Testing dogs for immunity against Canine Parvovirus, Canine Distemper Virus and Infectious Canine Hepatitis

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Summary

The aim of the study was 1) to describe the distributions of scores for the level of immunity against Parvovirus (CPV), Canine Distemper Virus (CDV), and Infectious Canine Hepatitis (ICH) for dogs tested in the veterinary clinic of the Danish Raw Feeding Center, 2) to describe the relationships between the levels of immunity against these diseases, and 3) to identify factors influencing the risk of insufficient immunity.

The study includes 322 dogs of which 225 were tested once, 67 were tested twice, 24 were tested three times, 5 were tested four times, and 1 was tested five times. Thus, a total of 456 tests were performed.

When tested for the first time the proportions of dogs found protected against CPV, CDV and ICH individually were 88.5 %, 87.3 % and 85.1 %, respectively, corresponding to estimated true prevalences of dogs protected against these diseases of 91.2 %, 91.9 % and 87.8 %, given the sensitivity and specificity of the tests. The proportion of dogs found protected against all diseases was 74.8 %.

Pure bred dogs had significantly lower risk of insufficient immunity against ICH as compared to dogs of mixed breeds (OR = 0.41). Additionally, pure bred dogs tended to have lower risk of insufficient immunity against CPV (OR = 0.47; p = 0.056). Male dogs had significantly higher risk of insufficient immunity against ICH as compared to females (OR = 1.94).

Furthermore, significant associations were found between age and immunity status. Compared to dogs more than 3 y old, dogs younger than 1 y had higher risk of insufficient immunity against CPV (OR = 5.90) and ICH (OR = 3.10), and dogs >1 to 3 y old had higher risk of insufficient immunity against CDV (OR = 3.29) and ICH (2.77). The proportions of dogs younger than 1 y found unprotected were 25.3 %, 12.6 % and 20.7 % for CPV, CDV and ICH, respectively. The corresponding estimated true prevalences of unprotected dogs were 23.0 %, 8.0 % and 19.3 %, given the sensitivity and specificity of the tests.

Our results indicate that about 3/4 of the dogs revaccinated against CPV, CDV and ICH every year will have adequate immunity against these diseases and do not need to be revaccinated. Test of dogs for immunity against CPV, CDV and ICH once a year in the clinic may help to ensure that these dogs are not over-vaccinated. Furthermore, dogs not responding adequately to vaccination may be identified, if the immunity status of vaccinated dogs is tested 1 mo after vaccination. However, to ensure that no dogs receive vaccines against diseases for which, they are already protected, monovalent vaccines against CDV and ICH are relevant.

Keywords: dog; vaccination; immunity; canine parvovirus; canine distemper virus; infectious canine hepatitis

Introduction

Vaccination of dogs

According to the Danish legislation (BEK nr 1466 af 12/12, 2007) dogs from commercial breeders have to be vaccinated against Canine Parvovirus (CPV), Canine Distemper Virus (CDV) and Infectious Canine Hepatitis (ICH), before they are 12 weeks old. If the dogs are sold before they are vaccinated, the breeders are obliged to inform the buyer that the dog should be vaccinated against these diseases.

In Denmark traditionally veterinarians often recommended that dogs were revaccinated once a year in relation to the yearly health examination. In recent years, however, the longevity of the immunity in vaccinated dogs and the side effects of medical treatment in general have received more focus, and more effective vaccines have been produced.

Published data from studies by the major companies manufacturing canine vaccines for the U.S. market show that the minimum duration of vaccinal immunity for the core products CPV type 2, CDV and ICH (canine adenovirus-2) is 3 y or longer (Schultz, 2006). Generally, however, the effectiveness of non-core products is less than the effectiveness of the core products.

The American Animal Hospital Association (AAHA) has issued a set of canine vaccine guidelines first released in 2003 and later revised with new information in 2006 (Paul et al., 2006). AAHA recommends that vaccine decisions are made on an individual basis for each dog considering breed, age, environment, lifestyle and travel habits.

Presently, for vaccination of dogs with live attenuated vaccines against CPV, CDV and ICH (Nobivac DHP Live Vet) the Danish Medicines Agency (DMA, 2008) recommends a basis

vaccination at the age of 12 weeks and revaccination every third year. If the basis vaccination is given at the age of 6 – 8 weeks, the dog should be revaccinated at the age of 12 weeks.

