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AN ANALYSIS OF TITER TESTING AS PART OF THE VACCINATION GUIDELINE FOR DOGS

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Abstract

Most of the viral vaccines that are in use have an official registration for the duration of immunity (DOI) of 3 years, including the vaccines against Canine Distemper Virus, Canine Adenovirus and Canine Parvovirus. However, even 3 years after the last immunization many dogs might still have sufficient antibody levels for protection; revaccination against those pathogens was shown not to be necessary. Antibody titer testing is shown to be a useful tool to monitor immunity of dogs to decide if vaccination is needed or beneficial. The VacciCheck is such an antibody titer test, available for use in clinical practice. Data of the VacciCheck were collected from different veterinary practices and different patients. The percentage of antibody positive dogs at the time that booster vaccination is recommended (3 years after last vaccination), based on the vaccination schedule, was determined. The percentage of antibody positive dogs within different groups (breed, age, gender vaccination history), was determined and differences were statistically analyzed. From 16 Dutch veterinary clinics, data was collected. This data consisted of: breed, gender and neutering status, date of birth, date of the last vaccination given before the performance of the VacciCheck and the type of this vaccine, date of the execution of the VacciCheck and the results of the performed VacciCheck (S score 0 – 6) and finally, the remaining vaccination history (e.g. earlier core vaccinations and Leptospirosis vaccinations). Data from 929 dogs were received. The data showed for the group of dogs with a vaccination less than 3 years ago that the percentage of positive-tested dogs was 90.7% for CDV, 91.6% for CAV and 97.5% for CPV. The group of dogs with a vaccination more than 3 years ago, had a percentage of positive-tested dogs of 84.3% for CDV, 84.3% for CAV and 95.5% for CPV. In litters of puppies, differences in VacciCheck S-values between individual puppies were found. Also, for several other puppies the phenomenon was observed, that the Parvo S-value was still low after the dog had received the first core vaccination when the VacciCheck value was S0.

Results of this study show that most of the dogs that received last core vaccine more than 3 years ago still has a protective level of antibodies against CDV, CAV and CPV. Based on those results, the use of antibody titer testing can be supported. Also, titer testing in puppies might be a useful tool to determine whether the maternal antibodies have vanished and to decide about the optimal vaccination time. However, further research must be done about this optimal vaccination time.

Content

1. Introduction

Dogs are routinely vaccinated against Canine Distemper Virus, Infectious Canine Hepatitis, Canine Parvo Virus and Leptospirosis. For many years, veterinarians used the traditional one-year revaccination interval for all 4 vaccines. However, yearly revaccination against all 4 pathogens was shown not to be necessary. In several studies, the duration of immunity was shown to be longer for the viral vaccines based on the presence of protective antibody titers, for many years after vaccination. (1–4) Consequently, the use of an antibody titer test to determine the level of immunity has been increasing in the past few years.

1.1. Vaccination guideline for dogs

Distemper, hepatitis and parvo vaccines are considered to be core vaccines according to the WSAVA Guidelines and the AAHA Canine Vaccination Guidelines. A core vaccine indicates that all dogs throughout the world must receive this vaccine. (3,5) In the Netherlands, Leptospirosis is also considered a core vaccine. (1) To have a better understanding of the vaccination guidelines, the three different viruses and the diseases they cause will be discussed first.

Canine Distemper Virus (CDV) is a morbillivirus and causes a systemic disease which is highly contagious. (6) The virus mostly exists in respiratory exudates and is disseminated by aerosols or droplets. Also, transplacental transmission can take place. The prevalence is highest between the age of 3 to 6 months, which correlates with the maternal derived antibodies being decreased and thus being unprotected. After infection, the virus multiplies in macrophages and disseminates in these cells through the lymphatic system. The viral amount increases and virus disseminates to several tissues, such as the skin, central nervous system, urinary tract, gastrointestinal tract and the respiratory tract. (7) The disease is clinically associated with conjunctivitis, fever, leukopenia, anorexia, enteritis, and respiratory and neurologic complications. (6,7) Acute disease can eventually lead to death. (8)

Canine Adeno Virus type 1 (CAV-1) causes infectious canine hepatitis (ICH), a systemic disease. The infection is established by oronasal exposure and the virus disseminates through the lymphatic system before reaching the blood. This viremia leads to a fast spread to different tissues and body secretions. The hepatic cells and the central nervous system are the main targets of this virus. (7) CAV-1 produces an acute necrohemorrhagic hepatitis and clinical signs such as fever, abdominal pain, vomiting, diarrhea and diffuse hemorrhages. (9) Also, corneal edema and anterior uveitis are signs correlated with ICH. (7) ICH is mostly seen in dogs younger than 1 year. Moreover, the mortality can increase up to 100%. Dogs with severe infection can die in a few hours after the start of showing clinical signs. (7,8) The vaccine against ICH contains modified live CAV-2, which induces cross-protective immune responses against infection with CAV-1, but lacks the side effects, such as nephropathy and corneal opacity that can be observed with the CAV-1 containing vaccines. (8,10)

Canine Parvo Virus (CPV) causes a very contagious viral disease which is often fatal for dogs. The disease is caused by strains of CPV-2. CPV can spread rapidly from dog to dog through oronasal exposure to contaminated feces. After infection, the virus replicates in the oropharynx, lymph nodes and the thymus and disseminates to the intestinal crypts of the small intestine. The virus destroys cells within the crypts of the villi (the intestinal villus). (7) A consequence of a CPV infection is severe gastroenteritis and death. The most common clinical signs are vomiting, diarrhea with blood and weight loss. The disease is particularly common in puppies between 6-20 weeks of age, but adult dogs may be affected as well. (11)

The vaccines used against those three viruses are modified live virus (MLV) or attenuated vaccines. This implies that the viruses are intact and viable, but weakened. The purpose of this vaccine is to induce immunity by inducing a mild infection, which ensures that the virus can replicate within the animal without producing tissue damage or clinical signs of infection. Attenuated vaccines induce immunity more effectively than inactivated (killed) vaccines and appear to induce a cell-mediated and humoral immunity. (3)

Vaccination can reduce the three diseases described above effectively and can induce a good herd-immunity. In the first weeks of life, puppies are protected by maternal derived antibodies. After 8-12 weeks of age those maternal antibodies will decrease and active immunization can take place. A low (maternal) antibody titer is needed for an immune response to occur. This means that the level of maternal antibodies in newborns must be low, otherwise the vaccination has no effect. The reason for this vaccination failure, is that maternal antibodies neutralize the vaccine virus before it can stimulate immune cells. However, individual differences occur. Some puppies might have fewer maternal antibody and are vulnerable at an earlier age and capable of responding to vaccination, while other puppies might have many maternal antibodies and are not responding to vaccination until 12 weeks of age. No standard vaccination scheme can cover all possible circumstances. Therefore, the Vaccination Guidelines Group of the WSAVA advises to give the first vaccination at an age of 6-8 weeks. (3,12)

The most common vaccination schedule used in Dutch veterinary practices consists of vaccination at 6 weeks of age with CPV and CDV, at 9 weeks of age with leptospirosis, CPV and sometimes CAV, and at 12-14 weeks of age with CPV, CDV, leptospirosis and CAV. A revaccination occurs at an age of 1 year (or 1 year and 3 months) against all four pathogens. After that, a yearly revaccination with leptospirosis and every 3 year revaccination with CPV, CDV and CAV are recommended. (1)

The main reason to vaccinate dogs is to protect the individual dog against three diseases that commonly occur and are fatal. The above-mentioned viruses are also highly contagious; therefore, herd immunity is often mentioned as a reason as well. Vaccination of the individual dog leads to a decrease of the number of susceptible dogs in a population and thus reduces the prevalence of the disease. (3) Herd immunity can be described as the proportion of the vaccinated population that has developed sufficient immunity against the infection. The impact of herd immunity induced by vaccination depends on different factors, namely, the vaccine coverage (i.e. the proportion of the vaccinated dogs that develop sufficient immunity) and the market shares of vaccines. Also, the duration of vaccine-induced immunity of individual animals and their average life expectancy play a role. (13)

1.2. Duration of immunity

Most of the viral vaccines that are in use have an official registration for the duration of immunity (DOI) of 3 years. However, even 3 years after the last immunization many dogs might still have sufficient antibody levels for protection. (1,2,4)

The efficacy and the DOI of a vaccine are dependent on multiple factors, such as: vaccine factors, animal factors and pathogen factors. Vaccines from different manufacturers can vary a lot, for instance, in potency, efficacy and DOI. Furthermore, dogs will differ in their immune response, one will respond more strongly to a vaccine than one other. (13) Also, the size of the dog might play a role, since viral antigen is known to sequester in subcutaneous fat which might influence the immune response. (14) Pathogen factors include the amount of pathogen in the environment of the dog, the resistance of the pathogen and its distribution. Moreover, lifestyle factors influence the exposure of a dog to an agent. Lifestyle factors include the frequency of a dog being exposed to other susceptible animals and the density of the population. For example, dogs in single pet households usually have a lower risk for exposure to an infectious agent compared with dogs that are hold in animal shelters. An adequate immunity in a population of dogs can prevent an agent from being transmitted within the population, and thereby decreasing the exposure for individual dogs. (10)

The higher the antibody titer of a dog, the more protection the dog has against the viruses. However, dogs with low antibody titers are not necessarily susceptible to infection. The reason for this is that the dogs still have persistence of immune memory cells and also cell-mediated and mucosal immunity may play a role. (10,15,16) The DOI depends on two mechanisms. The first mechanism is the persistence of memory B and T cells, which are stimulated during vaccination or natural infection. The other mechanism is the persistence of long lived plasma cells, also known as 'memory effector B cells', which produce antibody for many years after vaccination or infection. (4,17) The population of memory cells can rapidly expand when exposed to an infectious agent and can persist for many years. Revaccination is not essential to maintain these cells. (16)
Controversially, a dog with a high or normal antibody titer might occasionally still become ill when contact with the infectious agent is established. This is a result of challenge, probably as a consequence of overwhelming exposure or immune suppression (16)

