

# Evaluation of antithyroglobulin antibodies after routine vaccination in pet and research dogs

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**Objective**—To determine whether routine vaccination induces antibodies against bovine thyroglobulin and autoantibodies against canine thyroglobulin in dogs.

**Design**—Prospective study.

**Animals**—20 healthy research Beagles and 16 healthy pet dogs.

**Procedure**—For the research Beagles, 5 dogs were vaccinated with a multivalent vaccine and a rabies vaccine, 5 dogs received only the multivalent vaccine, 5 dogs received only the rabies vaccine, and 5 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 per year. Blood was collected from all dogs at 8, 16, and 26 weeks of age and then 4 times yearly. Assays for antibodies directed against bovine and canine thyroglobulin were performed prior to and 2 weeks after each yearly vaccination. For the pet dogs, blood was collected prior to and 2 weeks after 1 vaccination.

**Results**—In the research Beagles, there was a significant increase in anti-bovine thyroglobulin antibodies in all vaccinated dogs, compared with control dogs. There was a significant increase in anti-canine thyroglobulin antibodies in the 2 groups of dogs that received the rabies vaccine but not in the group that received the multivalent vaccine alone. In the pet dogs, there was a significant increase in anti-canine thyroglobulin antibodies after vaccination but no significant change in anti-bovine thyroglobulin antibodies.

**Conclusions and Clinical Relevance**—Recent vaccination may result in increased anti-canine thyroglobulin antibodies. Whether these antibodies have a deleterious effect on canine thyroid function is unknown. (*J Am Vet Med Assoc* 2002;221:515–521)

**H**ypothyroidism is a common endocrinopathy in dogs. At least 50% of cases of canine hypothyroidism are believed to be caused by autoimmune thyroiditis.<sup>1,2</sup> Measurement of serum autoantibodies against canine thyroglobulin is considered a specific and sensitive test for the diagnosis of thyroiditis in dogs.<sup>2,3</sup> While the specific cause of canine thyroiditis is unknown, thyroiditis is heritable in Beagles and

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Borzoi, and a large number of other breeds have a high prevalence of this disease.<sup>4-6</sup> Intensive vaccination protocols have been suggested as partially responsible for an increased prevalence of autoimmune diseases in dogs in recent years.<sup>7-9</sup> The evidence for this relationship is primarily anecdotal. However, a case control study found a significant temporal relationship between vaccination and subsequent development of immune-mediated hemolytic anemia in dogs.<sup>10</sup>

In previous studies, Beagle puppies developed antibodies against a variety of bovine, murine, and porcine antigens after routine vaccination.<sup>11</sup> The authors suggested these antibodies were a normal immune response to heterologous antigens that contaminate most commonly used vaccines as a result of the tissue culture process or when added as stabilizers. We hypothesized that, with repeated immunizations, the antibodies against highly conserved heterologous antigens may cross-react with homologous canine antigens and could eventually result in autoimmune disease in predisposed individuals. Compelling evidence in support of this hypothesis comes from experiments in rodents and rabbits in which autoimmune diseases can be induced by injection of conserved heterologous antigens in combination with adjuvants. The purpose of the study reported here was to determine whether routine vaccination induces antibodies against bovine thyroglobulin and autoantibodies against canine thyroglobulin.

## Materials and Methods

**Laboratory dogs**—Twenty female Beagles were obtained from 5 litters and allocated to 4 experimental groups according to litter of origin and body weight at 8 weeks of age. The dogs were housed indoors in groups of 1 to 3 dogs/run and fed a standard laboratory ration<sup>a</sup> and water free choice. The day-light 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>mt</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 following the same schedule as dogs in group V<sup>mt</sup>. Groups V<sup>mt</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. The rabies vaccine was administered at 16 and 52 weeks of age and then once per year, as required by law in Indiana. Blood was collected from all dogs at 8, 16, and 26 weeks of age and then 4 times yearly (immediately prior to

vaccination and 2 weeks after vaccination). An appropriate response to vaccination was determined by measurement of antibody titers 2 weeks after each vaccination. Antibody titers against distemper and parvovirus infection were determined by use of serum neutralization and hemagglutination inhibition tests, respectively. Antibody titers against rabies were determined by use of the rapid fluorescence focus inhibition assay. Complete blood counts, serum biochemical profiles, and thyroid profiles (thyroxine [ $T_4$ ], triiodothyronine [ $T_3$ ], thyroid-stimulating hormone [TSH]) were evaluated at 8, 16, and 26 weeks of age and then every 6 months prior to each vaccination. Canine and bovine antithyroglobulin antibodies were measured at 8, 16, and 26 weeks of age and then immediately prior to each yearly vaccination and 2 weeks after each vaccination.

