PAPER

Long-lived immunity to canine core vaccine antigens in UK dogs as assessed by an in-practice test kit

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Objectives: **To determine the utility of an in-practice test kit to detect protective serum antibody against canine distemper virus, canine adenovirus and canine parvovirus type 2 in a sample of the UK dog population.**

Materials and Methods: **Serum samples from 486 dogs, last vaccinated between less than 1 month and 124 months previously, were tested with the VacciCheck™ test kit for protective antibodies against distemper, adenovirus and parvovirus type 2.**

Results: **A high proportion of the dogs tested (93·6%) had protective antibody against all three of the core vaccine antigens: 95·7% of the dogs were seropositive against canine distemper virus, 97·3% against canine adenovirus and 98·5% against canine parvovirus type 2. The small number of dogs that were seronegative for one or more of the antigens ($n = 31$) may have had waning of previous serum antibody or may have been rare genetic non-responders to that specific antigen.**

Clinical Significance: **UK veterinarians can be reassured that triennial revaccination of adult dogs with core vaccines provides long-lived protective immunity. In-practice serological test kits are a valuable tool for informing decision-making about canine core revaccination.**

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INTRODUCTION

Guidelines for the vaccination of dogs developed by the World Small Animal Veterinary Association (WSAVA) Vaccination Guidelines Group and the American Animal Hospital Association (AAHA) define the canine core vaccines as those that protect against infection by the canine distemper virus (CDV), canine adenovirus (CAV) and canine parvovirus type 2 (CPV2) (Welborn *et al.* 2011, Day *et al.* 2016; www.aaha.org/guidelines/canine_vaccination_guidelines.aspx). Both sets of guidelines recommend that revaccination of adult dogs with a combination of modified live virus core vaccines from any of the major international manufacturers be done no more frequently than every 3 years; this advice is now entirely consistent with the minimum licensed duration of immunity (DOI) for canine core vaccines marketed in the UK and many other countries.

Although canine core vaccines are now administered routinely to adult dogs at triennial intervals, it is clear that these modified live virus vaccines confer protection for considerably longer than 3 years. The presence of serum antibody against CDV, CAV and CPV2 is a robust correlate of protection for these vaccines such that seropositive dogs are deemed protected against infection by these viruses. Numerous serological studies have demonstrated the long-lived persistence of vaccine-induced antibody and, consequently, immune protection, for up to 15 years after last administration of a core vaccine (McCaw *et al.* 1998, Twark & Dodds 2000, Bohm *et al.* 2004, Mouzin *et al.* 2004, Ottiger *et al.* 2006, Schultz 2006, Schultz *et al.* 2010, Taguchi *et al.* 2011,

Mitchell *et al*. 2012). Moreover, experimental challenge studies with CDV and CPV2 demonstrate a minimum DOI for canine core vaccines of 9 years (Schultz 2006, Schultz *et al*. 2010).

The correlation between seropositivity for CDV, CAV and CPV2 and immune protection is so strong that the latest iteration of WSAVA guidelines supports the use of triennial serological testing in lieu of automatic core revaccination every three years (Day *et al*. 2016). The guidelines also suggest that as a precautionary measure, for geriatric dogs (aged over 10 years), such serological testing should be performed annually. Until recently, determination of serological protection for these core vaccine antigens relied on the 'gold standard' virus neutralisation (for CDV and CAV) or haemagglutination inhibition (for CPV2) tests as performed in specialist diagnostic laboratories. More recently, in-practice test kits have become available. These can rapidly, and sometimes semi-quantitatively, determine the presence of serum antibody against canine core vaccine antigens. These kits are developed to give a positive reading that correlates with a minimum protective titre as determined by the gold standard tests, and some have been validated independently, with supportive evidence published in the scientific literature (Waner *et al*. 2006, Belsare *et al*. 2014).