Multiple studies demonstrate that there is a correlation between the presence of antibodies against the core canine viral diseases, Canine Distemper Virus, Canine Parvovirus and Canine Adenovirus, and protective immunity. Therefore, antibody titer testing might be a useful tool to monitor immunity of dogs to decide if vaccination is needed or beneficial. (3,5,16,18)

1.3. Antibody titer test

An antibody titer test measures the number of antibodies against a specific virus. The purpose of a titer test is to establish whether a dog has a sufficient immune response to a specific agent and to examine whether the dog requires a new vaccination. (15) An advantage of performing an antibody titer test is that a more precise revaccination interval can be established in the individual dog. This, in turn, leads to less unnecessary vaccinations. Although the associated side effects, such as anaphylaxis, autoimmune reactions and immunosuppression occur relatively seldom, reduction of the number of vaccinations will further decrease its prevalence. (2)

For individual dogs, revaccination will not be necessary if sufficient antibody levels are present. For this reason, owners often ask for an antibody test instead of routine vaccination. (1) An antibody test could also be used for confirmation that a dog has responded to vaccination. This is mostly seen with puppies after the series of primary vaccinations. According to the WSAVA Guidelines, a newborn should be tested 4 weeks after the final vaccination at 16-18 weeks of age. (3) Puppies with a last vaccination that tested seropositive are protected and should not be revaccinated more often than once in every 3 years. Seronegative puppies should be revaccinated and retested 4 weeks later. When the test is negative again, the pup might be considered as a non-responder. (Figure 1) (3) A titer test is also recommended for dogs that are genetically or physiologically predisposed to developing adverse reactions after vaccination. (15) The WSAVA Guidelines also suggest that for geriatric dogs (aged over 10 years) serological testing should be performed annually instead of triennially, as a precautionary measure, (3) since it is suggested that aging affects the immune system. (19)

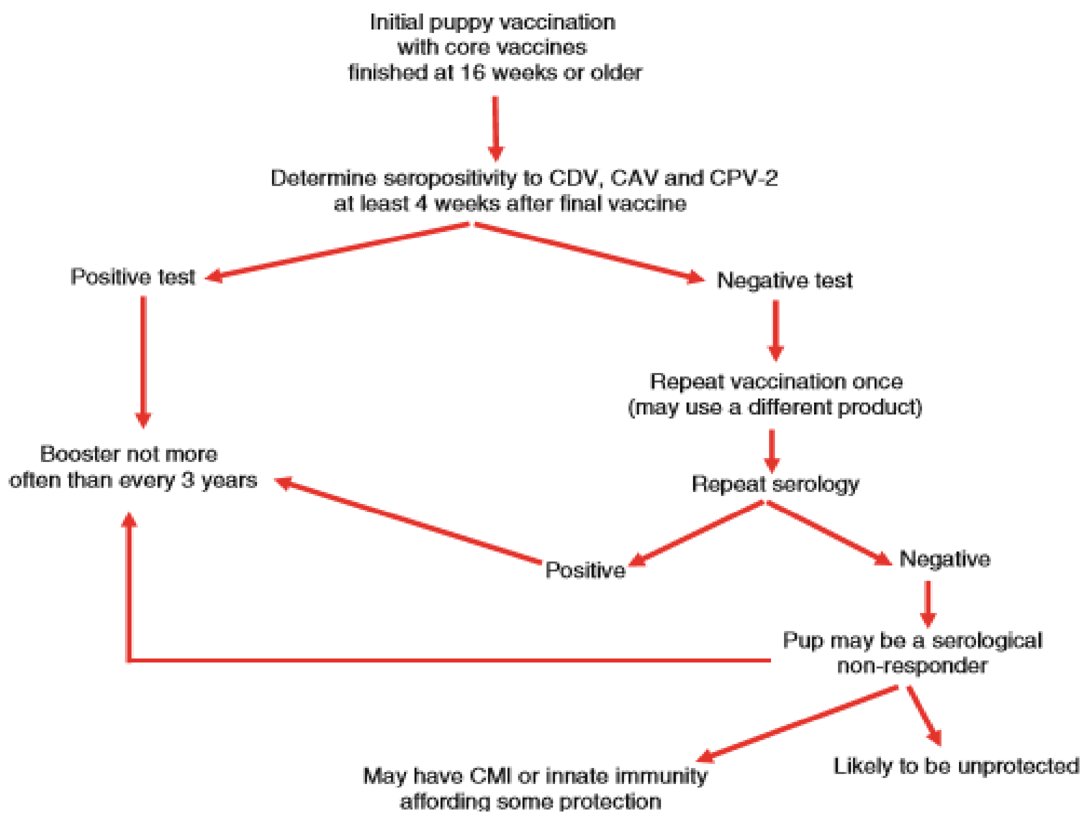

Figure 1: Flowchart for serological testing of puppies. Adapted from Day *et al.* (2016) (3)

Antibody titers can be determined either by sending a blood sample to a diagnostic laboratory or by a commercial test, the VacciCheck, which is available for use in clinical practice. VacciCheck is an ELISA-based system that requires several steps to be performed and gives a semi-quantitative result after about 20 minutes. It is a dot-ELISA and the intensity of the color of the dots with the different viral antigens correlate with antibody titers. If the color is equal or higher than the control dot, the dog is considered to be protected and vaccination is not recommended. The results will be scored with a so-called 'S' value. The control dot is referred to as S3. This means that S0, S1 and S2 have a lighter color intensity than the control dot and are referred to as a negative result (S0) or weakly positive result (S1, S2). S3, S4, S5 and S6 are positive and are darker or have the same color as control dot. (20)

Commercial test methods must correlate with the gold standard tests, which are in this case serum neutralization (SN) tests and hemagglutination inhibition (HI) tests. Studies demonstrated that there is a good correlation between CPV HI titers and CDV SN titers, compared to the 'S' value of the VacciCheck. (21,22) Also, a study by Mazar *et al.* has been conducted showing that there is a correlation between the antibody levels determined by the VacciCheck and neutralizing antibodies (needed for protection), determined in a laboratory setting. (23)

1.4. Aim of this study

The VacciCheck is increasingly being used in veterinary practice. Recently, a consensus statement has been published to answer some of the questions related to reliability of the test and interpretation of the results. (1) Some of the questions that arise are: how long after vaccination can antibody titers persist; what percentage of dogs still have antibody titers 3 years after their last vaccination; do differences exist between titers against the 3 different viruses; what is the preferred interval of antibody testing?

Antibody titer testing is already practiced in many veterinary clinics. Some of these clinics have gathered data over a long time period. This data will be collected from the veterinary database software systems. A pilot was already done which showed that it is relatively easy to extract this data. The specific aim of this project is to analyze the results of antibody titers, obtained from different veterinary practices, in order to increase the amount of data on the results of VacciCheck from veterinary clinics and to obtain information on the level and persistence of antibody titers of vaccinated dogs in the field. Differences in antibody titers (i.e. results of the VacciCheck) will be determined between breeds (small and large breeds), between age and between dogs with a different vaccination history (e.g. time between last vaccination and testing, and number of previous vaccinations).

2. Materials and Methods

Data of antibody titer testing will be collected from different veterinary practices and different patients. This data will be recorded in an excel file format and further analyzed. The percentage of antibody positive dogs at the time that booster vaccination is recommended, based on the vaccination schedule, will be determined. The percentage of antibody positive dogs within different groups (breed, age, vaccination history), will be determined and differences will be statistically analyzed.

2.1. Recruitment of veterinary clinics

Dutch veterinary practices were asked to contribute to this project and to deliver data. The first group of veterinary practices that was approached were reported on the website of NML Health. (24) NML Health is a Dutch company that produces and supplies many veterinary products. In the Netherlands, NML Health is the supplier of the VacciCheck. The practices mentioned on the website of NML Health are known to use the official VacciCheck. The second group of veterinary clinics that was approached were mentioned on the website of NML Health, as well as on the so-called Facebook page "Titeren met VacciCheck". (25) This Facebook page is set up by owners, to provide other owners with information about the antibody titer test. This group uses a list of veterinary clinics who can professionally use the VacciCheck and can interpret the results well, according to the owners.

In total, 87 clinics were approached by e-mail. However, only 34 clinics responded and were willing to cooperate with this project. Those clinics received a 'standard excel file' to fill in (See Appendix I). The data needed for an appropriate analysis were the following: breed, gender and neutering status, date of birth, date of the last vaccination given before the performance of the VacciCheck and the type of this vaccine, date of the execution of the VacciCheck and the results of the performed VacciCheck (S score 0 – 6) and finally, the remaining vaccination history (e.g. earlier core vaccinations and Leptospirosis vaccinations). Animal number or chip number was also asked to be able to recognize the animals in the future. From 16 clinics, a completed excel file was received. Data from 929 dogs were received.

2.2. Data Analysis

Statistical analysis of data was performed using STATA for Windows version 14.2 (StataCorp LLC, USA).

The age of the animals was determined at the time of the last vaccination and at the time of the VacciCheck. The ages were classified in three different groups, <1 year, 1-7 years and >= 7 years. Puppies (dogs younger than 1 year at the time of the performed VacciCheck) were excluded for the statistical analysis. Also, the time interval between the last vaccination and the performed VacciCheck was determined and classified in two groups: <3 years and >= 3 years. This threshold was chosen because 3 years after the last vaccination the booster vaccination is recommended. Therefore, the percentage of dogs with a positive and negative test result at the time that the booster vaccination is recommended, can be determined. Breeds were classified into two groups, large breeds if the average body weight was 10 kg or more and small breeds if the average body weight was less than 10 kg. For the results of the VacciCheck, the score was called 'positive' if the S value was S3 or higher and the score was called 'negative' if the S value was lower than S3.

The data were assessed by a gamma regression analysis and a logistic regression analysis. Differences were considered significant at $P < 0.05$.

