Final Report to the Hayward Foundation and
The Great Dane Health Foundation of a Study Titled

Vaccinosis in Great Danes

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Background: There is great concern among dog owners and veterinarians that some currently used vaccines or vaccine practices (e.g., yearly vaccination) adversely affect the health of dogs. Although few question the effectiveness of current vaccines for preventing common infectious diseases such as parvovirus, distemper, and rabies, the potential adverse effects of these vaccines are just becoming apparent. More and more owners are asking whether some vaccines used today could be responsible for the increasing prevalence of autoimmune diseases such as hypothyroidism, Addison's (hypoadrenocorticism), and hemolytic anemia, especially when the vaccines are administered annually. The situation has become so troublesome for some dog owners; they have stopped vaccinating their dogs entirely. The risk versus benefits of vaccinating however cannot be fully evaluated without a clearer understanding of the frequency and types of adverse events that result from vaccination. *The term vaccinosis describes the abnormal physiologic changes or clinical diseases associated either directly or indirectly, with the administration of a vaccine*. Such adverse events may be obvious when they occur soon after vaccination, but are more difficult to identify when their onset is delayed by months or even years following vaccination. Yet, there has been little research conducted on the frequency or severity of vaccine-related adverse reactions in dogs.

Specific Objective: to compare the health of fully vaccinated, partially vaccinated, and unvaccinated, Great Dane dogs with respect to their immune status and to identify specific vaccine types associated with a higher concentration of auto-antibodies. Of particular interest was the concentration of antibodies produced against the dogs' own thyroid gland, since an increased level of thyroid auto-antibodies has been associated with a greater probability of developing thyroid disease. For example, in one study, approximately 20% of anti-thyroglobulin positive dogs without overt signs of hypothyroidism developed thyroid dysfunction within 1-year.

Specific Hypotheses Tested: Vaccinated compared with unvaccinated Great Dane dogs have:

1) A significantly higher serum concentration of antibodies directed against their own tissues

2) A significantly higher serum concentration of TSH and lower concentrations of T3 and T4 hormones

3) A significantly higher proportion of dogs with a history or clinical signs of an autoimmune disease, particularly hypothyroidism

Materials and Methods

Great Danes for the proposed study were recruited through personal contacts, the Purdue University School of Veterinary Medicine website and the website of the Great Dane Health Foundation of America. The intent was to enroll 100 dogs divided among the following groups: 1) Dogs with no history of vaccination (never vaccinated), 2) Dogs with a history of vaccination only in the first year of life (partially vaccinated), and 3) Dogs that were vaccinated regularly throughout their life (fully vaccinated). The dogs were required to be 2 years of age or older and of either sex or neuter status to enter the study. Each owner was asked to complete a 15-page questionnaire that was developed specifically for this study. It included questions about their dog's age, weight, sex and neuter status, coat color, vaccine history, previous illnesses, diet and nutritional supplements used, medications, flea and tick control, housing, environment, etc. Each owner was also asked to take his or her dog(s) to a veterinarian to collect 10ml of blood, centrifuge the blood specimen, and send the serum on ice by express mail to the laboratory of Dr. Harm HogenEsch at Purdue University in W. Lafayette, IN. The veterinarian was also asked to conduct a physical examination and record any current conditions or diseases on a form provided to them by Purdue.

The serum specimens were assayed for the following:

- Total immunoglobulins IgA, IgG, and IgM
- Antibodies against bovine fibronectin, bovine thyroglobulin, canine thyroglobulin, bovine DNA, murine laminin, and porcine insulin
- Acute phase protein serum amyloid
- Hormones including TSH, T3, and T4
- Antibodies against canine distemper, parvovirus, and rabies, in order to validate the vaccination status of the dog

The test results and owner reported information were compared between the three groups using Analysis of Variance and Covariance, T-tests, and Chi-square tests. A p-value of <0.05 was considered to be statistically significant. All of the information collected from owners and veterinarians are being kept strictly confidential.

Results: A total of 75 Great Danes who met the eligibility requirements were enrolled in the study. A majority of these 75 dogs were female (47 dogs), neutered (41 dogs), and in average or optimum body condition (59 dogs). The mean (standard deviation) age was 5.7 (2.3) years with a minimum of 2.2 years and a maximum of 10.5 years; the median age was 5.0 years. Only 15 of the dogs had never been vaccinated during their life Eighteen dogs had never received a distemper vaccine, 19 dogs had never received a parvovirus vaccine, and 23 had never been vaccinated against rabies. It should be noted that totally unvaccinated dogs were much harder to recruit into the study than vaccinated dogs, because many owners of unvaccinated dogs did not have a regular veterinarian. Therefore, they either could not or would not provide us with a serum sample or a questionnaire completed by a veterinarian. Very few dogs, whether vaccinated or not, had a history of either an endocrine or autoimmune disorder. The disease most commonly reported by owners (7 dogs) or their veterinarians (6 dogs) was hypothyroidism.

