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 we use 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.

The objective of our study in Great Danes, a breed thought to be at high risk of developing certain autoimmune diseases, was to identify immunization schedules or specific types of vaccines that provoke autoimmunity and immune-mediated disease. The long-term goal of our research is to provide scientific knowledge that can be used by pharmaceutical companies to produce safer canine vaccines.

Proposed Research

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. (Appendix 1) 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. (Appendix 2)

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

These are the same tests we performed in our laboratory at Purdue University to monitor a colony of Beagle dogs for their response to vaccination. These tests were standardized and the methods previously reported in the literature. Testing using an immunofluorescence assay for the detection of anti-adrenal gland antibodies was included in the proposal, but was deleted when the test proved unreliable.

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 by removing any personal identifiers and by storing them in a locked cabinet. Results for individual dogs will be shared with the owner of the dog, only if they indicate the presence of disease or a greater likelihood of developing disease in the future. Otherwise, information from all dogs was pooled for data analysis and reporting of results.

Results

A total of 75 Great Danes who met the eligibility requirements were enrolled in the study. A description of these dogs is provided in Table 1. 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 (Table 2). 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 (Table 3). 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. (Table 4, Figure 1). A similarly strong relationship was found for rabies. (Table 5, Figure 2). However, there was no clear-cut relationship between the parvovirus vaccine history and antibody titers to parvovirus. (Table 6, Figure 3) 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 (Table 7), age, weight, and height (Table 8, Figure 4). 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. (Table 9) 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. (Table 10)

There were 7 adverse reactions reported by Great Dane owners. Three reactions were to vaccinations while four were to drugs or anesthesia (Tables 11 & 12). 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.

The relationship between auto-antibody titers to several tissue antigens and the dogs' status with respect to a history of distemper, parvovirus, or rabies, vaccination are shown in Figures 5A—10C. 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 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 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, but they do suggest a possible causal role.

Summary of pattern of association between frequency of vaccination as reported by owners and a dogs' auto-antibody titer			
	Vaccine		
Antibody directed against:	**Distemper**	**Parvovirus**	**Rabies**
Bovine fibronectin	None	None	Positive (strong)
Bovine thyroglobulin	Positive (strong)	Positive (strong)	Positive (strong)
Canine thyroglobulin	None	Positive (weak)	None
Calf DNA	None	Positive (weak)	Positive (weak)
Murine laminin	None	None	None
Porcine insulin	None	None	None

The concentration of anti-bovine thyroglobulin antibodies in the serum was significantly correlated with the concentration of anti-canine thyroglobulin antibodies (correlation coefficient = 0.27; p = 0.02). 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 (Table 13; Figures 15A-15C) and serum T4 (Table 14; Figures 16A-16C) 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 (Table 15) 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 ($p = 0.44$) or T4 ($p = 0.95$) 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 have either used veterinary services regularly or would have taken 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 consequences of vaccine-related autoantibodies 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.

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.

Table 1—Description of 75 Great Danes enrolled in the vaccinosis study

	N	%
Gender		
Female	47	62.7
Male	28	37.3
Neuter status		
Neutered	41	54.7
Intact	34	45.3
Body condition as categorized by owners		
Underweight	9	12.0
Average / optimum	59	78.7
Overweight	7	9.3
Body condition as categorized by veterinarians		
Underweight	2	2.7
Average / optimum	59	78.7
Overweight	5	6.7
Obese	1	1.3
Missing	8	10.7

Table 2—Vaccine history of 75 Great Danes enrolled in the vaccinosis study

	N	%
Any vaccinations ever?		
No	15	20.0
Yes	60	80.0
Pattern of distemper / measles vaccination		
Never	18	24.0
As puppy / sporadic / irregular	20	26.7
Regularly every one or two years	37	49.3
Pattern of parvovirus vaccination		
Never	19	25.3
As puppy / sporadic / irregular	17	22.7
Regularly every one or two years	39	52.0
Pattern of rabies vaccination		
Never	23	30.7
As puppy / sporadic / irregular	13	17.3
Regularly every three years	23	30.7
Regularly every one or two years	16	21.3

Table 2—(cont'd)

	N	%
Pattern of adenovirus vaccination		
Never	35	46.7
As puppy / yearly for a while	15	20.0
Regularly every one or two years	25	33.3
Pattern of leptospirosis vaccination		
Never	49	65.3
As puppy / sporadic / once	11	14.7
Regularly every one or two years	15	20.0
Pattern of parainfluenza vaccination		
Never	29	38.6
As puppy / sporadic / yearly for a while	17	22.7
Regularly every one or two years	29	38.6
Pattern of hepatitis vaccination		
Never	52	69.3
As puppy / yearly for a while	7	9.4
Regularly every one, two or three years	16	21.3
Pattern of coronavirus vaccination		
Never	41	54.7
As puppy / sporadic / yearly for a while	17	22.6
Regularly every one or two years	17	22.6
Pattern of lyme disease vaccination		
Never	61	81.4
As puppy / once / yearly for a while	7	9.3
Regularly every year	7	9.3
Pattern of bordatella vaccination		
Never	36	48.0
As puppy / sporadic / yearly for a while	17	22.7
Regularly every year	21	28.0
Pattern of giardia vaccination		
Never	72	96.0
Sporadic	1	1.3
Regularly every three years	2	2.7

Table 3—History of endocrine or autoimmune diseases in 75 Great Danes in the vaccinosis study

	Owner-reported		Veterinarian-reported	
	N	%	N	%
Endocrine diseases				
Hypothyroidism	7	9.2	6	7.9
Hyperthyroidism	0	0.0	0	0.0
Cushing's	0	0.0	0	0.0
Addison's	0	0.0	0	0.0
Diabetes mellitus	0	0.0	0	0.0
Autoimmune diseases				
Autoimmune hemolytic anemia	0	0.0	0	0.0
Thrombocytopenia (ITP)	0	0.0	0	0.0
Systemic lupus erythematosis	0	0.0	0	0.0
Arthritis immune-mediated	0	0.0	3	3.9
Thyroiditis	1	1.3	3	3.9
Other (?)	1	1.3	0	0.0

Table 4—Distribution of distemper titer in vaccinated and unvaccinated Great Danes

	N	Distemper titer					
		Mean	± (SD)	Median	Min, Max	Test statistic	P-value
Any vaccination ever?							
No	15	83.2	231.2	-4.0	-8.0, 768.0	Mann W	0.000
Yes	60	323.9	376.6	192.0	-4.0, 1536.0	U = 110.0	
Any distemper / measles vaccination ever?							
No	18	70.2	212.0	-4.0	-8.0, 768.0	Mann W	0.000
Yes	57	340.7	379.0	192.0	4.0, 1536.0	U = 98.5	
Pattern of distemper / measles vaccination							
Never	18	70.2	212.0	-4.0	-8.0, 768.0	Kruskal	0.000
As puppy / sporadic / irregular	20	255.0	379.0	112.0	4.0, 1536.0	Wallis =	
Regularly every one or two years	37	387.0	376.1	256.0	48.0, 1536.0	31.5	

Figure 1—Distribution of distemper titer based on pattern of distemper / measles vaccination

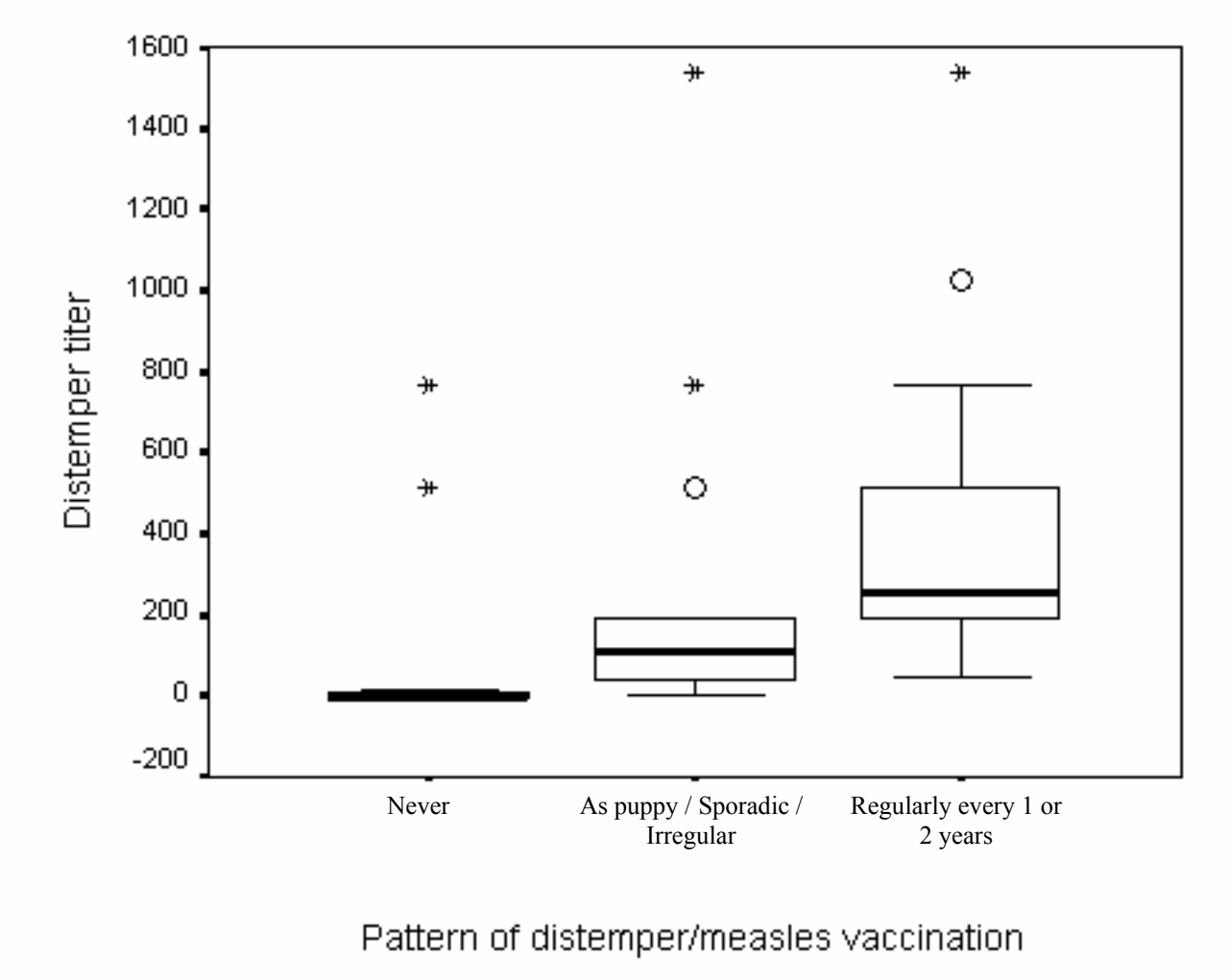

Table 5—Distribution of rabies titer in vaccinated and unvaccinated Great Danes

	N	Rabies titer					
		Mean	± (SD)	Median	Min, Max	Test statistic	P-value
Any vaccination ever?							
No	15	5.0	0.0	5.0	5.0, 5.0	Mann W	0.000
Yes	60	902.9	1709.0	167.5	5.0, 7000.0	U = 112.5	
Any rabies vaccination ever?							
No	23	5.0	0.0	5.0	5.0, 5.0	Mann W	0.000
Yes	52	1041.1	1798.1	300.0	5.0, 7000.0	U = 80.5	
Pattern of rabies vaccination							
Never	23	5.0	0.0	5.0	5.0, 5.0	Kruskal	0.000
As puppy / sporadic / irregular	13	53.1	96.0	17.0	5.0, 360.0	Wallis =	
Regularly every three years	23	1157.0	1837.1	340.0	5.0, 6300.0	53.2	
Regularly every one or two years	16	1677.2	2176.4	1100.0	95.0, 7000.0		

Figure 2—Distribution of rabies titer based on pattern of rabies vaccination

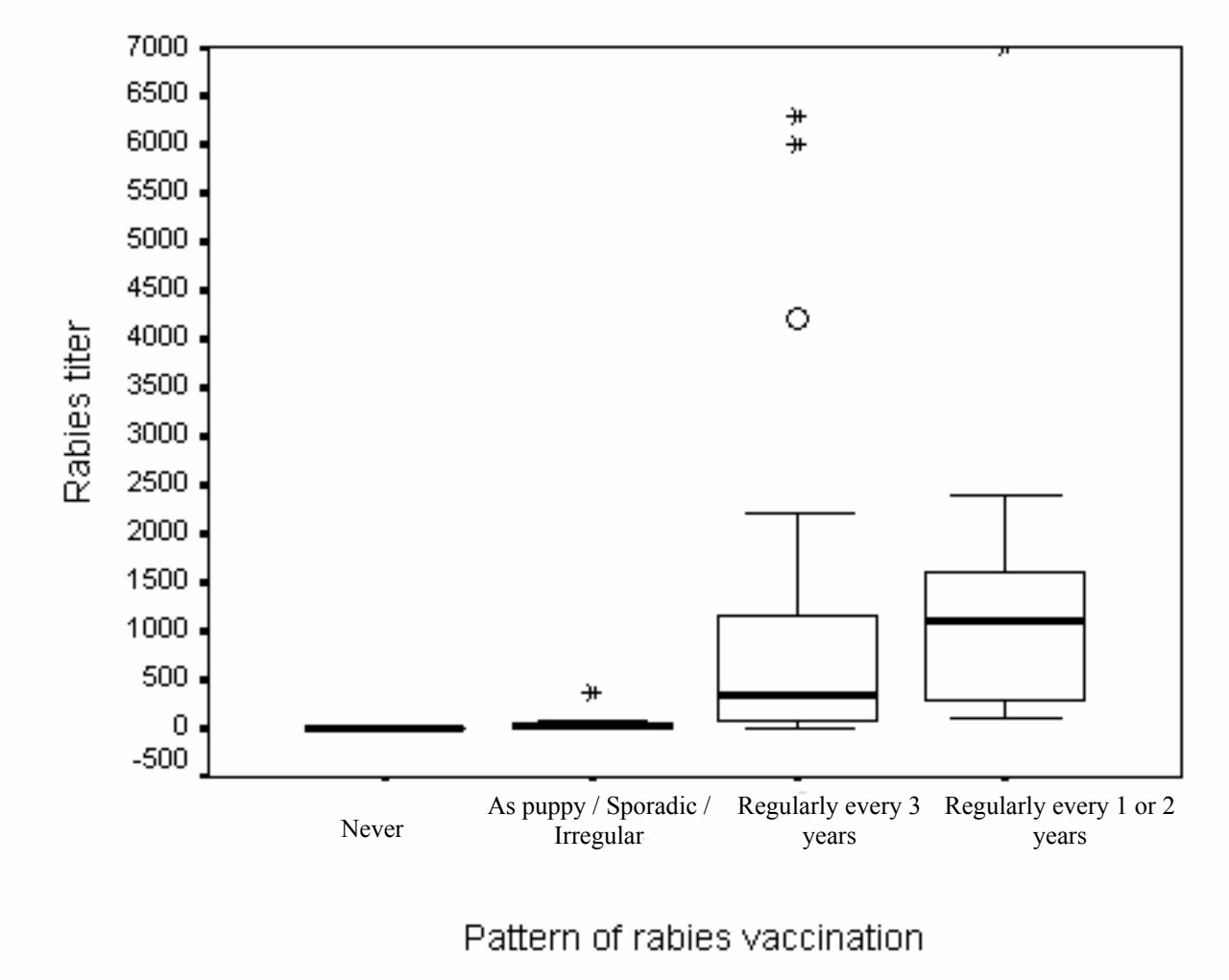

Table 6—Distribution of parvovirus titer in vaccinated and unvaccinated Great Danes

	N	Parvovirus titer					
		Mean	± (SD)	Median	Min, Max	Test statistic	P-value
Any vaccination ever?							
No	15	1063.3	1802.9	20.0	-10.0, 5120.0	Mann W	0.11
Yes	60	856.8	1212.3	320.0	-10.0, 5120.0	U = 329.0	
Any parvovirus vaccination ever?							
No	19	976.8	1681.4	20.0	-10.0, 5120.0	Mann W	0.03
Yes	56	871.4	1218.6	320.0	10.0, 5120.0	U = 358.5	
Pattern of parvovirus vaccination							
Never	19	976.8	1681.4	20.0	-10.0, 5120.0	Kruskal	0.103
As puppy / sporadic / irregular	17	1035.3	1339.6	640.0	10.0, 5120.0	Wallis =	
Regularly every one or two years	39	800.0	1173.3	320.0	40.0, 5120.0	4.55	

Figure 3—Distribution of parvovirus titer based on pattern of parvovirus vaccination