**Pet dogs**—Sixteen healthy adult pet dogs of various breeds were studied before and after vaccination. All dogs were  $\geq 2$  years of age and had been vaccinated at least once as an adult  $\geq 12$  months prior to entry into the study. At the time of entry into the study, the owners of these dogs were asked to complete a questionnaire regarding previous vaccinations, adverse reactions to previous vaccinations, and other medical history. A physical examination was performed, and blood and urine samples were collected for a CBC, serum biochemical profile, thyroid profile ( $T_4$ , TSH) and measurement of canine and bovine thyroglobulin antibodies. Dogs were then vaccinated with a multivalent vaccine<sup>b</sup> and a rabies vaccine<sup>c</sup> according to label recommendations. The dogs were reexamined 14 days after vaccination and any adverse reaction to vaccination was noted. Samples were then collected for the same tests performed at entry into the study.

**Hormone assay techniques**—Serum  $T_4$  and canine TSH concentrations were measured by use of chemiluminescent

enzyme immunoassays validated for use in dogs.<sup>12,d,e</sup> Reference range for  $T_4$  was 1.3 to 4.0  $\mu\text{g}/\text{dl}$  and for TSH was 0 to 0.65 ng/ml. Serum  $T_3$  was measured by use of a radioimmunoassay validated for use in dogs.<sup>13,f</sup> Reference range for  $T_3$  was 0.7 to 1.3 ng/dl.

**Antibody assay techniques**—Anti-bovine thyroglobulin antibodies were measured by use of an indirect ELISA as described elsewhere.<sup>11</sup> Briefly, high-binding ELISA plates<sup>g</sup> were coated with 10  $\mu\text{g}$  of bovine thyroglobulin/ml in 0.1M bicarbonate buffer. The wells were rinsed and incubated for 1 hour with phosphate-buffered saline (PBS)-0.1% Tween solution. Serum samples were diluted 1:100 in PBS and added to the wells in triplicate. After incubation, the wells were rinsed and incubated with alkaline phosphatase-labeled goat anti-dog IgG.<sup>h</sup> Alkaline phosphatase activity was measured after addition of p-nitrophenyl phosphate disodium substrate at 405 nm in a microplate reader.<sup>i</sup> Anti-canine thyroglobulin antibodies were measured via essentially the same procedure, except that a microtiter plate precoated with purified canine thyroglobulin from a commercial kit<sup>j</sup> was used as the ELISA plate.<sup>2</sup> The positive and negative control sera provided with the test kit were included in each assay. Results were expressed as optical density (OD) of sample minus OD of blank wells.

**Statistical analyses**—For the Beagles, a general linear model<sup>14</sup> was used to determine whether there were significant differences among the 4 treatment groups for OD values of canine and bovine antithyroglobulin antibodies and the concentration of  $T_3$ ,  $T_4$ , and TSH, at 8 weeks and 4 years of age. A repeated measures ANOVA<sup>15</sup> was performed to determine whether there were significant differences among treatment groups in the absolute change in OD values of anti-canine and anti-bovine thyroglobulin antibodies before versus after

