

## Lack of Association between Repeated Vaccination and Thyroiditis in Laboratory Beagles

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**Background:** Intensive vaccination protocols have been suggested as partially responsible for an increased prevalence of autoimmune diseases in dogs in recent years. The aim of this study was to determine whether repeated routine vaccination in dogs is associated with an increased prevalence of thyroiditis.

**Methodology/principal findings:** We conducted a prospective experimental study with 20 healthy purpose-bred Beagles. Five dogs were vaccinated with a multivalent vaccine and a rabies vaccine. Five dogs received only the multivalent vaccine, and 5 dogs received only the rabies vaccine. Five dogs were unvaccinated controls. The multivalent vaccine was administered at 8, 10, 12, 16, 20, 26, and 52 weeks of age and every 6 months thereafter. The rabies vaccine was administered at 16 and 52 weeks of age and then once a year. Blood samples were collected 1 week before euthanasia for evaluation of thyroid profiles and measurement of antibodies directed against canine thyroglobulin. Dogs were euthanized at 5.5 years of age, and the thyroid glands were evaluated histopathologically.

Thyroiditis was present in 8 of 20 (40%) dogs at postmortem examination. No association was found between a dog being vaccinated and the prevalence of thyroiditis at postmortem examination. However, the power of the study to detect such an association was low because of the unexpected high prevalence of thyroiditis in the unvaccinated control dogs. Thyroid function tests were abnormal in 2 of 8 dogs with thyroiditis but were normal in all dogs without thyroiditis.

**Conclusions/significance:** There was no evidence to support an association between routine vaccination and thyroiditis at postmortem examination in beagle dogs after repeated vaccination.

**Key words:** Thyroglobulin; Thyroid gland; Thyroiditis; Vaccination.

Lymphocytic thyroiditis is believed to be present in approximately 50% of cases of canine hypothyroidism.<sup>1,2</sup> Although the specific cause of canine thyroiditis is unknown, it is heritable in Beagles and Borzois.<sup>3,4</sup> Other breeds have an increased prevalence of this disease, suggesting that heredity plays a part in its pathogenesis.<sup>5</sup> Intensive vaccination protocols have been suggested as partially responsible for an increased prevalence of autoimmune diseases in dogs in recent years. Although the evidence is primarily anecdotal,<sup>6–8</sup> one case control study found a significant temporal relationship between vaccination and subsequent development of immune-mediated hemolytic anemia in dogs, but other studies did not confirm this association.<sup>9,10</sup>

Our group reported previously that beagles developed antibodies against canine thyroglobulin after vaccination with a rabies vaccine.<sup>11</sup> These antibodies were hypothesized to be the result of a normal immune response to heterologous antigens that contaminate most commonly used vaccines. The objective of the study reported here was to determine whether vaccination of these same dogs was associated with an increased prevalence of thyroiditis at postmortem examination.

This study reports on the last 18 months of a 5.5-year study. Results from the 1st 4.5 years of this study have been reported previously.<sup>11</sup>

## Materials and Methods

Twenty female Beagles were allocated to 4 experimental groups of 5 dogs each, such that the groups were equally distributed according to litter of origin and body weight at 8 weeks of age. The dogs were housed indoors in groups of 1–3 dogs per run, fed a standard laboratory ration,<sup>a</sup> and provided water, ad libitum. The light/dark cycle was 12/12 hours. The study was approved by the Purdue University Animal Care and Use Committee.

### Vaccination Schedule and Serum Collection

Five dogs (group V<sup>mr</sup>) were vaccinated SC with a multivalent vaccine<sup>b</sup> and a rabies vaccine.<sup>c</sup> Five dogs (group V<sup>m</sup>) received only the multivalent vaccine, and 5 dogs (group V<sup>r</sup>) received only the rabies vaccine. Five dogs (group U) were unvaccinated but received 1 mL of sterile saline (0.9% NaCl) solution SC according to the same schedule as dogs in group V<sup>mr</sup>. Groups V<sup>mr</sup> and V<sup>m</sup> were housed in a similar, but separate, room from groups V<sup>r</sup> and U.