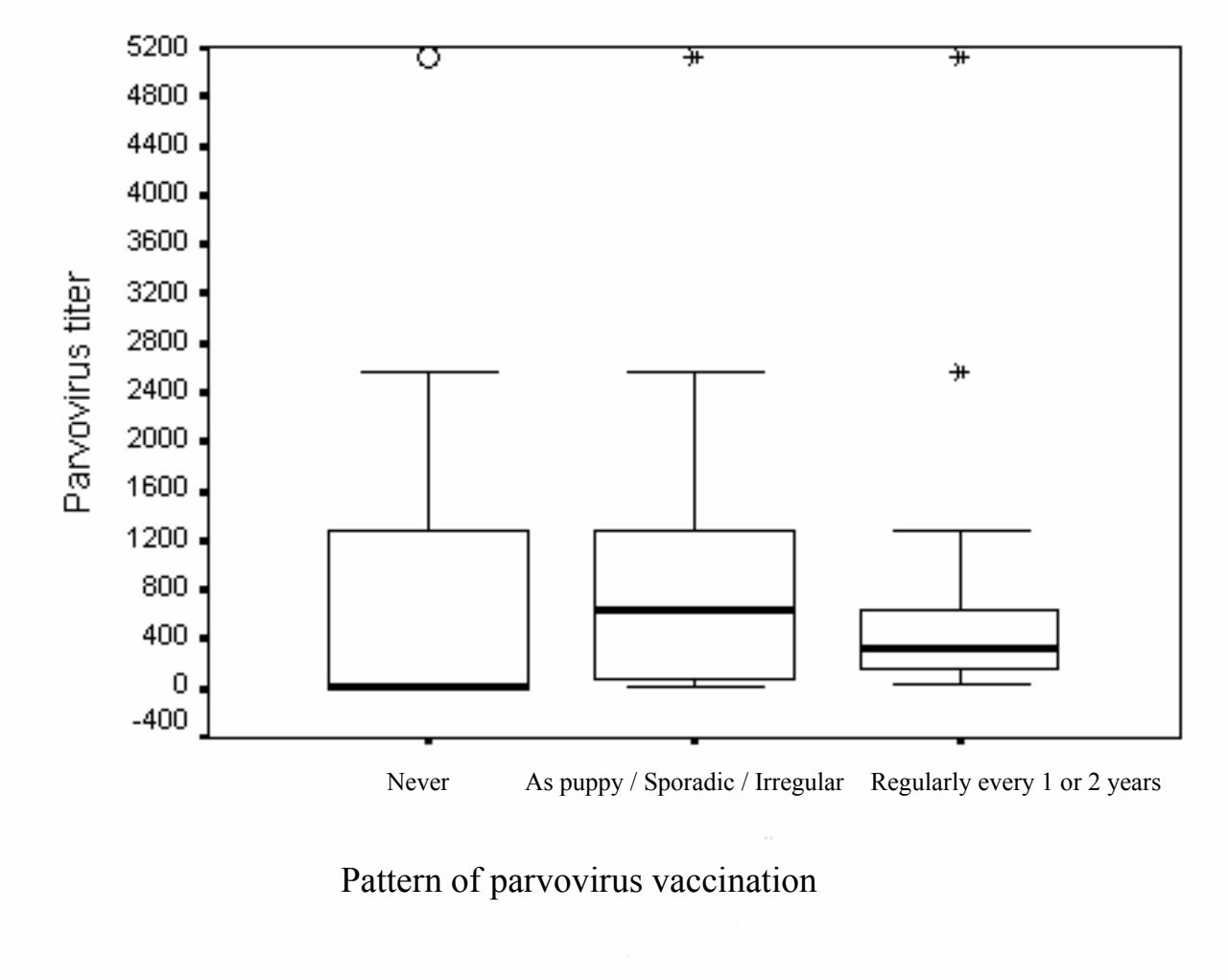

Table 7—Comparison of host characteristics (gender, neuter status and body condition) in vaccinated and unvaccinated Great Danes

	Never vaccinated		Vaccinated at least once in dog's lifetime		Test statistic	P-value
	N	%	N	%		
Gender						
Female	9	60.0	38	63.3	$\chi^2 = 0.06$	0.81
Male	6	40.0	22	36.7		
Neuter status						
Neutered	4	26.7	37	61.7	$\chi^2 = 5.93$	0.02
Intact	11	73.3	23	38.3		
Body condition						
Overweight	0	0.0	7	11.7	$\chi^2 = 2.71$	0.26
Average/optimum	14	93.3	45	75.0		
Underweight	1	6.7	8	13.3		

Table 8—Comparison of host characteristics (age, weight and height) in vaccinated and unvaccinated Great Danes

	N	Mean	± (SD)	Median	Min, Max	Test statistic	P-value
Age, years							
Never vaccinated	15	6.0	2.4	5.9	2.5, 10.2		
Vaccinated at least once in dog's lifetime	60	5.6	2.3	4.9	2.2, 10.5	t-test = -0.587	0.56
Weight, kgs							
Never vaccinated	15	62.2	9.8	61.4	50.0, 85.0		
Vaccinated at least once in dog's lifetime	60	61.2	9.7	59.1	44.5, 90.9	t-test = 0.33	0.74
Height, cms							
Never vaccinated	15	83.1	5.3	82.5	72.5, 92.5		
Vaccinated at least once in dog's lifetime	60	82.9	5.9	82.5	67.5, 95.0	t-test = 0.12	0.91

Figure 4—Age distribution of unvaccinated and vaccinated dogs

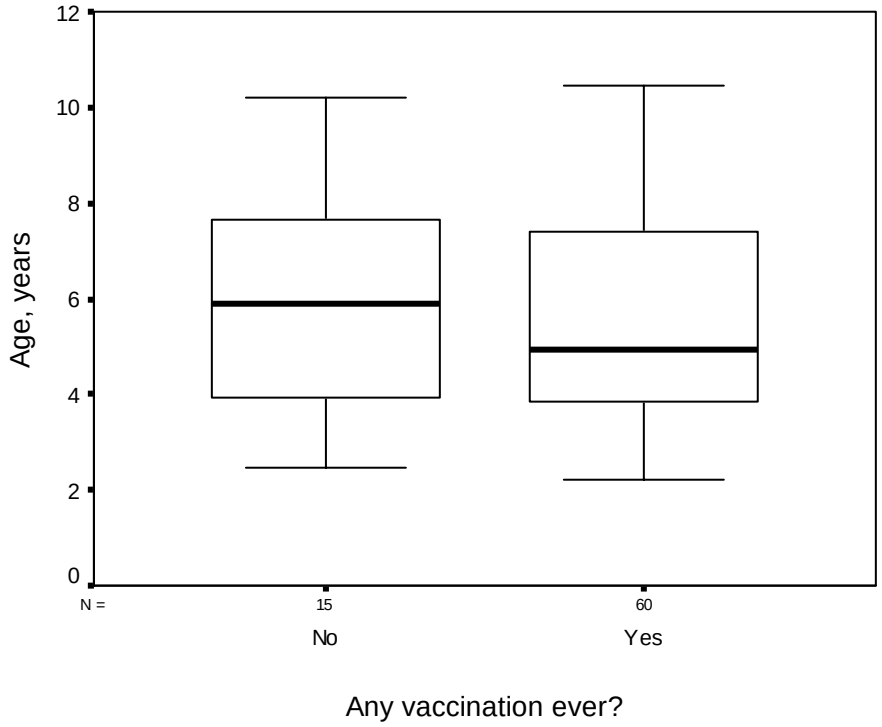

Table 9—Differences in the management practices of owners of unvaccinated and vaccinated dogs

	Never vaccinated		Vaccinated at least once in dog's lifetime		Test statistic	P-value
	N	%	N	%		
Dog has a regular vet						
No	11	73.3	2	3.3	$\chi^2 = 58.3$	<0.001
Yes	1	6.7	58	96.7		
Unclear	3	20.0	0	0.0		
Whether wormed						
No	2	13.3	8	13.3	$\chi^2 = 0.0$	1.00
Yes	13	86.7	52	86.7		
Frequency of worming medications						
Never/As puppy only	10	66.7	23	38.3		
Sporadic based on fecal examinations	4	26.7	30	50.0	$\chi^2 = 3.91$	0.14
Yearly/Monthly with heartworm medications	1	6.7	7	11.7		
Whether given heartworm medications						
No	6	40.0	5	8.3	$\chi^2 = 9.62$	0.002
Yes	9	60.0	55	91.7		
Any flea / tick products used						
No	13	86.7	9	15.0		
Yes	1	6.7	51	85.0	$\chi^2 = 32.9$	<0.001
Missing information	1	6.7	0	0.0		

Table 10—Distribution of (owner-reported) diseases by system/organ type between unvaccinated and vaccinated dogs

	Never vaccinated		Vaccinated at least once in dog's lifetime		Test statistic	P-value
	N	%	N	%		
Any malignant neoplasm						
No	15	100.0	59	98.3	$\chi^2 = 0.25$	0.62
Yes	0	0.0	1	1.7		
Any nonmalignant neoplasm						
No	9	60.0	48	80.0	$\chi^2 = 2.63$	0.11
Yes	6	40.0	12	20.0		
Any allergies						
No	15	100.0	53	88.3	$\chi^2 = 1.93$	0.17
Yes	0	0.0	7	11.7		
Any endocrine diseases						
No	15	100.0	53	88.3	$\chi^2 = 1.93$	0.17
Yes	0	0.0	7	11.7		
Any autoimmune diseases						
No	15	100.0	58	96.7	$\chi^2 = 0.51$	0.47
Yes	0	0.0	2	3.3		
Any urinary tract / renal diseases						
No	15	100.0	53	88.3	$\chi^2 = 1.93$	0.17
Yes	0	0.0	7	11.7		
Any neurological diseases						
No	15	100.0	56	93.3	$\chi^2 = 1.06$	0.30
Yes	0	0.0	4	6.7		
Any musculoskeletal diseases						
No	12	80.0	39	65.0	$\chi^2 = 1.24$	0.27
Yes	3	20.0	21	35.0		
Any genetic diseases						
No	14	93.3	43	71.7	$\chi^2 = 3.09$	0.08
Yes	1	6.7	17	28.3		

Table 11—Details of three owner-reported vaccine reactions.

Dog #	Vaccine type or brand	Age of dog	Reaction seen by a vet?	Reaction treated by a vet?	How treated?	Signs of reaction	When did the signs begin to manifest?	Dog revaccinated ?	Any precautions taken during revaccination?	Any long-term effects?
1	Rabies+da2lpp	5 m	Yes	Yes	Aspirin; Changed food	Weakness Couldn't stand Joints hot	3 days after vaccination	Yes	Yes; vaccinated less often	No
2	Rabies	6 y	Yes	Not needed	--	Swelling	2 days after vaccination	Yes	No	No
3	Rabies Imrab1 Merial	4 y 4 m	Yes	Yes	Antibiotics	Injection site eruption; subsequent infection	12 hours after vaccination	Yes	Yes; changed brand. Separated days given rabies, dapp.	No

Table 12—Details of four owner-reported drug reactions. All four dogs belong to the vaccinated group.

Dog #	Drug	Age of dog	Reaction seen by a vet?	Reaction treated by a vet?	How treated?	Signs of reaction	When did the signs begin to manifest?	Any long-term effects?
1	Advantage	1 y	No	No	--	Chewing	After 1 day	No
2	Carprofen	4 y	Yes	Yes	Imodium; Changed to Etogesic	Vomiting /diarrhea Bloody stool	After 2 days	No
3	Anesthesia	8 y	Yes	Yes	Tablet & injection	Vomiting / diarrhea	After 30 minutes	No
4	Proheart 6 Ft Dodge	2 y	Yes	No	--	Swelling	After 5 minutes	No

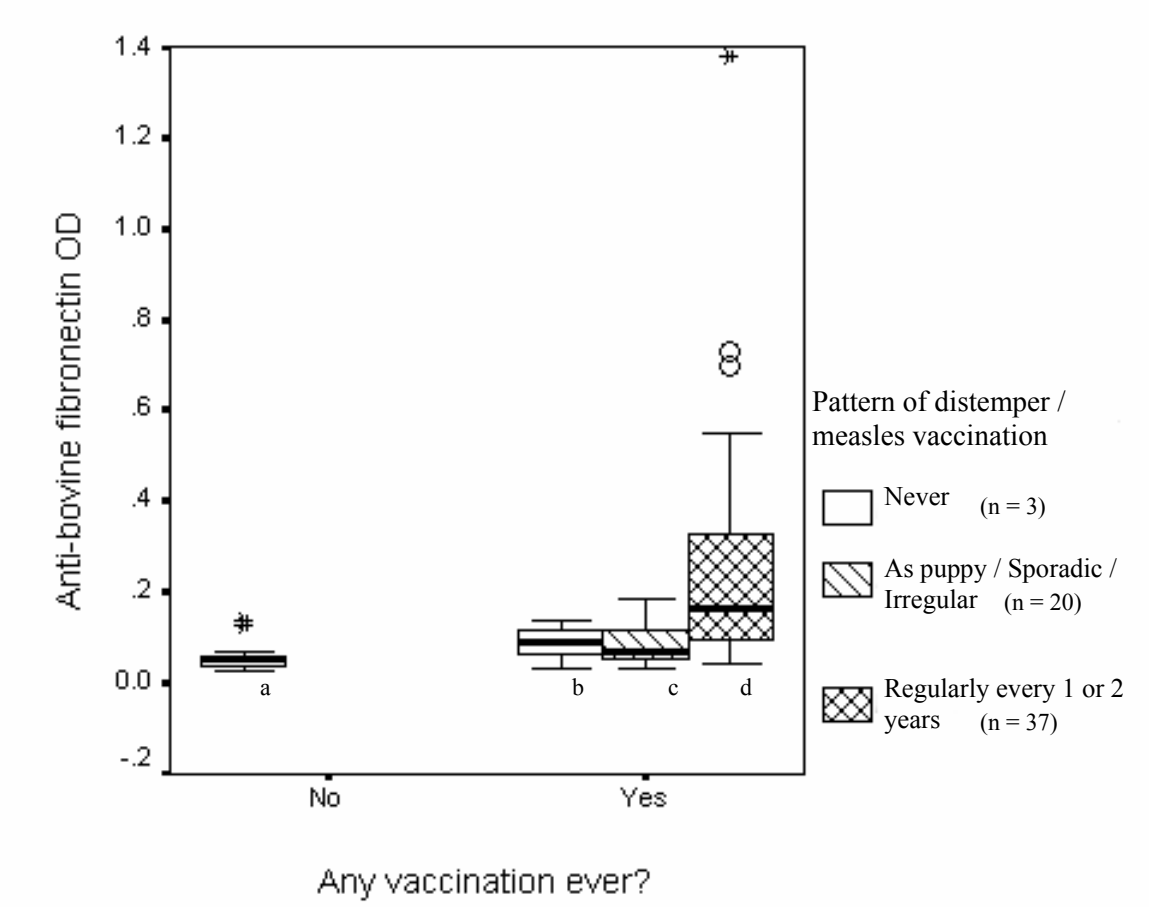

Figure 5A—Boxplots of optical density (OD) values of anti-bovine fibronectin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *d*, *c* and *d.*

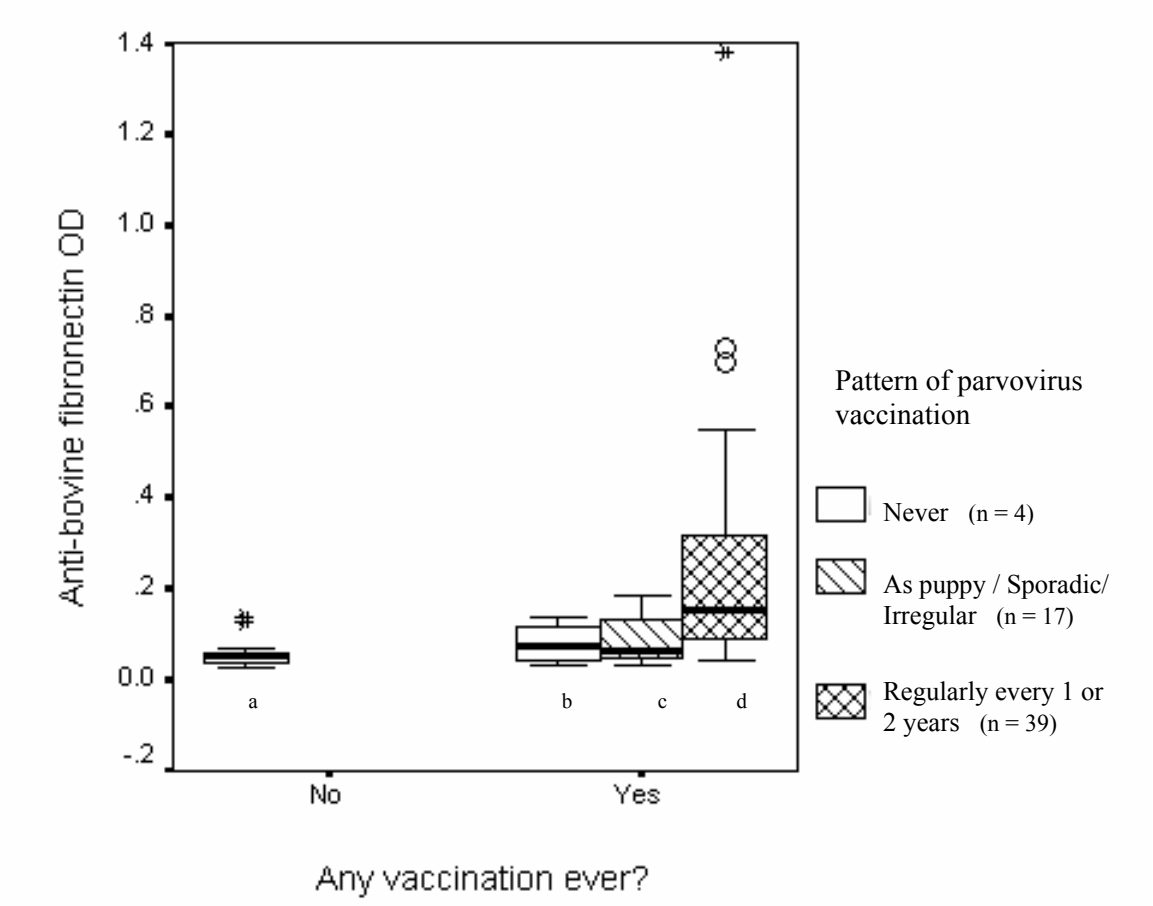

Figure 5B—Boxplots of optical density (OD) values of anti-bovine fibronectin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *d*, *c* and *d*.