Vaccine-associated adverse events

In a large study of adverse reactions diagnosed within three days after vaccination More et al. (, 2005) found a rate of 38.2 vaccine-associated adverse events (VAAEs) per 10.000 dogs. Of the VAAEs recorded, 65.8 % were coded as vaccine reactions, 31.7 % as allergic reactions, 1.7 % as anaphylaxis, 0.7 % as urticaria, and 0.1 % as cardiac arrest. The risk of VAAE increased as the body weight of the dogs decreased. The risk VAAEs in males and females did not significantly differ. The risk of VAAE was, however, greater for neutered as compared to sexually intact dogs. Furthermore, a linear relationship was found between the risk of VAAE and the number of vaccine doses administered per office visit.

Other studies have indicated possible delayed effects associated to vaccine such as immune-mediated haemolytic anemia (Duval and Giger, 1996), fibrosarcomas developed at the vaccination sites (Vascellari et al., 2003), and autoimmune diseases affecting the thyroid gland (Scott-Moncrieff et al., 2002).

Immunity tests in the clinic

In the clinic of the Danish Raw Feeding Center dogs are always tested for immunity against CPV, CDV and ICH prior to revaccination. The test is performed in order to avoid unnecessary vaccination of dogs already protected against these diseases. The dogs are called in for testing once a year, and the cost of the test for the owners corresponds to the cost of a normal vaccination. If the

level of antibodies, however, indicates that the dog is no longer protected, the dog is vaccinated for free.

Aim of the study

The aim of the present study was 1) to describe the distributions of scores for the humoral immunity against CPV, CDV and ICH obtained using a test kit designed for use in the veterinary clinic, 2) to describe the relationships between the levels of humoral immunity against these diseases, and 3) to identify factors influencing the risk of insufficient humoral immunity.

Materials and methods

Dogs tested

The study included 322 dogs tested in the veterinary clinic of the Danish Raw Feeding Center for immunity against CPV, CDV, and ICH in the period from 2007-09-01 to 2011-04-30. All dogs were previously vaccinated in our or other clinics prior to the immunity test.

The dogs included 263 (81.5 %) purebred dogs distributed on 78 different breeds and 59 (18.5 %) dogs of mixed breeds (Table 1). The proportion of males to females was about 1:1. The age of the dogs at the time of the first test ranged from 2 mo to 13 y and 4 mo. The mean age was 2 y and 11 mo, and 41.7 % of dogs were 1 y or younger. The age distribution is shown in Figure 1.

Test kit used

The test kit used was ImmunoComb® Canine VacciCheck IgG Antibody Test Kit, Biogal – Galed Labs. The test is based on solid phase "dot"-ELISA technology, and antigens are applied to test spots on a comb-shaped plastic card (Biogal, 2007).

The blood samples to be tested are mixed with diluents in the first row of wells of a multi-chamber developing plate. The test spots on the comb are then incubated with the sample in the developing plate. Specific IgG antibodies from the samples, if present, bind to the antigens at the test spots.

After incubation unbound antibodies are washed from the antigen spots on the comb in the second well of the developing plate. In the third well the spots are allowed to react with an anti-dog IgG alkaline phosphate conjugate, which will bind to antigen-antibody complexes at the test spots. After two more washes in the fourth and the fifth well, the test spots are allowed to develop color by an enzymatic reaction in the last well. The intensity of the color directly corresponds to the level of antibodies in the test sample.

The immunity against CPV, CDV and ICH is scored individually on a scale from 0 to 6. The score of 0 means that the dog has no detectable antibodies against the disease, and scores of 1 - 2 means a low level of antibodies not considered to be protective. Scores of 3 - 4, however, are consistent with a protective level of antibodies, and the score of 5 - 6 reflects a high level of humoral immunity. Thus, for dogs with scores of 3 or higher revaccination is not needed.

According to the producer of the test (Biogal, 2007) the specificity and sensitivity for CPV are 100 % and 97 %, respectively. For CDV the specificity is 100 % and the sensitivity 95 %, and for ICH the specificity is 86 % and the sensitivity 95 %.

