WHAT WE NEED TO KNOW ABOUT VACCINATION AND TITRE TESTING

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INTRODUCTION

The delivery of vaccination to dogs and cats has undergone fundamental change in the past decade. In response to questions over vaccine safety, guidelines groups have introduced new vaccination schedules that have been accommodated by vaccine manufacturers introducing products with extended duration of immunity (DOI) and products with fewer antigenic components. The newest advance in vaccinology is the availability of simple in-practice test kits that demonstrate whether an individual animal has serological evidence of protection. These test kits can now inform decision making about vaccination in practice. This presentation briefly reviews currently recommended vaccination schedules and focuses on the potential applications for in-house serological testing.

VACCINATION GUIDELINES

The WSAVA Vaccination Guidelines as endorsed by BSAVA consider vaccines as CORE or NON-CORE. CORE vaccines are those that every dog or cat should receive as they confer protection against diseases that are life threatening or of significant morbidity. Even in developed countries, these diseases have not been eliminated and the occurrence of regional outbreaks indicates the important of maintaining herd immunity through vaccination. The CORE vaccine-preventable diseases of the dog are those induced by canine distemper virus (CDV), canine parvovirus-2 (CPV) and canine adenovirus-1 (CAV). The CORE vaccine-preventable diseases of the cat are those induced by feline parvovirus (FPV), feline calicivirus (FCV) and feline herpesvirus-1 (FHV). WSAVA guidelines encourage use of NON-CORE vaccines after making an appropriate benefit-risk analysis tailored to the lifestyle and risk of exposure of the individual pet. For that reason, in the UK practitioners generally include vaccines for the prevention of leptospirosis as a CORE canine vaccine and may chose to include feline leukaemia virus (FeLV) vaccines in the CORE feline vaccination schedule.

Modified live virus (MLV) or 'infectious' CORE vaccines should be administered to puppies and kittens from 8 – 9 weeks of age, with a second dose given 3 – 4 weeks later and a final dose between 14 – 16 weeks. This change ensures that all pups and kittens can mount at least a primary immune response to vaccination – even when maternally-derived antibody (MDA) persists to 12 weeks of age. Pups and kittens should also receive a booster CORE vaccine either 12 months after the final of the early life series, or at 12 months of age.

Adult dogs and cats should receive MLV CORE vaccines no more frequently than every three years and WSAVA recommendations are also for triennial revaccination with FeLV. In the UK, the majority of canine CORE vaccines on the market now carry a licensed 3-year DOI and at least some FPV vaccines are licensed for triennial use. NON-CORE or 'non-infectious' vaccines other than FeLV must

still be given annually to adult animals where these products are incorporated into an individualized vaccination programme.

The over-arching new concept in vaccination is that vaccines be delivered as one component of an 'annual health check' consultation that addresses all aspects of the health and well being of the individual animal. Vaccination programmes should be tailored to the requirements of the specific pet (individualized medicine) based on thorough assessment of the lifestyle and risk factors of that animal. The majority of UK practitioners will currently administer triennial MLV CORE vaccines to the dog with an annual leptospirosis vaccine. Practitioners will also use a triennial programme for feline CORE vaccines, but where an individual cat is perceived as having higher risk for the feline infectious respiratory disease complex, that cat may receive annual FCV and FHV components, which are available as a separate combination product.

CORRELATES OF PROTECTION

For licensing studies it is still necessary for animals to be used in experimental challenge studies in which vaccinated animals are challenged with the virulent form of a pathogen some time (e.g. 3 or 4 years) after vaccination to demonstrate either sterilizing immunity (failure of the pathogen to infect) or amelioration of clinical disease (infection occurs but the effects are limited). Studies over decades have shown that there are strong correlates of protection in such challenge studies.

For CDV, CPV, CAV and FPV the presence of serum antibody able to neutralize infectious virus and prevent infection and disease provides an extremely strong correlate of protection. This correlation is so strong that it is possible to state that the presence of serum antibody to one of those viruses equates definitively with protective immunity. Some regulatory authorities are now beginning to accept seroprotection rather than experimental challenge in modulating licence claims. The presence of serum antibody does not, however, provide a correlate of immunity for FCV and FHV protection. For respiratory pathogens such as FCV, the presence of mucosal secretory IgA provides a correlate of protection, but it is not possible to measure these antibodies routinely. For FHV there is a stronger correlation between protection and cell-mediated immunity (CMI), but again it is difficult to measure CMI on a routine basis.

GOLD STANDARD TESTS

Traditionally, correlation between challenge immunity and seroprotection has been measured by two gold standard tests for the CORE virus infections. These are the virus neutralization (VN) and haemagglutination inhibition (HAI) tests. A positive VN test indicates that serum from the animal contains antibodies that will neutralize infectious virus particles *in vitro* and prevent them from subsequently producing infection and cell damage (as assessed by cytopathic effect on cells *in vitro*). A positive HAI test indicates that serum from the animal contains antibodies that will bind to infectious virus particles and neutralize the ability of that virus to subsequently cause agglutination of erythrocytes from particular animal species.