In the statistical analysis, clusters were used to take into account factors that can cause different results between veterinary practices, such as different experiences, protocols, interpretation of the color intensity of the VacciCheck and the dog population. Cluster analysis means that objects in the same group (cluster) are more similar to each other than objects in other groups. In this study, the groups (or clusters) are the 16 different veterinary practices. Cluster-robust standard errors were used in this statistical analysis.

The group of dogs with an interval of 3 years or more between last core vaccination and performed VacciCheck was further analyzed. For the dogs that had a negative result in this group, age and breed were determined for CDV, CAV an CPV. Also, it was determined for which, and for how many, of the vaccine antigens those dogs are classified as seronegative.

Puppies were analyzed separately. The data of those puppies were placed in a separate excel file. Data from litters and data from puppies that had multiple VacciCheck tests were extracted. From the data of litters, three examples were taken. The choice of those examples was based on the completeness of the data and the aim to demonstrate the best result. Data of puppies that had multiple titer tests were listed in another excel file. One example of this was taken and this choice was again based on the completeness of the data and because it showed a clear development of antibody titers.

3. Results

In total, 1094 results of the VacciCheck were derived from 929 dogs. However, not all data were complete. 313 (28.6%) data entries were very incomplete and showed only the date and the results of the VacciCheck. From 475 dogs (51.1%), the breed was identified, of which 111 (23.4%) were small breeds and 338 (71.1%) were large breeds. The remaining 26 dogs (5.5%) were crossbred dogs and it was not known if the dogs were small or large crossbreds. There was a wide variety of breeds, with no specific breed being over-represented in the population. The gender and neutering status were known for 390 out of 929 dogs (42.0%). Out of the 929 dogs, 19.5% were male, 22.5% were female and for the remaining 58.0% the gender was unknown. From the dogs for which gender was recorded, 30 dogs were male neutered (7.7%), 142 male intact (36.4%), 9 male with an unknown neutering status (2.3%), 65 bitch neutered (16.7%), 134 bitch intact (34.4%) and 10 bitch with an unknown neutering status (2.5%). The birth date was known for 620 out of 929 dogs (66.7%). All dogs were aged between 3 months and 15 years. The date of the last vaccination was identified for 505 dogs (54.4%), of which 36 (young) dogs that never had a vaccination (7.1%). At the time of the performed VacciCheck, dogs had last received a core vaccination between less than one month and 131 months.

3.1. Adult Dogs

Figures 2-4 show the number of adult dogs with a positive (S-value of 3 or higher) or negative (S-value lower than 3) VacciCheck result for CDV, CAV and CPV plotted against the interval of the last core vaccination and the performed VacciCheck. The population existed of 412 adult dogs with an identified last vaccination date and date of the performed VacciCheck, through which the interval between last vaccination and VacciCheck could be determined. For CDV, 89.3% of the population of 412 dogs were seropositive. For CAV, 90.0% were seropositive and for CPV this were 97.1%. For the group of dogs with a last vaccination less than 3 years ago, the percentage of positive dogs were 90.7% for CDV, 91.6% for CAV and 97.5% for CPV. Finally, the group of dogs with a last vaccination more than 3 years ago, had a percentage of positive dogs of 84.3% for CDV, 84.3% for CAV and 95.5% for CPV (Figure 5).

The time elapsed since the last vaccination appeared not to be significantly correlated with gender, age and breed. The results of a gamma regression analysis showed that generally, dogs with an interval of 3 years or more between the last core vaccination and the performed VacciCheck have a lower VacciCheck S-value than dogs with an interval of less than 3 years ($P = 0.004$ for CDV, $P = 0.000$ for CAV and $P = 0.025$ for CPV) (See Appendix III). Also, a significant difference was found between age and the S-value for CPV ($P = 0.012$). The results showed that older dogs have a lower antibody titer for CPV than younger dogs. For CDV and CAV this result was not significant. No significant relationship between S-value and gender and breeds was found. The logistic regression analysis showed that dogs with an interval of 3 years or more between the last core vaccination and the performed VacciCheck have a significant lower odd to have a positive VacciCheck result for CAV than dogs with an interval of less than 3 years ($P = 0.032$) (See Appendix IV). For CDV and CPV this relationship was not significantly found.

Figure 5 shows the percentage of dogs with a positive (S-score of 3 or higher) or negative (S-score lower than 3) VacciCheck result 3 years after their last core vaccination, determined for CDV, CAV and CPV. Figure 6 shows the percentage of dogs with a positive (S-score of 3 or higher) or negative (S-score lower than 3) VacciCheck result that had their last core vaccination less than 3 years ago, determined for CDV, CAV and CPV.

In total, 89 dogs had a last core vaccination more than 3 years ago (Figure 7). Of those 89 dogs, 65 dogs (73%) were seropositive for all three vaccine antigens. Only 24 dogs (27%) were seronegative for one or more antigen. Of these 24 dogs, 13 dogs (3.2% of the total population of 412 adult dogs) had a negative VacciCheck result for CDV, 13 dogs (3.2% of the total population of 412 adult dogs) had a negative VacciCheck result for CAV and 3 dogs (0.7% of the total population of 412 adult dogs) had a negative VacciCheck result for CPV. One dog in the population (0.2% of the total population of 412 adult dogs) had less protective antibodies to all three vaccine antigens. This dog was 9 years of age at the time of the performed VacciCheck, with the last core vaccination received 4 years ago. Breed, gender and vaccination history were not identified for this single dog. Of the 24 dogs with one or more negative results, age ranged between 4 years and 12 years and the time elapsed since last core vaccination ranged between 3 years and 10 years. There was no breed or gender being over-represented. Figure 8 shows the VacciCheck S-values of the 24 dogs with one or more negative results. This figure shows that the majority of the dogs had a S-value of 2.

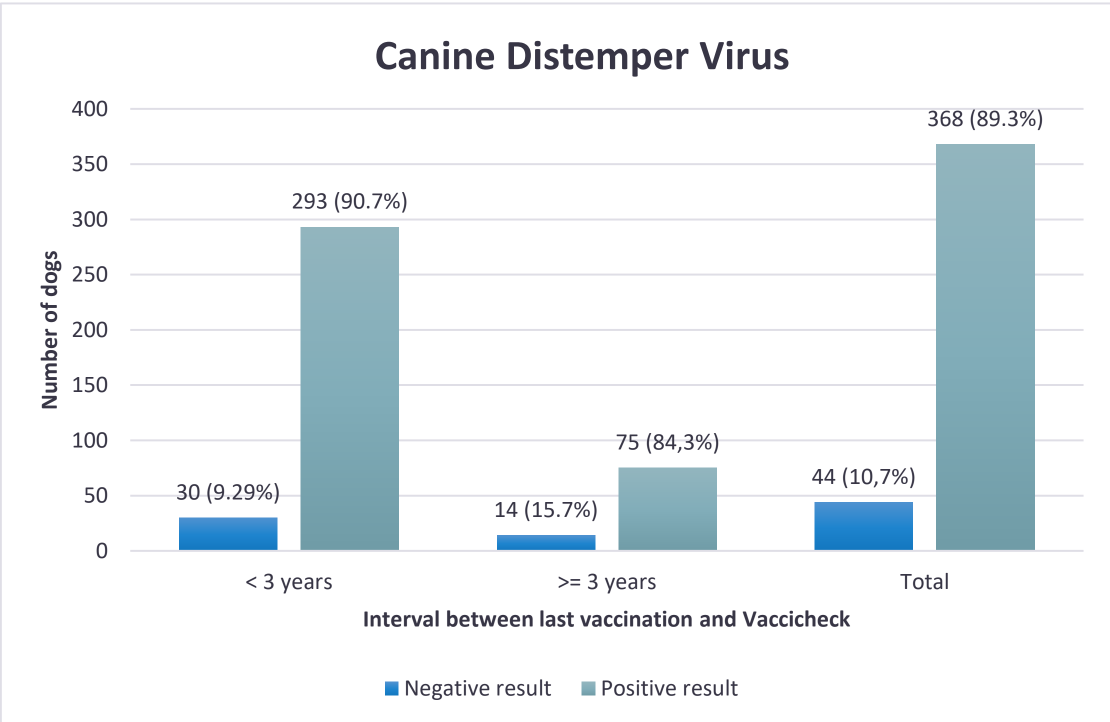

Figure 2: Number of dogs with a positive or negative VacciCheck result for CDV with an interval of less than 3 years and 3 or more years between the last core vaccination and performing the VacciCheck. In brackets, the relative percentages are shown.

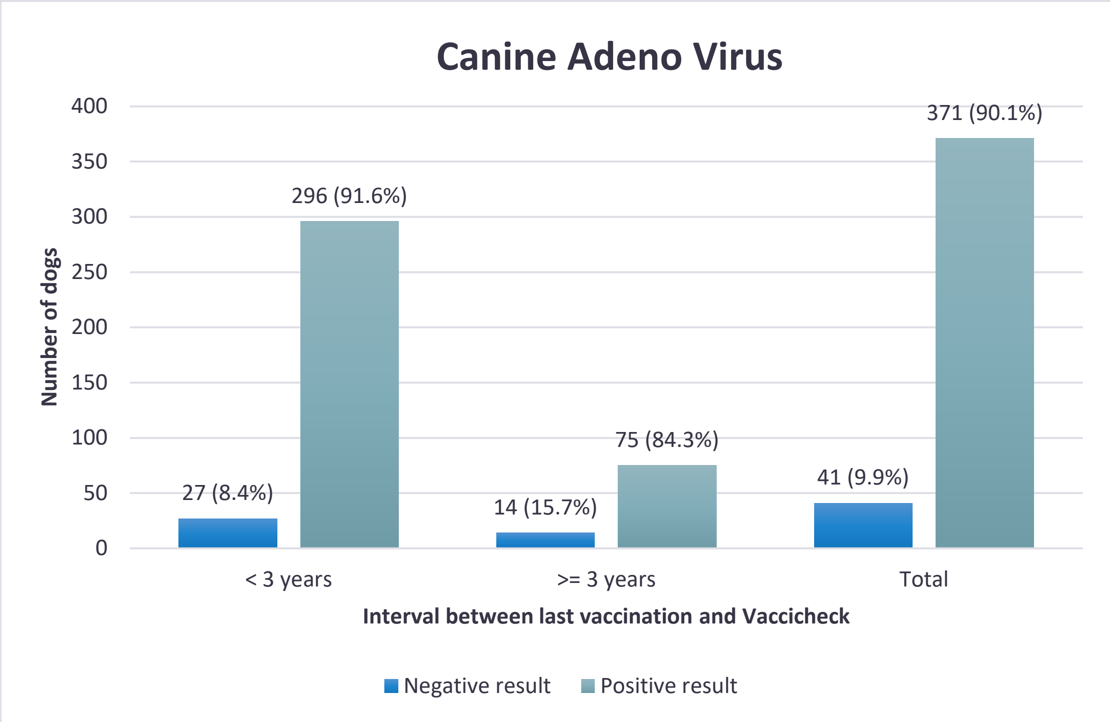

Figure 3: Number of dogs with a positive or negative VacciCheck result for CAV with an interval of less than 3 years and 3 or more years between the last core vaccination and performing the VacciCheck. In brackets, the relative percentages are shown.