Figure 2 shows the relationships between the true prevalence of protected dogs and the expected proportions of false negatives and false positives among the dogs tested. Knowing the sensitivity and specificity of the test and the proportion in the sample testing positive (*Pr*(T+)) the true prevalence of protected dogs may be estimated (Petrie et al., 2002):

Estim. true prevalence = (Specificity + *Pr*(T+) – 1)/(Sensitivity + Specificity – 1)

Vaccines used

Dogs no longer protected against CPV, CDV and/or ICH were revaccinated with Nobivac DHP Live Vet, Nobivac Puppy DP or Nobivac Parvo Live Vet depending on the level of immunity against each of these diseases. Monovalent vaccines against CDV and ICH were not available.

Statistical analysis

All statistical analysis was performed using the software package Statistical Analysis Systems version 9.1. Distributions were compared using the Wilcoxon Two-Sample Test (proc NPAR1WAY). Correlations between scores for the immunity against CPV, CDV and ICH were calculated using Spearman Correlation Coefficients (proc CORR). Odds ratios and confidence intervals for potential risk factors for insufficient immunity against CPV, CDV and ICH were calculated using logistic regression (proc GENMOD). Additionally, logistic regression (proc LOGISTIC) was used calculating the predicted probabilities of test scores < 3 for immunity against these diseases as a function of age. Based on these probabilities the true prevalences of unprotected dogs in the study population were estimated.

Results

Tests performed - overview

Of the 322 dogs tested, 225 were tested once, 67 were tested twice, 24 were tested three times, 5 were tested four times, and 1 was tested five times. Thus, a total of 456 tests were performed. The mean time interval between first test and the second test was 13.3 mo (STD = 5.88 mo), and mean interval between the second and the third test was 11.7 mo (STD = 5.08 mo).

The results of the first, second and third test are shown in Table 2. Of the 241 (74.8 %) dogs found protected against CPV, CDV and ICH in first test 74 were tested again, and 79.7 % of these dogs were still found protected in the second test. Of the 81 (25.2 %) dogs found unprotected against one or more of the diseases and consequently revaccinated 23 were tested again. Of these dogs only 65.2 % were found protected in the second test. However, the difference between the two groups in the proportions of protected dogs was not statistically significant ($p = 0.153$, Pearson chi-square test).

Six dogs were subjected to a fourth test. Two dogs were found protected against CPV, CDV as well as ICH in all 4 tests. One dog was found protected in first test only, and one dog in the first 3 tests. The fifth dog was found unprotected in the first and the third test. In the second and the fourth test, however, the dog was found protected. The sixth dog was found protected in the third test only. One of the dogs found protected in all 4 tests was tested 5 times. This dog was still found fully protected.

Dogs tested for the first time

Figure 3 shows the distributions of scores for the immunity against CPV, CDV and ICH among the 2322 dogs tested for in clinic for the first time. Of these dogs 88.5% were found fully protected (score >= 3) against CPV, 87.3 % fully protected against CDV, and 85.1 % fully protected against ICH. The corresponding estimated true prevalences of immunity against these diseases given the sensitivity and specificity of the tests were 91.2 %, 91.9 % and 87.8 %, respectively.

The distributions of scores for the immunity against these diseases, however, significantly differed (pair wise comparison: p = <0.001). Thus, for immunity against CPV 54.0 % of the dogs achieved maximum score (Figure 3a). For immunity against CDV and ICH, however, maximum score was achieved by only 15.8 % and 9.9 % of the dogs, respectively (Figure 3b & 3c). Furthermore, for immunity against CPV only 2.8 % of the dogs achieved the lowest acceptable score for fully protection (score = 3). For immunity against CDV and ICH the lowest acceptable score was achieved by 9.6 % and 17.4 % of the dogs, respectively.

Low correlation was found between CPV immunity scores and CDV immunity scores ($r = 0.26$, $p < 0.001$) and moderate correlations were found between CPV immunity scores and ICH immunity scores ($r = 0.41$, $p < 0.001$) and between CDV immunity scores and ICH immunity scores ($r = 0.42$, $p < 0.001$).

Of the 81 dogs found unprotected against CPV, CDV or ICH only 7 dogs (8.6 %) were not protected against any of them (Table 3).

