die sufficiently to be practically useful
- Prognosis is poorer if there’s an outbreak in a breeding kennel (Kurt de Cramer, personal communication): instead of a 20-25% mortality you’re expecting a 20-25% survival rate

Vaccination - and why parvo vaccines can fail
Back to basics: The immune response
Initial exposure to a pathogen or vaccine triggers a primary adaptive immune response during which IgM synthesis predominates. Continued or repeated exposure to the same pathogen stimulates a secondary adaptive response during which antibody synthesis escalates and changes to the IgG type. When using killed vaccines, the responses
appear separately because the vaccine virus does not multiply inside the host. During a natural infection or vaccination with a modified live antigen, the pathogen continues to multiply, so both responses are triggered and overlap (see figure 1).

Figure 1 Antibody response to vaccination and infection
Blue: primary adaptive immune response with IgM predominating. Note time delay. Green: secondary adaptive immune response resulting in much higher titres of IgG after a shorter delay. Black: response to modified live virus (MLV) vaccine or infection (reproduced with permission)

Maternal derived antibodies (MDA) and the window of susceptibility:
Neonates absorb antibodies from colostrum. The amount of antigen available in colostrum depends on the immune status and disease history of the mother. The amount absorbed varies between individuals in the litter according to how much colostrum is taken in. MDA half-life varies according to antigen (7-15 days) and is approximately 10 days for CPV antibodies.

Because vaccine virus is attenuated, it is also less infective than wild virus. Thus all neonates that have any MDA go through a stage where they have enough antibody to block effective vaccination, but not enough to prevent infection with more virulent field strains of the virus. This is the window of susceptibility. A single dose of a highly effective vaccine like a MLV CPV vaccine will effectively immunise a susceptible puppy IF GIVEN WHEN MDA have fallen. Multiple doses of such vaccines are given because it is
not known when MDA will fall sufficiently to allow immunisation and it is preferable to minimise the window of susceptibility.

Figure 2 Maternal derived antibodies (MDA) and the window of susceptibility
The amount of MDA each puppy absorbs from the colostrum varies throughout the litter. Antibody levels then have every 10 days in the case of CPV: Puppy 1’s antibody levels are indicated in blue, puppy 2’s in red. The level of MDA necessary to protect against wild virus infection is higher than the level of MDA below which vaccination is possible (intersection between the two horizontal lines and the individual puppy’s graph) The time that it takes for the antibody to decline between these two points represents the window of susceptibility. This time period is indicated by the coloured blocks for each puppy. Thus the window of susceptibility occurs at different times for different individuals. The time at which it will occur is determined by the initial amount of MDA absorbed (reproduced with permission ²). The horizontal vaccine bar can move up and down depending on how good the vaccine is a breaking through immunity eg if you used an old FPV-based parvo vaccine, MDA would have to be close to 0 before the pup would seroconvert. The horizontal bar for infection by wild virus will also vary depending on infection pressure (compare exposure during an outbreak in a kennel vs exposure from owner bring a few virus particles into the house on the soles of their shoes)

Current vaccination guidelines
American Animal Hospital Association ¹⁹, World Small Animal Veterinary Association⁴, South African Veterinary Council (www.savc.co.za under “policies and guidelines”)

DOGS
### Puppies:
- Age when start vaccinating against core antigens (CPV, canine distemper, canine adenovirus and rabies) and interval between vaccinations determined by likely maternal antibodies, local disease risk and manufacturer’s recommendations
- Last vaccine at 14-16 weeks depending on genetics, disease risk

### Adolescents:
- Booster against core antigens at 12-15 months essential

### Adults:
- Vaccinate against core viral antigens every three years
- Leptospira vaccines annually in endemic areas
- Local disease outbreaks or individual susceptibilities determine use of non-core vaccines / more frequent vaccination with core antigens

| Use non-core vaccines only if specifically indicated for that individual | Use MLV if possible unless reversion to virulence is a risk (e.g. pregnancy, immunosuppression) |

Note: There are challenge studies showing that some canine core vaccines can protect the majority of vaccinated animals for 3 years. The efficacy of vaccines is likely to differ between manufacturers.