There is excellent correlation between a positive VN test and protection for CDV, CPV, CAV, FPV and rabies. There is an excellent correlation between a positive HAI test and protection for CPV and FPV. For FCV the correlation between a positive VN test and protection is considered only good to fair (as secretory IgA provides a better measure) and for FHV the correlation between positive VN test and protection is only fair (as CMI is a better correlate of immunity).

Other serological test methods (e.g. ELISA or IFA based) must in turn be correlated with the gold standard VN or HAI tests. Both of the in-house test systems that will be described below have had such correlation and validation with sera derived from animals in challenge studies tested by the gold standards.

THE CONCEPT OF TITRE

A titre provides a means of measuring the concentration of antibody present in a serum sample. Obtaining a titre involves performing an immunological test in which the serum sample is subject to a series of doubling dilutions (that progressively reduces the antibody concentration). Each serum dilution is tested and the highest dilution that gives an unequivocally positive reaction in the test provides the titre. The titre is the reciprocal of that dilution (e.g. a dilution of 1/10 provides a titre of 10).

Testing laboratories have traditionally provided a titre for serological tests of antibody related to vaccine protection. The actual number may sometimes differ for the same sample tested by different laboratories. In reality, the number is relatively arbitrary as the titre is not a single defined number, but rather represents a range. For example, a sample with a titre of 10 actually indicates that the value is not less than 5 and not more than 20 (one doubling dilution above and below the titre). A sample with a titre of 1280 has a titre that is not less than 640 and not more than 2560. In this regard, a titre of 640 from one laboratory is actually the same as one of 2560 from another testing laboratory.

For this reason, the WSAVA Vaccination Guidelines Group encourages practitioners to consider that any titre (above the cut-off for the gold standard test; typically 20 for HAI and 100 for VN) should be considered positive, and protection in challenge studies is still conferred throughout a wide spectrum of titres. It is also for this reason that the new in-house test kits either provide a simple yes-no answer or a semiquantitative score – as the presence of antibody above control levels correlates with protection (for those diseases defined above).

IN-HOUSE TEST KITS

There are now two companies that produce in-house test kits for determination of protective serum antibody to CORE infectious diseases post-vaccination. Both test kit systems are simple to use, provide a rapid answer (protection or not) within 20 – 30 minutes, and are relatively inexpensive (costing around the same for testing as for revaccinating the animal). Both test kit systems have been validated independently and correlated with gold standard tests by a number of diagnostic laboratories. The test kits are the TiterCHEK™ system (manufactured by Synbiotics and now owned

and distributed by Pfizer) and the VacciCheck™ system (produced by Biogal Laboratories). The TiterCHEK™ system provides a yes-no (protected or not protected) answer for CDV and CPV. The VacciCheck™ system provides a semiquantitative score for serum antibody titres against CDV, CAV and CPV. A feline VacciCheck™ system provides scores for serum antibody titres against FPV, FCV and FHV. The kits have very good overall sensitivity (detection of samples with antibody from those seropositive by gold standard) and specificity (detection of samples without antibody by those seronegative by gold standard).

A set of excellent 'You Tube' videos produced by the US Charity 'Maddies Fund' is available on the web that provide very clear instruction in how to perform and interpret each of these test systems. Minor differences between the two systems are summarized below:

TiterCHEK	VacciCheck
Can run up to 96 samples at a time	Can run up to 12 samples at a time
Requires serum or plasma (cannot use whole blood)	Can use serum, plasma or whole blood
Can use haemolysed serum or plasma	Can use haemolysed serum or plasma
Provides a simple yes-no answer	Provides a semiquantitative score
Available for CDV and CPV only	Available for CDV, CAV and CPV
No feline test system	Available for FPV, FCV and FHV
Test discarded after use	Test combs may be stored for future reference
CDV sensitivity 94%, specificity 91%	CDV sensitivity 100%, specificity 83%
CPV sensitivity 96%, specificity 98%	CPV sensitivity 88%, specificity 100%
	CAV sensitivity 96%, specificity 82%
	FPV sensitivity 85%, specificity 98% [one recent study reports a sensitivity of 49% in shelter cat samples]
	FCV sensitivity 75%, specificity 67%
	FHV sensitivity 76%, specificity 84%

APPLICATIONS OF IN-HOUSE TESTING

To Determine Puppy Protection and Detect Genetic Non-Responders

The use of in-house test kits provides a simple measure of whether a puppy (CDV, CAV, CPV) or kitten (FPV) is protected after the initial series of early life vaccinations. This has the benefit of identifying animals that may not have responded to early life vaccination (particularly where a 14 – 16 week vaccine is not given) and may remain unprotected until the time of the 12 month booster. An animal that is seropositive and protected at this stage may not actually require the 12 month booster and could go straight to a triennial CORE vaccination programme.