Retesting of dogs

Figure 4a compares the distributions of scores for the immunity against CPV for dogs tested twice excluding dogs revaccinated due to inadequate immunity against CPV, CDV and/or ICH found in the first test (N = 74 dogs). Of these dogs 68 (91.9 %) were still found protected against CPV. For 36.5 % of the dogs, however, the score found in the second test was lower than the score found in the first test. For only 5.4 % of the dogs the score found in the second test was higher than the score found in the first test.

In Figure 4b the scores for the immunity against CDV in dogs tested twice are compared. Of the dogs found protected against CPV, CDV and ICH in the first test 66 (93.2 %) were still found protected against CDV in the second. The proportion of dogs scoring lower in the second test and the proportion of dogs scoring higher were 37.8 % and 20.3 %, respectively.

The scores for the immunity against ICH in dogs tested twice are compared in Figure 4c. Of the dogs found protected against CPV, CDV and ICH in the first test 60 (81.1 %) were still found protected against ICH in the second test. However, for 71.6 % of the dogs the scores found in the second test differed from the scores found in the first test. Thus, for 59.5 % of the dogs the score was lower, and for 12.2 % the dogs the score was higher.

Figure 5 shows the difference in score between the first and the second test for immunity against CPV, CDV and ICH. The correlations between these differences in score reflect the extent to which a change in the immunity score for one disease is accompanied by a change in score for one of the other diseases.

The correlation between the difference in score for immunity against CPV and the difference in score for immunity against CDV only approached significance ($r = 0.22$, $p = 0.055$; Figure 6a). The difference in CPV scores was, however, significantly correlated with the difference in ICH scores ($r = 0.43$, $p < 0.001$; Figure 6b). Furthermore, the difference in CDV scores was significantly correlated with the difference in ICH scores ($r = 0.36$, $p = 0.002$; Figure 6c).

Revaccination of dogs found unprotected

Eighty one dogs in the first test, 23 dogs in the second test, 5 dogs in the third test, and 3 dogs in the fourth test were found unprotected against CPV, CDV and/or ICH. All healthy dogs found unprotected against CPV and/or CDV were revaccinated. However, in 11 (40.7 %) of the 27 cases of dogs found unprotected against ICH only, the owner chose not to have the dog revaccinated.

Of the 81 dogs found unprotected in the first test 37 (45.7 %) were unprotected against CPV, 41 (50.6 %) were unprotected against CDV, and 48 (59.3 %) were unprotected against ICH. Of the dogs found unprotected against CPV 12 (32.4 %) were tested twice. Three dogs (25.0 %), however, were still found unprotected against CPV in the second test. Ten (24.4 %) of the dogs found unprotected against CDV were tested twice, and three of these dogs (30.0 %) were still found unprotected. Similarly, 11 (22.9 %) of the dogs found unprotected against ICH were tested twice. Of these dogs 4 (36.4 %) were still found unprotected in the second test.

Influence of pedigree, gender, body weight and age

As shown in Table 4 pure bred dogs had a significantly lower risk of insufficient immunity (score < 3) against ICH as compared to dogs of mixed breeds (OR = 0.41). Additionally, pure bred dogs tended to have a lower risk of insufficient immunity against CPV (OR = 0.47; $p = 0.056$).

Male dogs had significantly higher risk of insufficient immunity against ICH as compared to females (OR = 1.94).

Compared to dogs older than 3 y dogs between 0 and 1 y old had significantly higher risk of insufficient immunity against CPV (OR = 5.90) and ICH (OR = 3.10). Furthermore, dogs between 1 and 3 years old had significantly higher risks of insufficient immunity against CDV (OR = 3.29) and ICH (OR = 2.77).

Figure 7 shows the relationships between age and the predicted probabilities of test scores < 3 for immunity against CPV, CDV and ICH. The estimated true prevalences of insufficient immunity against these diseases based on these predicted probabilities are shown in Figure 8.

Discussion

Bias

The dogs tested in the veterinary clinic of the Danish Raw Feeding Center were not a random sample representative of the Danish dog population. Many of the dog owners may have come to the clinic, because The Danish Raw Feeding Center produce and sell raw food for dogs, and the owners often see raw feeding as the last chance of helping their dogs having serious skin and fur problems or weight loss and lack of appetite. Often raw feeding has been recommended by other dog owners raw feeding their own dogs with good results.