What is also stressed again and again is that there cannot be a universally applicable vaccination policy. Rather, the protocol should be adapted for each particular individual’s situation.

### Is it safe to adopt first world guidelines in South Africa?

The efficacy of vaccines at preventing disease depends on a number of factors

1. **Intrinsic qualities of the vaccine, as well as how it was handled**: Some pathogens trigger a durable immunity. Some pathogens are more difficult eg Leptospira bacterins result in immunity for 18 months at best. MLV vaccine contain live organisms that need to multiply - allowing them to warm up for long periods or to come into contact with alcohol may kill MLV thus markedly decreasing the vaccine’s ability to trigger a protective immunity.

2. **Genetics of the dog**: The Rottweiler, Doberman, German Shepherds (GSD) and American Staffordshire Bullterrier breeds are said to respond poorly to vaccination when compared with other breeds. Current research is looking at MHC Class II haplotypes and polymorphisms to explain this phenomenon.

3. **General health status and concurrent diseases**: Ill dogs may mount a suboptimal immune response. In particular, humans and animals on immunosuppressive or cytotoxic medication are known to have a poorer response to vaccination.

4. **Diet**: Studies have shown that addition of antioxidants to the diet can increase mean antibody titres following vaccination. One study showed antibody titres increasing by 0.5 - 1 dilution and even unsupplemented dogs had protective antibody titres. Conversely, it is likely that malnutrition will dampen the immune response. I could find no data quantifying the effect of malnutrition on vaccine response. Thus it is not clear whether these effects are clinically relevant.

5. **Concurrent worm burdens**: It has been shown that worm infestations decrease the antibody synthesis in response to vaccination in people. It is likely that this
occurs in dogs too. There are no studies that quantify the magnitude of this effect, so it is impossible to say whether concurrent worm burdens significantly compromise seroconversion the South African population.

6. Environmental challenge: the amount of virus in the environment as well as its relationship with the vaccine strain will affect how much antibody is needed to protect an individual from showing clinical signs. It will also determine how readily natural boosting will occur.

There are no South African studies in peer reviewed journals on vaccination of pet dogs against core antigens other than rabies. South African dogs will differ from their UK and USA fellows in the relative proportion of breeds. An increased proportion of poorly responding breeds in a population, should not, however, necessitate a general increase in vaccination frequency. An increased awareness of the need to confirm seroconversion in puppies of affected breeds should suffice. Thus relative breed proportions are relevant only in as much as there may be additional breeds common in South Africa and rare in the UK and USA that respond poorly to vaccination that have not yet been identified.

There are several multivalent vaccines that have challenge studies to support an extended vaccination interval of 3 years (Nobivac range, Intervet-Schering Plough and Duramune Adult range, Fort Dodge Animal Health) for the core canine antigens CPV, CDV and CAV. Pfizer and Merial have serological data supporting a 3 year duration of immunity (DOI) for the core antigens in Vanguard and Recombitek dog vaccines respectively. Fort Dodge and Pfizer still recommend annual vaccination against core antigens in South Africa. In Fort Dodge’s case this is because the Duramune Max marketed in South Africa is a different vaccine which has only had one year challenge studies done on it. The Pfizer vaccine marketed in South Africa is the same as the one on which the serological study was done in the USA. The company continues to recommend annual vaccination because they feel that South African dogs are subjected to a higher environmental challenge and more immunosuppressive disease.