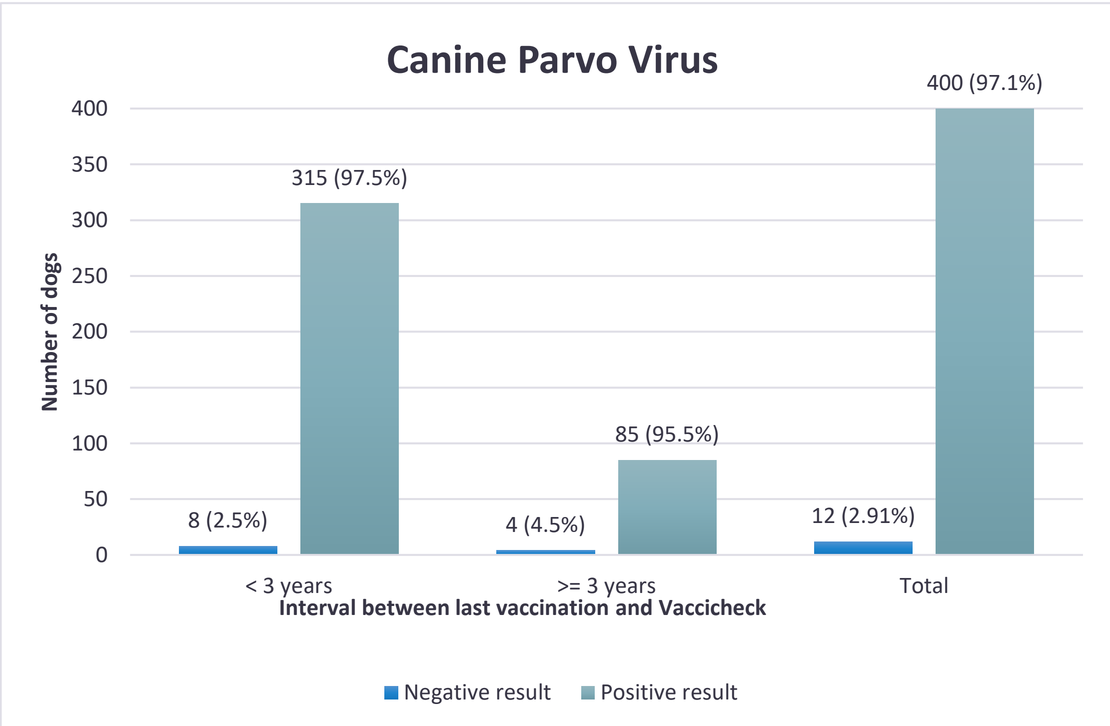

Figure 4: Number of dogs with a positive or negative VacciCheck result for CPV with an interval of less than 3 years and 3 or more years between the last core vaccination and performing the VacciCheck. In brackets, the relative percentages are shown.

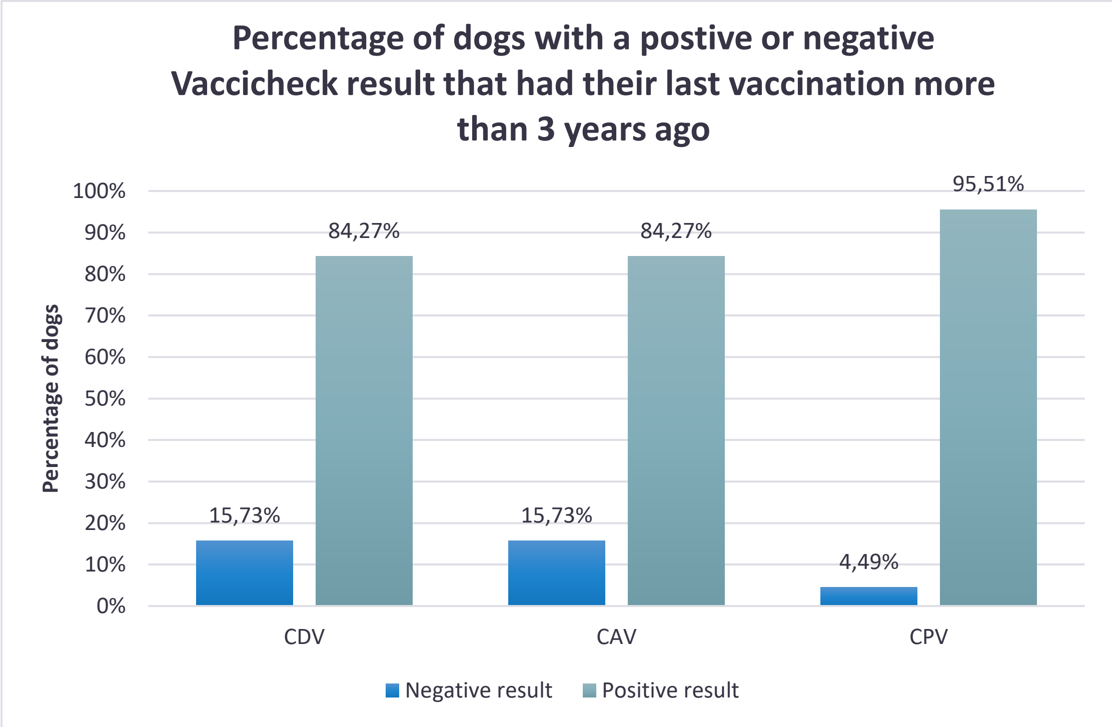

Figure 5: Percentage of dogs with a positive or negative VacciCheck result that had their last vaccination more than 3 years ago, determined for CDV, CAV and CPV.

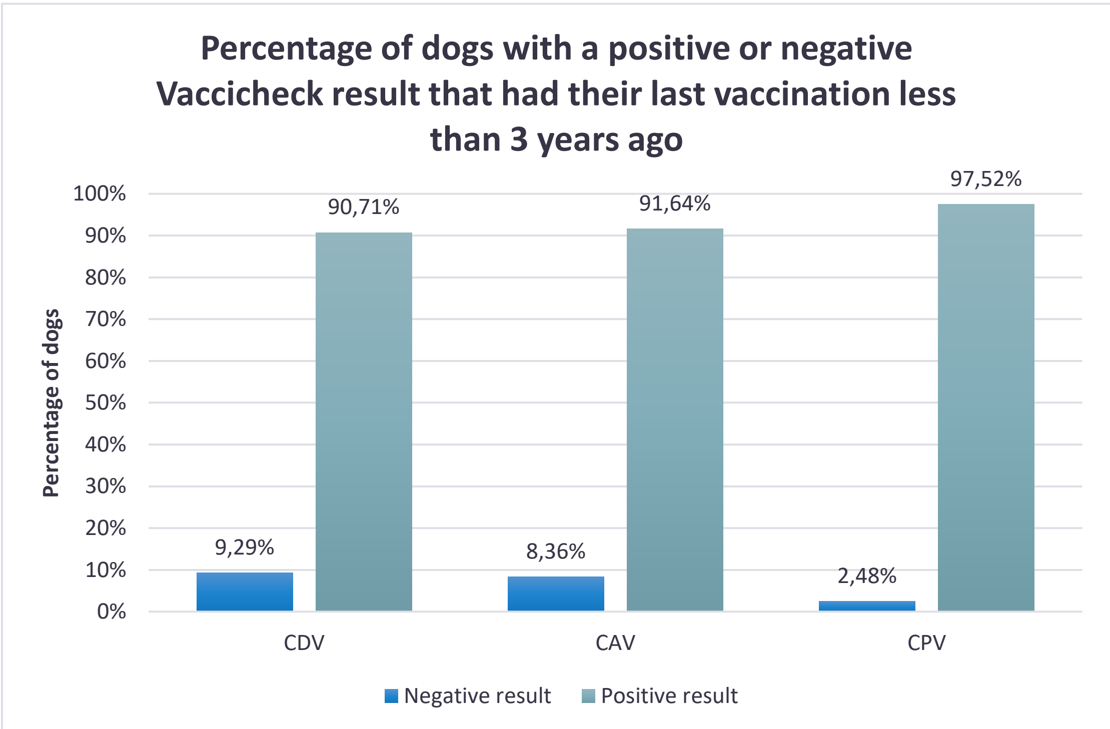

Figure 6: Percentage of dogs with a positive or negative VacciCheck result that had their last vaccination less than 3 years ago, determined for CDV, CAV and CPV.

