Feb 2009
VetNews

www.vetcpd.co.za

HOW FREQUENTLY SHOULD WE BE VACCINATING DOGS (IN SOUTH AFRICA)?

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Background

This question was first raised in the UK and USA in the mid 1990s. Duval and Giger (1996)[1] showed that a large proportion of their dogs with auto-immune haemolytic anaemia (AIHA) developed signs in the 3 months following vaccination. They asked whether vaccination could be involved as a trigger for the disease. At the same time, reports of a dramatic increase in fibroscarcoma at injection sites in cats emerged from the USA[2] and Catherine O'Driscoll published a book called 'Who killed the darling buds of May – what vets don't tell you about vaccination'[3]. She was convinced that the deaths of her two Golden Retrievers were related to vaccination. The book received a great deal of publicity by mainstream press in the UK, probably because the association between MMR vaccines and autism in humans was also being hotly debated. Subsequent work has not been able to support the association between AIHA and vaccination, but the circumstantial evidence linking injection site sarcoma to vaccination is strong.

This issue is now starting to reach owners in South Africa. There are really two questions:

1. Are vaccines causing harm to pets?
2. Are annual vaccinations a waste of money for the owner?

1. Vaccine side effects

The most common adverse reactions associated with vaccination are general malaise for a few days following vaccination, localised swelling, type I (immediate) hypersensitivity reactions and reversion to virulence of particular batches, resulting in signs of the disease the vaccine was supposed to protect against. Immune-mediated haemolytic anaemia, thrombocytopenia, uveitis and corneal oedema, cutaneous vasculopathies resulting in alopecia, metaphyseal osteopathy and polyradiculoneuritis and encephalitis are also reported.

A retrospective study in a large US hospital group showed that 38.2/10 000 canine vaccine doses were associated with an adverse reaction. The majority showed urticaria, facial oedema or pruritus (65%) and 13.5% showed either a localised swelling / malaise[4]. The same group reported 20.34 adverse events /10 000 doses in cats[5]. Seventy-five to 80% of these adverse events were limited to lethargy with or without fever or a localised swelling. In the UK, the Veterinary Products Committee reported vaccine reactions in 0.21-0.61/10 000 doses. As the VPC relied on voluntary reporting of adverse reactions by vets and industry, it appears likely that not all suspected vaccine reactions were reported.

In summary: side effects from vaccination are rare and usually mild. In a small proportion of cases, they can be severe or even life threatening. In a country like South Africa where parvoviral enteritis and distemper are common, the risk to a puppy's health from not vaccinating far outweighs the risk to their heath from vaccine reactions. In the case of rabies vaccinations, the risk to humans from not vaccinating pets (exposure to bites from rabid animals, side effects of post-exposure prophylaxis following bite from an animal with unknown disease status) should outweigh any concerns about potential side effects in animals.

2. Annual vaccination: a necessity or a racket?

Historically, annual vaccination had been recommended. There were two reasons. The most important was that vaccine manufacturers had proof that the core vaccines provided immunity for at least a year. To prove this, vaccinated animals were challenged with virulent pathogens a year after inoculation and were shown to remain healthy while unvaccinated controls became ill or died. Prior to the 1990s, challenge studies were not performed after more than a year because of the cost of keeping animals in isolation for several years and because of concerns about the welfare of study dogs. In recent years, driven by the concerns about over-vaccination, several companies have performed challenge studies 3 or even 4 years after initial vaccination.

More of this later.
The second reason used to justify annual vaccination was that pets benefit from an annual health check – usually given at the time of vaccination. This check facilitates the early detection of heart disease, renal disease and tumours and is an ideal opportunity to remind owners about parasite control, discuss management of skin disease, neutering and the like. There is no doubt that an annual health check is an excellent idea, but this does not provide proof that annual vaccination *per se* is actually necessary.

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 fig.1).

Maternal-derived antibodies (MDA) and the window of susceptibility:
Neonates absorb antibodies from colostrum. The amount of antibody 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). 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. 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.