CPV causes severe disease particularly in puppies and adolescent dogs. Adult dogs may shed virus, but do not usually show severe signs. Thus protecting puppies is most important. A serological survey in UK pet dogs 3-15 years post vaccination showed that 94.4% maintained protective CPV titres for this period. More frequent vaccination may thus benefit about one in 20 dogs. CPV is ubiquitous in the environment which has two consequences: adult dogs are likely to boost immunity naturally, usually showing mild / no signs. In addition, the effects of a poorly performing vaccine will rapidly become obvious with an increase in CPV in puppies. It appears likely that most currently available vaccines probably work fairly well and that clinical CPV cases are usually the result of natural infection developing before vaccines are able to break through maternal immunity. In my opinion, it is unlikely that adhering to the WSAVA guidelines in South Africa will result in an increase in CPV diagnoses in adult dogs.

Concurrent diseases, worm burdens and poor diets are likely to affect township dogs and farm dogs rather than the suburban pet population. Environmental exposure to pathogens is likely to vary dramatically from area to area. Nevertheless, the typical South African pet dog that is currently presented annually for vaccination is likely to be well fed, regularly dewormed and is usually confined to a yard. It seems reasonable to expect that such a dog will respond much like dogs in first world countries. More
frequent vaccination of ‘at risk’ dogs especially in townships may be desirable - but as these dogs rarely belong to vets’ clients, this becomes a mute point.

Vaccines brand - does it matter?
Historically, there are several examples where clear differences between vaccines were demonstrated: In 1994 Larson and Schulz showed that three of the 6 CPV vaccines on the market in the USA failed to immunise the majority of the puppies 15. These vaccines were replaced.

The Merial Primodog vaccine was able seroconvert significantly more pups at 6 w of age than the monovalent parvo vaccines produced by Intervet, Pfizer and Virbac8. The Merial vaccine was able to seroconvert a 62% of Boerbul, 80% of Rottweiler and 90% of GSD puppies at 4 weeks of age5. This has not been shown for any other vaccine as far as I’m aware.

Do current vaccines work against CPV 2c?
To date, the only experimental evidence offered has shown that current vaccines DO cross-protect against CPV 2c. Specifically, the work has been published on Vanguard (Pfizer)22, Nobivac16,23, and Galaxy16. In all 3 experiments, between five and ten SPF or at least maternal antibody free pups were vaccinated and then challenged with CPV 2c after 3-5 weeks. Vaccinated pups showed no clinical signs, no significant leukopenia, rarely shed CPV in faeces and antibody levels did not change following challenge. Similar work has been done on Eurican and Recombitek but has not been published to my knowledge. So the only available experimental evidence supports the ‘yes’ answer.

Dissenters have pointed to CPV2c cases in vaccinated dogs (which occur occasionally with any strain)12,20 and CPV 2c causing disease in adult dogs in a kennel (without details on the vaccine or vaccination program used)7.

Playing devil’s advocate, the number of pups used in the above experiments is small, the fact that they had no MDA and that the pups were challenged at the time of peak antibody levels post vaccination made it easy for the vaccines to work. What these experiments cannot tell us is whether a CPV 2c vaccine would be better antigen against CPV2c infections (possibly) and against CPV 2a and 2b infections (much more doubtful) - eg by breaking through MDA more quickly or by providing a higher titre response that persisted for a more protracted period.

Common questions on vaccination:

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<thead>
<tr>
<th>Vaccination while neutering</th>
<th>Vaccination while on prednisolone treatment</th>
<th>Vaccinating a dog with auto-immune disease</th>
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<tbody>
<tr>
<td>Not ideal, but studies in rescue centres show that animals are still effectively immunised in most cases</td>
<td>Not ideal, but as long as doses low (0.5 mg/kg daily or below), this should not affect antibody synthesis in dogs.</td>
<td>Consider titre testing. If titres borderline or low, consider lifestyle, likelihood of exposure and likely consequences of exposure before deciding whether to vaccinate or not</td>
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</tbody>
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References
1  Abdelmagid OY, Larson L, Payne L, et al.: 2004, Evaluation of the efficacy and duration of immunity of a canine combination vaccine against virulent parvovirus,