The conditions of many of the dogs coming to the clinic for the first time may have affected their ability to make an adequate immunity response to vaccination or their ability to retain immunity.

Furthermore, the age distribution of the tested dogs was strongly biased towards young dogs a large proportion of which may not have responded adequately to the first vaccination as puppies. On the other hand, a large proportion of the dog tested in the clinic have changed diet from commercial dry food to raw meet and bones supplemented by vitamins and minerals coming from natural sources. This may have changed the status of immune system of the dogs. Thus, the distribution of scores for the immunity against CPV, CDV and ICH found in the present study may differ from what could be found in at true random sample of the Danish dog population.

Relationships between immunity against CPV, CDV and ICH

In the first test the great majority of dogs were found protected against CPV, CDV and ICH, if looking at each of these diseases separately (85.1 – 88.5 %). The proportion of dogs protected against all three diseases, however, was somewhat lower (74.8 %). Of the dogs found unprotected against one or more of these diseases only 8.6 % were not protected against any of them. This indicates that the ability to respond to vaccination and retain immunity depends on the disease in question.

With respect to immunity against CPV 63.5 % of the dogs scored the same or higher in the second test than in the first. For immunity against CDV and immunity against ICH the proportions of dogs scoring the same of higher in the second test were 62.2 % and 40.5 %, respectively. Thus, the dogs were better to retain (or increase) immunity against CPV and CDV than immunity against ICH. It should be noted, however, that dogs seemed to make a stronger immunity response to CPV than to

CDV and ICH and a very large proportion (74.3 %) of the dogs tested twice had maximum score for immunity against CPV in the first test. Thus, only a few dogs were able to show an increased score from the first to the second test.

Only to some degree changes in the level of immunity against one of the diseases were accompanied by changes in the level of immunity against the other diseases. Thus, the immunity against CPV and immunity against CDV seemed to change independently of each other, whereas moderate correlations were found between changes in immunity against ICH and changes in immunity against CPV as well as immunity against CDV.

This may indicate that the level of immunity against these diseases is influenced by factors affecting general health and status of the immune system as well as factors influencing immunity against CPV, CDV and ICH individually. Interestingly, some dogs were found to increase test score from the first to the second test, even though they were not revaccinated. Thus, the time course of the level of immunity may be divided in two components: 1) a long term decrease, and 2) short term fluctuations depending on actual challenges to the immune system like changes in health status and exposure to pathogens. If protected dogs are exposed to CPV, CDV and ICH, the level of immunity against these diseases is expected to increase.

Influence of pedigree, gender and age

Previous studies by Twark & Dodds (2005) and Eghafona et al. (2007) on testing of dogs for immunity against CPV and CDV found no significant influence of breed, gender and age on the level of immunity. McCaw (1998) also found no significant influence of breed and gender (N = 24122). They did, however, find a significant association between age and CPV titer with younger

dogs having higher titers, but age was not significantly associated with CDV titer. Furthermore, Hougaard (2005) found that dog in the age group 5 – 14 y did not respond to vaccination as well as younger dogs.

In agreement with previous studies pedigree and gender were neither significantly associated with CPV nor CDV immunity scores in the present study. Pure bred dogs, however, tended to have a lower risk of insufficient immunity against CPV as compared to dogs of mixed breeds. Furthermore, we found that pure breed dogs had a lower risk of insufficient immunity against ICH as compared to dogs on mixed breeds, and male dogs had a higher risk of insufficient immunity against ICH as compared to females.

In contrast to the previous studies significant associations were found both between age and CPV scores and between age and CDV scores. For immunity against CPV the proportion of dogs having low scores (score < 3) was directly related to age. For CDV, however, although dogs between 0 and 1 y old did not have significantly higher risk of insufficient immunity against CDV as compared to dogs older than 3 y, dogs between 1 y and 3 y old did. Furthermore, we found a significant association between age and ICH score.

Although Twark & Dodds (2005) found no significant influence of age, the results presented in their paper (Table 1) indicate that significant association between age and CPV antibody response may have been found with larger proportions younger dogs having low titers, if the data was analysed using a logistic regression model.