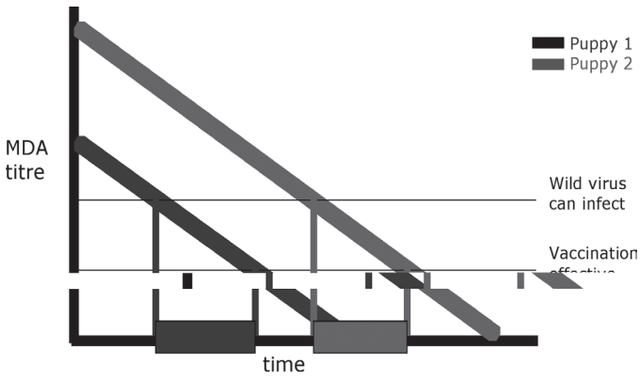
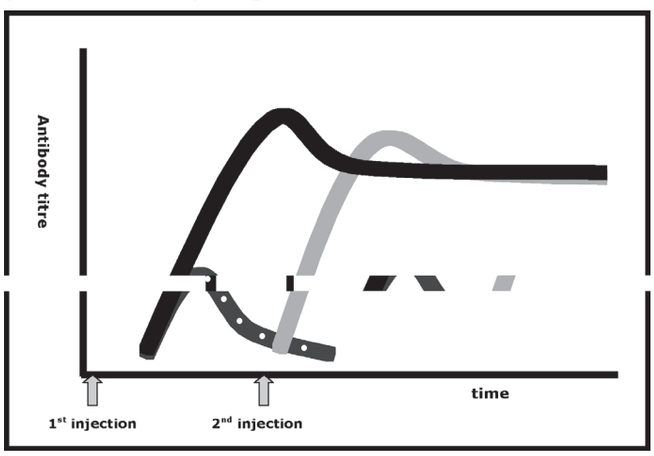


Figure 2 **Maternal derived antibodies (MDA) and the window of susceptibility**

The amount of MDA each puppy absorbs from the colostrum varies throughout the litter. Antibody levels then decrease at a uniform rate: Puppy 1's are indicated in black, puppy 2's in grey. 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[6])

Figure 1 **Antibody response to vaccination and infection**



■ primary adaptive immune response with IgM predominating. Note time delay.
■ secondary adaptive immune response resulting in much higher titres of IgG after a shorter delay.
■ response to modified live virus (MLV) vaccine or infection (reproduced with permission[6])

Table 1 **Primary vaccinations and adult boosters are fundamentally different:**

Primary vaccine course (puppies and kittens)	Booster vaccination (adults)
MDA present (half life of 7-15 days)	Own antibodies usually present (persist for months / years)
No specific cell-mediated immunity (CMI) present prior to vaccination	Concurrent specific CMI probably present
Vaccination triggers a primary (IgM) and a secondary (IgG) response	Vaccination triggers further IgG synthesis if levels have fallen
>90% of vaccinates expected to respond to primary course	<5–40% of vaccinates expected to respond in the case of canine core vaccines. Much higher percentages expected to respond to boosters of vaccines with a short duration of immunity, e.g. *Leptospira* bacterins and intra-nasal vaccines
Window of susceptibility (see below)	No window of susceptibility

(reproduced with permission[6])

***Different vaccine types*:**

Vaccines made from attenuated live organisms differ from killed vaccines (see Table 1). **Genetic modification** has been used to try and combine the benefits and decrease the risks of ML and killed vaccines. A variety of modifications have been used, each with advantages and limitations.

Table 2: **Differences between modified live and killed vaccines**

Modified live / attenuated	Killed
Multiplies in host, so initial amount of antigen in vaccine less important	Does not multiply in host, so need high antigen mass
Correct handling is essential to avoid killing vaccine virus	More stable than MLV
Vaccine virus may mutate and become more virulent – causing the disease against which the vaccine is designed to protect	Cannot mutate. This is why all small-animal rabies vaccines on the market in South Africa are killed vaccines
May cause disease in foetuses or immunosuppressed patients	Safe in immunosuppressed / pregnant animals
Potential for transmission of pathogenic cell-culture contaminants	Decreased likelihood that contaminants could be transmitted
Generally result in higher titres of antibodies	Lower than MLV vaccine against the same antigen
Generally longer lasting immunity	Shorter duration of immunity than MLV vaccine against same antigen
More effective at stimulating CMI	Primarily stimulates antibody synthesis
No need to use adjuvants	Usually need adjuvants to stimulate reasonable immunity. Adjuvanted vaccines are thought more likely to result in - Allergic reactions - injection site sarcoma - pain at injection site
Single dose may be sufficient. Because vaccine virus multiplies in the host, the two curves in figure 1 combine	Need at least 2 doses for maximal protection. (see figure 1)
Vaccination interval should not be less than 2 weeks or interferon produced in response to the first vaccine will inhibit antibody synthesis in response to the second	Vaccination interval should not be < 2 weeks for the same reason. With most antigens, vaccination interval should not be > 4 weeks or the priming effect will be lost (see figure 1)
Vaccine of choice for healthy animals, where available	Vaccine of choice for pregnant or immunocompromised animals

