



Age and Long-term Protective Immunity in Dogs and Cats

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Summary

Vaccination can provide an immune response that is similar in duration to that following a natural infection. In general, adaptive immunity to viruses develops earliest and is highly effective. Such anti-viral immune responses often result in the development of sterile immunity and the duration of immunity (DOI) is often lifelong. In contrast, adaptive immunity to bacteria, fungi or parasites develops more slowly and the DOI is generally short compared with most systemic viral infections. Sterile immunity to these infectious agents is less commonly engendered. Old dogs and cats rarely die from vaccine-preventable infectious disease, especially when they have been vaccinated and immunized as young adults (i.e. between 16 weeks and 1 year of age). However, young animals do die, often because vaccines were either not given or not given at an appropriate age (e.g. too early in life in the presence of maternally derived antibody [MDA]). More animals need to be vaccinated to increase herd (population) immunity. The present study examines the DOI for core viral vaccines in dogs that had not been revaccinated for as long as 9 years. These animals had serum antibody to canine distemper virus (CDV), canine parvovirus type 2 (CPV-2) and canine adenovirus type-1 (CAV-1) at levels considered protective and when challenged with these viruses, the dogs resisted infection and/or disease. Thus, even a single dose of modified live virus (MLV) canine core vaccines (against CDV, cpv-2 and cpv-2) or MLV feline core vaccines (against feline parvovirus [FPV], feline calicivirus [FCV] and feline herpesvirus [FHV]), when administered at 16 weeks or older, could provide long-term immunity in a very high percentage of animals, while also increasing herd immunity.

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Introduction

Age has a profound effect on the development and the decline of the immune system including innate and adaptive components. Clearly, the innate immune system is more mature at birth than the adaptive immune system; however, neither is fully developed and only after several weeks to months of life does the immune system become immunologically mature. The young of all species are dependent on immunity that is passively acquired from the dam. Thus, the very young of all mammalian species are at greater risk of developing disease and these diseases, once

they develop, will often be more severe during the first weeks to months of life than they would be in older, immunologically naïve animals. However, age not only affects the quality of the immune response in young animals, but also impacts on immune function in very old animals. The decline of immunity in older animals ('immunosenescence') may make them more susceptible to certain infectious diseases. Studies on immunosenescence in the dog and cat have suggested a decline in the immune system with age, but the significance of the decline with regard to increased susceptibility, especially to infectious agents, has not been shown (Schultz, 1984; Schultz and Conklin, 1998; Campbell *et al.*, 2004; HogenEsch *et al.*, 2004; Blount *et al.*, 2005; Greeley *et al.*, 2006; Day, 2007).

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Active Immunization

In general, adaptive immunity following vaccination with modified live virus (MLV) vaccines develops earliest and most effectively in that it is often complete (e.g. sterile immunity is induced) and duration of immunity (DOI) is often lifelong. In contrast, adaptive immunity to bacterial, fungal or parasite vaccines develops more slowly, rarely induces sterile immunity and the DOI is generally shorter compared with viral vaccines.

In the studies presented herein, we determined the antibody response that developed after immunizing dogs of different ages to different kinds of 'novel' antigens including sheep red blood cells (SRBCs), multiple *Leptospira* serovars and killed bovine viral diarrhoea virus (BVDV).

Beagle dogs were placed into three groups: animals 5–7 weeks of age ($n = 25$), animals 6–10 months of age ($n = 24$) and animals 7–9 years of age ($n = 20$). They were immunized three times at 2-week intervals and serum was collected at the time of immunization and again 2 weeks after the last dose of antigens was administered. There was no difference in the titre of haemolytic and agglutinating antibody induced after administration of SRBCs to dogs in the different age groups (Fig. 1).

Serum antibody to heterologous bovine and guinea pig red blood cells was also measured and there were no differences based on the age of the dog in the heterogeneity of antibody responsiveness (Fig. 2) in the different age groups. Similarly, when serum antibody specific for ovine, bovine and caprine albumin was measured (by enzyme linked immunosorbent assay; ELISA) in dogs immunized with sheep serum, the responses were again very similar among the age groups (Fig. 3).