Only one study, published in 2004, has examined the persistence of immunity to canine core vaccines in UK dogs (Bohm *et al*. 2004). This investigation utilised gold-standard tests to test sera from 144 dogs that had not been vaccinated for between 3 and 15 years. Protective antibody concentrations against CDV, CAV and CPV2 were present in 71·5%, 82% and 95% of the population, respectively. Given the changes in advice regarding core revaccination and serological testing since that time (Heayns & Baugh 2012), and the more recent availability of in-practice test kits, the aim of the present study was to use such a kit to determine current levels of seroprotection in a sample of the UK dog population.

MATERIALS AND METHODS

The present study is based on the analysis of data accumulated by a large UK veterinary practice group (Medivet, Watford, Hertfordshire) and veterinary practice (Hyde Park Veterinary Centre, London), which had been using in-practice core vaccine serological testing over a seven-year period (2009 to 2016). Both practice groups use the same brand of canine core vaccine and both routinely employ the Canine VacciCheck™ (Biogal Laboratories, Kibbutz Galed, Israel) test kit to inform decisions about core revaccination of dogs.

The VacciCheck™ test kit uses a dot-ELISA-based system to provide a semi-quantitative assessment of the concentration of antibodies against proprietary CDV, CAV and CPV2 antigens. The canine VacciCheck™ test kit has been approved by the US Food and Drug Administration (2011), the Canadian Food Inspection Agency (2013) and the Japanese Ministry of Agriculture, Food and Fisheries (2017).

A protective level of antibody is defined by this test kit as a reaction (coloured spot) calibrated to an antibody titre obtained using the gold-standard methods of haemagglutination inhibition (for CPV2) or virus neutralisation (for CDV and CAV). For CPV2 and CDV, a protective titre with the VacciCheck™ kit is defined as 80, and for CAV, a protective titre is considered to be 40 (Waner *et al*. 2006, Mazer *et al*. 2009). For the purposes of this study, we recorded each animal as being either seropositive or seronegative (*i.e.* having antibody above a gold-standard minimum titre of 20 as defined by the VacciCheck™ kit) as recommended by WSAVA guidelines (Day *et al*. 2016). WSAVA guidelines state that the simple presence of antibody is more important than the titre of that antibody (Day *et al*. 2016). This advice is based on the fundamental immunological principle that the presence of an antigen-specific antibody necessitates the presence of long-lived memory B lymphocytes and plasma cells and antigen-specific helper T lymphocytes and the ability to mount a rapid and effective secondary (anamnestic) immune response on challenge. It is also well-recognised that defining protective titres is challenging (Chen *et al*. 2013) and that inter-laboratory variation occurs in test methodology and definition of the protective titre (Greene & Levy 2012). For example, protective titres against CDV and CPV2 are defined by one testing laboratory as greater than 8 or greater than 20, respectively, consistent with the levels applied in the present study (www.vetmed.wisc.edu/lab/cavids/).

Age, breed and gender of each animal was recorded, together with full vaccination history and any relevant medical history. For many of the dogs, results of sequential serological tests performed over time were available. A total of 486 dogs were included in the study.

RESULTS

The population of 486 dogs was aged between 3 months and 19 years. There was a wide range of breeds, with no particular breed being over-represented in the population. The population included 56·6% male and 42·8% female dogs, with gender not recorded in 0·62% of the records. Collectively, 61·9% of dogs where gender was recorded were neutered, and 31·8% were entire. The dogs had last received a core vaccine between less than 1 month and 124 months previously. Figs 1–3 show the number of dogs that were seropositive or seronegative for CDV, CAV and CPV2, respectively, broken down by months elapsed since the last core vaccination. The majority of the dogs had last been revaccinated up to 42 months previously, with fewer animals having longer intervals since last revaccination.