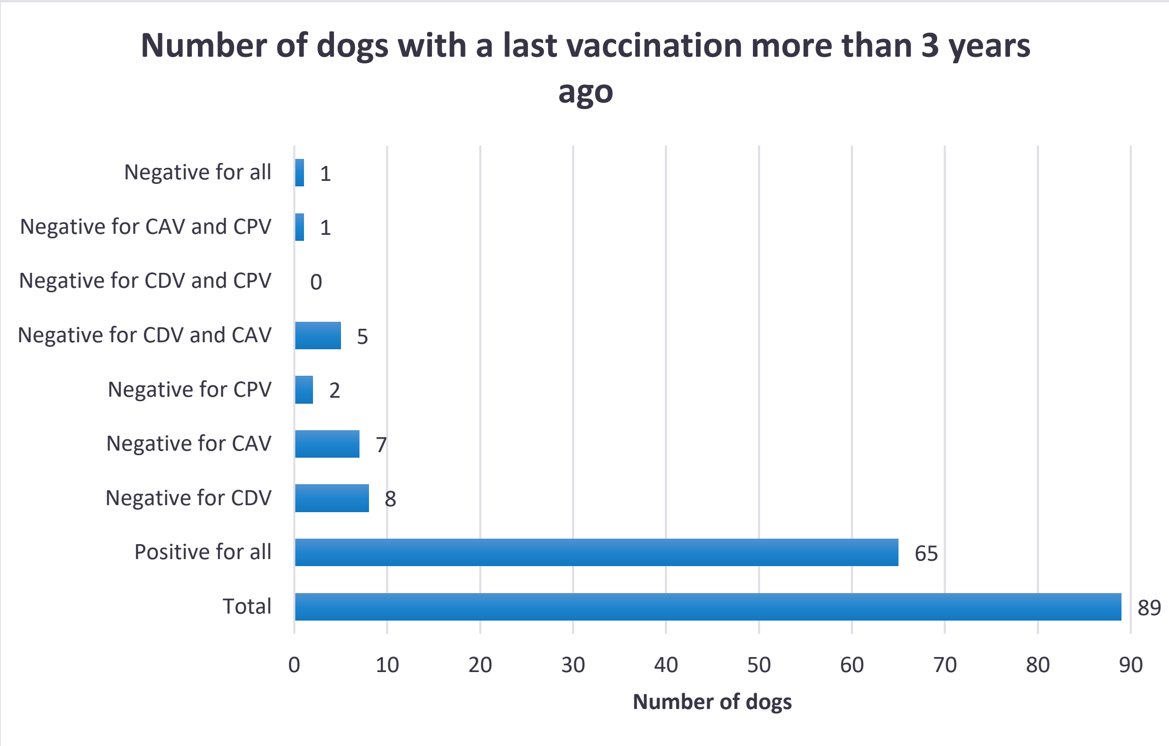

Figure 7: This figure shows the 89 dogs with a last core vaccination more than 3 years ago and for which antigen the VacciCheck was negative.

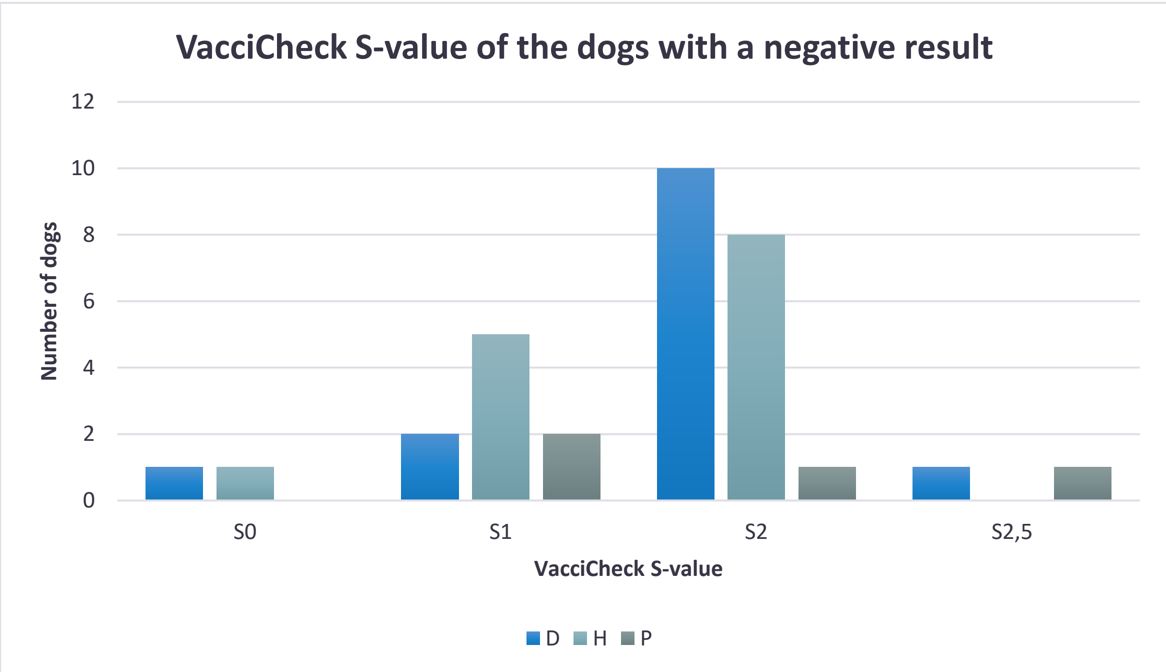

Figure 8: VacciCheck S-values of the 24 dogs with a last vaccination more than 3 years ago, which had a negative result for one or more viral antigens. D = Canine Distemper Virus, H = Infectious Canine Hepatitis (Canine Adeno Virus), P = Canine Parvovirus.

3.2. Puppies

Of the 929 dogs, 145 (15.6%) were puppies of an age between one month and one year. For the analysis in this study only the results of 9 litters and 45 puppies that had a performed VacciCheck more than once, were analyzed.

Of those litters, three litters contained 2 dogs, two litters contained 3 dogs, two litters consisted of 4 dogs and the remaining two litters consisted of 6 dogs. It was not known if the litters that consisted of 2 or 3 dogs were that small, or that only 2 newborns of a bigger litter were tested. Differences in VacciCheck S-values between individual newborns were found in the litters. Figure 9 shows the S-value differences between dogs for three different litters. In the first litter of Shih Tzu's the values are not very divergent. This litter was tested at 8 weeks of age and the newborns had never have a vaccination before. In the litter of Bernese Mountain dogs and Australian Shepherds the values of individual newborns are much more divergent. In the Bernese Mountain litter, especially the values of Distemper vary a lot, from S1 to S5. The same applies for the Australian Shepherd litter, with the Distemper value varying from S0 to S6 and the Parvo value from S1 to S4. The dog with the lowest Distemper value had also the lowest Parvo value. It should be noted that the dogs in those two litters never had a vaccination before and that the VacciCheck was performed at 6 weeks of age.

Another interesting result occurred in a small litter Drentsche Patrijsdogs, consisting of two dogs. Both dogs were tested with the VacciCheck at the age of 6 weeks and still had sufficient antibody titers for all three antigens. At the age of 9 weeks, a second test was done and both dogs had values of S0 and S1 for all antigens and had a value of S1 for Parvo. As a result of this test, the dogs were vaccinated with a DHP vaccine. Four weeks later, a third (control) VacciCheck was performed and for both dogs the values for Distemper and Hepatitis were a S6, while the value for Parvo was S0. After this, a Parvo vaccine was given and 4 weeks later both dogs had a sufficient antibody titer for all antigens.

This occurrence as described above, that the Parvo value was still low after giving the first core vaccination when the VacciCheck value was S0 or S1, arose for several of the puppies. The VacciCheck was performed more than once for 45 out of the 145 puppies. Those results can show the development of the VacciCheck S-values during the first weeks of life. Then, of those 45 puppies, 12 puppies (26.7%) had a VacciCheck result of S0 or S1 for Parvo after their first core vaccination a few weeks earlier. Those dogs were all large breeds, except for two dogs with an unknown breed. The dogs were of 8 different pure breeds. Also, all dogs were vaccinated with a core vaccine only when the Parvo titer had a value of S0 or S1. Figure 10 shows the development of the VacciCheck S-values of a Golden Retriever pup. The dog was female and four VacciChecks were performed, at 9, 12, 17 and 22 weeks of age. The first DHP vaccination was given at 12 weeks of age (first arrow) when the VacciCheck values were low (Distemper S2, Hepatitis S0, Parvo S1). A control test was performed 5 weeks later that showed that the VacciCheck values increased for Distemper and Hepatitis, but for Parvo the value decreased from S1 to S0. At that moment, the dog received a Parvo vaccine (second arrow) and a control test 5 weeks later showed a decent protection for all three antigens.

For Hepatitis, 3 dogs (6.7%) lacked antibodies after the first core vaccination. The breeds of those dogs consisted of one beagle and two unknown breeds. For Distemper, only 2 puppies (4.4%) had a low antibody titer after vaccination, one Labrador Retriever and one Flatcoated Retriever.