Vaccination of puppies

Puppies receive antibodies from their mother that protects them against disease. A minor part of the antibodies is transferred through the placenta. The major part, however, is transferred through absorption of collostrum within the first 24 h after birth (Mitchell, 2010). Because these antibodies are not replaced, they gradually disappear with a half life of about 10 days. The level of antibodies transferred to the puppies and thus the length of the period, where the puppies stay protected, depend on the level antibodies in the blood of their mother.

The maternally derived antibodies interfere with vaccination (Waner et al., 1996). Thus, a high level of maternal antibodies blocks the effectiveness of the vaccine. When the level has sufficiently declined, the immunity may be achieved through vaccination. However, a window of susceptibility opens, when the level of antibodies is too low to offer adequate protection, but high enough to interfere with active immunity from the vaccine (Rashid et al., 2009). This window may be open for several days to several weeks.

In a study by Waner et al. (2003) puppies were vaccinated according one of two protocols depending on their age at presentation at the clinic (6 – 8 weeks versus after 8 weeks). Two weeks after the last vaccination the puppies were tested for CPV and CDV IgG antibodies. Of the dogs vaccinated according to protocol 1, 13 % failed to respond to the CPV vaccine, 13 % to the CDV vaccine, and 2 % (1 dog) to both vaccines. Of the dogs vaccinated according to protocol 2, 14 % failed to respond to the CPV vaccine, 20 % to the CDV vaccine, and 5 % to both vaccines.

In the present study a surprisingly large proportion of the group of dogs from 0 to 1 y of age was found unprotected against CPV (25.3 %), CDV (12.6 %) or ICH (20.7 %) - despite the fact that these dogs were expected to be vaccinated less than a year ago. For comparison the proportions of

dogs found unprotected in the group of dogs more than 3 years old were 5.4 %, 7.0 % and 7.8 % for CPV, CDV and ICH, respectively. Even more surprising, large proportions of the group of dogs from 1 to 3 y of age were found unprotected against CDV (19.8 %) or ICH (18.9 %). The proportion of dogs not protected against CPV, however, did not significantly differ from the unprotected proportion among dogs more than 3 years old.

Our results indicate that a large proportion of dogs did not respond adequately, when they were vaccinated as puppies. Thus, they are potentially at risk of CPV, CDV and ICH. The estimated prevalences of unprotected dogs among dogs < 1 y were 23.0 %, 8.0 % and 19.3 % for these diseases respectively. Although, all dogs were previously vaccinated prior to the first test, some dogs may have been vaccinated against CPV and CDV only. This partly may explain the high proportion of dogs found unprotected against ICH.

To ensure that the puppy has developed an immune response after vaccination with products containing CDV and CPV-2 American Animal Hospital Association (AAHA) recommends that the puppy is serologically tested 2 or more weeks after completion of a puppy series at 14 to 16 weeks of age (Paul et al., 2006). This way low responders and non responders can be identified.

More attention should be given to factors, which may influence the ability of the dog to make an adequate immunity response to vaccination - not only in the laboratory, but also in real life. Around the time, when puppies are vaccinated for the first time, they are exposed to a whole series of stressful events. They are removed from their mother and litter mates, they experience large changes in their environment, they have to adapt to a new “family”, and they visit the veterinarian. All this happen in a period of life, when puppies are very vulnerable.

Most puppies are vaccinated with multivalent vaccines like DHP, DHPi and DHPiL and, thus, they are challenged with up to 5 attenuated pathogens at the same time. The question is, if a larger proportion of the puppies will respond adequately, if the individual pathogens are given separately as monovalent vaccines, and the puppies are given time to respond after each vaccination.

Vaccination of dogs not protected

Of the 322 dogs tested for the first time 81 were not protected against CPV, CDV and/or ICH. The 97 dogs neither protected against CPV nor CDV and ICH were vaccinated with Nobivac DHP Live Vet (Table 3). The 16 dogs protected against CDV and ICH, but not CPV, were vaccinated with Nobivac Parvo Live Vet. And the 5 dogs protected against ICH, but not CPV and CDV, were vaccinated with Nobivac Puppy DP Vet.