Overall, 93·6% of this population was seropositive to all three of the infectious agents when last vaccinated up to 124 months previously. Only 31 dogs (6·4%) were seronegative for one or more of the core vaccine antigens. These dogs were of 16 different pure breeds or were crossbred dogs. Of these 31 dogs, 21 (4·3% of the total population of 486 dogs) lacked serum antibody to CDV, 13 of the 31 dogs (2·6% of the total population of 486 dogs) lacked serum antibody to CAV, and 8 of the 31 dogs (1·6% of the total population of 486 dogs) lacked serum antibody to CPV2. Three dogs (0·6% of the total population of 486 dogs) lacked serum antibody to all three vaccine antigens. Of 100 dogs that had last been vaccinated more than 3 years previously,

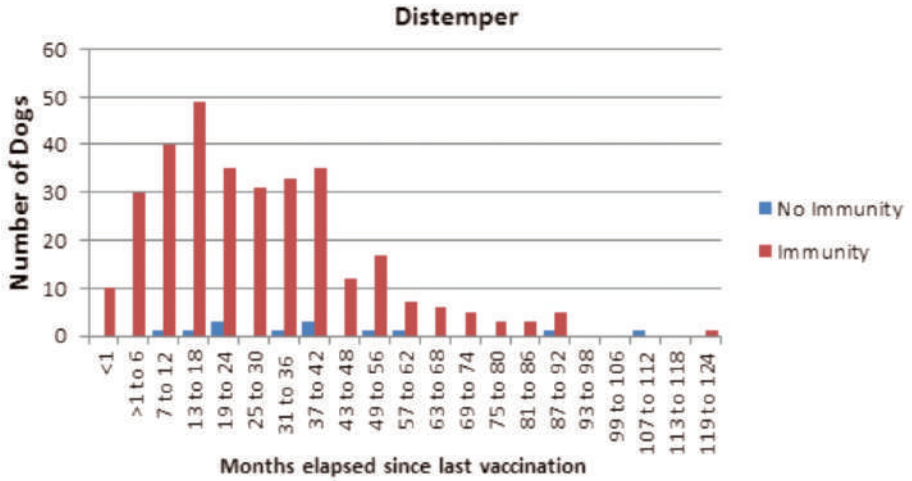

FIG 1. Number of dogs seropositive or seronegative for CDV *versus* months elapsed since last vaccination

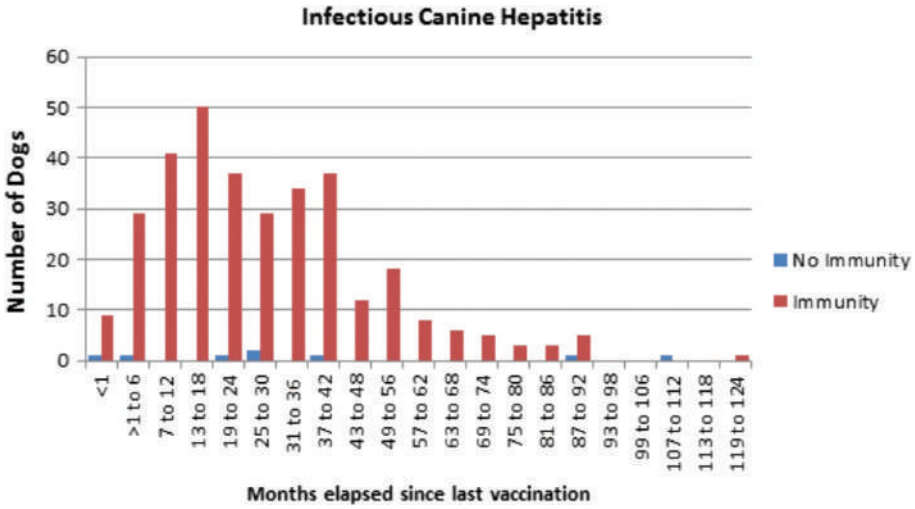

FIG 2. Number of dogs seropositive or seronegative for CAV *versus* months elapsed since last vaccination