The dogs were also vaccinated with a bovine leptospirosis vaccine, which included the serovars *canicola*,

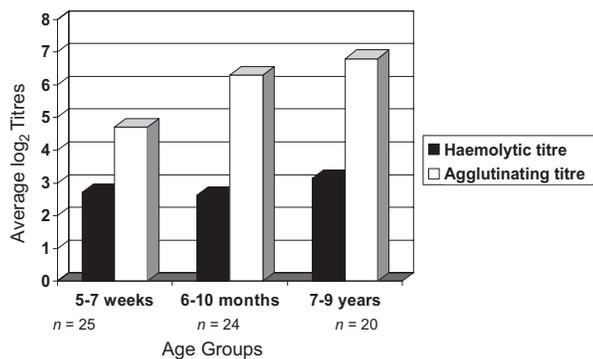


Fig. 1. Haemolytic and agglutinating antibody titres to sheep red blood cells (SRBCs) in dogs injected three times with SRBC at 2 week intervals.

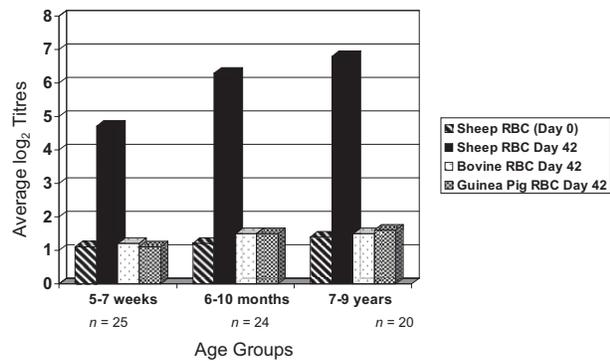


Fig. 2. Agglutinating antibody titre to ovine, bovine and guinea pig red blood cells (RBCs) in dogs injected with sheep RBCs three times at 2-week intervals. Serum was collected 2 weeks after the final injection.

grippityphosa, *hardjo*, *icterohaemorrhagiae* and *pomona*. Apart from *hardjo*, which is never used in canine vaccines, the same four serovars are used in a commercially available canine leptospirosis vaccine that is available in the USA. Serum from the vaccinated dogs was tested for the presence of antibody to each of the five vaccine serovars, as well as to *bratislava* and *autumnalis* (two serovars not found in canine vaccines.) The microscopic agglutination test (MAT) showed an age-related difference: serum from the youngest dogs, which had not been given leptospira antigen prior to the study, showed no cross-reaction with *bratislava* (Fig. 4). However, serum from these young animals showed excellent cross-reactivity to *autumnalis*. The dogs of the 6–10-month-old age group, which had been vaccinated several months previously with the four component canine vaccine, showed cross-reaction against *bratislava* and *autumnalis*, as did the dogs of the mature age group, which had received several booster vaccinations during their lives with the four component canine vaccine. All age groups showed similar antibody responses to the five serovars of *Leptospira* in the

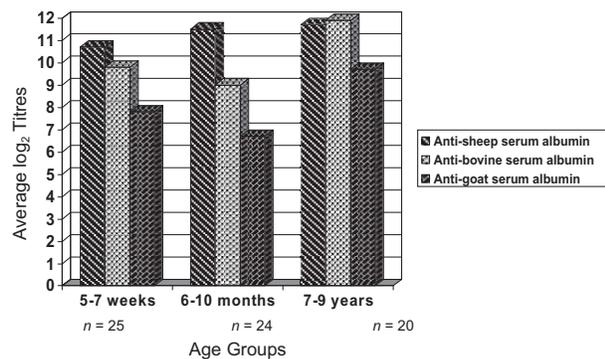


Fig. 3. Serum antibody titres to sheep, bovine and goat serum albumin in dogs immunized with sheep serum. Titres are determined by ELISA.