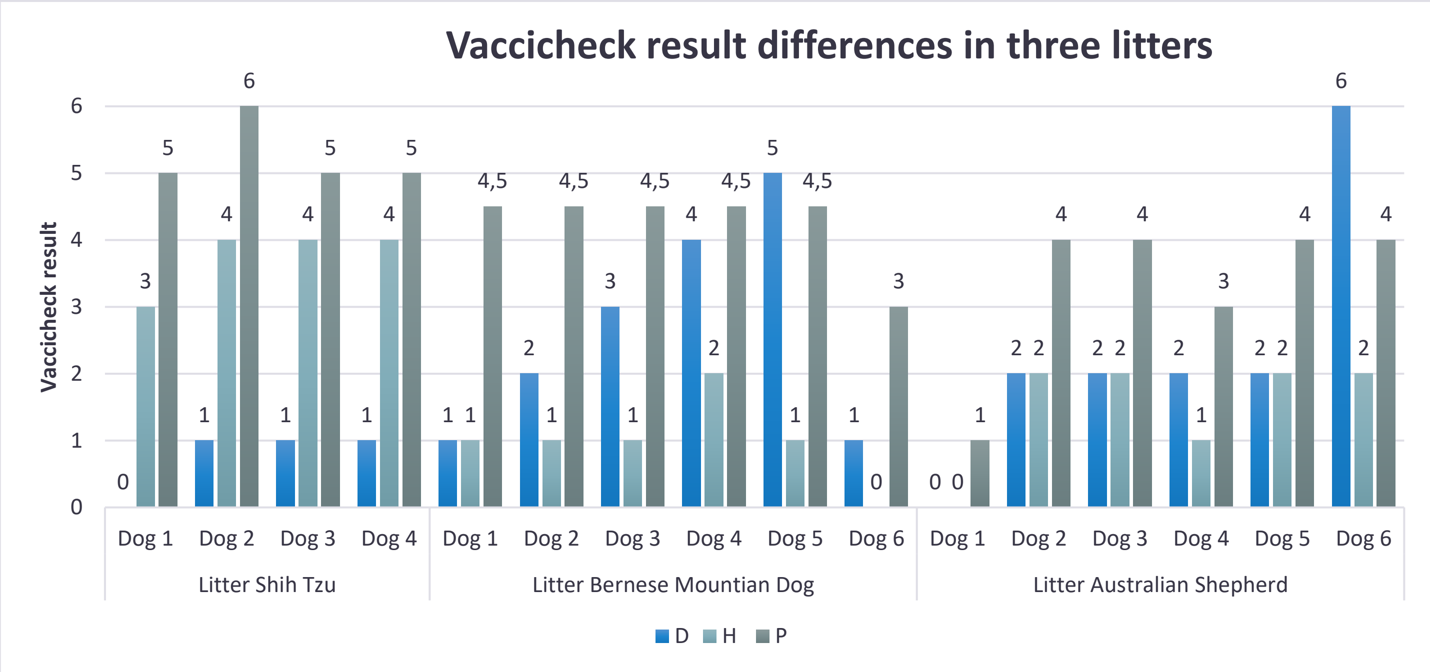

Figure 9: Differences in VacciCheck S-values between dogs in three different litters. D = Canine Distemper Virus, H = Infectious Canine Hepatitis (Canine Adeno Virus), P = Canine Parvovirus.

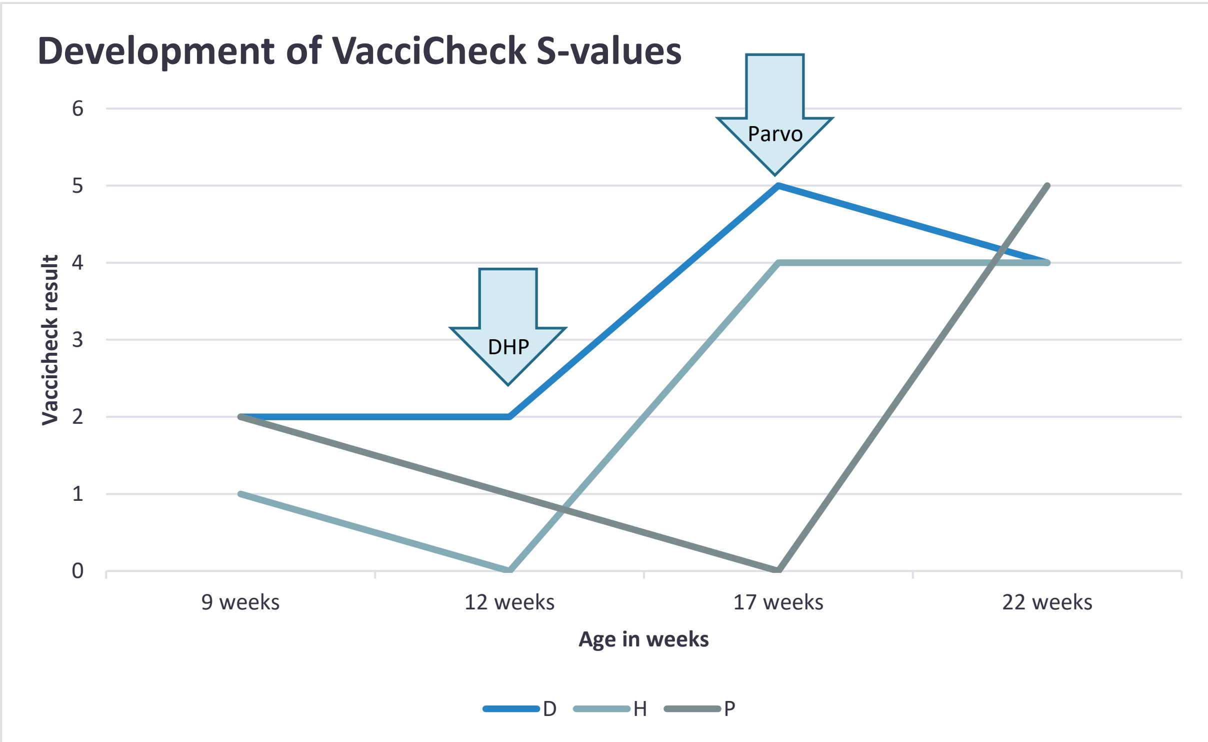

Figure 10: Development of the VacciCheck values of a Golden Retriever pup. The first arrow is the moment that the first DHP vaccination is given and the second arrow is the moment of the Parvo vaccination. DHP = Canine core vaccine with CDV, CAV, CPV. Parvo = Parvo vaccine

4. Discussion

The study shows that most of the dog population that received the last core vaccination more than 3 years ago still has a protective level of antibodies against CDV, CAV and CPV. This is in line with several other studies that support the observation that many dogs maintain protective antibodies against those viruses for many years. (1,2,4,16,17,26) A recent study performed by Killey *et al.* (2017) showed comparable results. (27) In that study, of the total population of 486 dogs, 93.6% were seropositive to all three of the infectious agents when vaccinated up to 124 months ago. Only 6.4% were seronegative for one or more of the viral antigens. Looking at each antigen, the percentage of positive dogs were 95.7%, 97.3% and 98.5% for CDV, CAV and CPV respectively. Also, in a population of 100 dogs with a last vaccination more than 3 years ago showed the results that 93% of those dogs had protective antibody levels against CDV, CAV and CPV. (27) The comparable results of our present study of 412 adult dogs demonstrate a protected antibody level for CDV, CAV and CPV of 89.3%, 90.0% and 97.1% respectively. Furthermore, in the population of 89 dogs with a last vaccination more than 3 years ago, 73% were seropositive against all three vaccine antigens. The two studies differ in the recruitment of veterinary practices (one large veterinary practice group and one veterinary practice versus 16 different veterinary practices) and in the recorded data (all data complete plus a relevant medical history versus not all data complete and no medical history). Another difference between the studies is that a VacciCheck result of S2 was considered positive in the study of Killey *et al.* In contrast, within the present study a VacciCheck result of S2 was considered negative and S3 positive. (27) This may account for the dissimilarity observed.

The results of this study support the use of the VacciCheck to monitor immune status of dogs and making decisions about revaccinating dogs. The percentage of the dogs with a last core vaccination more than 3 years ago that were seronegative (S-value lower than 3) for one or more core vaccine antigens (27%), is relatively small. To minimize this percentage further it is important to keep monitoring the vaccination status of dogs and revaccinate when the VacciCheck values are low (S-value below S3). Several reasons may account for the seronegative dogs in the population. The first reason is that the dogs had a decreasing serum antibody titer at the time of testing, however, decrease of immunity over the years is to be expected. (27) Furthermore, current disease might be involved in decreasing the vaccine-induced immunity, since a recent study showed that dogs hospitalized in an intensive care unit were more often seronegative for CDV and CPV. For example, dogs with sepsis had lower antibody titers for CDV and CPV, probably due to immunoparalysis which makes it unable for the dog to generate adequate antibody titers. However, further evidence must be collected. (28) Another reason for a seronegative test result might be that a rare number of dogs in the population can be a so-called ‘non-responder’. Those dogs cannot genetically induce immunity to specific viral vaccine antigens. (27) It is estimated that approximately 0.05% to 0.075% of dogs are non-responders to CDV-vaccines, 0.001% to 0.002% are non-responders to CAV-vaccines and 0.1% to 0.2% cannot respond to CPV-vaccines. (29) Finally, false-negative test results might also occur. The VacciCheck has a specificity of 92% for CDV, 88% for CAV and 100% for CPV. Specificity is the ability of the test to identify seronegative dogs. The sensitivity of the VacciCheck for CDV is 100%, for CAV 92% to 94% and 88% for CPV. The sensitivity of a test is the ability to identify seropositive dogs. A false-negative result means that the test shows that the dog is seronegative, but in fact the animal is seropositive. (23)

In the present study, age was not significantly correlated with the time elapsed since last core vaccination. However, for CPV, age was significantly correlated with the value of the titer. The results showed that older dogs have a significant lower VacciCheck S-value for CPV than younger dogs. This result was not significant for CDV and CAV. Multiple other studies did not find a significant association between the age and the antibody titer for CDV, CAV and CPV. (15,17,19) A study from Ottiger *et al*. (2006) on CPV-antibody titers found that dogs with an antibody titer value above the cutoff value of 80, were significantly younger than those with antibody titers below 80 (Hemagglutination inhibition (HI) test with a cutoff value of 80 was used). (30) This is in line with the results of the present study that older dogs have lower antibody titers for CPV than younger dogs. An explanation might be that younger dogs have received multiple vaccinations more recently. Additionally, the risk of exposure to wild CPV and the degree of natural boosting might be greater in younger dogs. (30) McCaw *et al*. (1998) support the observation that older dogs have lower antibody titers as well. (31)