The multivalent vaccine was administered at 8, 10, 12, 16, 20, 26, and 52 weeks of age and every 6 months thereafter until 5 years of age. The rabies vaccine was administered at 16 and 52 weeks of age and then once a year until 5 years of age. Blood was collected from all dogs at 8, 16, and 26 weeks of age and then 4 times yearly (immediately before vaccination and 2 weeks after vaccination). An appropriate response to vaccination was determined by measurement of antibody titers 2 weeks after each vaccination. Complete blood counts, serum biochemical profiles, and thyroid profiles (thyroxine [T<sub>4</sub>], thyroid-stimulating hormone [TSH]) were evaluated at 8, 16, and 26 weeks of age and then every 6 months before each vaccination. Anti-thyroglobulin antibody activity was measured at 8, 16, and 26 weeks of age and then immediately before each yearly vaccination and 2 weeks after each yearly vaccination. At 5.5 years of age (1 week before euthanasia), blood samples were collected for CBC, biochemical profile, thyroid profile, and anti-thyroglobulin antibody activity. One week later, all dogs were euthanized by overdose of pentobarbital. At postmortem examination, the thyroid glands, adrenal glands, kidneys, ovaries, and uterus were collected, weighed, and immediately fixed by immersion in 10% neutral buffered formalin. The tissues were embedded in paraffin and 5- $\mu$ m sections were stained with hematoxylin-eosin. The slides were examined by a board-certified pathologist (HH). The thyroid glands were evaluated for

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degree of inflammation with a semiquantitative scoring system ranging from 0 (least severe) to 3 (most severe).

### Hormone Assay Techniques

Serum T<sub>4</sub> and TSH concentrations in dogs were measured with chemiluminescent enzyme immunoassays previously validated for use in dogs.<sup>12,d,e</sup> Reference range for T<sub>4</sub> was 1.3–4.0 µg/dL and for TSH was 0–0.65 ng/mL.

### Antibody Assay Techniques

Anti-canine thyroglobulin antibody activity was measured by use of an indirect enzyme-linked immunosorbent assay as described previously.<sup>11</sup> Results were expressed as optical density (OD) of sample/OD of positive control × 100 (antibody score).

### Statistical Analyses

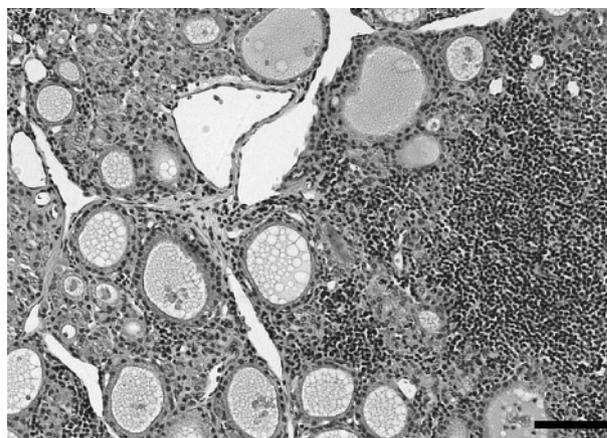
Analysis of variance was used to determine whether significant differences were found at the time of euthanasia between the vaccine groups with regard to anti-thyroglobulin antibody activity, T<sub>4</sub> concentration, TSH concentration, or thyroid weight as a percentage of the body weight. The Mann-Whitney test was used to determine whether significant differences were found at the time of euthanasia between dogs with and without thyroiditis with regard to anti-thyroglobulin antibody activity, T<sub>4</sub> concentration, TSH concentration, or thyroid weight. In all analyses,  $P < .05$  was considered significant.

### Results

All dogs were healthy for the 5.5-year duration of the study. Results of CBC and serum biochemical profiles were within reference ranges for all dogs during the study. All dogs in group V<sup>mr</sup> and V<sup>m</sup> developed high antibody titers against distemper virus and parvovirus, and all dogs in groups V<sup>mr</sup> and V<sup>r</sup> developed protective titers against rabies.<sup>13</sup>

Changes in anti-thyroglobulin antibody activity and T<sub>4</sub> and TSH concentrations during the study are reported elsewhere.<sup>11</sup>