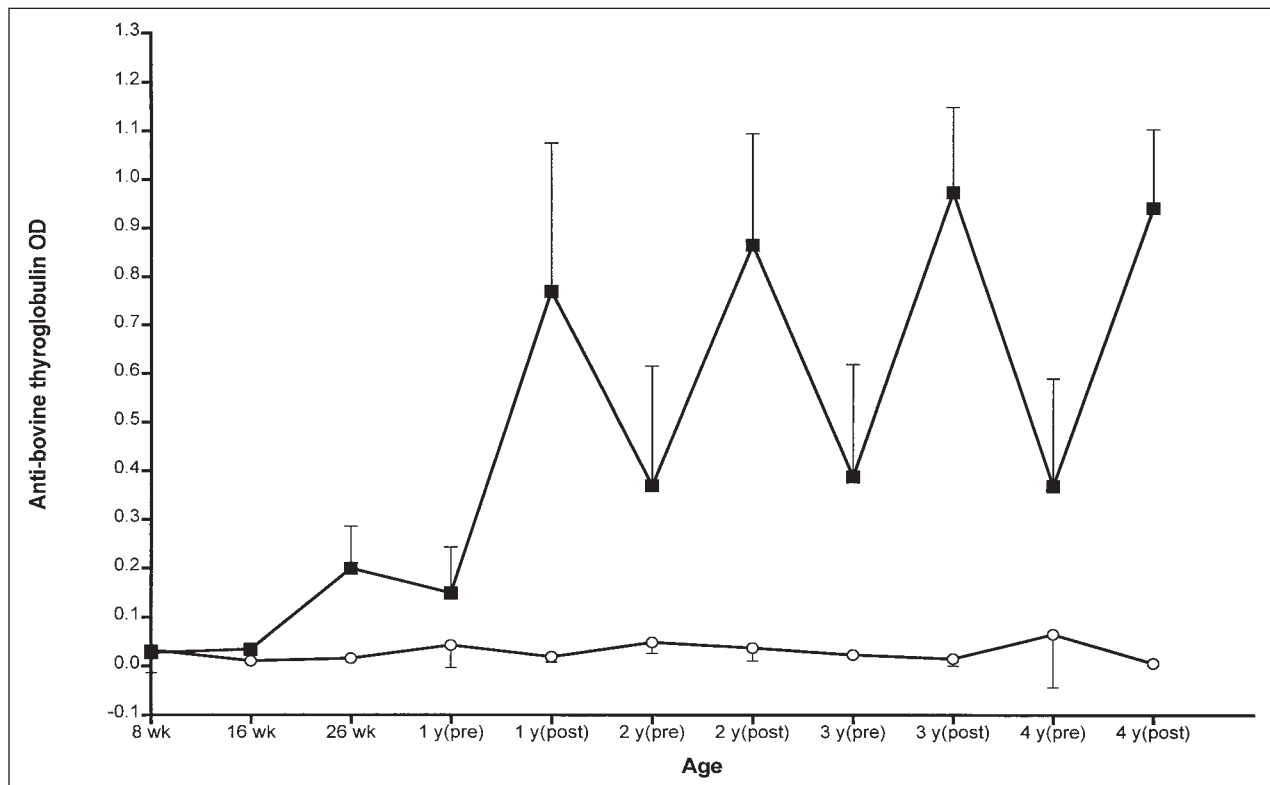


Figure 1—Optical density (OD) values (mean  $\pm$  SD) of anti-bovine thyroglobulin antibodies in 3 unvaccinated dogs (open circles) and 5 dogs (solid squares) that received multivalent vaccines (at 8, 10, 12, 16, 20, 26, and 52 weeks of age and every 6 months thereafter) and rabies vaccines (at 16 and 52 weeks of age and yearly thereafter). Antibodies were measured at 8, 16, and 26 weeks of age, and immediately prior to each yearly vaccination (pre) and 2 weeks after each yearly vaccination (post).

vaccination, at 1, 2, 3, and 4 years of age. Repeated measures ANOVA was also used to assess the change in concentration of  $T_3$ ,  $T_4$ , and TSH, among treatment groups over 7 time points. Treatment group  $\times$  time interactions were evaluated in each repeated measures model. In all analyses, a value of  $P < 0.05$  was considered to be significant.

For the pet dogs, linear regression analysis<sup>14</sup> was performed to test for an association between age and antibody OD values prior to vaccination. A Wilcoxon rank test<sup>16</sup> was performed to test for an association between sex and breed (purebred vs mixed breed) and antibody OD values prior to vaccination. A paired  $t$  test<sup>17</sup> was used to determine whether there was a significant difference in the CBC, biochemical profile, or thyroid profile between samples collected before and 14 days after vaccination. A repeated measures ANOVA<sup>15</sup> was used to assess differences in antibody OD values between samples collected before and 14 days after vaccination. In all analyses, a value of  $P < 0.05$  was considered to be significant.

## Results

**Laboratory dogs**—All dogs were healthy for the duration of the study (4.5 years). Results of CBC and serum biochemical profiles were within reference limits for all dogs during the study. All dogs in groups  $V^{mr}$  and  $V^m$  developed high antibody titers against distemper and parvovirus infection and all dogs in groups  $V^{mr}$  and  $V^m$  developed protective titers against rabies.<sup>18</sup>

**Subset of dogs with suspected spontaneous thyroiditis**—Three dogs (1 in group  $V^m$ , 2 in group U) developed evidence of spontaneous canine thyroiditis early in the study. These dogs were identified by a marked increase in autoantibodies against canine thyroglobulin

starting at 1 year of age, peaking at 1 to 2 years of age, and declining thereafter. There was no temporal association between vaccination and OD values of anti-canine thyroglobulin antibodies in these 3 dogs. These dogs also had similar but smaller increases in antibodies reactive with bovine thyroglobulin that paralleled the time course of the canine thyroglobulin autoantibodies. By the end of the study, all 3 of these dogs had developed some evidence of early hypothyroidism, characterized by a marked increase in TSH in 1 dog, a low  $T_4$  concentration in 1 dog, and increased TSH and low  $T_4$  in 1 dog. Increased concentrations of  $T_3$  were also detected at 2 to 3 years of age in these 3 dogs, presumably because of interference from anti- $T_3$  antibodies.<sup>19</sup> Because of the potential confounding effect of this distinct subset of dogs with spontaneous thyroiditis, all statistical analyses were performed both with and without them. Exclusion of these 3 dogs from the statistical analyses, however, did not significantly change the results except where noted.

