Purdue University and Hayward Foundation Study on Vaccines

For 15 years, some Great Dane breeders have postulated the adverse effects of the aggressive use of vaccines on the immature (puppy) immune system. This concern led these breeders to a more conservative approach to vaccinations, the monitoring of antibody titers before boosters were given, or in some cases safe alternatives to conventional practice were invoked.

Over the last two years a cutting edge scientific research study has been conducted at Purdue University, under the direction of Larry Glickman, VDM,PhD and his associate Dr. Harm HogenEsch (Curricula Vitae and the impressive credentials of these scientists are included for your review). The study proposal is to investigate the autoimmune responses to vaccines in dogs. This study, at Purdue, was conceptualized by Great Dane people, and subsequently funded (± $175,000.00) by a Great Dane friendly organization called the John & Winifred Hayward Foundation. Originally, it was considered ideal to conduct the study with a colony of Great Danes. However, the feasibility and practicality of maintaining and housing a controlled group of Great Danes was economically out of reach. Therefore, the study identified the Beagle as the canine of choice, but recognized the applications of any results to the canine population as a whole, including Great Danes.

The study has produced some dramatic results, with concrete and clear evidence that there are adverse events elicited as a result of the use of vaccines following manufacturer's recommendations. (Review the following article for a more complete picture of study results.)

In Dr. LaRosa's (Trustee of the Hayward Foundation) article (following), he states that a number of autoantibodies to several critical proteins and DNA were identified in the vaccinated group. Identifying these autoimmune antibodies, and monitoring their titers may lead, in addition to the thyroiditis conditions, a better understanding of the role of vaccines in soliciting adverse events that contribute to problematic conditions observed in the Great Dane, such as Cardiomyopathy, and Various Bone related disorders.

The positive outcomes of the study at Purdue will hopefully be the identification of a number of genetic markers that will facilitate a brighter and healthier future for our breed, as well as recommendations for safer vaccines and their uses.

The Hayward Foundation is limited by its charter to investigate Human Genetic Disorders, therefore its ability to significantly fund this study further falls outside of the realm of its charter. However, the Trustees of the Hayward Foundation have committed an additional $25,000.00 to help in the maintenance of this colony until further funding is identified.

Glickman has stated that the colony will be lost unless further and immediate funding is identified to maintain the colony . Dr. Glickman is applying to the AKC Foundation and the NIH (National Institute of Health) for funding. Two years of research will not be lost but the future of this work which relies on maintaining the colony for some time to come will be lost.

VACCINE SAFETY (?)

A Possible Etiology of Autoimmune Diseases

By : William R. La Rosa, M.D. (Trustee)

The Hayward Foundation is dedicated to research and eradication of human genetic diseases. There is much anecdotal hearsay about the safety of vaccines in dogs as well as in humans. The Vaccine Safety Committee recently emphasized the need for more definitive research on possible adverse effects during the development of new vaccines and vaccine combinations. (National Academy Press, Washington D.C. 1994).

When a dog vaccine safety issue was brought to our attention by Laura Kiaulenas, a prominent Harlequin Great Dane breeder, and after reading articles by Jean Dodds DVM, we decided to fund a study to prove or disprove the supposition of multiple diseases, acute and chronic, caused by vaccination. If indeed, many breeders are correct, then is the dog a canary sentinel, and are human similarly being affected, and if so can we identify the dog or human who is genetically susceptible to these reactions ?

We were fortunate that prominent and respected researchers, Drs. Larry T. Glickman, Harm HogenEsch, Juan I. Azona-Olivera, J. Catherine Scott-Montcrieff, and Paul W. Snyder of Purdue University, School of Veterinary Medicine, agreed to undertake the study. The results are enlightening and they are enthusiastically working on the second phase, a study of longer duration.

They presented a paper to the International Veterinary Vaccines and Diagnostics Conference, July 27 - 31, 1997, in Madison, Wisconsin, hosted by the University of Wisconsin. These proceedings have been published in Advances in Veterinary Science and Comparative Medicine . Another manuscript: Vaccine Induced Serum Autoantibodies in Young Beagles , has been submitted to a human immunology journal Clinical Immunology and Immmunopathology.

Their study was based on the increasing concern among veterinarians and breeders that current vaccination protocols adversely affect the health of dogs. This concern has largely been based on anecdotal and circumstantial evidence. They studied the effects of routinely used vaccination protocol on the immune and endocrine systems of Beagles.
One control group was not vaccinated and the other group was vaccinated with a commercial multivalent vaccine at 8, 10, 12, 16, and 20 weeks of age and with a rabies vaccine at 16 weeks of age.

A definition of autoimmune disease is now necessary. In dogs as well as humans, the body sometimes forms antibodies against itself (self antigens) which can lead to diseases of the pancreas (diabetes), thyroid (Hashimoto's Disease), collagen and fibronectin (Scleroderma, Lupus),cardiolipin (Cardiomyopathy), etc. The body literally attacks itself to cause the autoimmune disease.

The vaccinated group developed significant levels of autoantibodies against: fibronectin, laminin, DNA, albumin, Cytochrome C, transferrin, cardiolipin, collagen. The responses varied among individual animals, probably reflecting genetic differences. The clinical significance of those autoantibodies remains to be determined, but speculation must be that something in the vaccines is one of the etiologies (in the genetically susceptible dog) of such diseases as Cardiomyopathy, Lupus Erythematosus, Glomerulonephritis, etc. I apologise for using these technical terms but this is a complex study and adds validity to the report.