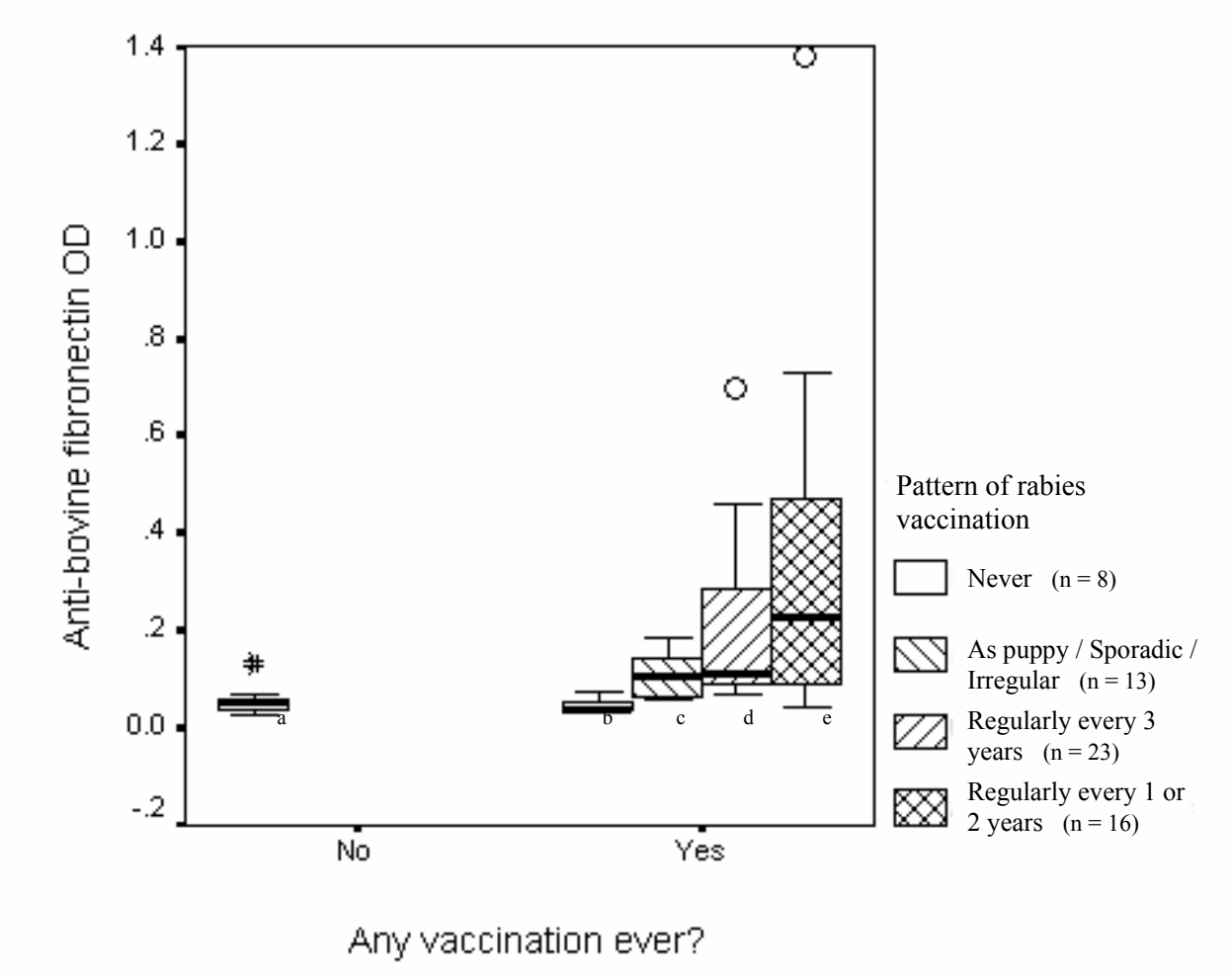

Figure 5C— Boxplots of optical density (OD) values of anti-bovine fibronectin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *c*, *a* and *d*, *a* and *e*, *b* and *c*, *b* and *d*, *b* and *e*.

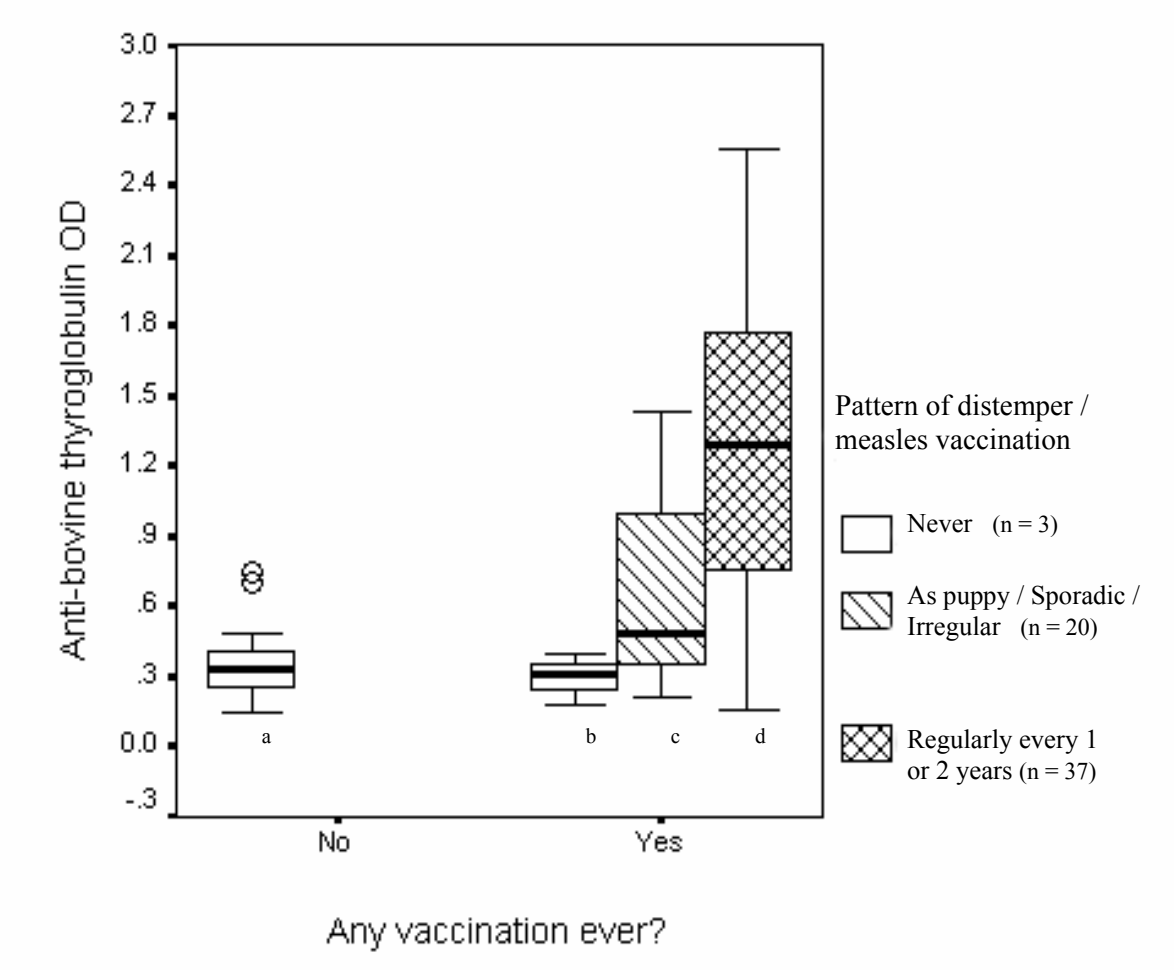

Figure 6A—Boxplots of optical density (OD) values of anti-bovine thyroglobulin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tukey's test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *d*, *b* and *d*, *c* and *d*.

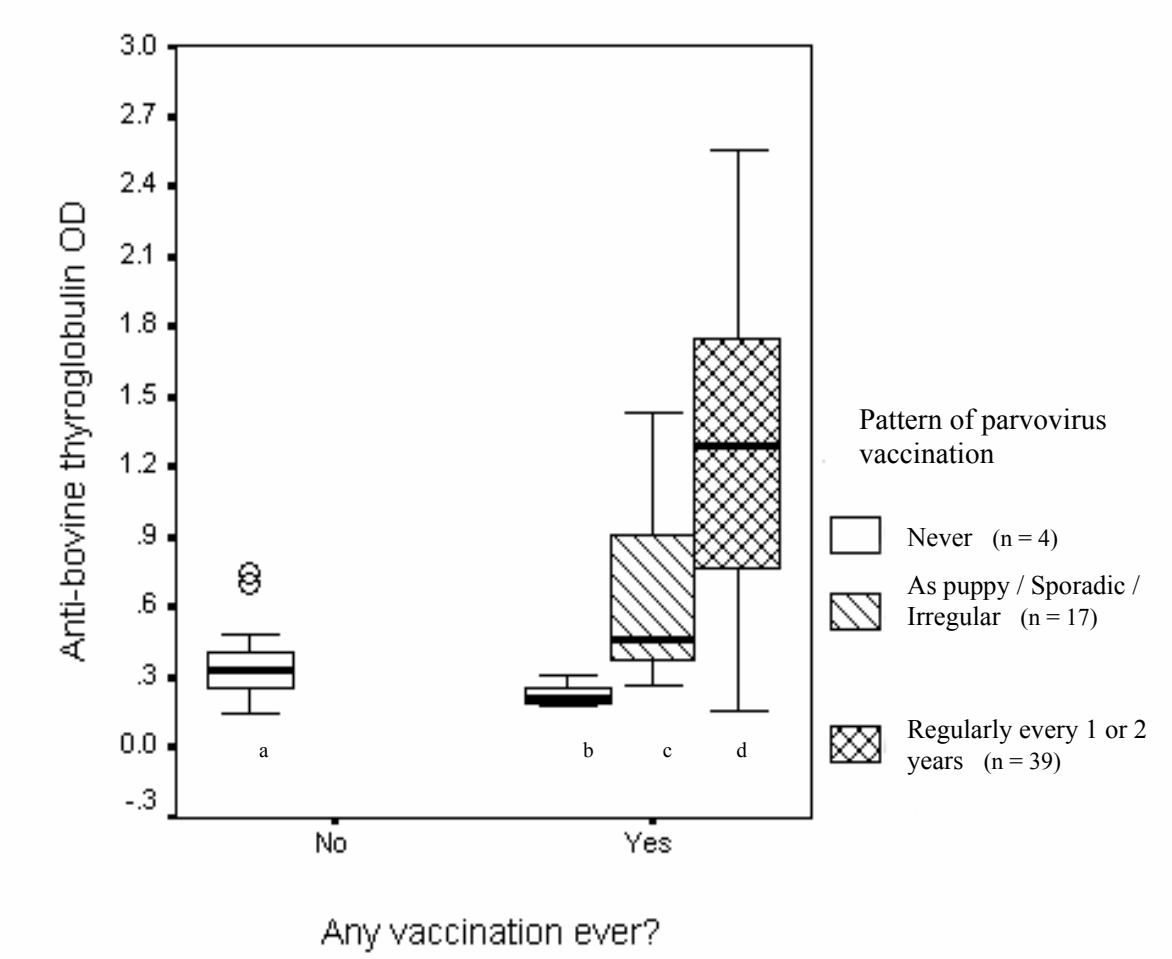

Figure 6B—Boxplots of optical density (OD) values of anti-bovine thyroglobulin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tukey's test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *c*, *a* and *d*, *b* and *c*, *b* and *d*, *c* and *d*.

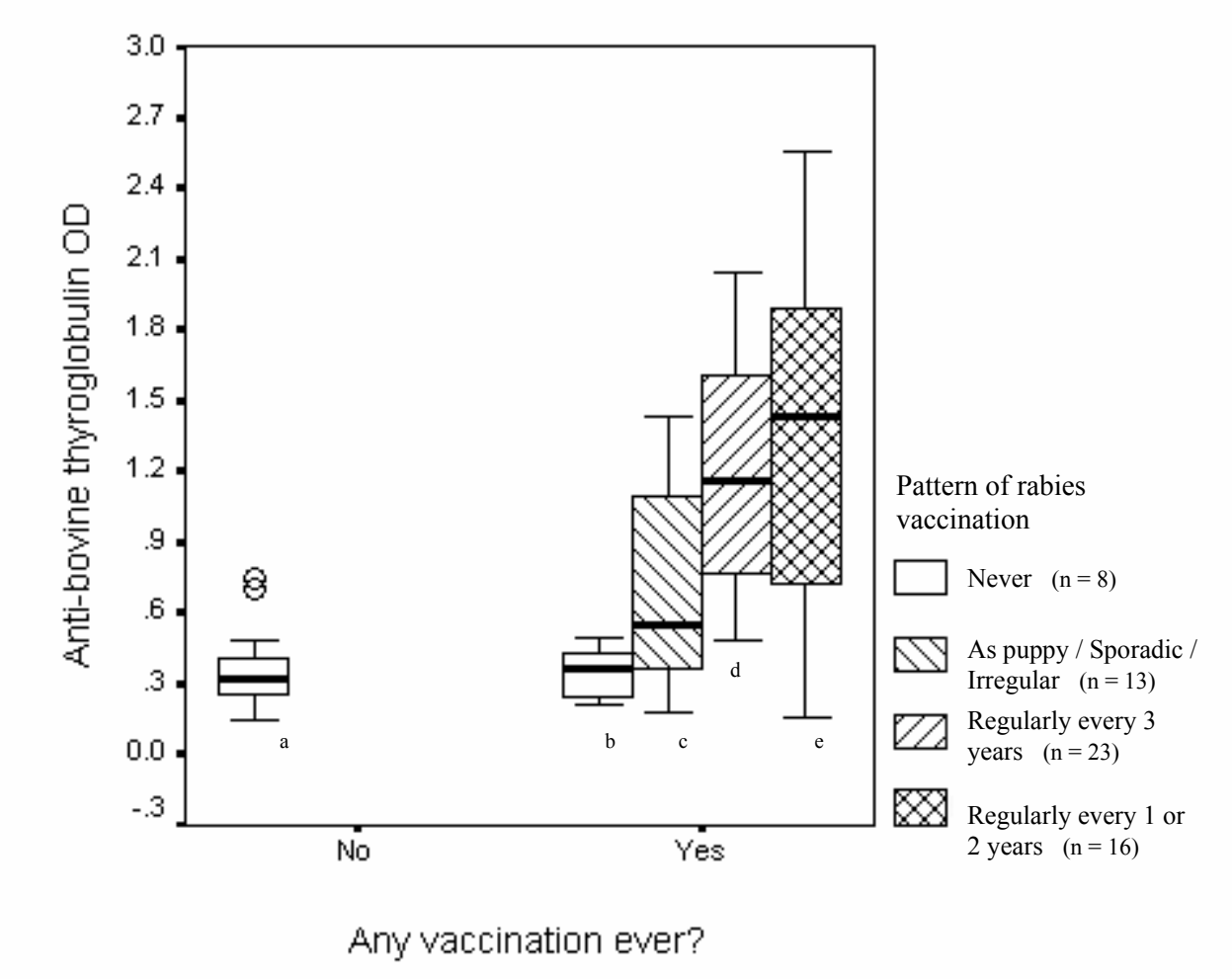

Figure 6C—Boxplots of optical density (OD) values of anti-bovine thyroglobulin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tukey's test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *d*, *b* and *d*, *c* and *d, a* and *e, b* and *e*.

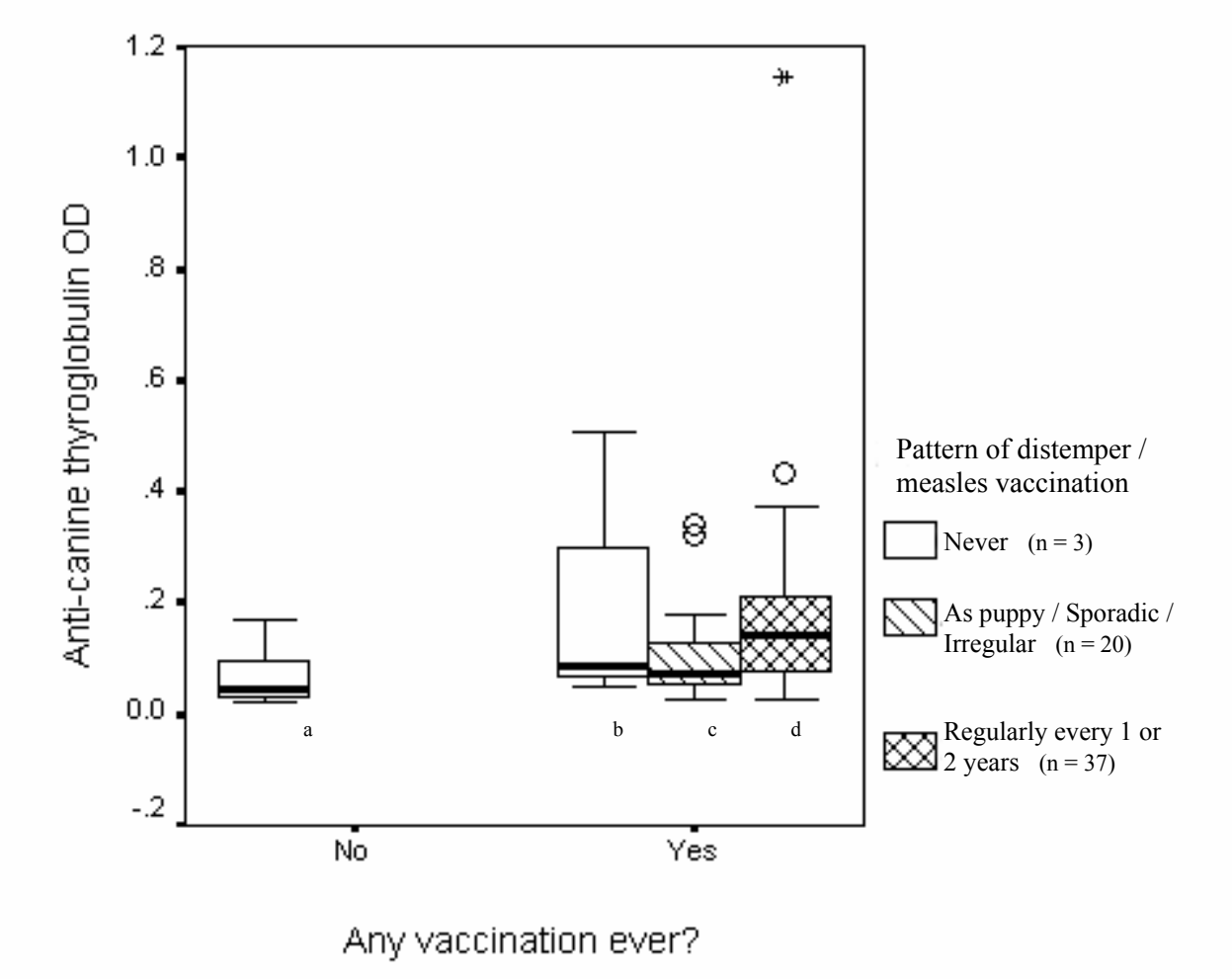

Figure 7A—Boxplots of optical density (OD) values of anti-canine thyroglobulin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tukey's test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *d*.