**Anti-thyroglobulin antibodies**—Prior to administration of the first vaccines at 8 weeks of age, there were no significant differences among the 4 groups in the OD values of antibodies against canine or bovine thyroglobulin ( $P = 0.86$  and  $0.43$ , respectively). At 4 years of age, prior to the last vaccination, there were no significant differences ( $P = 0.56$ ) among groups in regard to canine thyroglobulin autoantibodies. However, dogs in group U had significantly ( $P = 0.03$ ) fewer antibodies against bovine thyroglobulin, compared with the other 3 groups. This difference between the groups was no

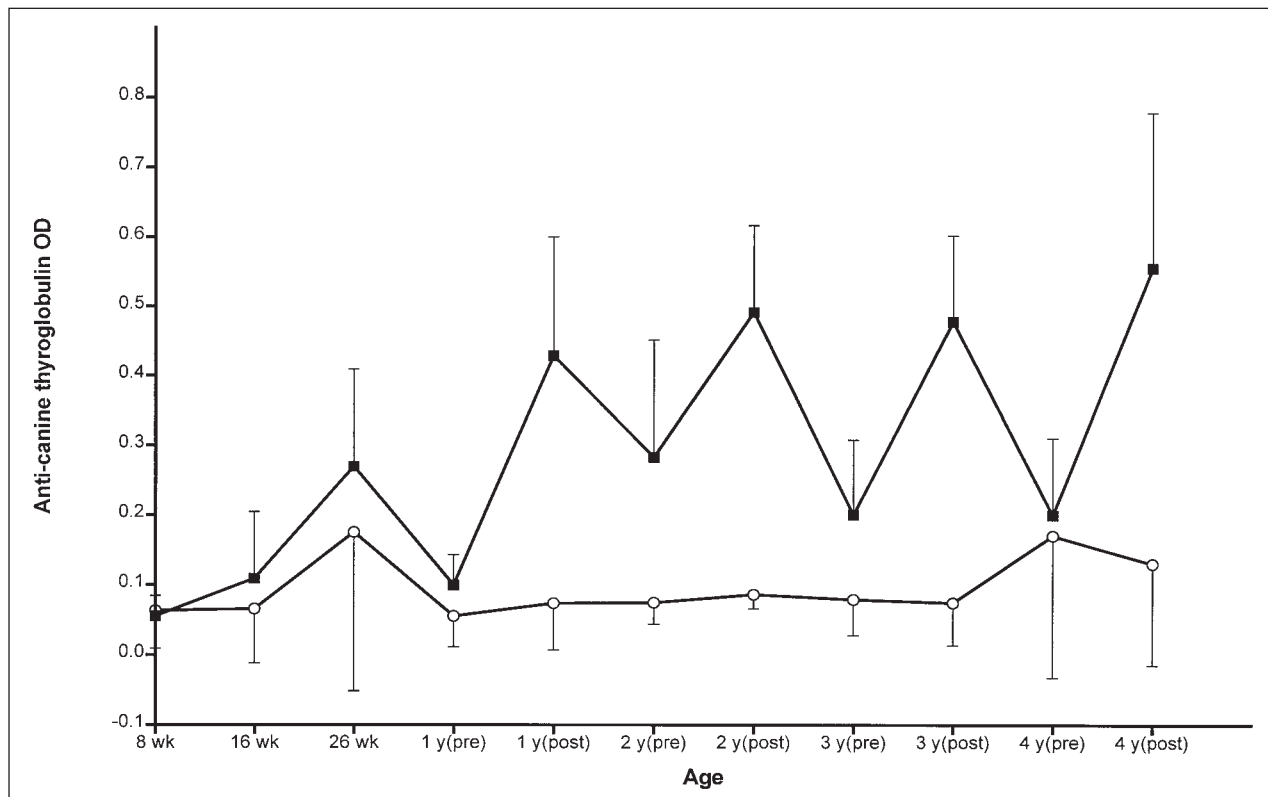


Figure 2—Optical density values (mean  $\pm$  SD) of anti-canine thyroglobulin antibodies in 3 unvaccinated dogs (open circles) and 5 dogs (solid squares) that received multivalent vaccines (at 8, 10, 12, 16, 20, 26, and 52 weeks of age and every 6 months thereafter) and rabies vaccines (at 16 and 52 weeks of age and yearly thereafter). Antibodies were measured at 8, 16, and 26 weeks of age, and immediately prior to each yearly vaccination (pre) and 2 weeks after each yearly vaccination (post).

longer significant however, when the 3 dogs with suspected spontaneous canine thyroiditis were excluded from the analysis ( $P = 0.24$ ).

In the 14 vaccinated dogs without evidence of spontaneous thyroiditis, there was an increase in antibodies directed against bovine thyroglobulin that was temporally associated with each vaccination (Fig 1). These changes were not observed in the unvaccinated dogs. In the 2 groups of dogs that received rabies vaccine (Groups  $V^{mr}$  and  $V^r$ ) there was also an increase in anti-canine thyroglobulin autoantibodies that was temporally associated with vaccination (Fig 2). However, this was not observed in the group of dogs that only received the multivalent vaccine ( $V^m$ ).