Of the remaining dogs (N = 53), 41 were found unprotected against ICH. These dogs had to be vaccinated with Nobivac DHP Live Vet to achieve protection, although 17 were already protected against CPV, 9 against CDV, and 15 against both CPV and CDV.

Furthermore, 12 dogs were protected against CPV and ICH, but not CDV. These dogs had to be vaccinated with either Nobivac Puppy DP Vet or Nobivac DHP Live Vet.

Thus, 65 % of the 81 dogs that needed to be vaccinated against CPV, CDV and/or ICH had to receive vaccine against one or two diseases against which, they were already protected.

Conclusion

About 3/4 of the dogs revaccinated against CPV, CDV and ICH every year will have adequate immunity against these diseases and do not need to be revaccinated. Test of dogs for immunity against CPV, CDV and ICH in the clinic once a year may help to ensure that these dogs are not over-vaccinated. Furthermore, some dogs may not give an adequate immune-response to vaccination. These dogs may be identified by testing them 1 mo after vaccination.

Our study indicates that a very large proportion of dogs in the Danish dog population do not make an adequate immune response to vaccination against CPV, CDV and/or ICH when vaccinated as puppies. Thus, they may be at risk for these diseases. Therefore, special attention should be given to the immunity response of puppies and young dogs.

If tested dogs with deficient immunity against CPV, CDV and/or ICH are revaccinated with the available vaccines 65 % of the dogs may be over-vaccinated with respect to one or more of these diseases. This over-vaccination may be avoided, if monovalent vaccines against CDV and ICH were available.

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References

Table 1. Breed distribution of tested dogs. Only breeds represented by 4 or more dogs are specified

Breed	Number	Percent
Golden Retriever	22	6.8
Labrador Retriever	18	5.6
West Highland White Terrier	18	5.3
Coton de Tulear	12	3.8
Cairn Terrier	9	2.8
French Bulldog	9	2.8
Mops	9	2.8
Rottweiler	9	2.8
Alsatian	8	2.5
Samoyed	7	2.2
Collie	6	1.9
English Bulldog	6	1.9
Welsh Springer Spaniel	6	1.9
American Bulldog	5	1.6
Danish-Swedish Farmdog	5	1.6
Eurasian	5	1.6
Flat Coated Retriever	5	1.6
Border Collie	4	1.3
Cocker Spaniel	4	1.3
Other breeds (N = 59)	96	29.8
Mixed breeds	59	18.5
Total	322	100.0

1*Table 2. Results of the first, second and third test for immunity against Canine Parvovirus (CPV),*

2*Canine Distemper Virus (CDV) as well as Infectious Canine Hepatitis (ICH).*

First test			Second test			Third test		
Protected	N	%	Protected	N	%	Protected	N	%
+	241	74.8	+	59	79.7	+	14	100.0
						–	0	0.0
			–	15	20.3	+	5	62.5
						–	3	37.5
–	81	25.2	+	15	65.2	+	3	60.0
						–	2	40.0
			–	8	34.8	+	3	100.0
						–	0	0.0
Total	322			97			30	

3*' + ' = Protected (Score >= 3); '– ' =Unprotected (Score < 3)*

Table 3. Protection against Canine Parvo Virus (CPV), Canine Distemper Virus (CDV), and/or Infectious Canine Hepatitis (ICH) found in the first test.

CPV	CDV	ICH	Number	Percent
+	+	+	241	74.8
–	+	+	16	5.0
+	–	+	12	3.7
+	+	–	15	4.7
–	–	+	5	1.6
–	+	–	9	2.8
+	–	–	17	5.3
–	–	–	7	2.2

' + ' = Protected (Score >= 3); '–' =Unprotected (Score < 3)

1*Table 4. Potential risk factors for insufficient immunity (score < 3) against Canine Parvovirus (CPV), Canine Distemper Virus (CDV)*

2*and Infectious Canine Hepatitis (ICH) in 322 dogs tested in the clinic for the first time*