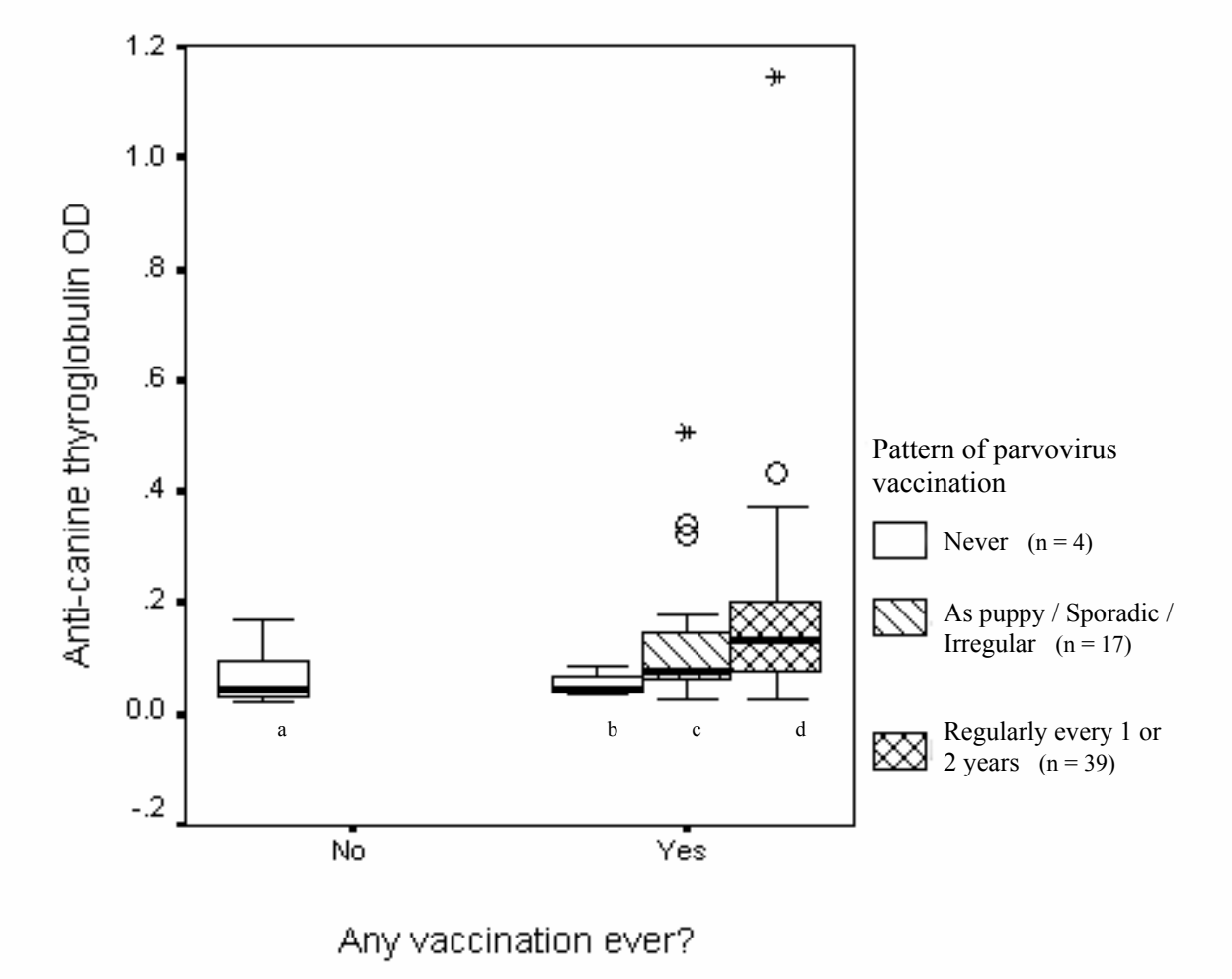

Figure 7B—Boxplots of optical density (OD) values of anti-canine thyroglobulin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tukey's test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *d*.

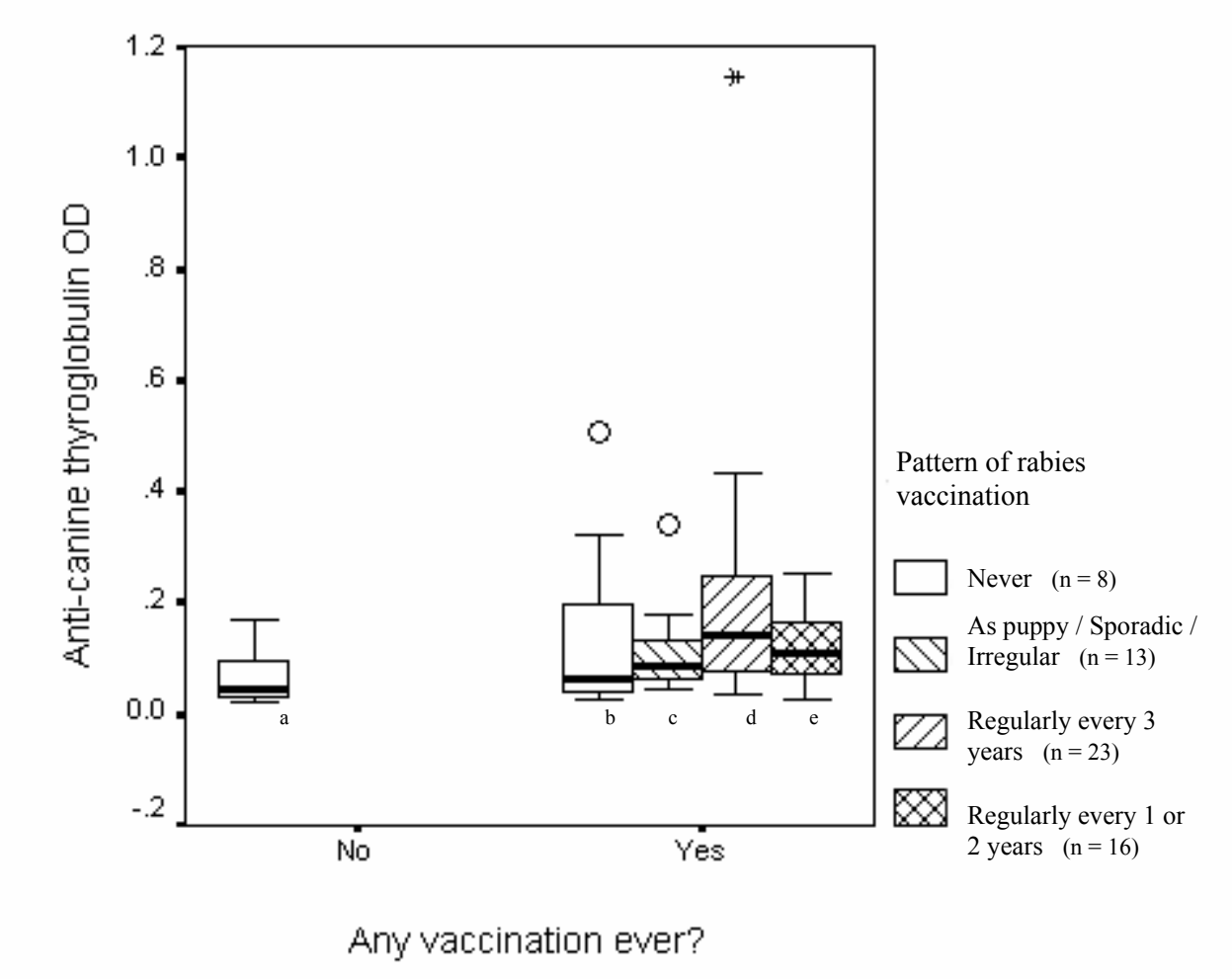

Figure 7C—Boxplots of optical density (OD) values of anti-canine thyroglobulin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tukey's test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *d*.

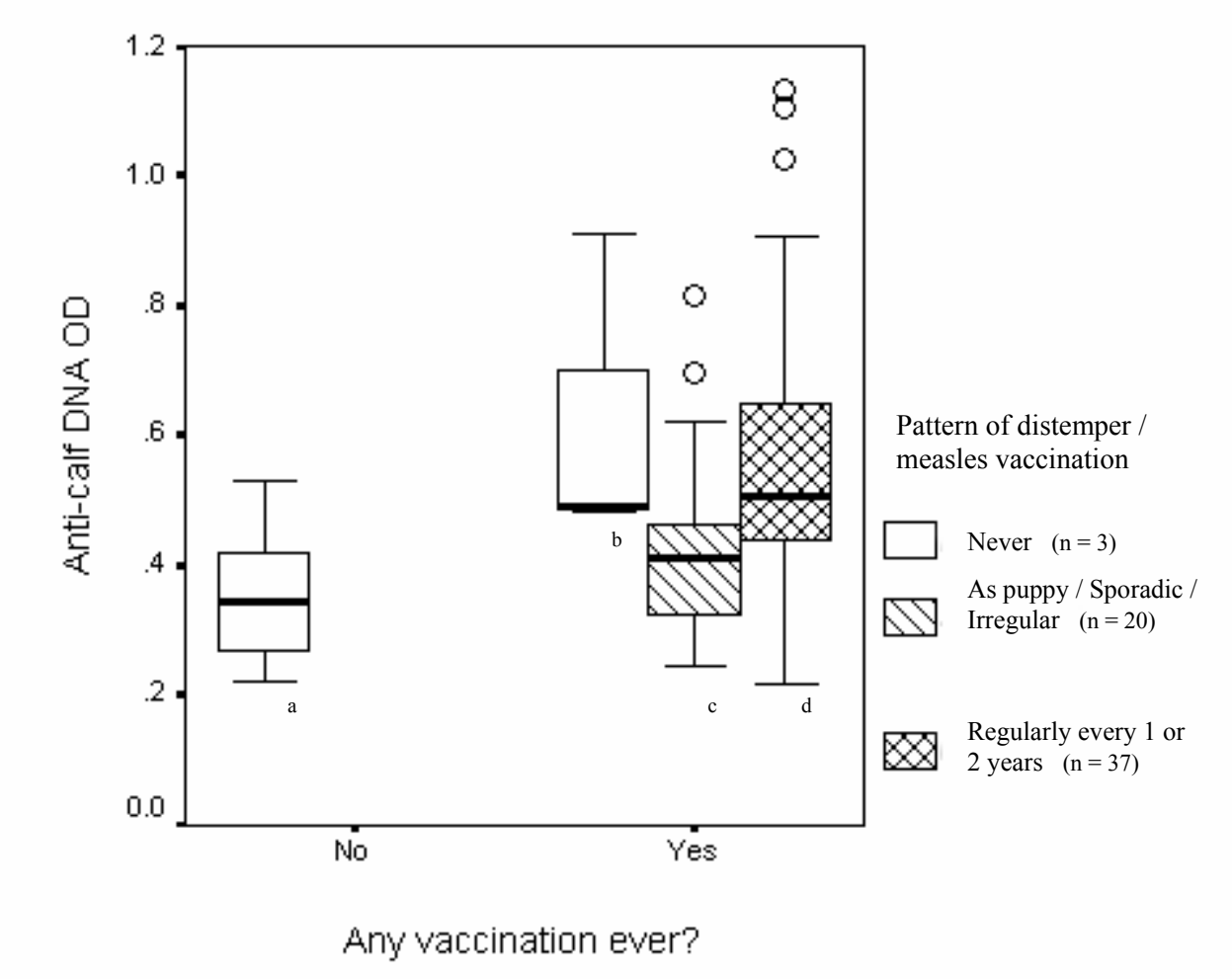

Figure 8A—Boxplots of optical density (OD) values of anti-calf DNA antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tukey's test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *b*, *a* and *d*, *c* and *d*.

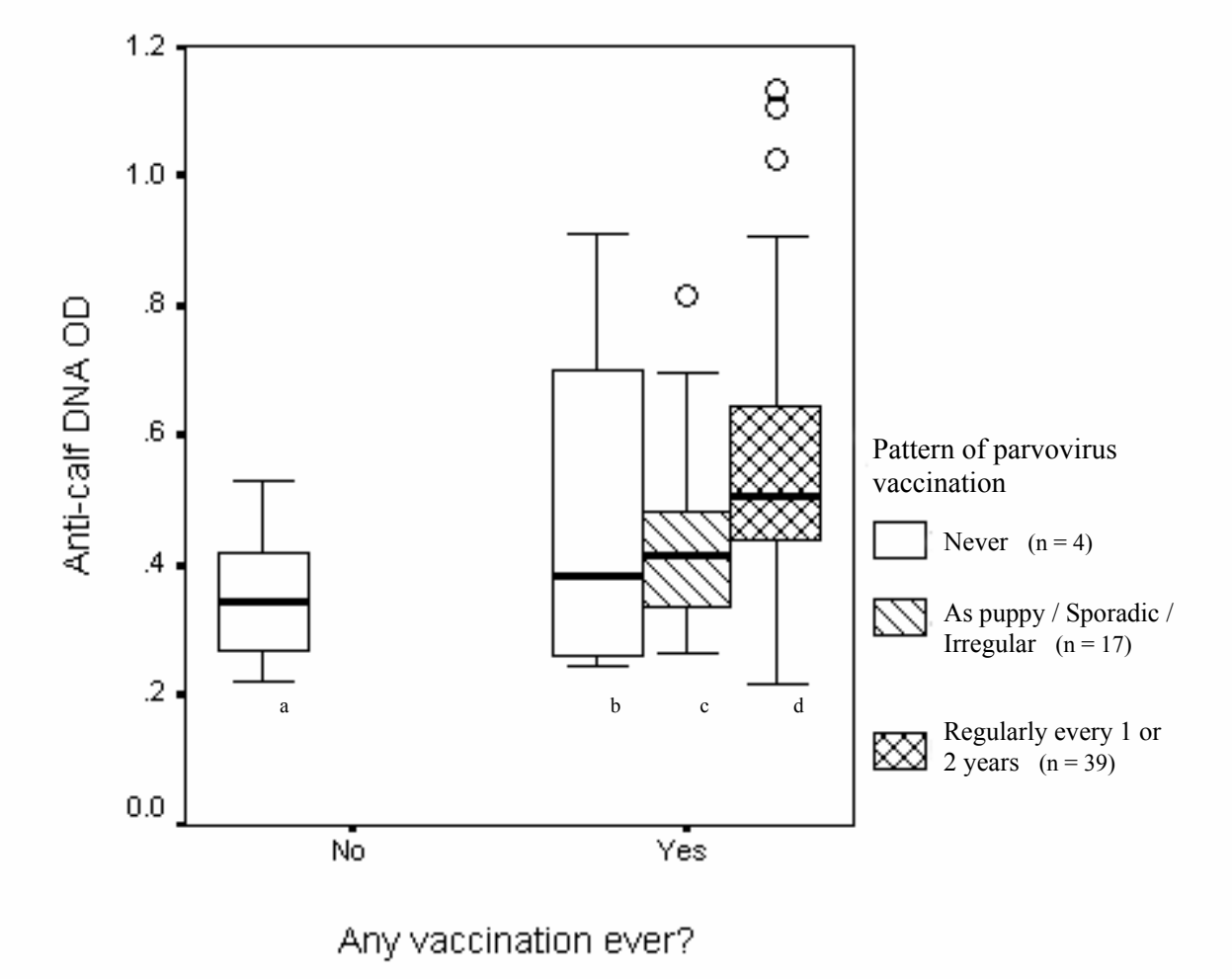

Figure 8B—Boxplots of optical density (OD) values of anti-calf DNA antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tukey's test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *d*.

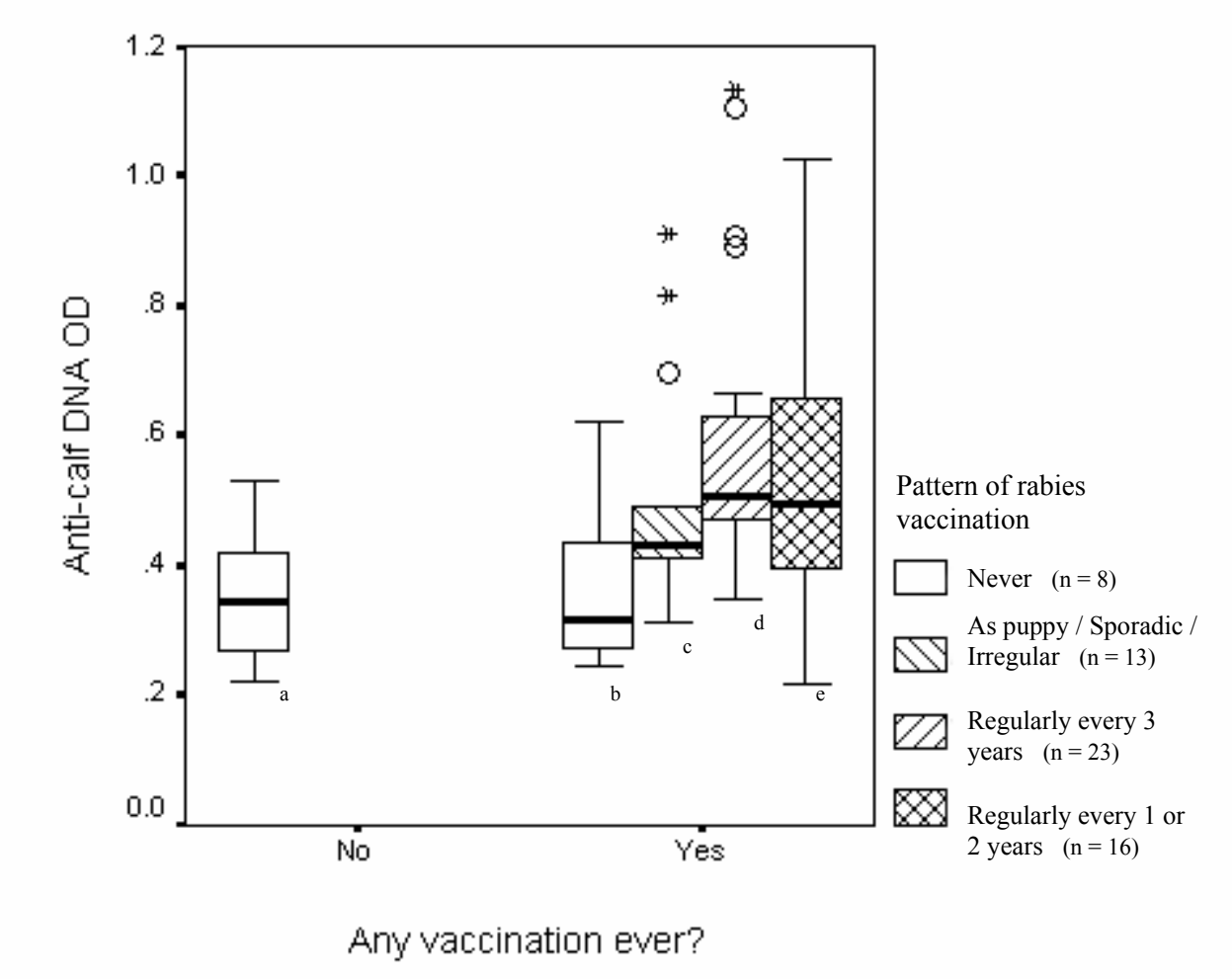

Figure 8C—Boxplots of optical density (OD) values of anti-calf DNA antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA and Tukey's test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *d*, *a* and *e*, *b* and *d*.

