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.
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 kits are developed to give a positive reading that correlates with a minimum protective titre as determined by 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, Mazet 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,
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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-respondor 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...
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(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.

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