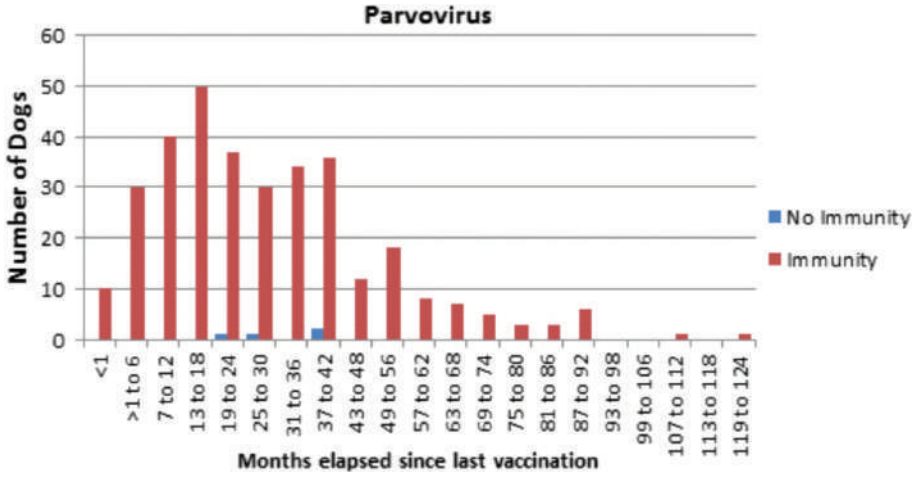

FIG 3. Number of dogs seropositive or seronegative for CPV2 *versus* months elapsed since last vaccination

93 animals (93%) were seropositive to all three core vaccine antigens. Of the 31 dogs with a one or more negative results, four had ongoing diseases at the time of serological testing (inflammatory enteropathy, pancreatitis, epilepsy and syringomyelia), while the remaining 27 animals were healthy and had been presented for a routine health check.

DISCUSSION

The present study provides the first evaluation of the longevity of canine core vaccine immunity in UK dogs since the 2004 study of Bohm *et al.* (2004). During the 13-year period since publication of that study, UK canine core vaccines have been licensed with a minimum DOI of 3 years, and a number of practice groups have begun to implement in-practice serological testing to inform decision-making about canine core revaccination (Heayns & Baugh 2012).

The results of this study compare favourably to those of Bohm *et al.* (2004). In that investigation, the percentages of a population of 144 adult dogs that were protected against CDV, CAV and CPV2 were 71·5%, 82% and 95%, respectively. The comparable figures for our study of 486 dogs demonstrate protection against CDV, CAV and CPV2 of 95·7%, 97·3% and 98·5%, respectively. The two studies differ with respect to the testing methodology (gold standard *versus* in-practice test kit) and definition of protection [achieving specific titres of 64 (CDV and CAV) or 128 (CPV2) *versus* being seropositive (any titre >20)], which may account for the variation observed. In both studies, the highest rates of seroprotection were for CPV2; it is possible that at least some of this is accounted for by field exposure to the virus (*i.e.* 'natural boosting' of the immune response), but it is less likely that such field exposure accounted for seropositivity to CDV and CAV. Overall, it would seem that the introduction of triennial canine core revaccination has not impacted seroprotection and 'herd immunity' against these diseases in the UK dog population. Moreover, the study provides further evidence for the long-lived protective immunity induced by canine core vaccines, with serological protection demonstrated for up to 142 months after last revaccination.

The results of the present study also clearly support the use of in-practice serological testing to monitor vaccine protection and inform decision making about core revaccination in the dog. The relatively small number of vaccinated dogs that did not have protective antibody against one or more core virus antigens is of note and justifies the use of serology to detect and monitor such animals. There are a number of reasons that may account for these seronegative dogs within the population. The most likely reason is that these individual dogs, for some unexplained reason, had waning of serum antibody on the occasion of testing. These animals may well have responded adequately to past vaccination (and therefore have immunological memory) and would be likely to seroconvert following revaccination. It is also possible that the concurrent medical illness in four of the 31 seronegative dogs may have impacted immune function and impaired antibody production. However, such dogs may still have had immunological memory of exposure to core vaccine antigens. There is little formal evidence that critical illness can affect vaccine-induced protection, although one recent study reported that dogs hospitalised in an intensive care unit were less often seropositive to CDV and CPV2 than expected from studies of healthy populations (Mahon *et al.* 2017). Of the 31 seronegative dogs in the present study, 27 were healthy animals presented for a routine health check. The majority of the 455 seropositive dogs in the study were also apparently healthy animals, but we cannot discount the possibility that a proportion of this group may have been affected by clinical or subclinical disease. Alternatively, some of these seronegative dogs may have been rare examples of true genetic non-responders, who simply lack the ability to respond immunologically to specific vaccine antigens. It is estimated that up to one in 1000 dogs may be a genetic non-responder to CPV2, and one in 5000 dogs may fail to respond to the CDV antigen (Day *et al.* 2016). It is well recognised, for example, that dogs of the Rottweiler breed are more often likely to be non-responders to CPV2 or the rabies vaccine (Houston *et al.* 1996, Kennedy *et al.* 2007). Analysis of the breeds of seronegative dogs in the present study did not demonstrate over-representation of any one breed, and none of the dogs were Rottweilers. Finally, it is also possible that some of these animals may have had false-negative results; the VacciCheck™ test kit has reported sensitivity of 88% to 100% (*i.e.* how well the test can identify seropositive dogs in a population of dogs defined as seropositive using the gold standard) and specificity of 92% to 100% (*i.e.* how well the test discriminates seronegative dogs in a population of dogs defined as seronegative using the gold standard) for the three different antigenic components (manufacturer's data). The reported ranges in sensitivity and specificity relate to validation studies performed with samples collected in three successive years (manufacturer's data).