The dogs in this study were divided into three vaccine groups (never vaccinated, partially vaccinated, and fully vaccinated) based on owner reports. Therefore, it was important to document the validity of these reports. This was done by measuring the antibody titer against distemper, parvovirus, and rabies, and comparing them with the owners' answer to the question "How frequently and when was your dog vaccinated against distemper, parvovirus, and rabies?" A very significant and strong correlation was found between the owners' responses regarding the pattern of administration of distemper vaccine and the distemper antibody titer. A similarly strong relationship was found for rabies. However, there was no clear-cut relationship between the parvovirus vaccine history and antibody titers to parvovirus. In fact, the parvovirus antibody titers of dogs belonging to owners who said they never vaccinated or only sporadically vaccinated their dog for parvovirus were not significantly different from dogs belonging to owners who claimed they had

never vaccinated their dog against parvovirus. The findings with regard to rabies and distemper antibody titers support the validity of the owners' answers on the questionnaire. It is not surprising however, that many dogs unvaccinated for parvovirus based on owners' reports had antibody titers as high or higher against parvovirus than dogs that were reported to have been vaccinated against parvovirus either regularly or sporadically. Parvovirus is commonly shed in dog feces (either the vaccine or natural strain) and contaminates the environment of parks, homes, kennels, etc. Once in the environment it is highly resistant to a wide range of climatic conditions and is readily transmitted from dog to dog, by fecal oral contact. In contrast, distemper and rabies virus are not stable in the environment and transmission from dog to dog requires closer contact between individuals. Therefore, we believe the vaccine groups (never vaccinated, partially vaccinated, and fully vaccinated) to which dogs were assigned in this study were valid.

The dogs vaccinated at least once in their lifetime did not differ significantly from those that were never vaccinated with respect to their gender, body condition, age, weight, and height. However, the unvaccinated dogs were significantly less likely to have been neutered. Also, there were significant differences between the vaccine groups related to whether the dog had a regular veterinarian and if it had received routine medication for heartworm or flea/tick prevention. In general, dogs in the never vaccinated group were less likely to have received routine preventive medical care or had been surgically neutered, compared with dogs in the vaccinated group. However, dogs reported by owners as never being vaccinated were not more likely to have a history of non-infectious conditions including cancer, allergies, endocrine abnormalities, autoimmunity, urinary tract problems, neurological disease, musculoskeletal disease, or genetic problems.

There were 7 adverse reactions reported by Great Dane owners. Three reactions were to vaccinations while four were to drugs or anesthesia. None of these adverse reactions had deleterious long-term consequences.

Specific Hypotheses Tested:

<u>Hypothesis 1</u>: Vaccinated compared with unvaccinated Great Dane dogs have a significantly higher serum concentration of antibodies directed against their own tissues, particularly the thyroid gland

Thyroiditis or inflammation of the thyroid gland is thought to be a precursor of clinical hypothyroidism in dogs. Approximately 40% of dogs with thyroiditis have an increased concentration of antibodies in their blood directed against thyroid tissue. It is not known what triggers production of these thyroid autoantibodies. Environmental factors such as estrogenic-like chemicals that disrupt hormone function and viruses have been suggested as causing the dog's immune system to produce antibodies that destroy its own tissues. Recently, evidence from experiments in Beagles and epidemiological studies of owned dogs indicate that commonly used vaccines may act to trigger autoimmune responses, particularly against the thyroid gland. For this reason we evaluated whether dogs receiving regular vaccinations for distemper, parvovirus, and rabies, had higher levels of auto-antibodies in their blood than dogs never vaccinated or partially vaccinated. Furthermore, we looked for a positive relationship between the number of vaccines a dog had received and the auto-antibody titer. While our primary interest was in auto-antibodies directed against the thyroid gland, we also measured those directed against connective tissue components (fibronectin), laminin, DNA, and insulin, since such antibodies have been associated with health disorders in people and dogs.

As in previous studies, the strongest positive relationship was shown between previous vaccination for rabies and an antibody response to bovine fibronectin and bovine thyroglobulin. A strong positive association was also observed between vaccination for distemper or parvovirus and bovine thyroglobulin. In contrast, there was only a weak positive relationship between previous vaccination for parvovirus and antibody to canine thyroglobulin. It thus appears that vaccinated dogs in this study were producing antibodies that reacted to bovine contaminants plus adjuvant in the canine vaccines. This reaction was evidenced by higher concentrations of antibodies against bovine thyroglobulin, but these antibodies only weakly cross-reacted with canine thyroglobulin. Similarly, the increasing antibody titers to bovine laminin in dogs with regular rabies

vaccination, was likely caused by contaminants in the rabies vaccine combined with the presence of adjuvant. *These findings alone do not tell us whether the serum auto-antibodies might be responsible for clinical autoimmunity in dogs.* They do however warrant further investigation o determine if they play a causal role in thyroid disease.