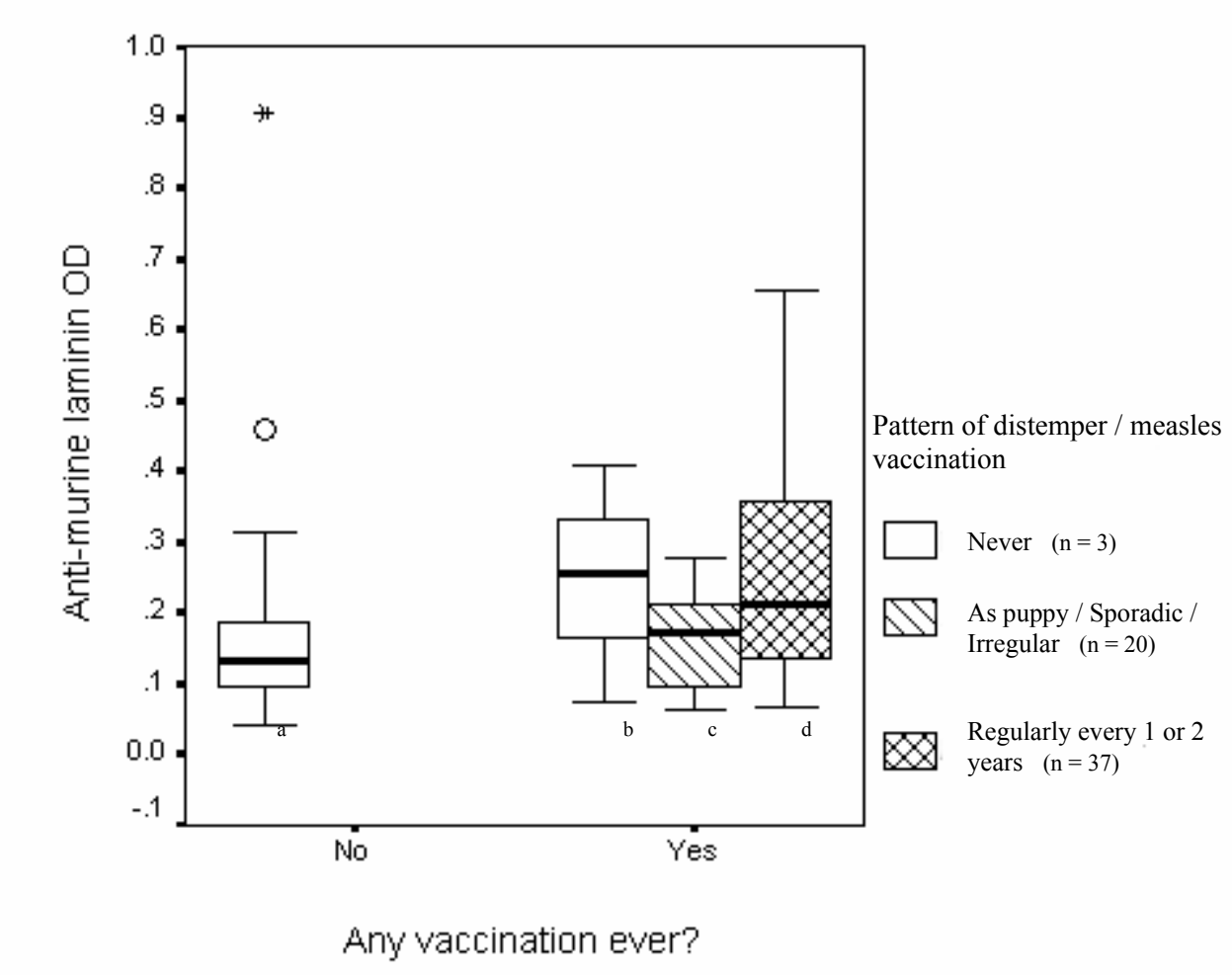

Figure 9A—Boxplots of optical density (OD) values of anti-murine laminin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

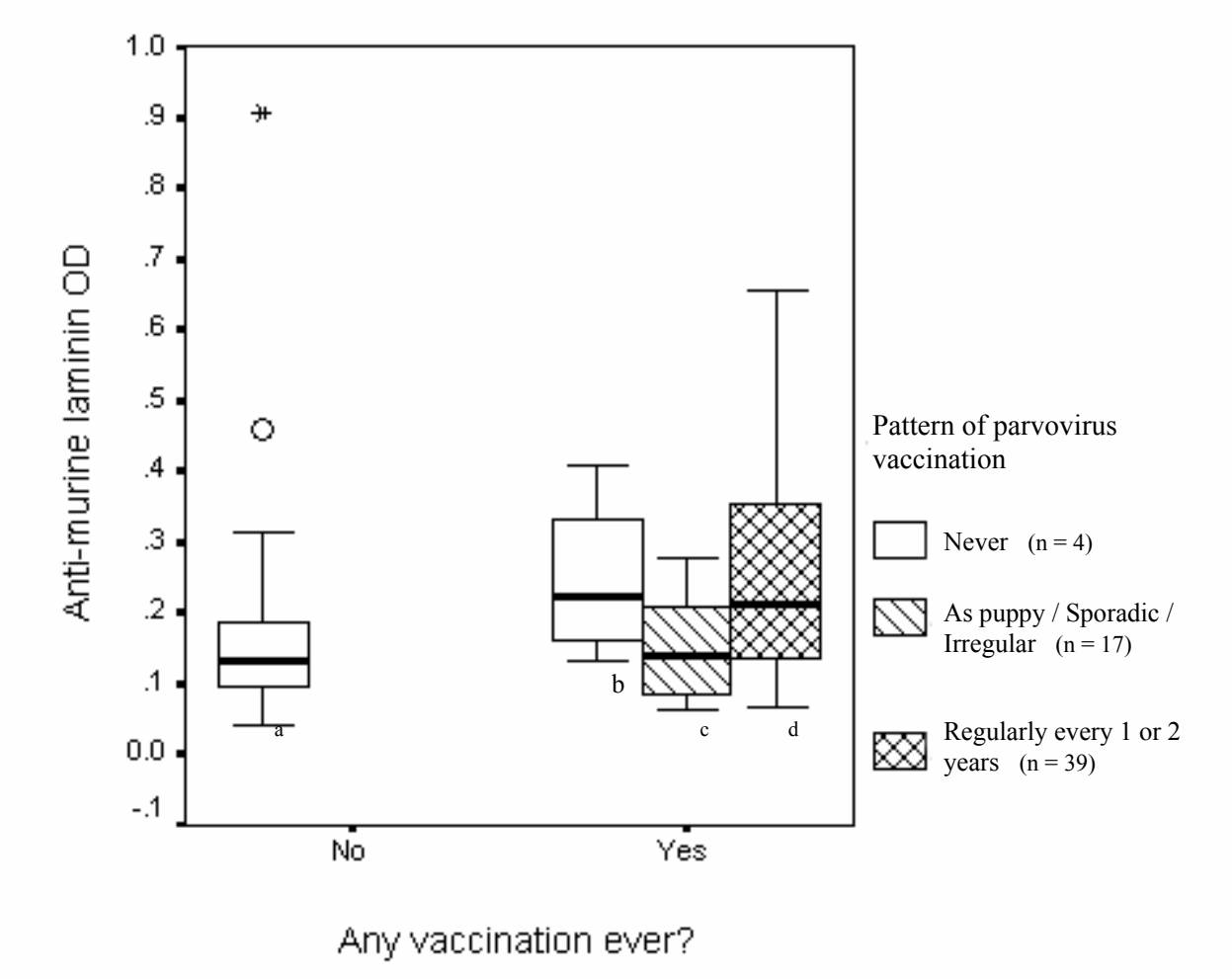

Figure 9B—Boxplots of optical density (OD) values of anti-murine laminin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

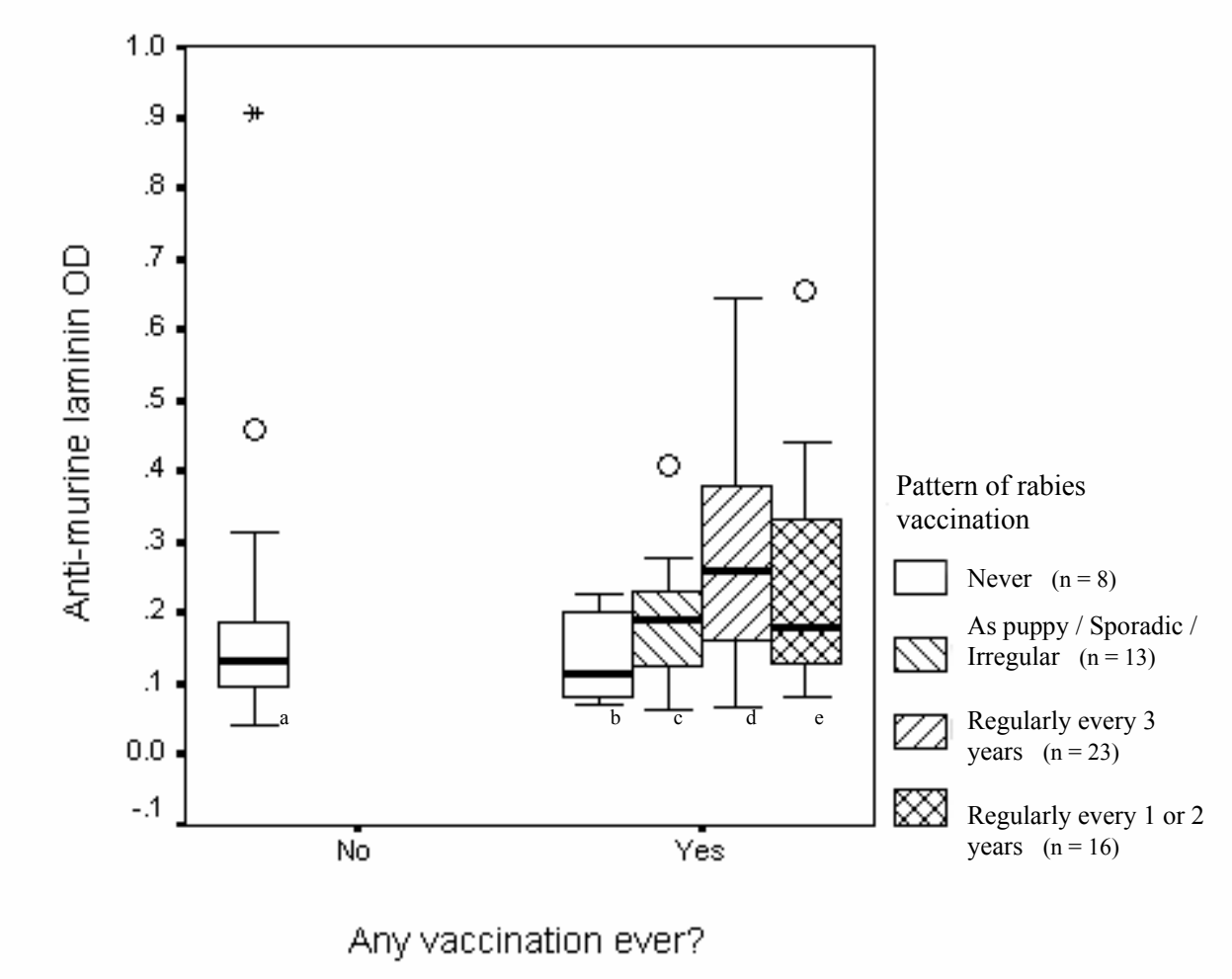

Figure 9C—Boxplots of optical density (OD) values of anti-murine laminin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

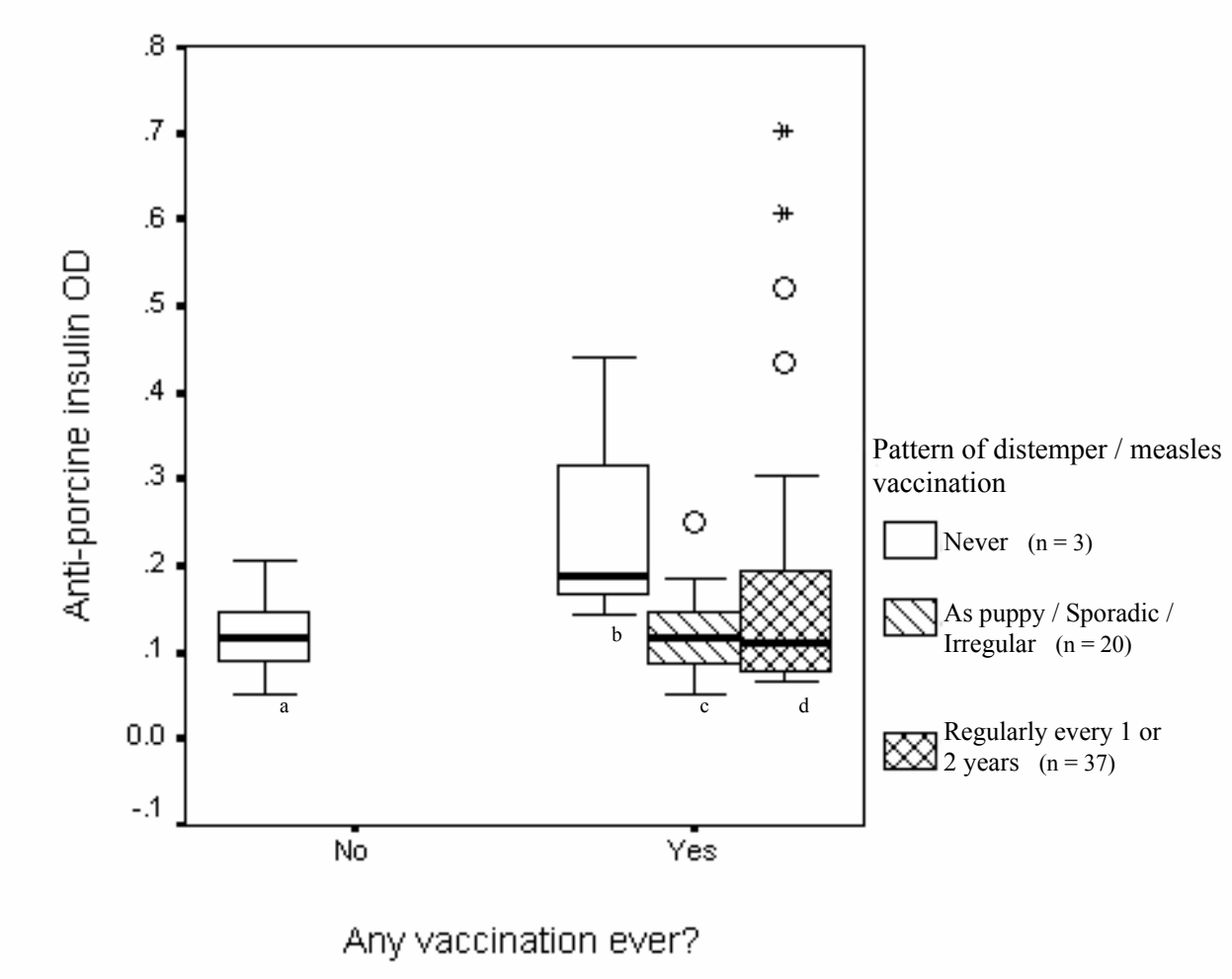

Figure 10A—Boxplots of optical density (OD) values of anti-porcine insulin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

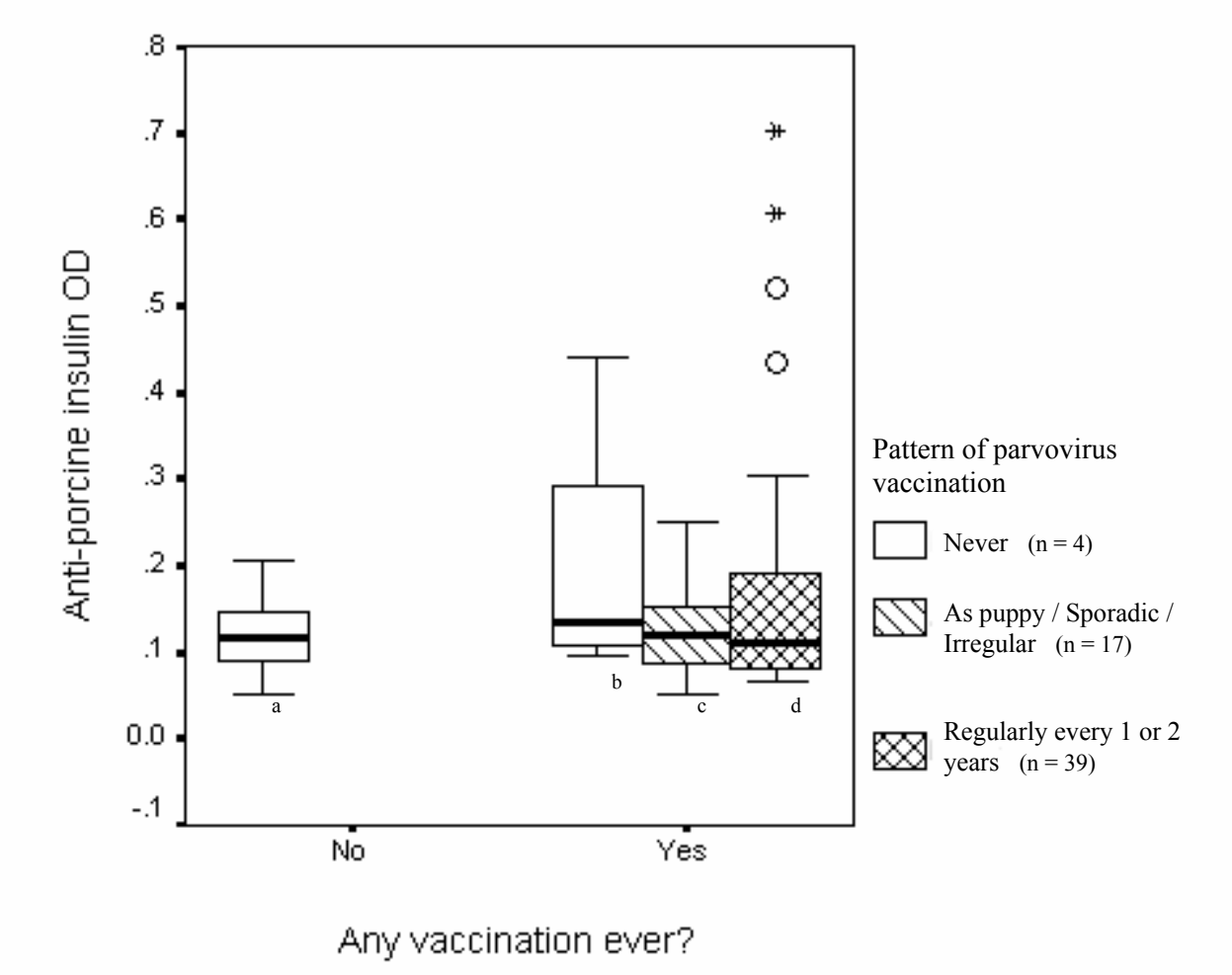

Figure 10B—Boxplots of optical density (OD) values of anti-porcine insulin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