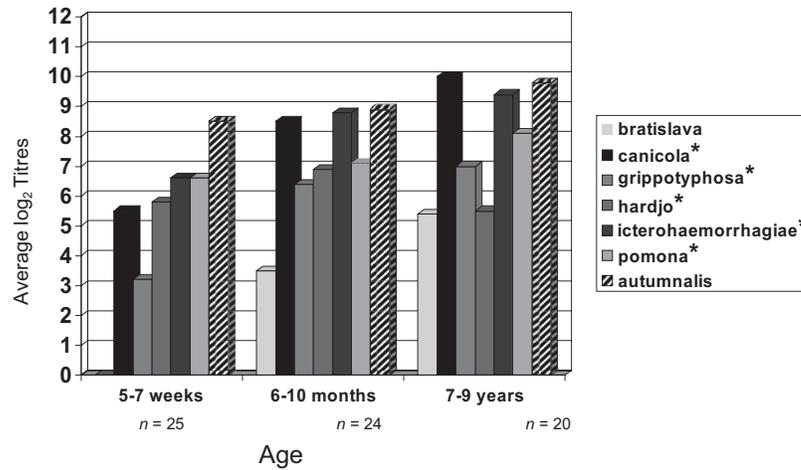


Fig. 4. Anti-*Leptospira* antibody titres against various serovars as detected by the microagglutination test (MAT) in dogs immunized with the bovine five component *Leptospira* vaccine. Those serovars included in the vaccine are indicated (*).

vaccine (Fig. 4). Regardless of age, the responses were similar and an anamnestic response was not apparent.

It would seem that the long-term immune memory for an immunoglobulin (Ig) E (type I hypersensitivity) response, as determined by skin tests, to the *Leptospira* antigens lasted for over 4 years in older dogs, while the *Leptospira* antigens only induced a short term IgG/IgM antibody response, as determined by MAT. The IgG antibody is believed to provide protective immunity against leptospirosis.

The dogs were also vaccinated with killed BVDV type 1, but no antibodies were produced by dogs in

any of the age groups. This would obviously not have been the case if calves had been vaccinated instead of dogs and it is not clear why the dogs failed to respond to this antigenic stimulus.

An earlier study demonstrated no association between age and blood lymphocyte response to stimulation with phytohaemagglutinin (PHA) in dogs of different ages (Schultz, 1984; Fig. 5). Similar studies of lymphocyte responses to mitogens by other groups reported no significant change with increasing age or showed a significant decline in response with age. However, this decline appeared to relate to the method

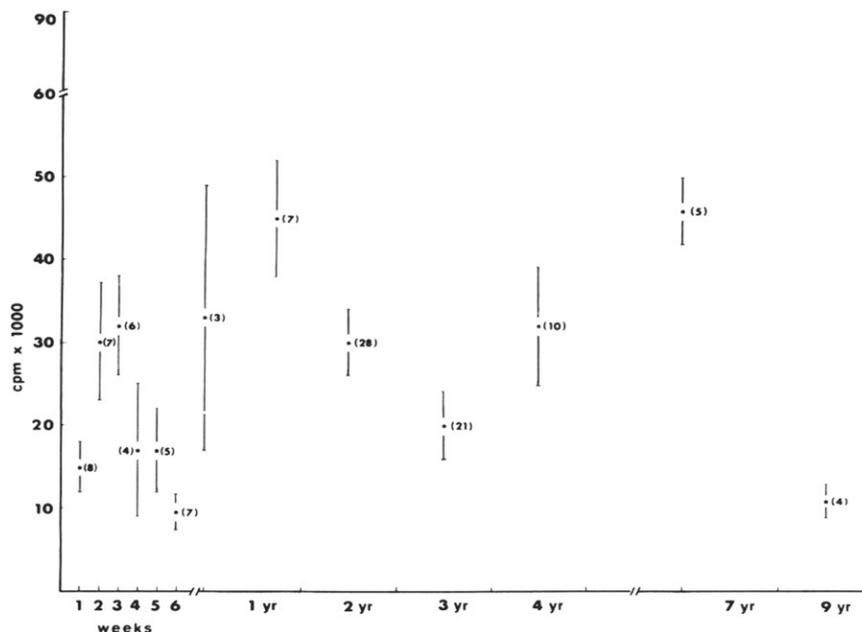


Fig. 5. Lymphocyte blastogenesis test response to phytohaemagglutinin in dogs of different ages (Schultz, 1984).