Between breed and antibody titer for CDV, CAV and CPV no significant associations were found in the present study. A study by Twark and Dodds (2000) suggested that certain breeds of dogs have difficulty to induce a proper immune response to CPV, including breeds such as Rottweiler, Doberman Pinscher, Labrador Retriever, Alaskan sled dog, Pomeranian and American Staffordshire Terrier. (15) In the current study one Rottweiler, two Labrador Retrievers and one Pomeranian were seronegative for CPV. One of the Labrador Retrievers puppies had two puppy core vaccinations but responded to none of them. According to current recommendations, dogs like this Labrador Retriever should be revaccinated and retested to determine if seroconversion had occurred. Another method is to re-test the dog with the gold standard procedure (serum neutralization (SN) tests and hemagglutination inhibition (HI) tests). Failure of seroconversion indicates that the dog might be a non-responder for CPV. (27)

Between gender and neutering status and antibody titer for CDV, CAV and CPV, no significant associations were found in this study. This is in line with other performed studies. (15,32)

The present study showed some interesting results concerning puppies. There is not much information available yet about performing antibody titer tests for puppies. In the Netherlands, the performance of an antibody titer test for puppies is increasing, since it is a method to determine whether the maternal antibodies have vanished or not and to decide about the optimal vaccination time. However, performing a titer test for newborns has disadvantages. One disadvantage is that the welfare of the animals could be harmed since they are subjected to multiple blood tests. This is a consequence of the differences in antibody titers between individual puppies of the litters, as seen in figure 9 of the results, due to which all the puppies in a litter should be tested every 2-3 weeks. A second disadvantage is that there is an increasing risk of infections because the puppies might stay unprotected for a longer period of time if the titer test is not performed within the required timeframe (every 2-3 weeks starting from 6 weeks of age). (1)

On the other hand, there are also advantages of performing a titer test in puppies. One big advantage is to determine the optimal vaccination time and to monitor if the vaccination induced immunity and provided seroconversion. The reason that the puppies in a litter have differences in antibody titer most probably has to do with the difference in ingestion of colostrum, since maternal antibodies are transferred to the puppies through the ingestion of colostrum in the first hours of life (first day). Another factor that might play a minor role is the passive transfer through the placenta. (33) The differences of ingestion of colostrum between littermates is related to the size and strength of the individual newborn and the maternal abilities of the bitch. There is also variation between the concentration of colostrum antibodies of the individual bitch and the vaccination status of the bitch, but this should be equal for all newborns within a litter. (34) Furthermore, the ability of the newborn to absorb the colostrum antibodies in the intestine plays also a role. (35) The differences of Distemper S-values within a litter, as seen in figure 9 (Bernese Mountain Dog litter and Australian Shepherd litter) are hard to explain. It can be concluded that dog number 1 from the Australian Shepherd litter had less colostrum than the other newborns or that the absorption of colostrum antibodies had failed, since the S-values of Hepatitis and Parvo are also low. The other newborns have comparable S-values for Hepatitis and Parvo; however, this is not the case for Distemper. This exception can possibly be explained by the fact that the titer test or the blood sample has been performed inadequately.

The present results also show the remarkable phenomenon that for CPV, the antibody titer of puppies remained negative after receiving a first core vaccination at a time point that the VacciCheck S-value was below S3 (even S1 or S0). This occurred while the maternal antibodies were almost absent. This observation provides little evidence that failure of the CPV vaccine to induce immunity has occurred several times. The first and most likely reason for vaccine failure is the interaction with maternal antibodies. (33) The results of this study indicate that even small amounts of maternal antibodies might still interfere with the immunization and that deciding about the optimal vaccination time is difficult. A study done by Altman *et al.* (2017) claims that the use of a final vaccination at less than 12 weeks of age leads to a predisposition for vaccine failure and that a final vaccination at 16 weeks of age will reduce this number of vaccine failure cases. (33) Another possible reason that the vaccination does not work can be explained by the fact that the strains of CPV used in the vaccine and the strains of CPV circulating in the wild are different, however this distinction between strains cannot be made with the VacciCheck. (33,36)

If it is decided to perform antibody titer tests for puppies, it is recommended – based on present results – to test the newborns every 2-3 weeks starting from 6 weeks of age and to vaccinate when the maternal antibodies have vanished (i.e. a VacciCheck S-value of S0 or S1, based on practical experiences). Also, it is important to perform control antibody titer test 3-4 weeks after vaccination to monitor whether the vaccination has induced immunity or not. Newborns do not have any cellular immunity yet, so if vaccination has failed, the newborn is susceptible for infection. The control antibody titer test 3-4 weeks after vaccination could prevent the newborn for staying unprotected for too long (if vaccination has failed and next vaccination is given one year later).

The present study had several limitations. First, this study was dependent on the work and the willingness to cooperate of the veterinarians. It turned out to be more work intensive for the veterinarians than expected, since they had to do this work next to their regular work. The reason why this study turned out to be more work intensive for the veterinarians, can be attributed to the fact that the required data were difficult to retrieve from the veterinary databases. It appeared to be impossible to extract this data in a single report, due to which the data had to be entered manually in an excel file. Because this work took a lot of time, many veterinary practices decided to drop out of this project. Moreover, the time period of the project was limited to only 12 weeks, which was rather short for a proper collection of data. With a longer duration of this project, more data could be collected and veterinary practices could be given more time to extract the needed data. Another limitation of this study was that many of the received data were not fully complete. In many cases, the date of the last vaccination was unknown and without this value the time interval between last vaccination and performed VacciCheck could not be calculated. Also, another 313 data entries were extreme incomplete, since only the date of the VacciCheck and the results of the VacciCheck were known.

During the analysis of the results, some assumptions have been made. Some data showed only years as date of last vaccination, lacking an exact time period (i.e. month and day). To determine the interval between last vaccination and performed VacciCheck, a full date was needed. Most adult dogs are vaccinated annually or triennially, so the assumption was made that the date was on the same day and month as the birth date. Also, some VacciCheck results did not show a regular S-value, but for example, S4+ or S4-. Those results were rounded off to a S4. Furthermore, in this study it was assumed that a S-value of S6 is the maximum value. However, some results showed higher values (S7 or S8). Those values were corrected to the maximum value of S6 for this study. During the statistical analysis, it was noticed that the data of the puppies disturbed the results of the analysis. Especially the puppies that never had a vaccination in their life were mostly considered as seronegative. This meant that the percentage of seronegative dogs were higher than expected. Therefore, since it is mostly maternal antibodies that is measured and not the antibodies induced after vaccination, all the puppies were excluded from the statistical analysis and were analyzed separately. Moreover, to not induce any confusion, those puppies (i.e. dogs younger than 1 year of age) were excluded and not only the not-vaccinated puppies.

Recommendations for further research are to include the health status of dogs in the data. It might be that disease correlates with a negative result of the titer test. Further research must be done to investigate this relationship. Another recommendation is to make a program for an easier extraction of data from the veterinarian’s database. This may take some time, but it ensures a more efficient process and veterinarians might be more willing to cooperate if the effort required is brought to a minimum. This could ensure more data and consequently more reliable results. Furthermore, a more complete data set would lead to an improved analysis.

Further research is planned to be performed later this year. Results of VacciChecks that will be performed in the upcoming time will be added to the database of this study and the results will be analyzed again when more results are available. Also, later this year another in-practice titer test will be available. The goal of this future research is to compare the VacciCheck and this other test kit with the standard laboratory tests, such as serum neutralization (SN) tests and hemagglutination inhibition (HI) tests.

5. Conclusion

Results of the present study show that most of the dog population that received last core vaccine more than 3 years ago still has a protective level of antibodies against CDV, CAV and CPV. Based on those results, the use of antibody titer testing can be supported. For this the VacciCheck might be used to monitor vaccination status of dogs and inform decision making about revaccinating dogs. It is important to keep monitoring the vaccination status of individual dogs in order that a more precise revaccination interval can be established in the individual dog. This, in turn, leads to less unnecessary vaccinations. Titer testing by puppies might be a useful tool to determine whether the maternal antibodies have vanished and to decide about the optimal vaccination time. However, further research must be done about this optimal vaccination time. Because of the strong variations between individual newborns in maternal antibodies, the recommendation is to test the newborns every 2-3 weeks, starting from the age of 6 weeks. The vaccine should be administered when the maternal antibodies have vanished (i.e. a VacciCheck S-value of S0 or S1, based on practical experience). Also, 3-4 weeks after the vaccination a control antibody titer test should be performed to monitor whether the vaccination has induced immunity. Further research will be done to investigate more about antibody titer testing and duration of immunity. Also, future research will give more clarity about the specificity and sensitivity of the VacciCheck, which will be compared to other laboratory tests.

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8. Appendix

8.1. Appendix I: Standard excel file

This standard excel file was send to the veterinary clinics that were willing to cooperate. In red, an example is shown of a dog that had the complete set of puppy vaccinations according to the vaccination guidelines. The VacciCheck has been performed on this dog at 4 years and 7 years of age.