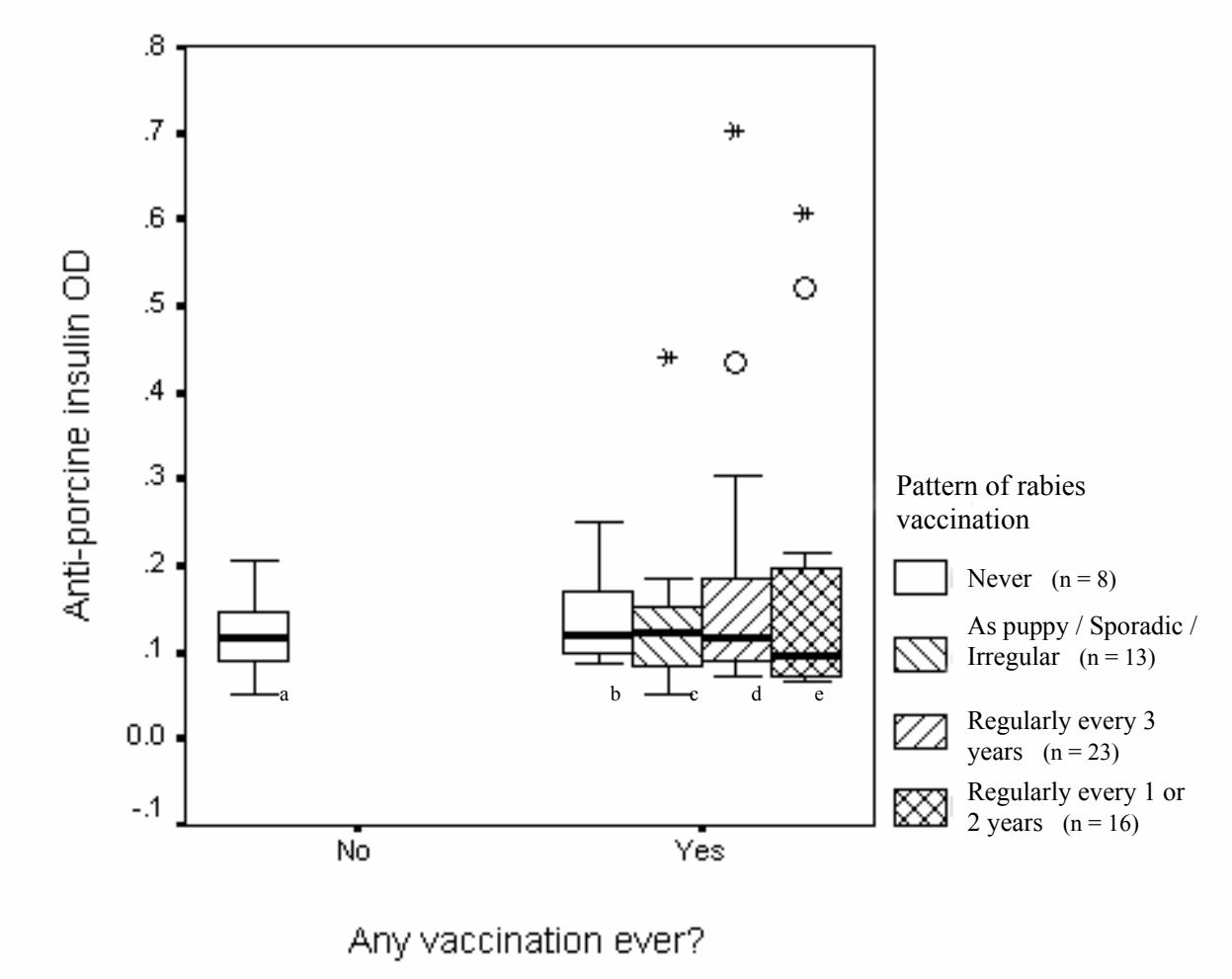

Figure 10C—Boxplots of optical density (OD) values of anti-porcine insulin antibodies in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

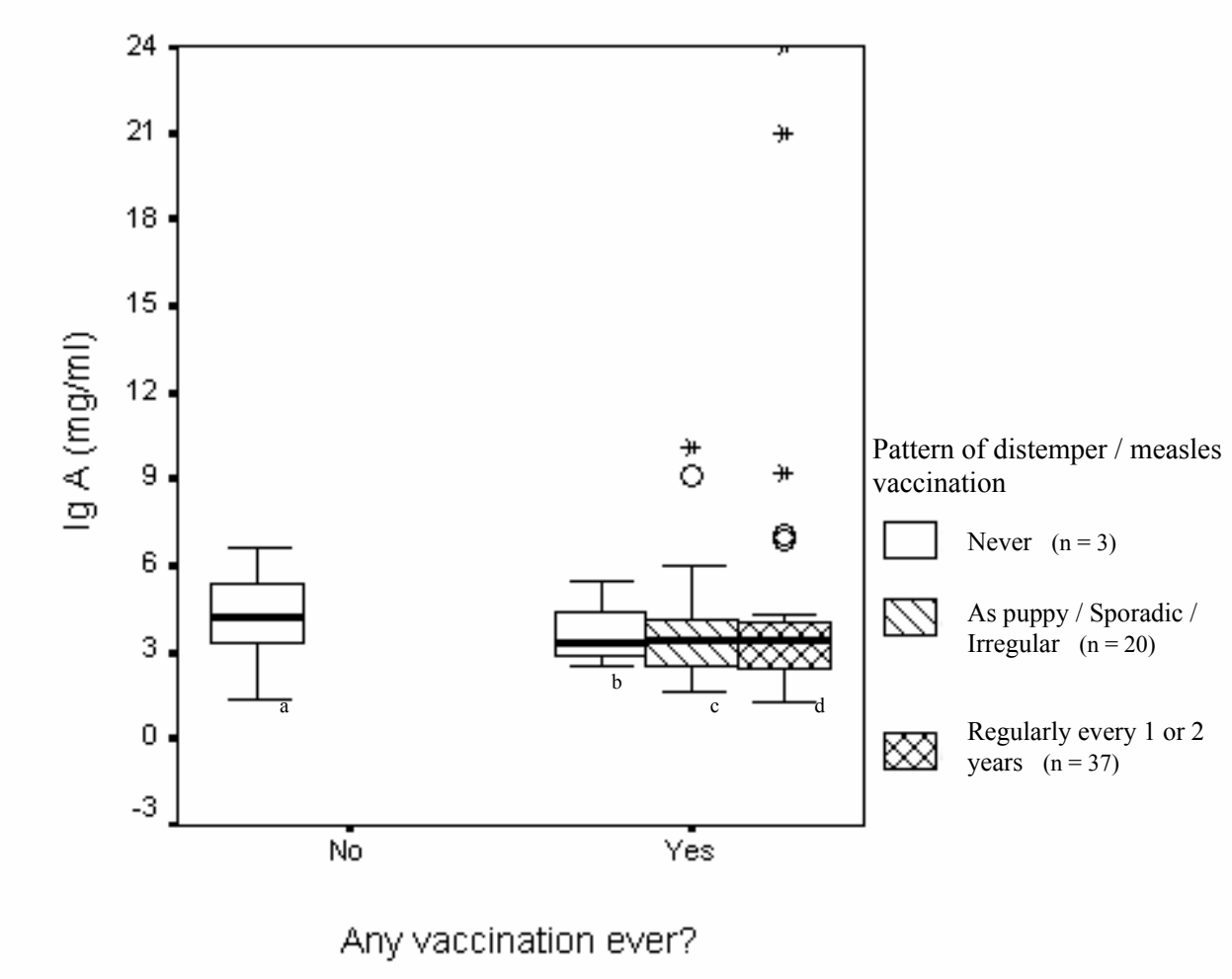

Figure 11A—Boxplots of serum concentration of IgA (mg/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

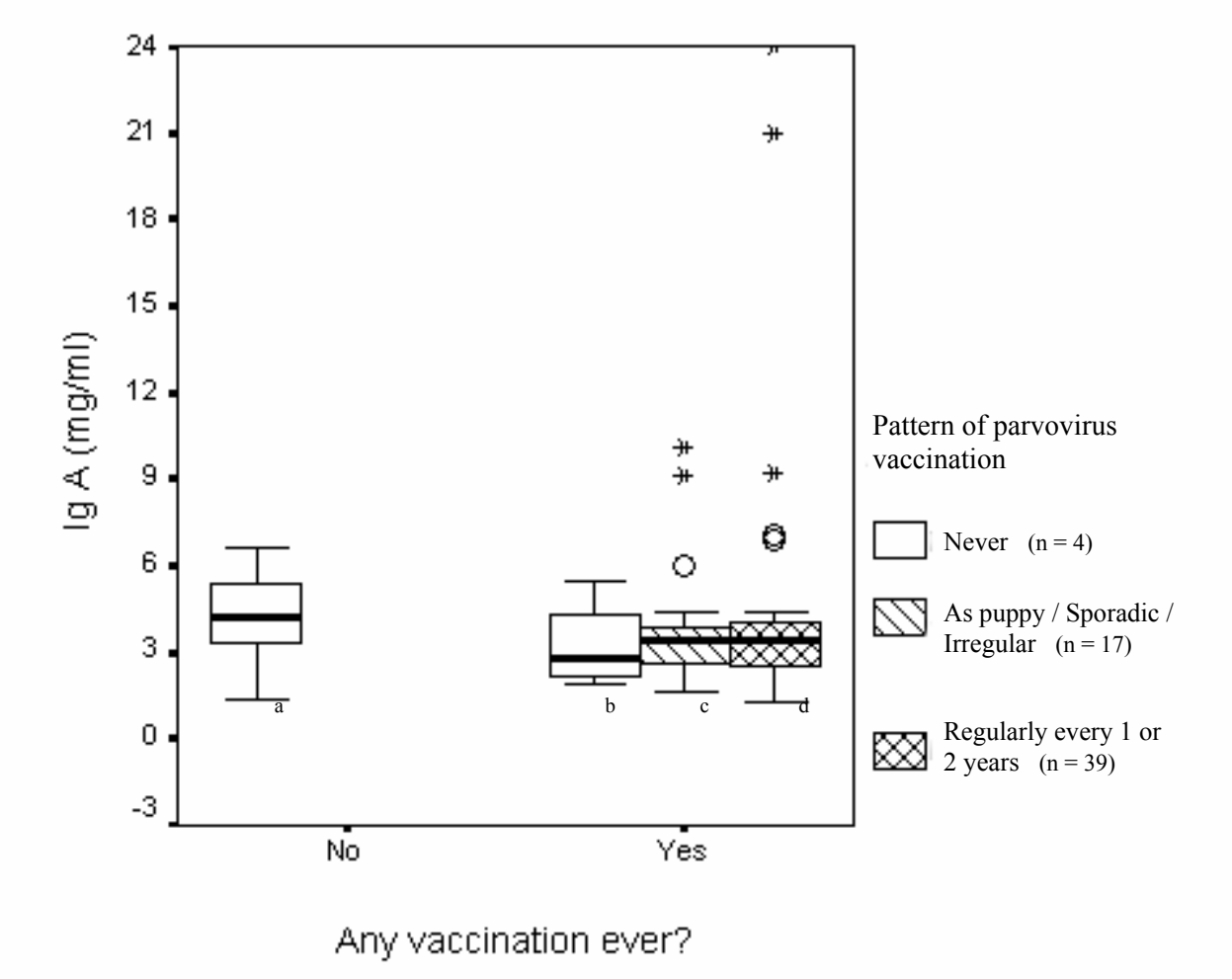

Figure 11B—Boxplots of serum concentration of IgA (mg/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

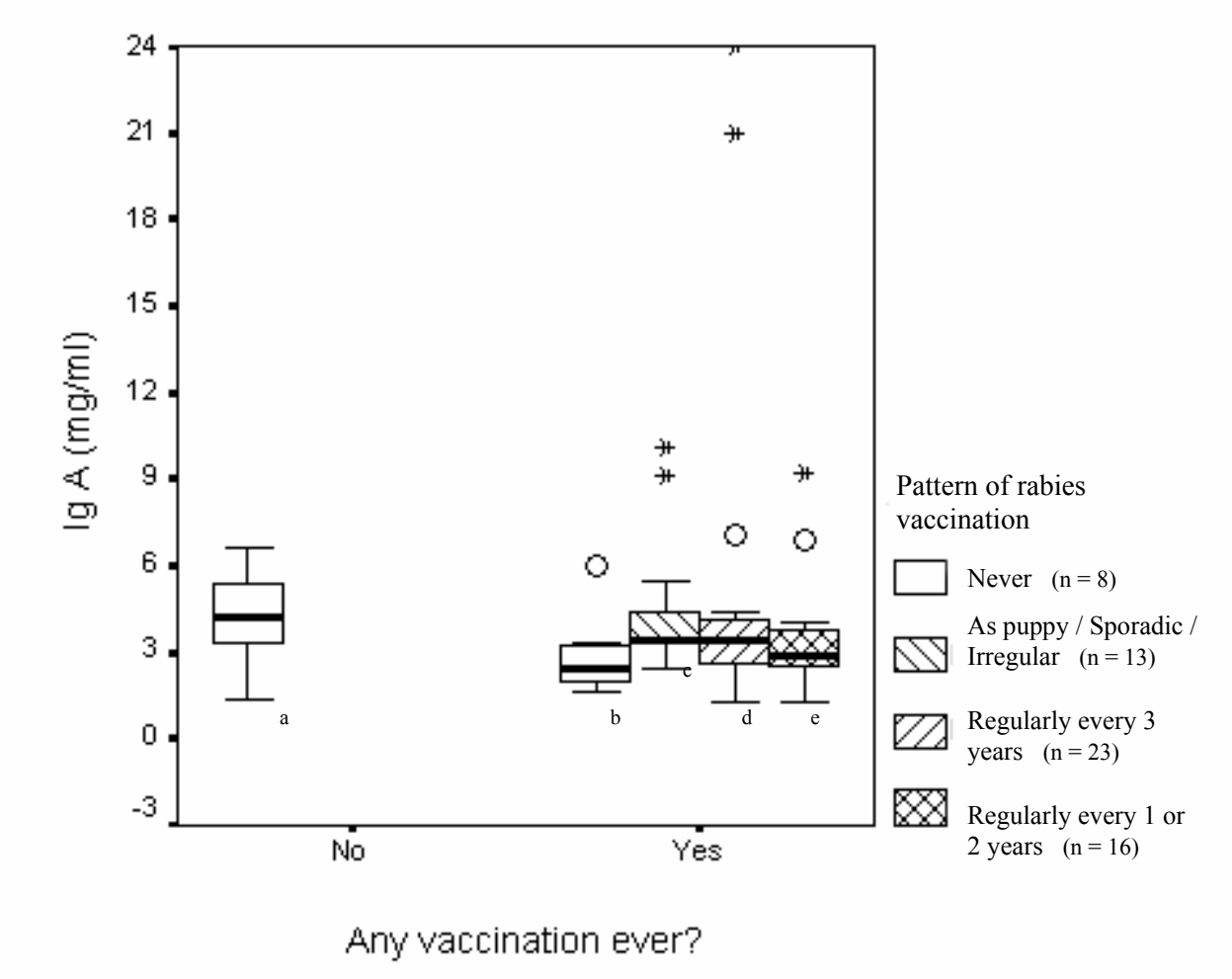

Figure 11C—Boxplots of serum concentration of IgA (mg/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

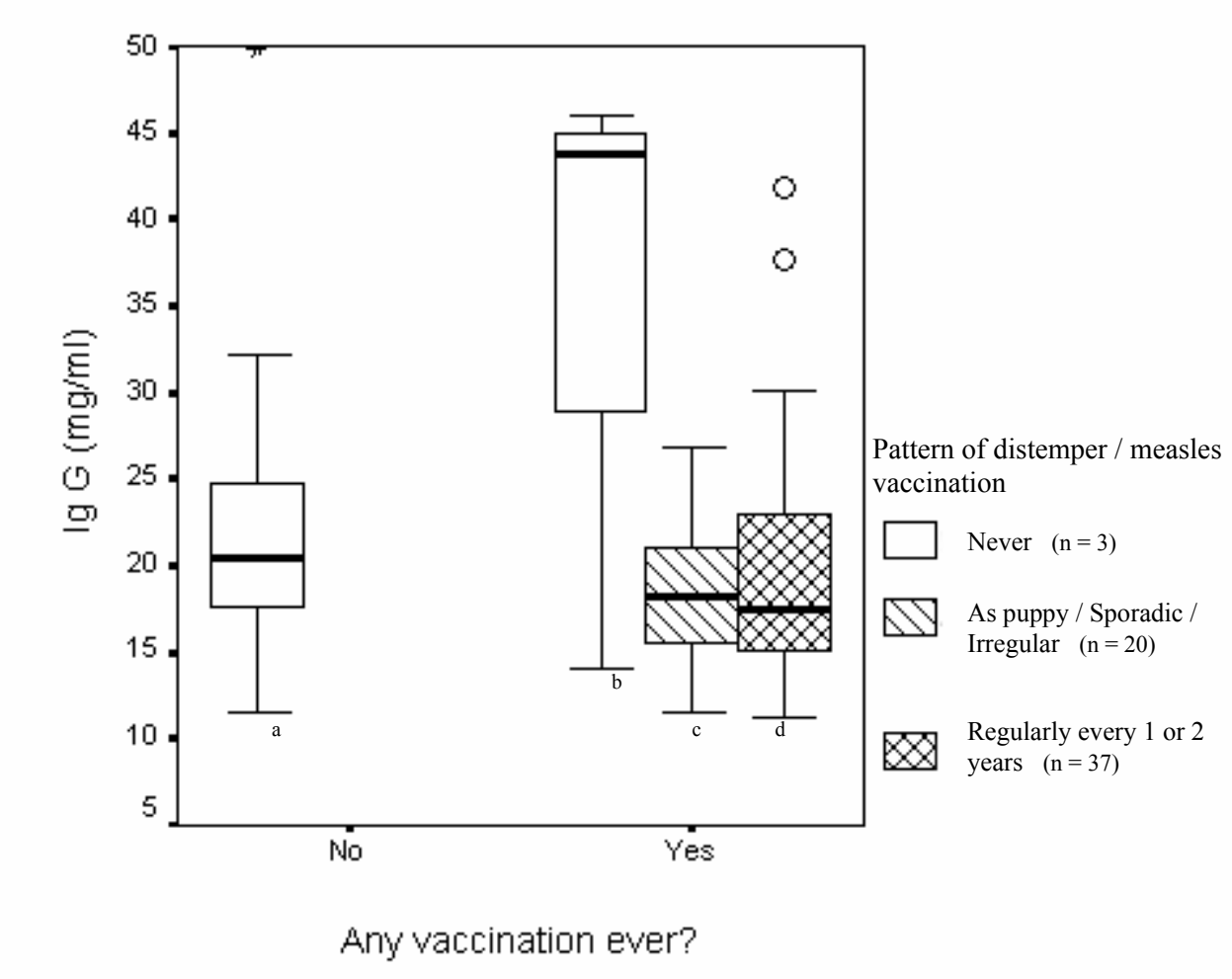

Figure 12A—Boxplots of serum concentration of IgG (mg/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