Table 1
Immunity as defined by antibody persistence after vaccination for canine distemper virus (CDV), canine adenovirus type-1 (CAV-1) and canine parvovirus type 2 (CPV-2)

Environment	Pathogen	DOI (antibody persistence) in years	Vaccine used
Not virus-free	CDV	14	CDV modified live virus (MLV)
Not virus-free	CAV-1	14	CAV-1/2 MLV
Not virus-free	CPV-2	10	CPV-2 MLV
Free of CDV, CPV-2	CDV	9	CDV MLV
Free of CDV, CPV-2	CDV	5*	CDV canarypox recombinant
Free of CDV, CPV-2	CPV-2	9	CPV-2 MLV

*Study still ongoing.

used to express the results. When a stimulation index (SI; i.e. the counts per minute [CPM] obtained from cultures of stimulated cells divided by CPM of control unstimulated cells) was used, a significant decline in response was seen. In contrast, when the mean difference in CPM between stimulated and control cells was used, as in the present study, a significant decline with age was not reported (Schultz, 1984; HogenEsch *et al.*, 2004; Blount *et al.*, 2005; Greeley *et al.*, 2006).

Duration of Immunity

An ideal means of estimating the DOI that could be expected from a vaccine would be to determine the DOI that develops after natural immunization (e.g. recovery from natural infection/disease). It is likely

that an effective MLV vaccine will provide a DOI similar to that following natural infection, but it is very unlikely that a vaccine will provide a longer DOI than would be achieved following natural infection. The DOI may persist via immunological memory involving B and T lymphocytes, long-lived plasma cells that continue to produce antibodies for several years ('memory effector B cells') and possibly long-lived 'memory effector T cells' (Schultz and Conklin, 1998).

When dogs recover from natural infection/disease due to CDV, CAV-1 or CPV-2, they develop life-long immunity to these diseases. Long-term immunity also develops in cats that have recovered from infection by FPV. Although immunity to the other core feline viruses (FCV and FHV) is less effective (no sterile immunity), immunity from severe disease does persist in most pet cats for years after vaccination (Scott and Geissinger, 1999; Schultz, 1998).

In our studies (Schultz 2006) we have examined the persistence of antibodies in vaccinated dogs kept in natural as well as virus-free environments. The longest period of time after initial vaccination that dogs were sampled and that antibody was found to persist was 14 years for CDV (vaccination with MLV), 14 years for CAV-1 (MLV against CAV-1 or CAV-2) and 10 years for CPV-2 (MLV) (Table 1). Similar studies have been reported in the cat (Scott and Geissinger, 1999).

In environments free from CDV and CPV-2, we have not been able to keep dogs for longer than 9 years, thus the minimum DOI as defined by antibody persistence was 9 years for CDV (MLV), CAV-1 (MLV) and CPV-2 (MLV). In the same environment, the minimum DOI measured thus far for the canarypox