The absolute change in anti-bovine thyroglobulin antibody OD values after each vaccination (OD after vaccination – OD before vaccination) during the study revealed the change after vaccination was greatest for groups  $V^{mr}$  and  $V^r$  and less marked in group  $V^m$ , whereas there was no change in the unvaccinated group (Fig 3). A similar pattern was observed for the absolute change in anti-canine thyroglobulin antibody OD values (Fig 4); however, there was no change in OD values after vaccination for either group  $V^m$  or group U. There was a significant ( $P < 0.001$ ) difference among the 4 groups in the absolute change for anti-bovine and anti-canine thyroglobulin antibodies after vaccination. For bovine thyroglobulin, all groups were significantly different from each other with the exception of groups  $V^{mr}$  and  $V^r$  (Fig 3). For canine thyroglobulin, groups  $V^{mr}$  and  $V^r$  were different from groups  $V^m$  and U (Fig 4); however, there was no difference between groups  $V^{mr}$  and  $V^r$  or groups  $V^m$  and U. There was a significant decrease in absolute change in OD values for anti-bovine thyroglobulin over time ( $P = 0.02$ ), howev-

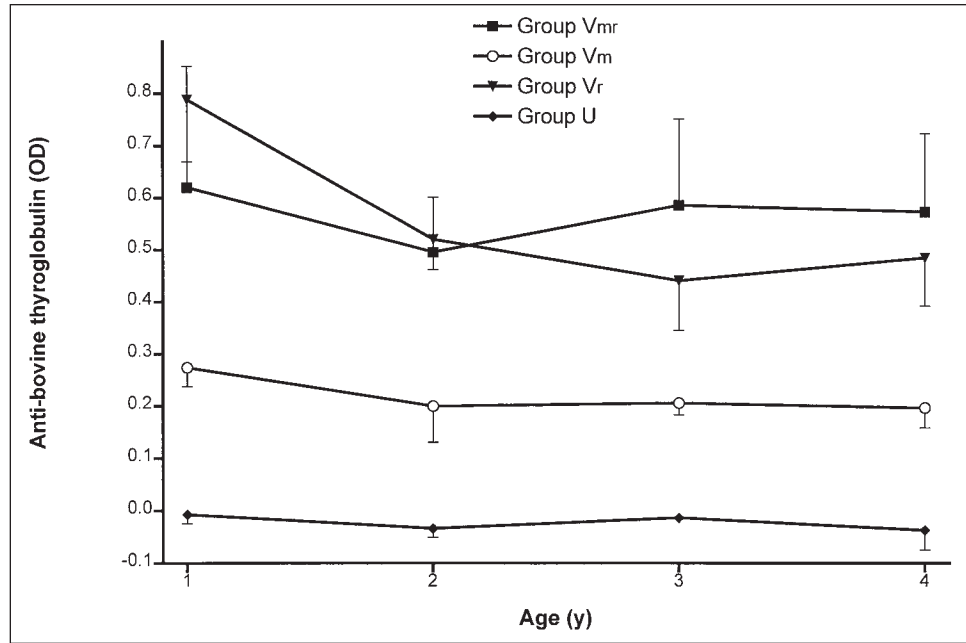


Figure 3—Absolute change in mean  $\pm$  SD OD over time for anti-bovine thyroglobulin antibodies in 4 groups of laboratory Beagles. Five dogs (Group  $V^{mr}$ ) received multivalent and rabies vaccines, 5 dogs (group  $V^m$ ) received multivalent vaccine alone, 5 dogs received rabies vaccine alone (group  $V^r$ ), and 5 dogs were unvaccinated (group U).

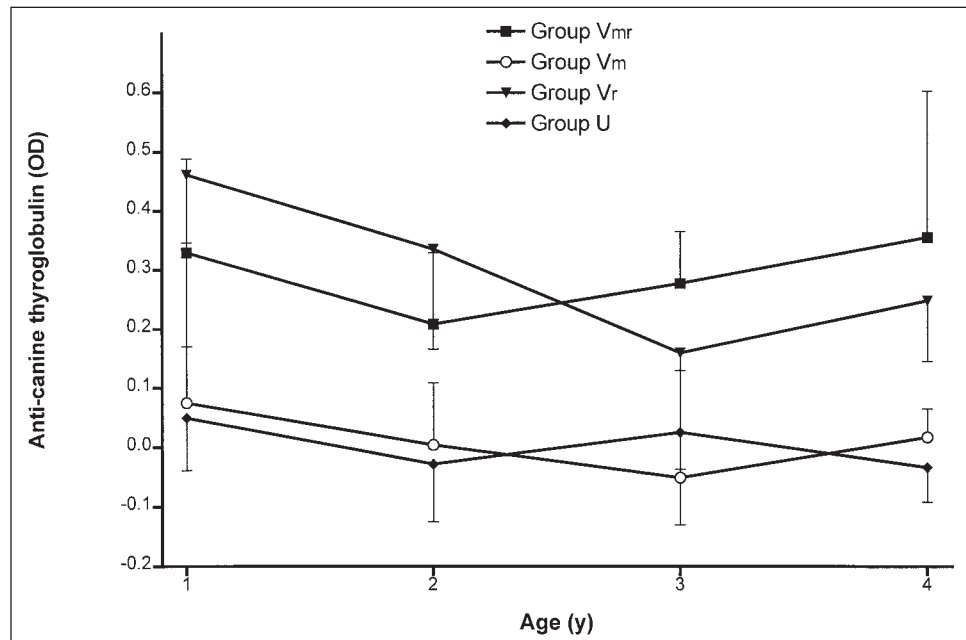


Figure 4—Absolute change in mean  $\pm$  SD OD over time for anti-canine thyroglobulin antibodies in 4 groups of laboratory Beagles. See Figure 3 for key.

er, this was no longer significant when the 3 dogs with spontaneous thyroiditis were excluded from the analysis ( $P = 0.09$ ). There was no significant time effect for absolute change in OD values for anti-canine thyroglobulin, and no significant group  $\times$  time interaction for absolute change in OD, for either anti-canine or anti-bovine thyroglobulin.