	CPV					CDV					ICH				
	Neg (%)	Estim (%)	OR	CI95	P	Neg (%)	Estim (%)	OR	CI95	P	Neg (%)	Estim (%)	OR	CI95	P
Pedigree															
Pure bred	10.0	7.1	0.47	0.22-1.02	0.056	12.2	7.6	0.76	0.34-1.69	0.497	12.6	9.4	0.41	0.21-0.83	0.012
Mixed	19.0	16.4	1.00	NA	NA	15.5	11.1	1.00	NA	NA	25.9	25.8	1.00	NA	NA
Gender															
Male	11.2	8.4	0.97	0.48-1.97	0.938	13.2	8.6	1.10	0.56-2.15	0.780	19.1	17.4	1.94	1.02-3.70	0.044
Female	11.5	8.7	1.00	NA	NA	12.1	7.5	1.00	NA	NA	10.8	7.2	1.00	NA	NA
Age															
0 – 1 y	25.3	23.0	5.90	2.39-14.54	<.001	12.6	8.0	1.93	0.76-4.87	0.164	20.7	19.3	3.10	1.35-7.10	0.007
>1 to 3 y	7.5	4.7	1.42	0.50-4.06	0.510	19.8	15.6	3.29	1.44-7.55	0.005	18.9	17.1	2.77	1.23-6.21	0.014
>3 y	5.4	2.5	1.00	NA	NA	7.0	2.1	1.00	NA	NA	7.8	3.4	1.00	NA	NA

3Neg = dogs found negative for sufficient immunity against the disease; Estim = estimated true prevalence of dogs without sufficient

4protection in the sample population given the specificity and sensitivity of the test; OR = Odds ratio; CI95 = 95 % confidence interval; P =

5probability.

Legends to the figures:

Figure 1. Age distribution of 322 dogs tested for immunity against Canine Parvovirus, Canine Distemper Virus and Infectious Canine Hepatitis in the veterinary clinic of The Danish Raw Feeding Center for the first time.

Figure 2. Expected proportions false negatives and false positives among dogs tested for immunity against Canine Parvovirus (CPV), Canine Distemper Virus (CDV) and Infectious Canine Hepatitis (ICH) as a function of the true prevalence of dogs protected against these diseases. No false positives may be found for immunity against CPV and CDV (specificity = 100 %).

Figure 3. Distribution of scores for the level of immunity against Canine Parvovirus (a), Canine Distemper Virus (b) and Infectious Canine Hepatitis (c) in the first test of 322 dogs. Solid columns represent protected dogs (score >=3), and unshaded columns represent dogs not protected (score < 3).

Figure 4. Comparison of scores for the level of immunity against Canine Parvovirus (a), Canine Distemper Virus (b) and Infectious Canine Hepatitis (c) found in the first (unshaded columns) and the second test (solid columns). The figure only includes dogs protected against all three diseases in the first test. As shown in some dogs were no longer protected in the second test.

Figure 5. Difference in scores between the first and the second test for the level of immunity against Canine Parvovirus (a), Canine Distemper Virus (b) and Infectious Canine Hepatitis (c). Hatched columns represent dogs scoring lower in the second test than in the first. Unshaded columns

represent dogs with the same score in both tests. Solid columns represent dogs scoring higher in the second test than in the first.

Figure 6. Relationships between the difference in scores between the first and the second test for the level of immunity against Canine Parvovirus, Canine Distemper Virus and Infectious Canine Hepatitis. The relationships between the differences in scores of these diseases are displayed pair wise: a) Canine Parvovirus (CPV) – Canine Distemper Virus (CDV); b) Canine Parvovirus (CPV) – Infectious Canine Hepatitis (ICH); c) Canine Distemper Virus (CDV) – Infectious Canine Hepatitis (ICH). The numbers refer to the numbers of observations plotting similarly. The regression lines are shown as solid lines. The dashed lines indicate perfect positive correlation.

Figure 7. Predicted probability of test scores < 3 as a function of age for immutity against Canine Parvovirus (CPV), Canine Distemper Virus (CDV) and Infectious Canine Hepatitis (ICH).

Figure 8. Estimated true prevalence of dogs unprotected against Canine Parvovirus (CPV), Canine Distemper Virus (CDV) and Infectious Canine Hepatitis (ICH) as a function age. The estimates are based on the predicted probabilities of test score < 3 shown in Figure 7. If the predicted probability is below, what may be expected given the sensitivity and specificity of the test, the estimated prevalence is zero.