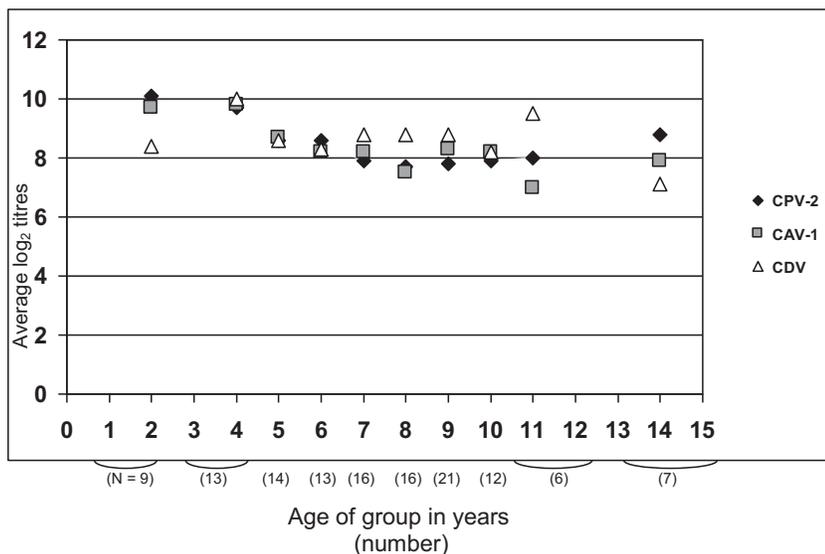


Fig. 6. The effect of age on antibody titre to canine parvovirus type 2 (CPV-2), canine adenovirus type-1 (CAV-1) and canine distemper virus (CDV).

Table 2
Dogs vaccinated against canine distemper virus (CDV) and canine parvovirus type 2 or 2a (CPV-2, -2a) and then challenged with CDV (intravenous) and CPV-2c or -2b (intranasal/oral)

<i>Challenge viruses</i>	<i>Number of dogs per group</i>	<i>Years since last vaccine given (average)</i>	<i>Type of CPV-2 vaccine component</i>	<i>CPV titre at PC Day 0 (average log₂)</i>	<i>CDV titre at PC Day 0 (average log₂)</i>	<i>Age at challenge in years: range and (average)</i>	<i>Outcome (% protection)</i>
CDV-SH, CPV-2b	10	4.5	CPV-2	6.3	6.6	4–8 (6.2)	100
CDV-SH, CPV-2c	10	5.5	CPV-2	7.5	8.4	5–9 (6.8)	100
CDV-SH, CPV-2c	10	5.9	CPV-2a	7.8	8.3	7–8 (7.3)	100
CDV-SH, CPV-2c	10	4.8	CPV-2	8.2	5.1	5–9 (6.8)	100

SH, Synder Hill strain; PC, post challenge.

recombinant virus-vectored CDV vaccine is 5 years (Larson and Schultz, 2007). These results suggest that the presence of overt antigenic stimulation may not be necessary for the persistence of immunological memory or serum antibody (Schultz and Conklin, 1998; Schultz, 1998, 2006).

Dogs maintained in a CDV and CPV-2 free environment were also shown to resist challenge at 9 years post-vaccination. The serum antibody levels in these dogs had decreased over time, but not significantly. However, when challenged all animals were completely protected regardless of antibody titre pre-challenge. In contrast, dogs that were not vaccinated and had no specific serum antibodies and were kept in the same environment as the vaccinated dogs, were susceptible to infection. These control animals shed virus and/or developed disease and/or died (Schultz, 2006).

Another study demonstrated that dogs that had been vaccinated once as puppies (at a time when they no longer had maternally derived antibodies [MDA]) and which were kept in an environment free of CDV, CAV-1/2 and CPV-2, maintained antibodies for at least 4.5 years and when challenged, were completely protected (Abdelmagid *et al.*, 2004). A study that measured the antibody titres in dogs of different ages (2–14 years old, $n = 127$) showed that there was no significant difference according to age in the antibody response to CPV-2, CAV-1 and CDV (Fig. 6; Schultz, 2006).

Thus, all studies based on persistence of antibody as well as challenge show that immunity to CDV, CPV-2 and CAV-1 persists for a lifetime after vaccination, similar to the persistence of immunity after natural infection (Schultz, 2006).

The Case of Canine Parvovirus

In view of the number of variants of canine parvovirus that are now described, the question arises as to whether older dogs vaccinated at an early age maintain protective immunity to all the variants of CPV-2 that are currently circulating (CPV-2a, b, c). Our

studies have shown that vaccination with any one of these variants provides cross-protection against the others (Table 2). Dogs that were vaccinated with CDV and CPV-2 or CPV-2a and then challenged 4–9 years later with both CDV (by intravenous injection) and CPV-2c or -2b (by administration intranasally and *per os*) showed 100% protection (Table 2). This study indicates that the CPV-2 variant used to vaccinate does not affect the minimum DOI (Larson and Schultz, 2008).