**Thyroid hormone concentrations**—There were no

significant differences among groups for  $T_4$  or TSH concentrations at 8 weeks of age ( $P = 0.5$  and  $0.29$ , respectively) or at 4 years of age ( $P = 0.56$  and  $0.47$  respectively). There was a significant difference among groups for  $T_3$  concentration ( $P = 0.02$ ). Concentrations of  $T_3$  were significantly ( $P = 0.02$ ) higher for group U than all other groups at 8 weeks of age, but were significantly ( $P < 0.001$ ) lower for group U than all other groups at 4 years of age. The difference at 8 weeks was no longer significant when the dogs with spontaneous thyroiditis were excluded from the analysis ( $P = 0.06$ ); however, the difference remained significant ( $P = 0.03$ ) at 4 years. There was a significant ( $P < 0.001$ ) decrease in  $T_4$  (Fig 5) and

$T_3$ , and an increase in TSH concentration during the study for all dogs; however, there were no differences among the 4 groups at any time point. There was also a significant group  $\times$  time interaction for  $T_4$ ,  $T_3$ , and TSH, indicating that the rate of change was different among groups. Concentrations of  $T_3$  and TSH were within the reference range for the duration of the study for all dogs, except for those with suspected spontaneous thyroiditis. Concentrations of  $T_4$  occasionally decreased slightly below the reference range in individual dogs later in the study, but were never  $< 1 \mu\text{g/dl}$ .

**Pet dogs**—The 16 dogs ranged in age from 1.5 to

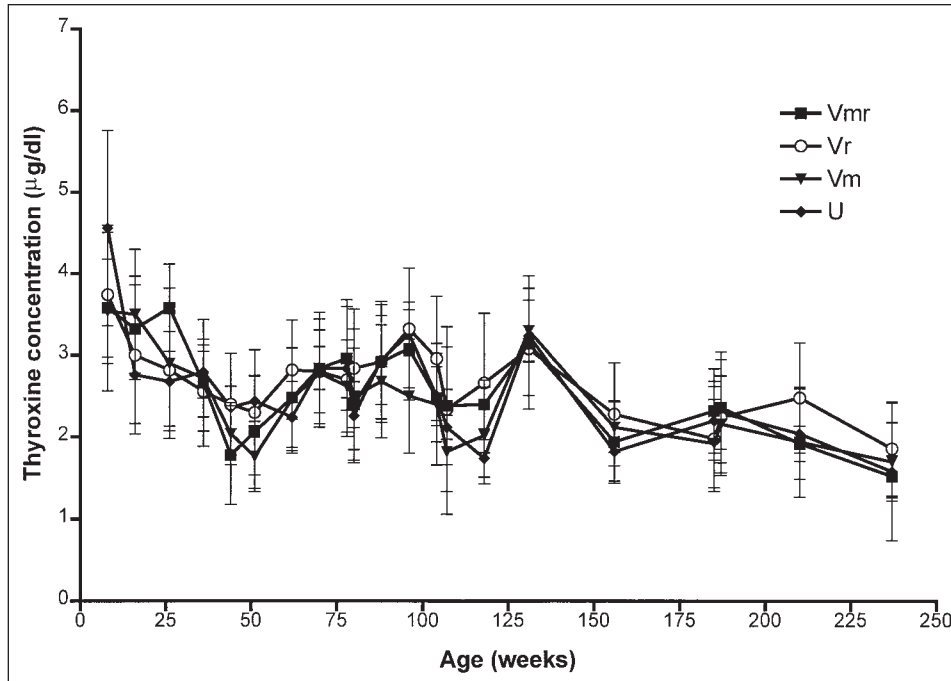


Figure 5—Change in mean  $\pm$  SD serum thyroxine concentration over time in 4 groups of laboratory Beagles. See Figure 3 for key.

14 years (mean, 6.3 years; median, 5 years) and from 4 to 57 kg (mean, 26.3 kg [57.9 lb]; median, 25.2 kg [55.4 lb]) in weight. No adverse responses to prior or current vaccines were reported. There was no association between age, sex, or breed and antibody OD values prior to vaccination. Statistical differences were not detected between results of the CBC, biochemical profile, or thyroid profiles for samples collected before and 14 days after vaccination. There was a significant increase in OD values 14 days after vaccination for canine thyroglobulin ( $P = 0.02$ ), but not for bovine thyroglobulin ( $P = 0.75$ ; Fig 6).