Current recommendations state that an animal testing seronegative for one of the three core viral antigens (truly negative or false negative) should be revaccinated and subsequently re-tested to determine whether seroconversion has occurred. Failure of an adult dog to seroconvert on this occasion may indicate genetic non-responder status for that specific antigen. The absence of serum antibody does not necessarily imply the absence of an immune response or the absence of immunological memory. Cell-mediated immunity may still be present, but it is not possible or practical to routinely test for cellular responses to canine vaccine antigens. Cellular testing has been reported experimentally for feline vaccinal immune responses but is more relevant for cats where protection against feline calicivirus and feline herpesvirus type 1 does not correlate well with the presence of serum antibody (Vermeulen *et al.* 2012).

The VacciCheck™ test will not distinguish those animals that may potentially show a false positive reaction for one or more of the three antigens tested. A true false-positive result may be interpreted to suggest that the dog has cross-reactive antibody specific for an antigen unrelated to the virus in question; however, to our knowledge, there are no studies that explore the nature of false-positive reactions in such test systems. Canine core vaccines are almost always delivered as a three-component combination and, as shown by the results of the present study, any lack of response

(including false-negative or false-positive reactions) will generally be related to only one of the component antigens.

Overall, the data presented here should reassure UK veterinarians that the currently licensed UK canine core vaccines provide a high level of long-lived protective immunity in vaccinated dogs. Serological testing, such as that undertaken with in-practice kits, can readily identify the rare individual dogs who may have not responded to a specific vaccine antigen. Such animals might be revaccinated and re-tested or re-tested using a gold-standard procedure; however, failure to seroconvert after repeated vaccination most likely indicates that the animal is a genetic non-responder to that specific vaccine component and may therefore be at risk of infection.

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Conflict of interest

The authors declare no conflict of interest with respect to publication of this manuscript. MJD is Chairman of the WSAVA Vaccination Guidelines Group (VGG), which is sponsored by MSD Animal Health. The VGG is an independent group of academic experts who formulate guidelines without consultation with industry. Representatives of the sponsoring company do not attend VGG meetings, and the company does not have the right of veto over VGG recommendations.

References

Belsare, A. V., Vanak, A. T. & Gompper, M. E. (2014) Epidemiology of viral pathogens of free-ranging dogs and Indian foxes in a human-dominated landscape in central India. *Transboundary and Emerging Diseases* **61**(Suppl. 1), 78-86

Bohm, M., Thompson, H., Weir, A., *et al*. (2004) Serum antibody titres to canine parvovirus, adenovirus and distemper virus in dogs in the UK which had not been vaccinated for at least three years. *Veterinary Record* **154**, 457-463

Chen, X., Bailleux, F., Desai, K., *et al*. (2013) A threshold method for immunological correlates of protection. *BMC Medical Research Methodology* **13**, 29

Day, M. J., Horzinek, M. C., Schultz, R. D., *et al*. (2016) Guidelines for the vaccination of dogs and cats. *Journal of Small Animal Practice* **57**, E1-E45