Autoimmune diseases are quite common in dogs as well as in humans, but much easier to study in dogs, especially since various breeds have genetic susceptibility or predisposition. The high prevalence of autoimmune disease in specific breeds makes it easier to search for the genetic markers. Humans are much more diverse and therefore more difficult to study.

We hope that many Breed Associations and the AKC Foundation will join us in expanding these studies with the needed research funds. Longer term studies are needed to determine the clinical importance of vaccine-induced autoantibodies and to identify markers of genetic susceptibility.

These are truly remarkable results. The next step is to study the development of safer vaccines, or possibly modify the recommended dosages, and the timing of vaccinations. Many vaccines, including a vaccine for use in humans, contain fibronectin. This appears to be a common contaminant. Other antigens will be studied.

A general theme of the Conference was that vaccine immunity lasts longer than the manufacturer claims; rabies is probably effective for at least 3 years and we are probably over-vaccinating our dogs. Even the vaccine industry tells you that there is never 100% protection. Therefore disease is seen even in vaccinated groups. In Europe, vaccines are prohibited once the disease is eliminated because the fear of reversion to virulence of the modified live virus.

Autoimmune diseases in dogs are clinically similar to those in humans. We hope that Veterinary and Medical Schools will continue and expand these preliminary research studies. Our companion dogs are crashing all around us and maybe we are now finding one of the sources of the problems. It has been so easy to point fingers at breeders but they may not be entirely at fault. Let us continue this important research to help our future generations of dogs and possibly children. Yes, indiscriminate breeding can genetically predispose the dog but is the trigger mechanism indiscriminate vaccinations

My personal interpretation of the above information in brief is: (from Dr. La Rosa MD)

1. These studies appear to support the conclusions of some breeders that multiple vaccinations may be harmful to dogs. Be cautious and keep current in the scientific literature.

2. Current vaccines induce autoantibodies. Contaminants may be part of the problem.

3. We need to research and explain the mechanism. Does it alter the DNA causing genetic abnormality or is the susceptible gene already in place? Can we find the genetic marker to avoid the adverse reaction of vaccination ?

4. The dog is a good model for study because different breeds already have susceptibility to specific diseases and genetic markers will be easier to find than in other diverse animals (and humans).

5. What is the solution or cure, and especially how is this applicable to humans.

6. Most current vaccines are effective in preventing disease, but they may be administered more frequently than is actually required.

This study certainly points out the preliminary conclusions that have also been done with the Canine Health Concern in the UK.

PROGRESS REPORT

Effects Of Vaccination On The Endocrine And Immune Systems of Dogs

Phase II

Purdue University, November 1, 1999

Drs. Harm Hogen Esch and Larry T. Glickman

Concern has been growing among owners, breeders, and veterinarians that current vaccines cause immune-mediated diseases in dogs. Vaccination is highly effective in preventing infectious disease, but the safety of many vaccines and the frequency of their administration are being questioned. The Vaccine Research Group at the Purdue University School of Veterinary Medicine has been conducting several studies to address these issues. In one such study, we are trying to determine if current vaccines cause changes in the immune system of the dog that will eventually result in life-threatening immune mediated diseases.

The Beagles being used in this study were bred by us and then vaccinated following a typical schedule used for pet dogs. These Beagles have been closely followed for three years with blood and other tests performed at regular intervals.

To date, routing vaccination of these Beagles has not caused any overt signs of clinical disease. However, the blood of all the vaccinated dogs contains significantly elevated concentrations of antibodies directed against proteins that are present in commercial vaccines as contaminants of the production process. None of the unvaccinated control dogs has had a similar increase in these antibodies. These proteins are typically of bovine origin since fetal calf serum is used to grow the viruses for vaccine production. The close similarity in structure of the bovine proteins to dog proteins results in a situation whereby the antibodies produced by the vaccinated dogs may cross-react with dog tissue proteins in a process similar to autoimmunity.

Experiments in other animal species suggest that these auto antibodies might eventually cause diseases in the vaccinated animals, but these Beagle dogs will need to be followed longer to determine if this is the case. In addition, the pattern of individual responses of the immune system to vaccination in this study suggests a possible genetic predisposition to autoimmunity.

The study described above is unique in that it attempts to determine if vaccinations that dogs routinely receive throughout their life have a cumulative adverse effect. The only way this is possible is under experimental conditions where one group of dogs remains unvaccinated.

The vaccine studies we are conducting both in Beagles and in pet dogs under natural conditions are designed to answer the question: "Does vaccination play a role in autoimmunity, how safe are currently used vaccines, and how often should these vaccines be administered?"

In March, 2000 I personally contacted Dr. Glickman regarding this study and in the course of events that followed; Dr. Glickman has agreed to extend the study to our breed, the Great Dane. In the initial conversations, Dr. Glickman postulated that to continue the study further would cost in the neighborhood of one to two million dollars. When I told him that we have a number of Great Danes that are totally unvaccinated and could act as 'controls'. he came back very excited and proposed the further study with Great Danes.

The Study will be divided into 3 groups: Unvaccinated..; Vaccinated without annual boosters and Vaccinated with annual vaccines

Final Report to the Hayward Foundation and

The Great Dane Health Foundation of a Study Titled

Vaccinosis in Great Danes

January 1, 2004

Submitted by:

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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:

Hypothesis 1:

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)

Hypothesis 2:

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 is posted at: **www.vet.purdue.edu/epi/gdhstudy.htm**