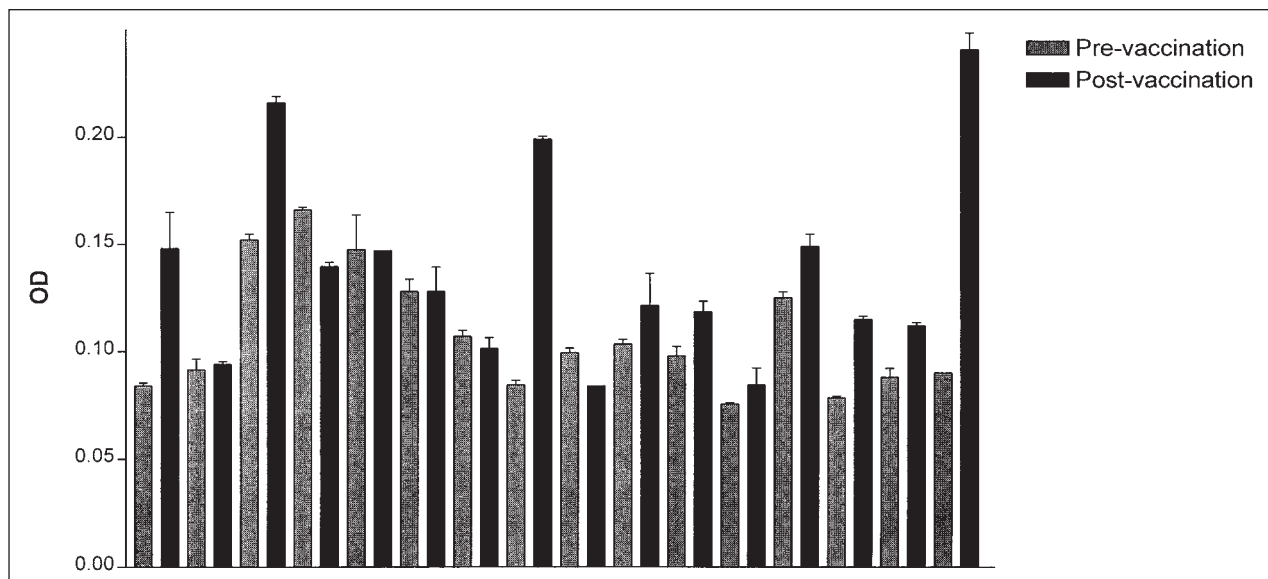


Figure 6—Optical density (mean  $\pm$  SD) of anti-canine thyroglobulin antibodies in 16 pet dogs before (shaded bars) and 2 weeks after (solid bars) vaccination with multivalent and rabies vaccines.

## Discussion

Autoimmune disease develops when the immune system mounts a pathologic response to self antigens. In healthy individuals, autoreactive B and T cells are either deleted during maturation or controlled peripherally by regulatory humoral and cellular mechanisms such as apoptosis, induction of anergy, or active suppression.<sup>20</sup> In certain circumstances, these control mechanisms are disrupted and autoimmune disease may result. Susceptibility genes have been identified for many autoimmune diseases, but whether a genetically susceptible individual will develop clinical disease depends on numerous endogenous and environmental factors.<sup>21</sup>

Spontaneous lymphocytic thyroiditis is an autoimmune disorder of dogs and humans that is characterized by multifocal to diffuse interstitial infiltration of the thyroid gland by lymphocytes, plasma cells, and macrophages associated with destruction of thyroid follicles.<sup>22</sup> Anti-thyroglobulin antibodies are present in Hashimoto thyroiditis, which is the most common form of autoimmune thyroid disease in humans, and also in canine lymphocytic thyroiditis. Risk factors for thyroid autoimmunity in humans include genetic susceptibility, low birth weight, female sex, pregnancy, stress, viral infection, changes in iodine intake, and environmental toxins such as cigarette smoke. There is also a breed predisposition for thyroiditis in dogs, and the genetic risk has been well characterized for Beagles.<sup>6,22</sup> However, endogenous or environmental risk factors have not been well defined.

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 are typically produced in tissue culture and are, therefore, likely to contain traces of heterologous cell and serum components. In addition, some proteins such as bovine serum albumin and gelatin are sometimes added to vaccines as stabilizers. In dogs, routine vaccination induces antibody responses to many protein contaminants in vaccines,<sup>11</sup> and because these proteins are highly conserved across species these antibodies may cross-react with host proteins and act as autoantibodies. However, direct evidence that vaccination is associated with increased risk of autoimmune disease in either dogs or humans is lacking. A temporal association has been observed between immune-mediated hemolytic anemia and recent vaccination in pet dogs in a controlled retrospective study.<sup>10</sup> Presently, more direct evidence for a causal relationship is needed.