Greene, C. E. & Levy, J. K. (2012) Immunoprophylaxis. In: Infectious Diseases of the Dog and Cat. 4th edn. Elsevier, St. Louis, MO, USA. pp 1163-1205

Heayns, B. J. & Baugh, S. (2012) Survey of veterinary surgeons on the introduction of serological testing to assess revaccination requirements. *Veterinary Record* **170**, 74-78

Houston, D. M., Ribble, C. S. & Head, L. L. (1996) Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982-1991). *Journal of the American Veterinary Medical Association* **208**, 542-546

Kennedy, L. J., Lunt, M., Barnes, A., *et al*. (2007) Factors influencing the antibody response of dogs vaccinated against rabies. *Vaccine* **25**, 8500-8507

Mahon, J. L., Rozanski, E. A. & Paul, A. L. (2017) Prevalence of serum antibody titers against canine distemper virus and canine parvovirus in dogs hospitalized in an intensive care unit. *Journal of the American Veterinary Medical Association* **250**, 1413-1418

Mazer, S., Larson, L. & Lavi, Y. (2009) Sensitivity–specificity–accuracy and difference between positive and negative mean results of the ImmunoComb® Canine VacciCheck antibody test kit for canine distemper, parvo and adenovirus. Bio-Gal Company data. http://vaccicheck.com/about-vaccicheck/ [accessed 6 September 2017]

McCaw, D. L., Thompson, M., Tate, D., *et al*. (1998) Serum distemper virus and parvovirus antibody titers among dogs brought to a veterinary hospital for revaccination. *Journal of the American Veterinary Medical Association* **213**, 72-75

Mitchell, S. A., Zwijnenberg, R. J., Huang, J., *et al*. (2012) Duration of serological response to canine parvovirus-type 2, canine distemper virus, canine adenovirus-type 1 and canine parainfluenza virus in client-owned dogs in Australia. *Australian Veterinary Journal* **90**, 468-473

Mouzin, D. E., Lorenzen, M. J., Haworth, J. D., *et al*. (2004) Duration of serologic response to five viral antigens in dogs. *Journal of the American Veterinary Medical Association* **224**, 55-60

Ottiger, H.-P., Neimeier-Forster, M., Stark, K. D. C., *et al*. (2006) Serological responses of adult dogs to revaccination against distemper, parvovirus and rabies. *Veterinary Record* **159**, 7-12

Schultz, R. D. (2006) Duration of immunity for canine and feline vaccines: a review. *Veterinary Microbiology* **117**, 75-79

Schultz, R. D., Thiel, B., Mukhtar, E., *et al*. (2010) Age and long-term protective immunity in dogs and cats. *Journal of Comparative Pathology* **142**(Suppl. 1), S102-S108

Taguchi, M., Namikawa, K., Maruo, T., *et al*. (2011) Antibody titers for canine parvovirus type-2, canine distemper virus, and canine adenovirus type-1 in adult household dogs. *Canadian Veterinary Journal* **52**, 983-986

Twark, L. & Dodds, W. J. (2000) Clinical use of serum parvovirus and distemper virus antibody titers for determining revaccination strategies in healthy dogs. *Journal of the American Veterinary Medical Association* **217**, 1021-1024

Vermeulen, B. L., Gleich, S. E., Dedeurwaerder, A., *et al*. (2012) In vitro assessment of the feline cell-mediated immune response against feline panleukopeniavirus, calicivirus and felid herpesvirus 1 using 5-bromo-2′-deoxyuridine labelling. *Veterinary Immunology and Immunopathology* **146**, 177-184

Waner, T., Mazar, S. & Keren-Kornblatt, E. (2006) Application of a dot enzyme-linked immunosorbent assay for evaluation of the immune status to canine parvovirus and distemper virus in adult dogs before revaccination. *Journal of Veterinary Diagnostic Investigation* **18**, 267-270

Welborn, L. V., DeVries, J. G., Ford, R., *et al*. (2011) 2011 AAHA canine vaccination guidelines. *Journal of the American Animal Hospital Association* **47**, 1-42