WSAVA guidelines recommend the final CORE vaccination at 14 – 16 weeks. The puppy can be tested from 2 weeks after this vaccination (typically at 18 weeks). Seropositivity at this stage indicates that the pup has made an endogenous immune response to vaccine as there can be no MDA remaining at this time. A puppy that is seronegative at 18 weeks should be revaccinated

(perhaps with an alternative product) and then tested again 2 weeks later. A positive result indicates protection. A second negative result may indicate that the pup is either a 'low responder' or a 'non-responder'. Performing a gold standard test at this stage may show the low antibody titre typical of a low responder dog. Such an animal will be protected from clinical disease but not from infection. Alternatively, the dog may lack antibody and be a genetic non-responder that is incapable of ever making an immune response to that particular antigen. Such dogs are therefore susceptible to infection and disease for life. Dogs of the rottweiler breed have a higher proportion of genetic non-responders to parvovirus and rabies virus vaccines. Although non-responder rottweilers are now no longer recognized in the US (the gene pool has selected against them), they are still seen in Europe. Note that genetic non-responders are generally unable to respond to one (rather than all) CORE vaccine antigens. The estimated prevalence of non-responders (US data) for CPV is 1 in every 1000 dogs and for CDV 1 in every 5000 dogs. CAV non-responders are very rare (estimated < 1 in every 100,000 dogs).

A recent Danish study has evaluated seroconversion in a population of 135 pups aged between 8 weeks and 12 months. Most of these dogs will have finished an early life protocol (unlikely to have included a 14 – 16 week vaccine) but have not yet received a 12 month booster. The prevalence of non-responders in this population was 25.3% for CPV, 20.7% for CAV and 12.6% for CDV as determined by VacciCheck™ testing.

To Decide about Vaccination of a 'Lapsed' Adult Dog

Much is currently made of revaccinating 'lapsed' adult dogs or adult dogs adopted without a vaccination history. Most current data sheets for MLV CORE vaccines suggest that it is necessary to treat such animals as puppies and give two injections 3 – 4 weeks apart. In fact, immunologically an adult dog can be primed, immunized and boosted from a single injection of MLV CORE vaccine as there is no inhibitory MDA. However, such dogs may not actually require vaccination at all – either because they have been previously vaccinated or in some instances have acquired natural immunity from field exposure to virus. Owners may therefore be offered serology rather than automatic vaccination in this circumstance. An adult dog with serum antibody to CDV, CAV and CPV is protected already and does not require revaccination at that time point. Similarly, a 'lapsed' or adopted adult cat with serum FPV antibody is protected and does not require that component of vaccine at that time point.

To Minimize Risk in an Animal previously having an Adverse Reaction to Vaccine

Adverse reactions of a wide spectrum are recognized post-vaccination in dogs and cats. The prevalence of these is low and most are mild and transient effects. However some (e.g. canine immune-mediated disease) are potentially life-threatening and if there is a suspicion that vaccination might have been a trigger for a disease then such animals should be subject to rigorous benefit-risk analysis before revaccination is considered. For CORE vaccine antigens, this decision is now made simpler by the availability of in-house serology. A dog with serum antibody to CDV, CAV

and CPV does not require revaccination with MLV CORE vaccines and serious consideration should be given to which NON-CORE products such an animal receives.

Serology Replacing Revaccination in an Annual Health Check

In the US and increasingly in Europe, the Annual Health Check concept is gaining momentum. So too is the adoption of triennial CORE revaccination schedules for adult animals. However, many US practices have now moved on again in this rapidly changing arena. Instead of offering triennial CORE revaccination, these practices are now offering the alternative of triennial serological testing using one of the in-house systems. Dogs that are seropositive (or cats seropositive for FPV) are not revaccinated with CORE vaccines as these are not required. NON-CORE vaccines may still be used annually and for cats at risk, FCV and FHV revaccination might be considered annually. Where this approach is used, the testing interval is reduced to annually for senior animals (dogs > 10 years and cats >15 years) to ensure that immunosenescence (aging of the immune system) is not an issue.

Management of Disease Outbreaks in Shelters

One of the most valuable applications of in-house serology has been in the management of infectious disease outbreaks in shelters – specifically for CDV, CPV and FPV outbreaks. The ability to rapidly and cheaply test populations in order to identify animals that are protected or susceptible has allowed many animals to live that might otherwise have been euthanised as they were of unknown status.

In the face of a disease outbreak, all animals currently resident within the shelter should be tested. Those that are seropositive are protected and will not become infected or die. This protected population should be separated from low or negative responder animals that should be isolated. The susceptible population should not be adopted out of the shelter until after at least 2 weeks for CPV or FPV or until after at least 6 weeks for CDV (reflecting the incubation periods of the diseases). The susceptible population might be retested after these intervals.

The second population to be considered are those animals that are wishing to enter the shelter. These should also be tested before considering admission. Seropositive animals may enter as they are protected from disease. Seronegative animals should be vaccinated and then ideally sent to foster homes and not allowed to enter the shelter until they have seroconverted (when retested 2 weeks later).

This approach has proven to be very successful in controlling infectious disease outbreaks in shelters. The approach is not applicable to outbreaks of feline infectious respiratory disease complex as serology is not correlated with protection.

FURTHER READING AND INFORMATION

http://www.wsava.org/VGG1.htm [WSAVA Vaccination Guidelines]

http://www.maddiesfund.org/ [videos on performing and interpreting in-house tests and use of serology in control of outbreaks in shelters]

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