Thyroglobulin is the major protein synthesized by the thyroid gland and is the main component of colloid in the thyroid follicle. Thyroglobulin is a large glycoprotein dimer containing iodotyrosines that serve as precursors for thyroid hormone synthesis. Thyroglobulin undergoes post-translational modifications including glycosylation, iodination, phosphorylation, and sulfation and these may be important in antigenicity.<sup>23</sup> There is considerable sequence homology for thyroglobulin between species and antibodies to thy-

roglobulin have some species cross-reactivity.<sup>23</sup> Thyroglobulin is believed to be an important autoantigen in the pathogenesis of thyroid disease in humans and dogs. Experimental autoimmune thyroiditis can be induced in susceptible mice by parenteral injection of either murine, bovine, or human thyroglobulin.<sup>23-25</sup> It is believed that thyroiditis is mediated predominantly by T cells. Whether anti-thyroglobulin antibodies are nonpathogenic antibodies generated secondary to tissue damage from thyroid infiltrating T-cells, or are directly involved in the pathogenesis of thyroiditis, is still disputed. However, there is growing evidence that anti-thyroglobulin antibodies play a direct role in initiation of autoimmune thyroiditis. In experimental autoimmune thyroiditis, passive transfer of thyroiditis by anti-thyroglobulin antibodies has been reported in some studies, but not in others.<sup>23</sup> Canine thyroiditis has been induced by injection of serum containing anti-thyroglobulin antibodies directly into the thyroid gland.<sup>26</sup>

In our study, there was a significant association between vaccination and change in the OD values of anti-canine and anti-bovine thyroglobulin antibodies. In certain vaccinated dogs, the OD values for anti-canine thyroglobulin antibody was in the range observed in the dogs with spontaneous thyroiditis; however the OD values decreased to background values prior to the next vaccination in most dogs. The change in OD values for anti-bovine thyroglobulin antibody in all 3 groups of vaccinated dogs was significantly greater than for the unvaccinated control dogs. However, the change in OD values of anti-canine thyroglobulin antibody for the group of dogs vaccinated with the multivalent vaccine alone was not greater than for the control group. This suggests that the multivalent and rabies vaccines both induced antibodies that were reactive with bovine thyroglobulin, but that only the rabies vaccine induced antibodies that were also reactive with canine thyroglobulin. The basis for this difference is presently unknown, but differences in processing and presentation of bovine thyroglobulin between the multivalent and rabies vaccine may be a factor. Such differences may be the result of various factors in the composition of the vaccines, including the use of aluminum adjuvant in the rabies vaccine and not the multivalent vaccine.<sup>18</sup> In the pet dogs, the change in OD values of anti-canine thyroglobulin antibody was significant, whereas the change in OD values of anti-bovine thyroglobulin antibody was not. The reason for this difference is unclear.

The clinical importance of these findings is unknown. It is clear that recent vaccination may result in positive results for anti-canine thyroglobulin antibodies as detected by use of the most commonly available commercial assay. How long the antibodies persists is unknown, because in this study samples for measurement of anti-thyroglobulin antibodies were only collected before and 2 weeks after each yearly vaccination. Whether these antibodies have a deleterious effect on canine thyroid function is also unknown. No dogs in the study, with the exception of the 3 dogs believed to have spontaneous thyroiditis, developed evidence of thyroid dysfunction by 4.5 years of age.

Anti-thyroglobulin antibodies are frequently found in healthy humans, and these antibodies differ from autoantibodies found in humans with autoimmune thyroid disease.<sup>23</sup> Whereas anti-thyroglobulin antibodies associated with thyroid disease are oligoclonal and restricted in their epitopic specificity, those in healthy humans are polyclonal in their epitopic recognition of thyroglobulin. There are antigenic differences between thyroglobulin from normal and diseased thyroid glands. The epitopic specificity of the antibodies detected in the dogs in this study and whether they play a role in the eventual development of thyroiditis in predisposed individuals was not determined.

Because it was hypothesized that anti-thyroglobulin antibodies detected in our study were the result of contamination of the vaccine by bovine thyroglobulin, it is important to detect bovine thyroglobulin in the vaccine that we used. This has been attempted unsuccessfully in our laboratory by use of a capture ELISA for human thyroglobulin and by use of western blot techniques, possibly because of the anti-human thyroglobulin monoclonal antibodies that were used in both tests. Unfortunately, a source of anti-bovine thyroglobulin antibodies has not been identified. Detection of bovine thyroglobulin in commercial vaccines is an important next step in order to prove the relationship between vaccination and antibody formation.

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

<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 Corp, Los Angeles, Calif.

<sup>e</sup>Immulite Canine TSH, Diagnostic Products Corp, Los Angeles, Calif.

<sup>f</sup>Coat-A-Count Total T3, Diagnostic Products Corp, Los Angeles, Calif.

<sup>g</sup>Costar, Cambridge, Mass.

<sup>h</sup>Kirkegaard and Perry, Gaithersburg, Md.

<sup>i</sup>Molecular Devices, Menlo Park, Calif.

<sup>j</sup>Canine Thyroglobulin Autoantibody Enzyme Immunoassay Kit, Oxford Biomedical Research, Oxford, Mich.

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