At the time of euthanasia, the vaccine groups did not differ with regard to anti-thyroglobulin antibody activity ( $P = .64$ ), T<sub>4</sub> concentration ( $P = .10$ ), TSH concentration ( $P = .50$ ), or thyroid weight as a percentage of the body weight ( $P = .56$ ). The median anti-thyroglobulin antibody OD in dogs with thyroiditis was 31 (range 10.6–74.9) and in the dogs without thyroiditis was 10.3 (range 2.3–41.1). Anti-thyroglobulin antibody activity in dogs with thyroiditis was significantly higher compared with those without thyroiditis at postmortem examination ( $P = .0098$ ). The median T<sub>4</sub> concentration in the dogs with thyroiditis was 1.7 µg/dL (range 1–2.4) and in the dogs without thyroiditis was 2.6 µg/dL (range 1.7–3.6). The T<sub>4</sub> concentration was significantly lower in the dogs with thyroiditis than in those without thyroiditis ( $P = .0062$ ). The median TSH concentration in the dogs with thyroiditis was 0.22 ng/mL (range 0.06–2.1) and in the dogs without thyroiditis was 0.17 ng/mL (range 0.07–0.28). TSH concentration between the dogs with and without thyroiditis was not significantly different ( $P = .25$ ). Thyroid weight as a percentage of the body weight was not different ( $P = .56$ ) between



**Fig 1.** Histopathologic section of thyroid gland from a Beagle (ID: 543) with lymphocytic thyroiditis. Lymphocytes have accumulated in the thyroid gland with loss of follicles. Remaining follicles contain pale colloid with vacuoles and cellular debris. Hematoxylin-eosin stain. Bar = 100 µm.

dogs with and without thyroiditis. At the end of the study, 2 dogs had evidence of thyroid dysfunction, 1 dog had increased TSH and normal T<sub>4</sub> (group V<sup>m</sup>), and 1 dog had low T<sub>4</sub> with a normal TSH (group U). Neither of these dogs had overt clinical evidence of hypothyroidism, but both dogs had evidence of thyroiditis at postmortem examination.

Histopathologic evaluation of the thyroid glands revealed multifocal lymphocytic thyroiditis in 8 of 20 dogs (40%; Fig 1). Thyroiditis was present in 3 of 5 unvaccinated dogs and in 5 of 15 vaccinated dogs (2 in group V<sup>mr</sup>, 2 in group V<sup>m</sup>, and 1 in group V<sup>r</sup>). The inflammation was bilateral and involved 10–25% of the gland. The severity of inflammation was scored as 1+ in 2 dogs (1 each in group V<sup>m</sup> and U), 2+ in 5 dogs (2 dogs in group V<sup>mr</sup> and 1 each in groups V<sup>m</sup>, V<sup>r</sup>, and U), and 3+ in 1 dog (group U). Histopathologic lesions were not detected in any other organs examined.

### Discussion

Spontaneous lymphocytic thyroiditis is an autoimmune disorder of dogs characterized by multifocal to diffuse interstitial infiltration of the thyroid gland by lymphocytes, plasma cells, and macrophages associated with destruction of thyroid follicles.<sup>14</sup> Endogenous and environmental risk factors for thyroiditis have not been well defined in dogs. There is a breed predisposition for thyroiditis in dogs, and the genetics for susceptibility have been characterized in beagles.<sup>4,14</sup> In this study of 20 beagles from 5 litters, 8 of 20 (40%) had evidence of thyroiditis at postmortem examination. This percentage is higher than the incidence of 16% reported in 1 large beagle colony.<sup>4</sup> However, certain litters in that colony had higher rates than others. In our study, dogs were distributed evenly among the groups on the basis of litter of origin and body weight, so the higher rate of thyroiditis in the control group cannot be explained by litter of origin. The numbers of dogs in each litter in the study were too small to detect evidence of clustering

of thyroiditis within litters, but the prevalence of thyroiditis in the litters ranged from 3 cases of thyroiditis in 1 litter of 4 dogs to 0 cases of thyroiditis in 1 litter of 5 dogs.