Protection and the Level of Antibodies

Antibody titre as it relates to protective immunity for CDV, CPV-2 and CAV-1 is of importance in passively immunized (unvaccinated dogs with MDA) dogs (Table 3). In contrast to passively immune pups, in actively immunized pups (either following natural or vaccine-induced immunization) the actual titre of antibody is not of importance, as long as the titre is detectable (Table 4). Actively immune dogs will develop an innate and a rapid anamnestic humoral and cell-mediated response, thus will be protected from infection and/or disease. The presence of antibodies, regardless of titre, in these dogs demonstrates protective immunity (Schultz, 1998, 2006; Schultz and Conklin, 1998).

Table 3
Minimum protective antibody titre required in passively immune dogs to protect against experimental challenge with canine distemper virus (CDV), canine adenovirus type-1 (CAV-1) and canine parvovirus type 2 (CPV-2)

<i>Challenge</i>	<i>Virus</i>	<i>Antibody titre: range and (mean)</i>	<i>Test</i>
IV/IN	CDV	16–64 (32)	VN
IV	CAV-1	32–128 (64)	VN
IN/oral	CPV-2	80–320 (160)	HI

IV, intravenous; IN, intranasal; VN, virus neutralization; HI, haemagglutination inhibition.

Table 4
Protective antibody titre required in actively immune dogs to protect against experimental challenge with canine distemper virus (CDV), canine adenovirus type-1 (CAV-1) and canine parvovirus type 2 (CPV-2)

Challenge	Virus	Antibody titre: range and (mean)	Test
IV/IN	CDV	4–16 (8)	VN
IV	CAV-1	2–8 (4)	VN
IN/oral	CPV-2	10–40 (20)	HI

IV, intravenous; IN, intranasal; VN, virus neutralization; HI, haemagglutination inhibition.

Conclusions

Based on experimental studies that have been ongoing since the 1970s, in which large numbers of vaccinated animals were challenged and/or tested for the titre of serum antibody, in addition to observations in the field, in particular in animal shelters experiencing outbreaks of CDV and/or CPV-2, it may be concluded that:

- Old dogs and cats do not die from vaccine-preventable infectious diseases. It is rare to see an old dog die from distemper, canine parvovirus or infectious canine hepatitis (CAV-1), unless it has never been vaccinated.
- Unlike elderly people, who often die from respiratory disease complex (i.e. pneumonia), old dogs and cats rarely die from canine/feline respiratory disease complex.
- In contrast to old dogs and cats, many younger dogs and cats do die from vaccine-preventable disease because they are not vaccinated or were not vaccinated at an appropriate age (i.e. at or after 16 weeks of age) or with effective vaccines.
- In spite of the relatively high percentage of vaccinated pets in the USA, only an estimated 25% of cats and 50% of dogs are ever vaccinated.
- Only one dose of the modified-live canine ‘core’ vaccine (against CDV, CAV-2 and CPV-2) or modified-live feline ‘core’ vaccine (against FPV, FCV and FHV), when administered at 16 weeks or older, will provide long lasting (many years to a lifetime) immunity in a very high percentage of animals (Schultz, 1998, 2000, 2006).
- Two doses of the core rabies vaccines given 3–4 weeks apart are likely to provide many years of immunity in both cats and dogs (Schultz *et al.*, 1977).
- If we wish to enhance the herd (population) immunity, we need to provide vaccination for animals that never see a veterinarian. When the percentage of vaccinated dogs or cats reaches or exceeds 50%, herd immunity will help protect many of the un-

vaccinated animals (domestic and wild) that are susceptible to the core diseases.

- It is strongly recommended that current vaccination guidelines for dogs and cats be followed whenever possible (Day *et al.*, 2007).

Conflict of Interest

The first author was an invited speaker at the Meril European Comparative Vaccinology Symposium and received travel expenses and an honorarium for this presentation.

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