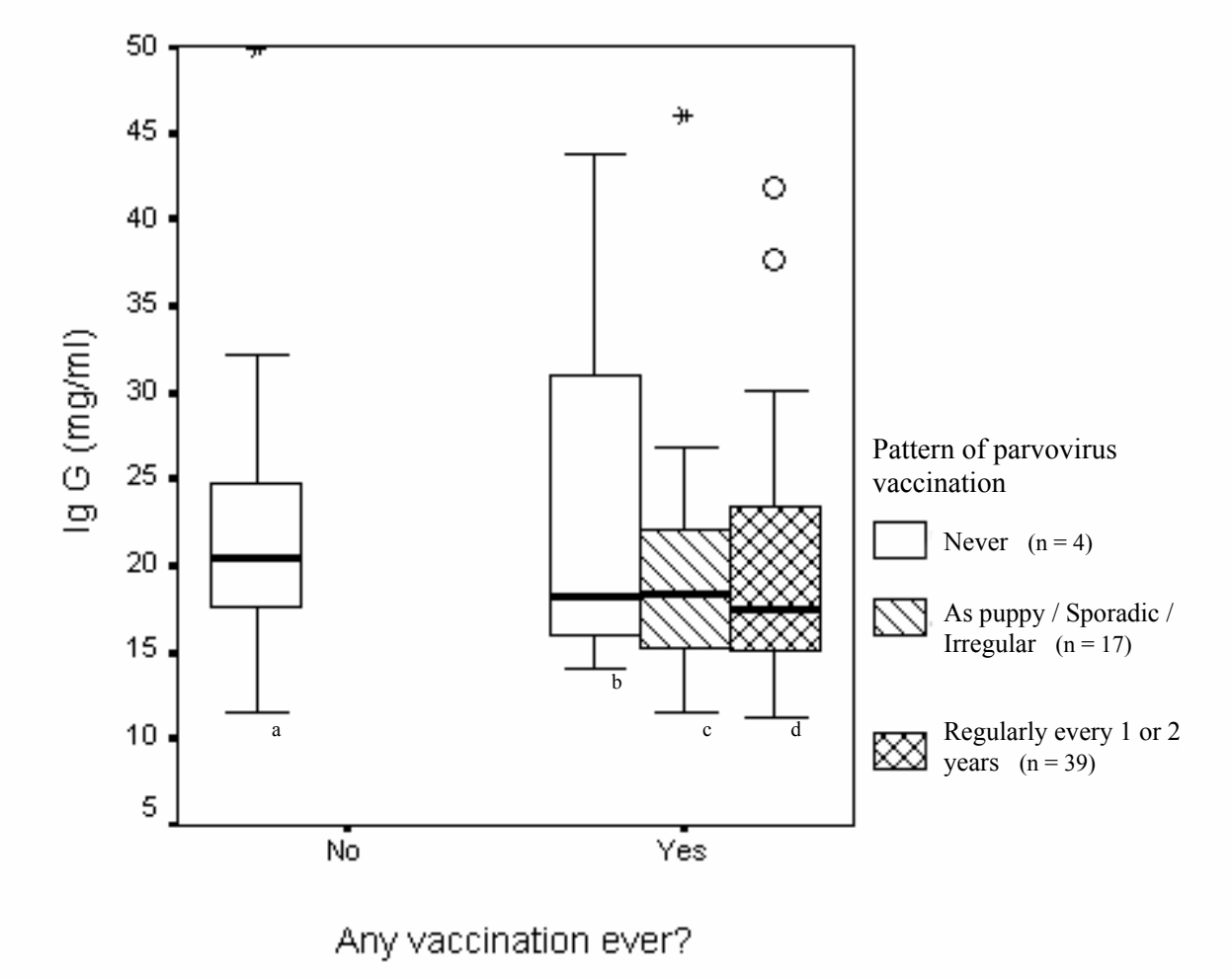

Figure 12B—Boxplots of serum concentration of IgG (mg/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

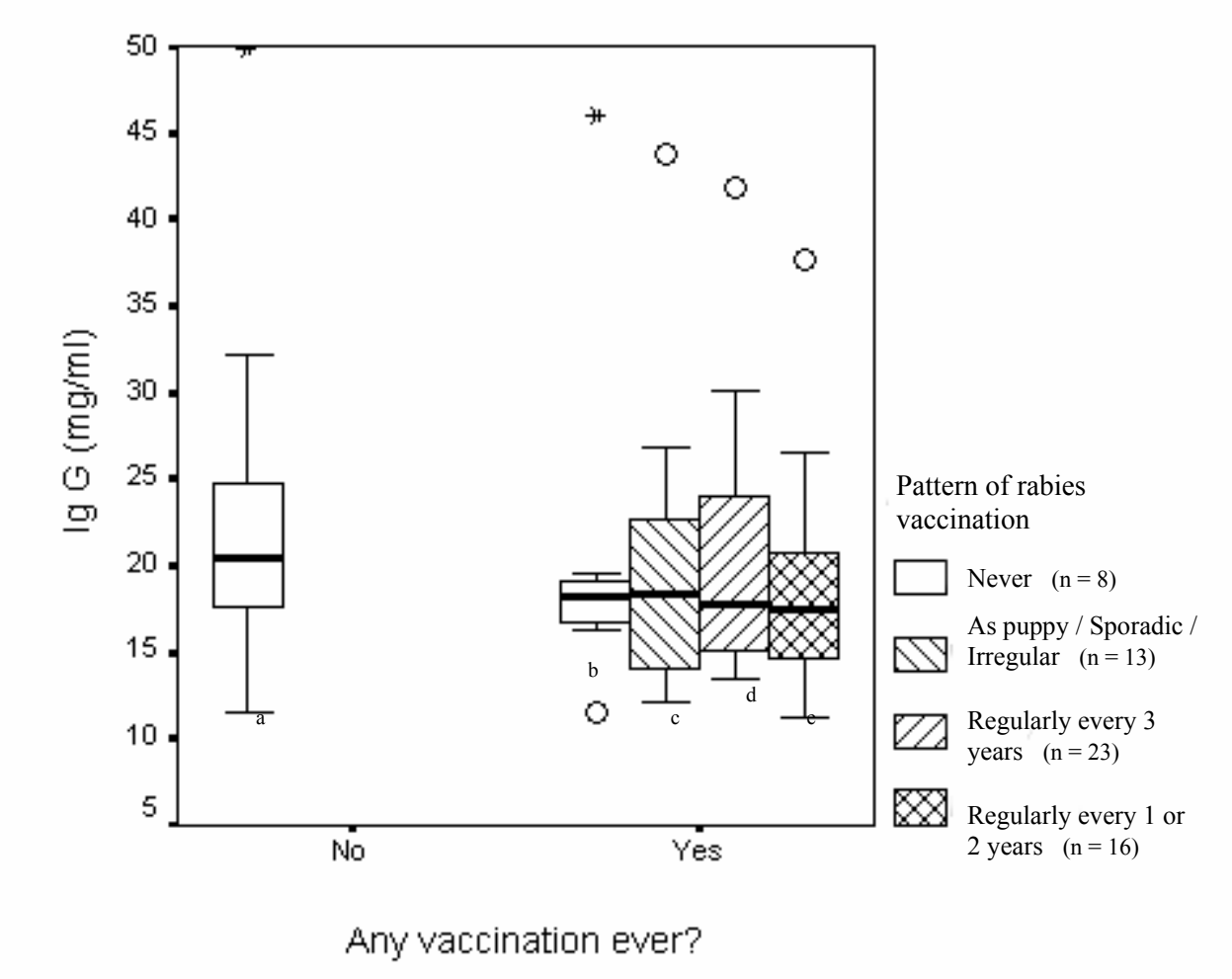

Figure 12C—Boxplots of serum concentration of IgG (mg/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

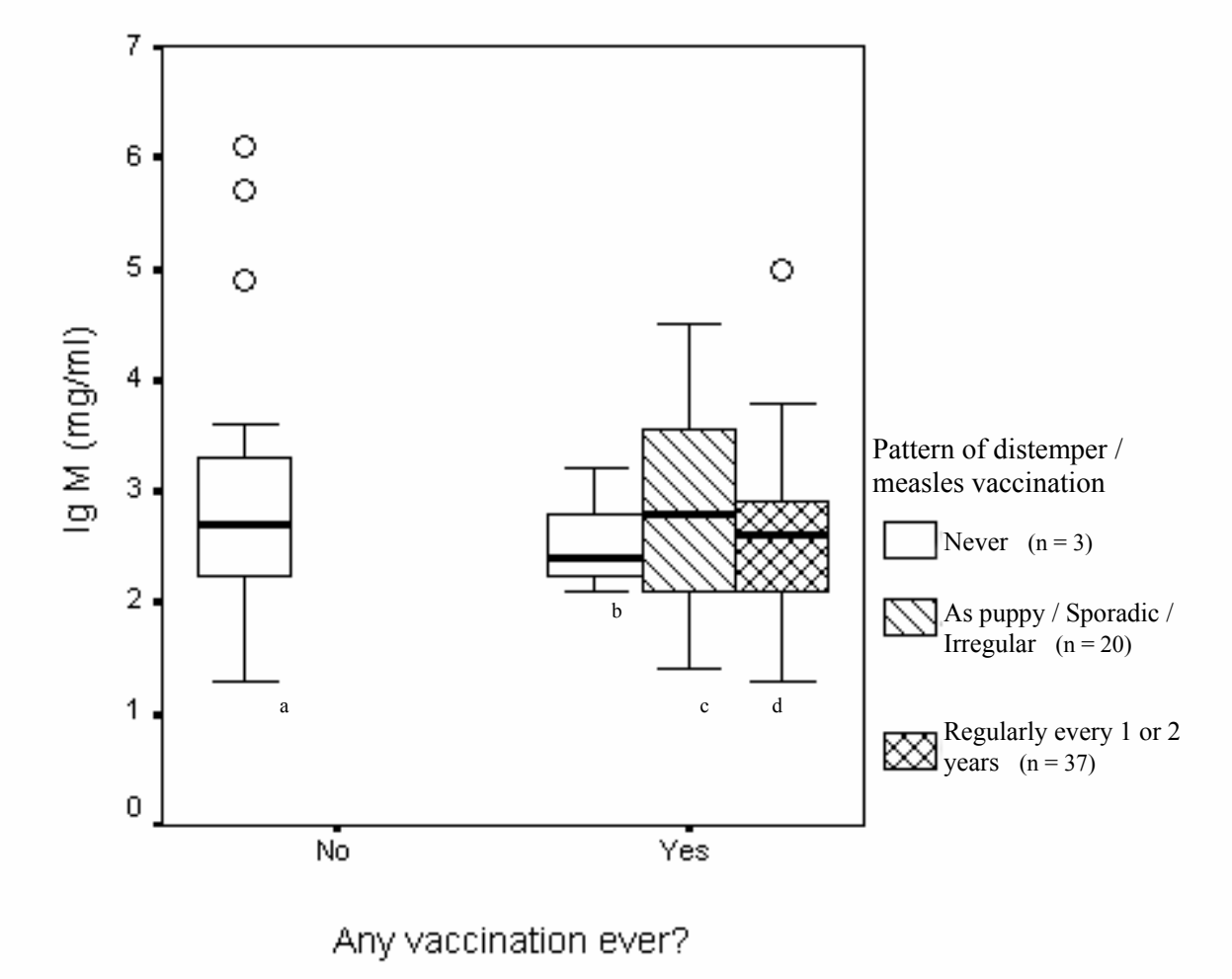

Figure 13A—Boxplots of serum concentration of IgM (mg/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

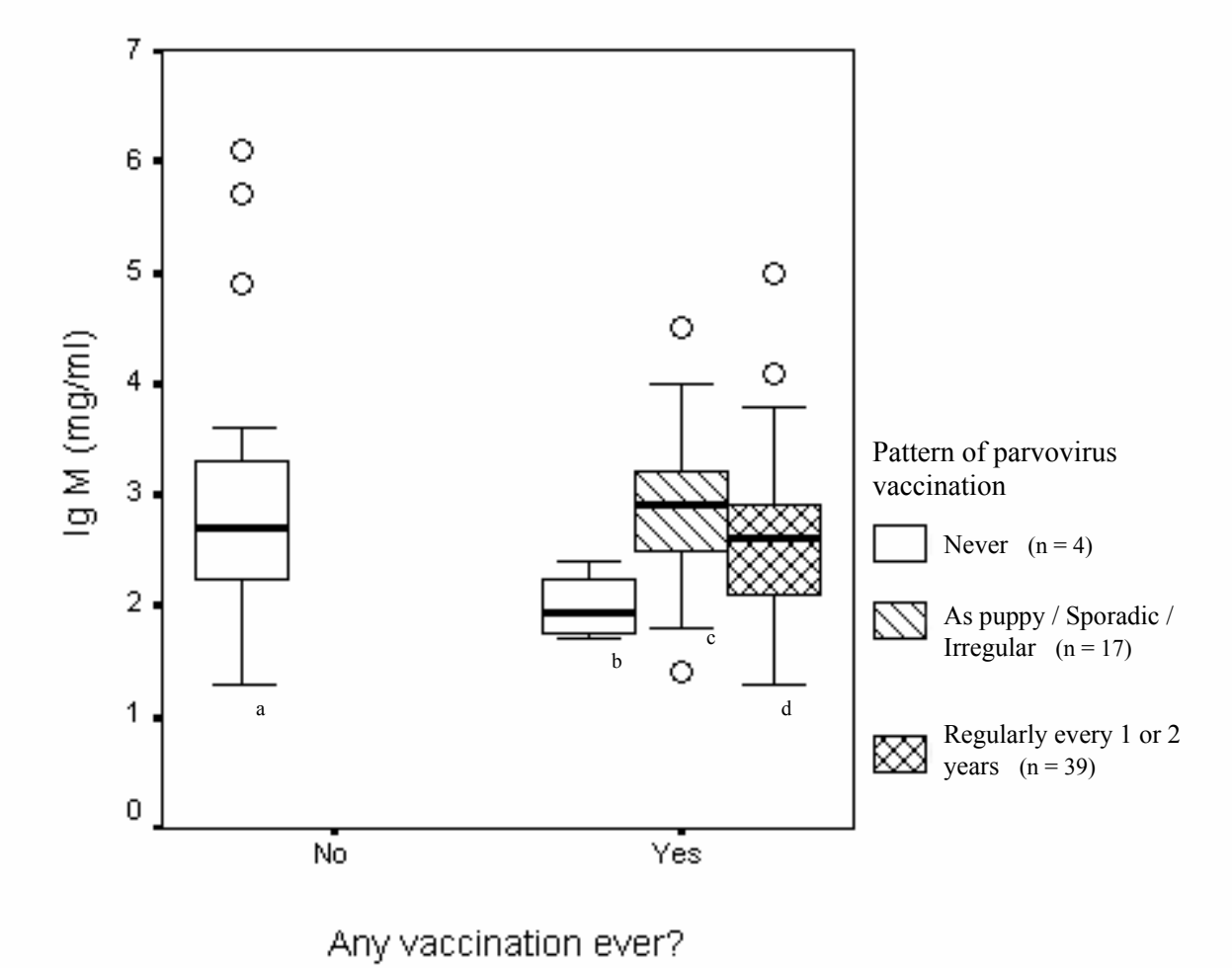

Figure 13B—Boxplots of serum concentration of IgM (mg/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