(modified and reproduced with permission[6])

Current international recommendations

In response to the above concerns, vaccination protocols were reassessed the concept of core and non-core vaccines was introduced.

Core vaccines are indicated in all animals, non-core ones only in individuals whose lifestyle, concurrent diseases or geographic situation places them at risk from rare or relatively less pathogenic agents.

Core vaccines
- Highly effective
- Safe
- Protect against diseases that have a high mortality/are highly infectious
- Protect against zoonoses

Non-core vaccines
- Protect against diseases with a low mortality, diseases that may be treated effectively
- Protect against diseases that only specific population segments are at risk from

Canine core vaccines in South Africa

Core vaccines	**Non-core vaccines**
Canine parvovirus (CPV)	*Leptospira* – a current study is looking for evidence of *Leptospira* exposure in South African dogs
Canine distemper virus (CDV)	Parainfluenza (Pi), *Bordetella* – pets being kennelled or shown
Canine adenovirus (CAV)– infectious canine hepatitis	Canine coronavirus (CCV) – in outbreaks, once CCV identified as cause
Rabies	*Babesia*
	Canine herpes virus

Current vaccination guidelines

American Animal Hospital Association[7], World Small Animal Veterinary Association[8], South African Veterinary Council (www.savc.co.za) under **"policies and guidelines")**

DOGS
Puppies: - age when start vaccinating and interval between vaccinations determined by likely maternal antibodies, local disease risk and manufacturer's recommendations - last vaccine at 12-16 weeks, depending on genetics, disease risk
Adolescents: - Booster 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[9-11]. 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' immunity. Some pathogens are more difficult, e.g. *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. 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.

Vaccine brands – does it matter?

Many colleagues assume that all vaccines are the same and select the brand used based on peripheral factors, e.g. costs, company discounts. In some cases, vaccines are exactly the same; e.g., in the UK, Virbac's Leucogen is sold by Intervet as Nobivac FeLV.

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[12]. These vaccines were replaced. In 1994-5 there was a CDV outbreak in Finland and distemper was common amongst vaccinated dogs. Two separate studies showed that there was a significant difference in the immunogenicity of the vaccines in use at the time[13,14]. Similar results were reported from

Sweden in 1995-6. Importation of one of the offending vaccines ceased. More recently, analysis of more than 10 000 serum samples collected for the UK Pet Travel Scheme (PETS) during 2002 showed that there were highly significant differences in the ability of different vaccine brands to trigger antibody synthesis in excess of 0.5 IU/ml three weeks after vaccination (the cut-off designated by the scheme). Failure rates varied between 0.7 and 20% depending on the rabies vaccine used[15]. There is little information available in peer-reviewed journals on the comparative efficacy of vaccines currently on the market and none is relevant to core vaccines.

Several multivalent vaccines have challenge studies to support an extended vaccination interval of 3 years (Nobivac range, Intervet-Schering Plough and Durammune 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 Durammune Max marketed in South Africa is a different vaccine on which only one-year challenge studies have been done. 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.

Looking specifically at core antigens

CPV: This virus causes severe disease, particularly in puppies and adolescent dogs. Adult dogs may shed virus, but usually do not 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[16]. 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 cases seen 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.