Vaccine administration has been hypothesized to be a contributing factor for autoimmune diseases, including thyroiditis, in dogs.<sup>7-9</sup> Vaccines contain not only the intended attenuated or inactivated bacterial or viral pathogen, but also adjuvants and other proprietary components. Vaccine viruses typically are produced in tissue culture and can contain traces of heterologous cell and serum components. In addition, proteins, such as bovine serum albumin and gelatin sometimes are added to vaccines as stabilizers. In dogs, routine vaccination induces antibody responses to many protein contaminants in vaccines, including thyroglobulin.<sup>11,15</sup> Because these proteins are highly conserved across species, antibodies can cross-react with host proteins and act as autoantibodies.

In an earlier study of this group of dogs undergoing vaccination, canine anti-thyroglobulin antibodies increased significantly in the 2 groups of dogs that received rabies vaccine but not in the group that received the multivalent vaccine alone, although there were no clinical signs or clinicopathologic evidence of hypothyroidism in any of the rabies-vaccinated dogs.<sup>11</sup> In this follow-up study, only 3 of 10 dogs vaccinated with the rabies vaccine had evidence of thyroiditis at postmortem examination.

Thyroglobulin is believed to be an important autoantigen in the pathogenesis of thyroid disease in dogs and humans. Experimental autoimmune thyroiditis can be induced in susceptible mice by parenteral injection of murine, bovine, or human thyroglobulin.<sup>16,17</sup> Whether anti-thyroglobulin antibodies are directly involved in the pathogenesis of spontaneous thyroiditis is still disputed.<sup>18</sup> Although it is believed that thyroiditis is mediated predominantly by T cells, there is some evidence that anti-thyroglobulin antibodies play a direct role in initiation of autoimmune thyroiditis. Thyroiditis has been induced by injection of canine serum containing anti-thyroglobulin antibodies directly into the thyroid gland of dogs.<sup>19</sup> It is possible that the anti-thyroglobulin antibodies detected in the dogs in this study differ from true pathogenic autoantibodies. Anti-thyroglobulin antibodies frequently are found in healthy humans, and these antibodies differ in their epitopic specificity from those found in humans with autoimmune thyroid disease.<sup>18</sup> The epitopic specificity of the antibodies detected in this study was not determined.

In this study, the highest rate of thyroiditis (3/5 dogs) was observed in the unvaccinated group of dogs. Because of the unexpectedly high rate of spontaneous thyroiditis in the control group, the power of this study to test for a positive association between vaccination and thyroiditis was low. The chance of detecting a 100% rate of thyroiditis in the vaccinated dogs was 80%, and anything less than 100% would likely not be detected. However it should be noted that the rate of thyroiditis in the vaccine groups in this study was actually lower than that of the control group. OD for canine anti-thyroglobulin antibody activity also was not different between vaccine groups at

the time of postmortem examination. In addition, both of the dogs with evidence of thyroid dysfunction were in groups that did not receive the rabies vaccine. Thyroid dysfunction in these 2 dogs was likely a consequence of spontaneous thyroiditis. Results of this study do not yield any evidence to support a causal relationship between vaccination and thyroiditis.

The presence of spontaneous thyroiditis in the dogs in this study is not surprising considering that thyroiditis is known to be hereditary in the beagle. The use of beagles was an intentional feature of the study design to maximize our chance of detecting an effect of vaccination. This was also the reason why vaccination with the multivalent vaccine was performed every 6 months, as is done in some kennel settings, rather than the more typical yearly frequency. The higher than expected rate of thyroiditis in the control group did have the unexpected effect of decreasing the power of the study. Because the pathogenesis of thyroiditis in beagles might be different from that in other breeds, the results of this study cannot necessarily be extrapolated to other breeds.

This experimental study yielded no evidence to suggest that routine immunization causes thyroiditis in dogs or is responsible for the high prevalence of thyroid disease in some dog breeds.

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## Footnotes

<sup>a</sup> Hill's Science Diet Canine Maintenance, Topeka, KS

<sup>b</sup> Vanguard 5/CV-L, Smith Kline Beecham, Exton, PA

<sup>c</sup> Imrab 3, Rhone Merieux, Athens, GA

<sup>d</sup> Immulite, Total T4, Diagnostic Products Corporation, Los Angeles, CA

<sup>e</sup> Immulite, Canine TSH, Diagnostic Products Corporation, Los Angeles, CA

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## Acknowledgment

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