							Uitslag VacciCheck (results VacciCheck)			**Vaccinatiegeschiedenis (Vaccination history)**		
Diernr (animal nr.)	**Ras (breed)**	**Geslacht (gender)**	**Geboortedatum (date of birth)**	**Datum laatste vaccinatie DHP voor VacciCheck (date of last vaccination)**	**Welk vaccin (type of the vaccine)**	**Datum Vaccicheck (date of the VacciCheck)**	**D**	**H**	**P**	**Datum/Vaccin (date/vaccine)**	**Datum/Vaccin (date/vaccine)**	**Datum/Vaccin (date/vaccine)**
48365	Labrador	Teef intact (bitch intact)	19-9-2008	21-12-2009	Nobivac DHP	14-1-2012	4	6	6	10-11-2008 / Nobivac Puppy DP	2-12-2008 / Nobivac Parvo + Lepto	28-12-2008 / Nobivac DHP
48365	Labrador	Teef intact (bitch intact)	19-9-2008	21-12-2009	Nobivac DHP	20-1-2015	2	4	5	10-11-2008 / Nobivac Puppy DP	2-12-2008 / Nobivac Parvo + Lepto	28-12-2008 / Nobivac DHP

8.2. APPENDIX II: STATISTICAL ANALYSIS RESULTS: TABLES

Key
Frequency Row percentage Column percentage

Distemper	Interval between last vaccination and VacciCheck		
	< 3	>= 3	Total
Negative result	30 68.18 9.29	14 31.82 15.73	44 100.00 10.68
Positive result	293 79.62 90.71	75 20.38 84.27	368 100.00 89.32
Total	323 78.40 100.00	89 21.60 100.00	412 100.00 100.00

Hepatitis	Interval between last vaccination and VacciCheck		
	< 3	>= 3	Total
Negative result	27 65.85 8.36	14 34.15 15.73	41 100.00 9.95
Positive result	296 79.78 91.64	75 20.22 84.27	371 100.00 90.05
Total	323 78.40 100.00	89 21.60 100.00	412 100.00 100.00

Parvo	Interval between last vaccination and VacciCheck		
	< 3	>= 3	Total
Negative result	8 66.67 2.48	4 33.33 4.49	12 100.00 2.91
Positive result	315 78.75 97.52	85 21.25 95.51	400 100.00 97.09
Total	323 78.40 100.00	89 21.60 100.00	412 100.00 100.00

8.3. Appendix III: Statistical analysis results: Gamma regression analysis

Distemper						95% Confidence Interval	
Parameter		Coefficient	Std. Error	Z	P	Lower Bound	Upper Bound
Interval between last Vaccination and VacciCheck	< 3	0	(base)				
	>= 3	-,1150556	,0402909	-2,86	0,004	-,1940243	-,0360869
	Unknown	-,047761	,0454697	-1,05	0,294	-,13688	,0413579
Age group	1-7	0	(base)				
	>7	-,0315656	,022574	-1,40	0,162	-,0758098	,0126768
	Unknown	,0262829	,0398309	0,66	0,509	-,0517843	,1043501
Gender and Neutering status	Bitch neutered	0	(base)				
	Bitch intact	,001152	,0515682	0,02	0,982	-,0999198	,1022238
	Bitch unknown	-,2386456	,1031554	-2,31	0,021	-,4408263	-,0364648
	Male neutered	,0458324	,0836972	0,55	0,584	-,1182112	,209876
	Male intact	,0322748	,0596318	0,54	0,588	-,0846015	,149151
	Male unknown	-,0458258	,0767977	-0,60	0,551	-,1963466	,104695
	Unknown	-,1228682	,0548687	-2,24	0,025	-,2304089	-,0153276
Breed	Small breed	0	(base)				
	Large breed	-,0071908	,0465391	-0,15	0,877	-,0984058	,0840243
	Unknown	,1221997	,0316755	3,86	0,000	,0601169	,1842826
	_cons	1,454575	,0479929	30,31	0,000	1,360511	1,54864

Hepatitis						95% Confidence Interval	
Parameter		Coefficient	Std. Error	Z	P	Lower Bound	Upper Bound
Interval between last Vaccination and VacciCheck	< 3	0	(base)				
	>= 3	-,1394241	,0292064	-4,77	0,000	-,1966676	-,0821807
	Unknown	-,0693238	,0545486	-1,27	0,204	-,1762371	,0375895
Age group	1-7	0	(base)				
	>7	-,0252997	,0194357	-1,30	0,196	-,063393	,0127936
	Unknown	-,0166611	,0561192	-0,30	0,767	-,1266526	,0933305
Gender and Neutering status	Bitch neutered	0	(base)				
	Bitch intact	-.0146177	,0368594	-0,40	0,692	-,0868608	,0576254
	Bitch unknown	,0476001	,1365332	0,35	0,727	-,2200001	,3152002
	Male neutered	-,0178415	,094624	-0,19	0,850	-,203301	,1676181
	Male intact	-,0676059	,0756395	-0,89	0,371	-,2158566	,0806447
	Male unknown	,1061111	,0482449	2,20	0,028	,0115528	,2006695
	Unknown	-,0312889	,0499845	-0,63	0,531	-,1292568	,066679
Breed	Small breed	0	(base)				
	Large breed	-,0010532	,0335738	-0,03	0,975	-,0668566	,0647502
	Unknown	,0937316	,0501458	1,87	0,062	-,0045524	,1920156
	_cons	1,543323	,0420056	36,74	0,000	1,460994	1,625653

Parvo						95% Confidence Interval	
Parameter		Coefficient	Std. Error	Z	P	Lower Bound	Upper Bound
Interval between last Vaccination and VacciCheck	< 3	0	(base)				
	>= 3	-,0372558	,0166279	-2,24	0,025	-,069846	-,0046657
	Unknown	-,0178313	,0213675	-0,83	0,404	-,0597109	,0240483
Age group	1-7	0	(base)				
	>7	-,0486912	,0193656	-2,51	0,012	-,0866471	-,0107352
	Unknown	-,0501342	,0187777	-2,67	0,008	-,0869377	-,0133307
Gender and Neutering status	Bitch neutered	0	(base)				
	Bitch intact	,0616833	,0259687	2,38	0,018	,0107857	,112581
	Bitch unknown	,1004404	,0270627	3,71	0,000	,0473986	,1534823
	Male neutered	-,0742069	,0515957	-1,44	0,150	-,1753326	,0269188
	Male intact	,0253946	,030796	0,82	0,410	-,0349645	,0857537
	Male unknown	,1461419	,0228733	6,39	0,000	,1013111	,1909727
	Unknown	,0384661	,0287484	1,34	0,181	-,0178797	,094812
Breed	Small breed	0	(base)				
	Large breed	,0003866	,0249607	0,02	0,988	-,0485355	,0493086
	Unknown	,0284475	,0166475	1,71	0,087	-,0041809	,061076
	_cons	1,665963	,0314356	53,00	0,000	1,604351	1,727576

8.4. Appendix IV: Statistical analysis results: Logistic regression analysis

Distemper						95% Confidence Interval	
Parameter		Odds Ratio	Std. Error	Z	P	Lower Bound	Upper Bound
Interval between last Vaccination and VacciCheck	< 3	1	(base)				
	>= 3	,5310184	,3005352	-1,12	0,263	,1751312	1,610111
	Unknown	,5216645	,2476134	-1,37	0,170	,2057594	1,322583
Age group	1-7	1	(base)				
	>7	1,290118	,418083	0,79	0,432	,6835715	2,434863
	Unknown	,7352619	,3543298	-0,64	0,523	,2859169	1,890794
Gender and Neutering status	Bitch neutered	1	(base)				
	Bitch intact	1,604263	,8377914	0,91	0,365	,5764402	4,464749
	Bitch unknown	,8639694	1,37102	-0,09	0,927	,0385248	19,37567
	Male neutered	1,421675	,9086446	0,55	0,582	,4062244	4,975474
	Male intact	2,009831	1,425293	0,98	0,325	,5006346	8,068601
	Male unknown	,7508133	,4507088	-0,48	0,633	,2315052	1,992222
	Unknown	,7740067	,3733566	-0,53	0,595	,3007128	1,992222
Breed	Small breed	1	(base)				
	Large breed	1,442742	,4267427	1,24	0,215	,8080072	2,576096
	Unknown	2,849954	1,28083	2,33	0,020	1,181109	6,876792
	_cons	5,301397	2,588624	3,42	0,001	2,035899	13,80462

Hepatitis						95% Confidence Interval	
Parameter		Odds Ratio	Std. Error	Z	P	Lower Bound	Upper Bound
Interval between last Vaccination and VacciCheck	< 3	1	(base)				
	>= 3	,5794071	,1475611	-2,14	0,032	,3517249	,9544749
	Unknown	,5740785	,1710881	-1,86	0,063	,3201064	1,029552
Age group	1-7	1	(base)				
	>7	,7596267	,2134375	-0,98	0,328	,4379578	1,317553
	Unknown	,5732095	,317139	-1,01	0,314	,1938073	1,69534
Gender and Neutering status	Bitch neutered	1	(base)				
	Bitch intact	,9875555	,4126884	-0,03	0,976	,4353653	2,240109
	Bitch unknown	,5558986	,7386925	-0,44	0,659	,0411052	7,51786
	Male neutered	1,048179	,7882762	0,06	0,950	,2400461	4,576949
	Male intact	,674174	,3521439	-0,75	0,450	,2421924	1,87665
	Male unknown	1	(empty)				
	Unknown	,887437	,6023467	-0,18	0,860	,2346311	3,356521
Breed	Small breed	1	(base)				
	Large breed	1,052303	,3192125	0,17	0,867	,5806734	1,906997
	Unknown	2,657711	1,881171	1,38	0,167	,6637638	10,64148
	_cons	9,69913	6,087598	3,62	0,000	2,834533	33,18823

Parvo						95% Confidence Interval	
Parameter		Odds Ratio	Std. Error	Z	P	Lower Bound	Upper Bound
Interval between last Vaccination and VacciCheck	< 3	1	(base)				
	>= 3	,8625925	,4995147	-0,26	0,799	,2772601	2,683638
	Unknown	,7493274	,4421125	-0,49	0,625	,2357502	2,381722
Age group	1-7	1	(base)				
	>7	,420269	,2252388	-1,62	0,106	,1470059	1,201489
	Unknown	,4524789	,3059443	-1,17	0,241	,1202423	1,702705
Gender and Neutering status	Bitch neutered	1	(base)				
	Bitch intact	1	(empty)				
	Bitch unknown	1	(empty)				
	Male neutered	,2152049	,1070804	-3.09	0,002	,0811551	,5706741
	Male intact	,886695	,5630593	-0,19	0,850	,2554193	3,078186
	Male unknown	1	(empty)				
	Unknown	,4545364	,3179735	-1,13	0,260	,1153734	1,790736
Breed	Small breed	1	(base)				
	Large breed	,822508	,5513512	-0,29	0,771	,2210827	3,060028
	Unknown	2,197485	1,170625	1,48	0,139	,7735372	6,242671
	_cons	58,33892	38,95947	6,09	0,000	15,75851	215,974