CDV: The case for CDV is less clear. The serological survey above showed that 71.5% of UK dogs 3-15 year post vaccination had protective CDV titres[16]. This is consistent with previous smaller studies. CMI plays a larger role in protection against this disease and is difficult to quantify. Several vaccine manufacturers have challenged laboratory dogs 3-4 years post vaccination and proven effective protection. Nevertheless, cases of CDV (usually showing only neurological signs) in vaccinated dogs are seen regularly at Onderstepoort and are a matter of concern. At this stage it is unclear whether vaccine brands perform variably well, whether maternal antibodies prevent a significant number of puppies seroconverting after their last vaccine, or whether CDV is just a poorer antigen and we cannot expect all animals to seroconvert. It is unclear whether increasing the vaccination interval will increase cases of CDV in vaccinated dogs.

CAV: Even less is known about infectious canine hepatitis. Occasional cases are still reported in South Africa. Again, several vaccine manufacturers have challenged laboratory dogs 3-4 years post vaccination and proven effective protection. In my opinion, adhering to WSAVA guidelines is unlikely to result in increased cases of infectious canine hepatitis in South Africa because infections are rare and because natural immunisation or boosting occurs when dogs contract kennel cough caused by CAV-2.

Rabies: Prior to the introduction of the PETS travel scheme regulating movement of dogs between the UK and Europe, there was little information on current rabies vaccines in peer-reviewed journals. Several studies have emerged since, documenting clear differences in the immunogenicity of different vaccines[15]. Unfortunately, they often do not identify the different vaccines by name. Human deaths were reported during the 2005/2006 rabies outbreak in Limpopo province[17]. Rabies remains endemic in

KwaZulu-Natal, resulting in human deaths every year[18]. By law, South African dogs and cats should be vaccinated twice 1-9 months apart, with the first vaccination when the animal is at least 12 weeks old. Thereafter, vaccinations every three years are mandatory. Rare adverse reactions to the rabies vaccine and the cost of an annual vaccination in endemic areas pale into insignificance against the risks to humans from contact with potentially infected dogs – and the risks to an unvaccinated pet showing neurological signs consistent with rabies (almost certain euthanasia). It is ironic that no one appears to question the wisdom of triennial vaccination against this important zoonosis.

Titre testing: the test that will allow you to vaccinate only those animals that need it?

Antibody titres assess the antibody-mediated immune response but ignore the cell-mediated (CMI) one. There is no easy way of assessing the CMI. In diseases where the antibody-mediated immune response is important and protective antibody levels have been determined by challenging puppies with MDA, antibodies provide a reasonably good estimation of immunity.

The most complete data are available for CPV: several challenge studies have shown that puppies with haemagglutination inhibition (HI) titres of 80 and above or 160 and above show no or only very mild signs of illness.

PETS has determined minimal antibody levels that prove an adequate response to rabies vaccination. These antibodies must be tested with 3-4 weeks of initial vaccination or 2-3 weeks of booster vaccination because levels peak during this time and fall dramatically within weeks. Thus titres could not be used to determine the protected proportion 3 years after vaccination with any accuracy.

There is some data on protective CDV antibody levels, with less on canine adenovirus (CAV). In diseases where CMI is important (Leptospirosis) or immunity is serosal (*Bordetella*), serum antibody titres are unlikely to assist in determining appropriate booster intervals.

If you are considering using antibody titres, remember that

1. Adequate antibodies prove protection. Low antibodies do not mean the animal will definitely get ill if exposed as CMI has not been assessed. But vaccination should be considered.
2. Consider what antibodies are tested. Haemagglutination inhibition (HI) is considered the gold standard for CPV, virus neutralisation (VN) the gold standard for CDV and CAV. These tests prove that the antibody measured is actively disabling virus because they look for a biological effect. It is often cheaper for laboratories to use ELISAs or immunofluorescent antibodies. These tests measure all antibody directed against the antigen, so levels are usually higher but don't correlate as well with protection.
3. The laboratory should have its own data validating the cut-offs they use.
4. Remember that you're testing something in a biological system that will be subject to variation. This means that the same serum sample submitted to the same laboratory may have antibody levels up to 2 titres higher or lower when the test is repeated. This means that although cut-off titres are accurate when applied to a population of dogs, borderline titres should be interpreted with caution in individual patients.