The concentration of anti-bovine thyroglobulin antibodies in the serum was significantly correlated with the concentration of anti-canine thyroglobulin antibodies Each unit increase in anti-bovine thyroglobulin antibodies was associated with a 0.07 increase in anti-canine thyroglobulin antibodies. This relationship is not surprising, since we previously hypothesized that dogs respond to impurities of bovine origin in canine vaccines by producing anti-bovine thyroglobulin antibodies that then cross-react with canine-thyroglobulin.

In contrast to the findings for specific auto-antibodies, no relationship was found between the vaccine history of dogs and the concentration of immunoglobulins IgG, IgM, IgA, or serum level of amyloid protein (SAA). (Figures 11A-14C)

<u>Hypothesis 2</u>: Vaccinated compared with unvaccinated Great Dane dogs have a significantly higher serum concentration of TSH and lower concentrations of T3 and T4 hormones

Dogs diagnosed with clinical hypothyroidism typically have lower serum concentrations of T3 and/or T4 hormones and an increased concentration of serum TSH hormone. The serum T3 and serum T4 concentrations were consistently lower for Great Danes in this study that were previously vaccinated compared with those never vaccinated, but these differences were not statistically significant. However, the vaccinated dogs in this study also had consistently lower serum concentrations of TSH compared with dogs that were never vaccinated. In addition, no significant correlation was found between the concentration of TSH in serum and either the T3 or T4 concentration. These findings when taken together, suggest the differences observed in thyroid hormone levels between dogs in the three vaccine groups, were more likely associated with non-thyroidal causes

rather than any abnormality in their thyroid function. It is also possible that vaccination results in substances in blood that interfere with the laboratory assay for T3, T4, and TSH. These findings warrant additional studies.

Hypothesis 3: Vaccinated compared with unvaccinated Great Dane dogs have a significantly higher risk of autoimmune diseases, particularly hypothyroidism

We were unable to test this hypothesis because only one owner of a dog in the unvaccinated group reported having a regular veterinarian or even using a veterinarian when their dog was ill. Since the diagnosis of an autoimmune disease requires specific tests that must be requested and or preformed by a veterinary laboratory, it was impossible to know if dogs in the unvaccinated group ever experienced an autoimmune disease. We did not anticipate this when the study was designed. We assumed that all Great Dane owners interested in participating in this health-related study would either use veterinary services regularly or would take their dog to a veterinarian when it was sick. Because many of the owners of dogs in the never vaccinated group had not established a veterinary-client relationship, it was difficult to even obtain blood samples for these dogs.

Conclusions

As in two previous studies we conducted, we confirmed that vaccinated dogs when compared with non-vaccinated dogs have a higher concentration of antibodies in their serum directed against bovine proteins such as thyroglobulin and fibronectin. These antibodies are probably produced in response to contaminants from fetal calf serum commonly used to make canine vaccines. These anti-bovine antibodies probably then cross-react with a dog's own thyroglobulin and fibronectin, resulting in detectable concentrations of autoantibodies in their serum. It would be difficult to design a study in pet dogs to prove this process of cross-reaction between bovine and canine proteins actually causes clinical signs of autoimmune disease in vaccinated dogs. There were too many differences between the vaccinated and unvaccinated Great Danes in the present study to further explore the clinical consequences of vaccine-related auto-antibodies produced against fibronectin or thyroglobulin.

The best way to determine if repeated vaccination of Great Danes causes autoimmune disease would be to prospectively follow a large number of regularly vaccinated and non-vaccinated dogs from birth, performing annual physical examinations and blood tests for autoimmunity. In our experience however, it is unlikely owners of unvaccinated Great Danes would actively participate in such a study. Therefore, the long-term potential adverse consequence of repeated vaccination is likely to remain unknown. Until further studies are done, we recommend that all Great Danes continue to be vaccinated using core vaccines as per guidelines published by the American Animal Hospital Association. You should discuss these guidelines with your veterinarian and work together to determine how they can be individualized to fit your dog's life style.

We would like to thank all of the Great Dane owners for both their participation and interest in this study. We enjoyed communicating with many of you over the past few years and appreciate your efforts. We plan to continue our research to make current canine vaccines safer for all dog breeds. We also thank the Great Dane Health Foundation and the Hayward Genetic Foundation for their sponsorship of this study and for their support of canine health research at the Purdue University School of Veterinary Medicine.

A more in-depth summary of this study will be posted at www.vet.epi.edu/epi