Titre testing could be considered in the following situations:

- Previous vaccine reaction
- To confirm seroconversion in a puppy of a poorly responding breed
- Auto-immune disease previously diagnosed
- (very worried owner)
- (after vaccinating a dog with a low titre – to prove seroconversion)

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Questions

1. The most common vaccine side effects in **dogs** are:
a. Fibrosarcoma, anaphylaxis, auto-immune haemolytic anaemia
b. Anaphylaxis, reversion to virulence, blue eye
c. Facial oedema, swelling, urticaria, pruritus, malaise
d. Local swelling, lethargy, pyrexia
e. Anaphylaxis, facial oedema, urticaria

2. Common vaccine side effects in **cats** are:
a. Fibrosarcoma, anaphylaxis, auto-immune haemolytic anaemia
b. Anaphylaxis, urticaria, malaise
c. Facial oedema, swelling, urticaria, pruritus, malaise
d. Local swelling, lethargy, pyrexia
e. Anaphylaxis, facial oedema, urticaria

3. The window of susceptibility:
a. Is the time during which a puppy has enough maternal antibodies to prevent effective vaccination but too few to prevent infection with virulent virus
b. Usually occurs when a puppy is between 6 and 8 weeks old
c. Lasts longer if the dam has high antibody titres
d. Occurs at the same time in all puppies of a litter
e. Occurs at the same time for all antigens.

4. Which statement is FALSE?
a. Primary vaccinations seroconvert most vaccinated animals
b. Boosters against core antigens seroconvert a minority of animals
c. Killed vaccines are usually safe in pregnant or immunosuppressed animals
d. Modified live vaccines are generally more stable and more tolerant to breaks in the cold chain than killed ones
e. Modified live vaccines multiply in the host resulting in a primary as well as a secondary adaptive immune response

5. Which is FALSE regarding the new SAVC guidelines?
a. They should be applied uniformly throughout South Africa: if we have different protocols for different pets we'll just have confused owners
b. They should be used as a guideline; a vaccination protocol should be adapted for each individual pet taking into consideration their specific disease exposure and concurrent problems.
c. Only vaccine brands that have challenge studies showing that they protect vaccinated dogs for at least 3 years should be used in this manner.
d. The booster 1 year after the primary course is essential to seroconvert those animals that did not respond to the puppy vaccinations.
e. All of the above

6. Canine core antigens are:
a. Distemper, hepatitis, parvo, parainfluenza, *Leptospira* and rabies
b. Distemper, hepatitis, parvo and rabies
c. Distemper, hepatitis, parvo, parainfluenza, coronavirus, *Leptospira* and rabies
d. Distemper, hepatitis, parvo, parainfluenza, *Bordetella*, *Leptospira* and rabies
e. Distemper, hepatitis, parvo, *Babesia* and rabies

7. Which is TRUE regarding titre testing?
a. All animals with low antibody titres are susceptible to infection
b. Titres can vary by 2 dilutions in either direction of the result because of vagaries of the test
c. Virus neutralisation is the method of choice for determining CPV titres
d. Titres from the same animal run by different laboratories may be compared with ease
e. Rabies serology can be used to time rabies boosters

8. Seroconversion following vaccination MAY be suboptimal in:
a. Genetically predisposed individuals (Rottweiler, Doberman, German Shepherds (GSD) and American Staffordshire Bullterrier)
b. Animals with high concurrent worm burdens
c. Animals on immunosuppressive therapy
d. Malnourished animals
e. All of the above

9. Titre testing is indicated:
a. In all patients
b. To prove seroconversion in known poorly responding breeds
c. In patients that have previously had a vaccine reaction
d. In patients with very concerned owners
e. B, C and D

10. Indicate which statement is FALSE: When purchasing vaccines for my practice I should:
a. Insist on seeing data published in a peer-reviewed journal proving the vaccine's efficacy
b. Assume that data presented in marketing material is just that: advertising, not a peer-reviewed study
c. Assume all vaccines are essentially equal
d. Realise that as the main buyer and user of vaccines, I (not the learned academics) have the greatest influence over vaccine quality
e. Check that the data presented on the vaccine marketed by the same manufacturer under the same name overseas is, in fact, the same vaccine being sold to me and not just one with the same name.

SAVC Accredation Code: AC/0305/08
To answer the questions please visit www.vetcpd.co.za and use the Vet CPD web system code: a14784 for this article. Phone 012 346 1150 for assistance.