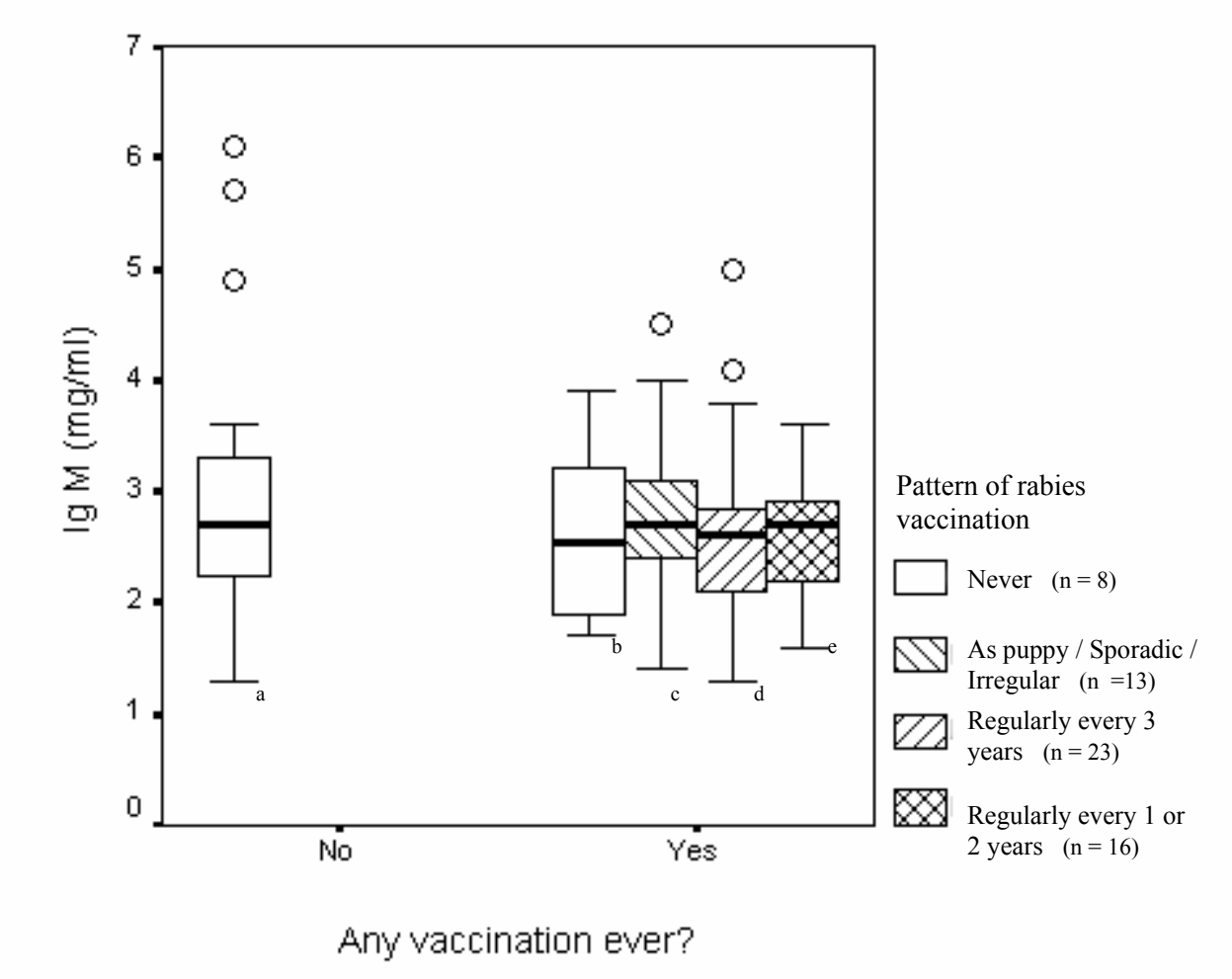

Figure 13C—Boxplots of serum concentration of IgM (mg/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

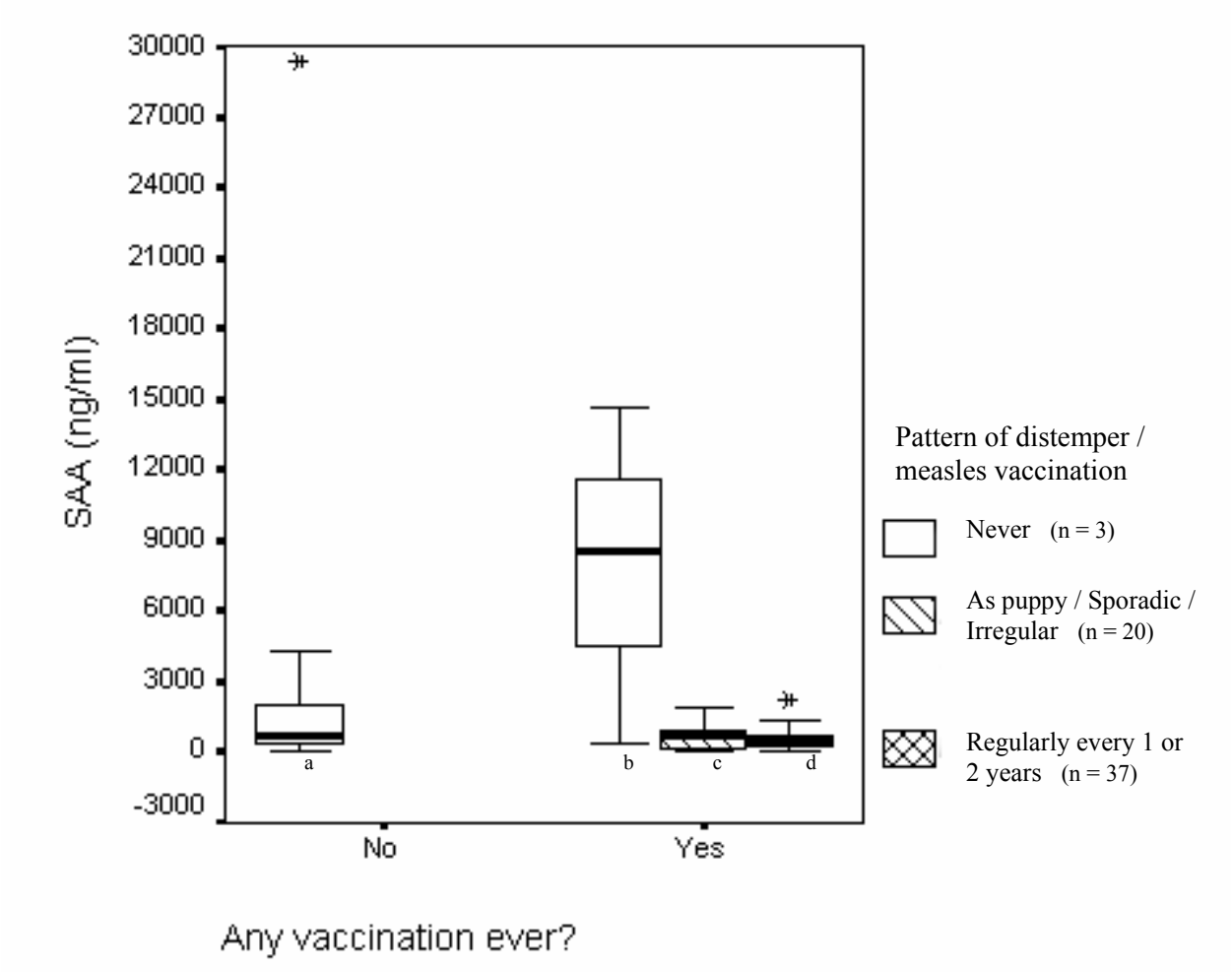

Figure 14A—Boxplots of serum concentration of SAA (ng/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

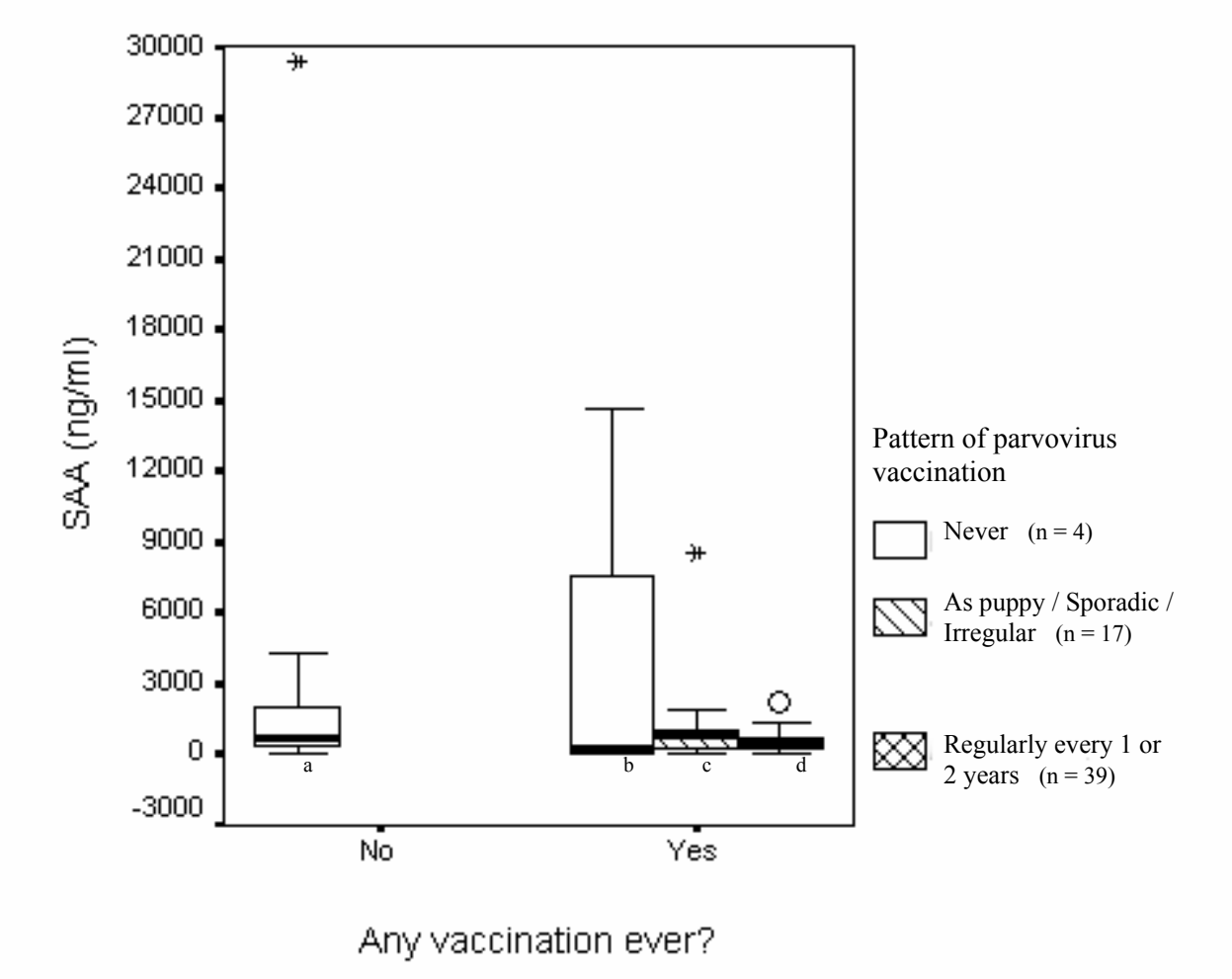

Figure 14B—Boxplots of serum concentration of SAA (ng/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

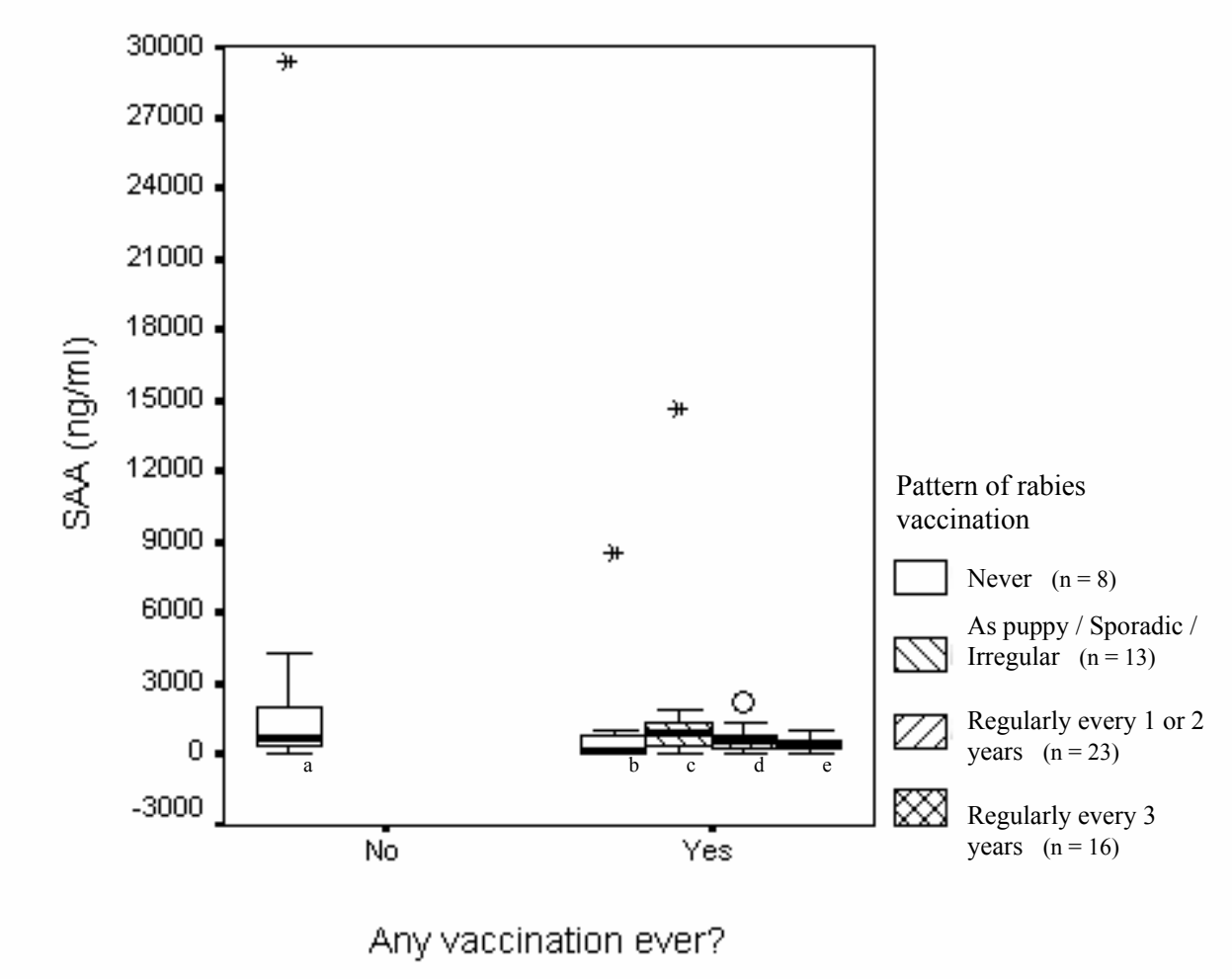

Figure 14C—Boxplots of serum concentration of SAA (ng/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means of log transformed values were compared using one-way ANOVA. None of the pairs of means were significantly ($p<0.05$) different from each other.

Table 13—Distribution of 3,5,3' Triiodothyronine (T3) in vaccinated and unvaccinated Great Danes

	N	3,5,3' Triiodothyronine (T3) µg/dl					
		Mean	± (SD)	Median	Min, Max	T-test	P-value
Any vaccination ever?							
No	15	0.91	0.32	0.85	0.50, 1.58	1.57	0.12
Yes	60	0.77	0.30	0.72	0.27, 1.96		
Any distemper / measles vaccination ever?							
No	18	0.86	0.32	0.83	0.46, 1.58	0.99	0.33
Yes	57	0.78	0.30	0.73	0.27, 1.96		
Any parvovirus vaccination ever?							
No	19	0.88	0.31	0.85	0.46, 1.58	1.31	0.19
Yes	56	0.77	0.31	0.70	0.27, 1.96		
Any rabies vaccination ever?							
No	23	0.84	0.29	0.84	0.47, 1.58	0.90	0.37
Yes	52	0.78	0.31	0.72	0.27, 1.96		

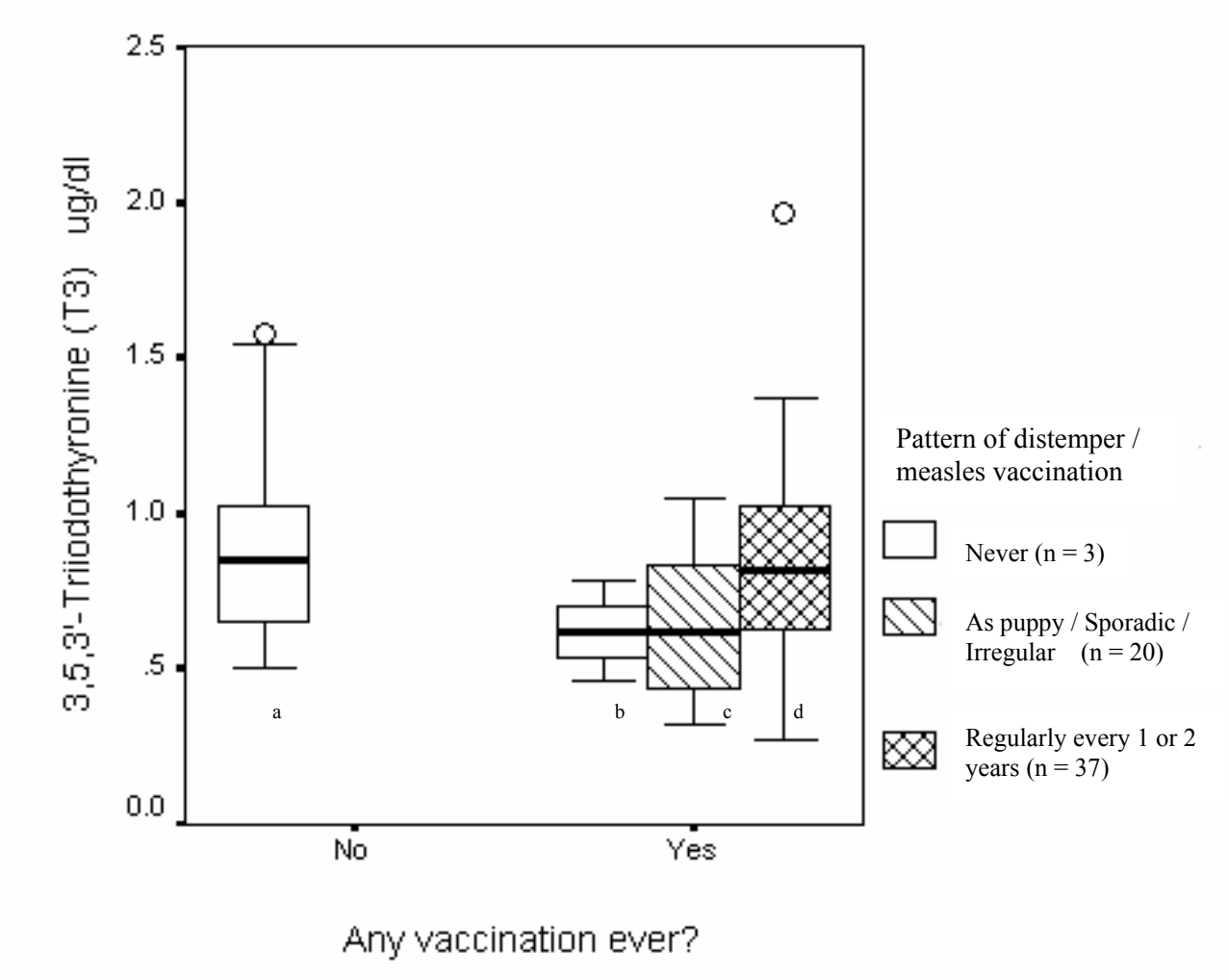

Figure 15A—Boxplots of 3,5,3'-Triiodothyronine (T3) (µg/dl) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers are represented as open circles. Means were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *c*, *c* and *d*.

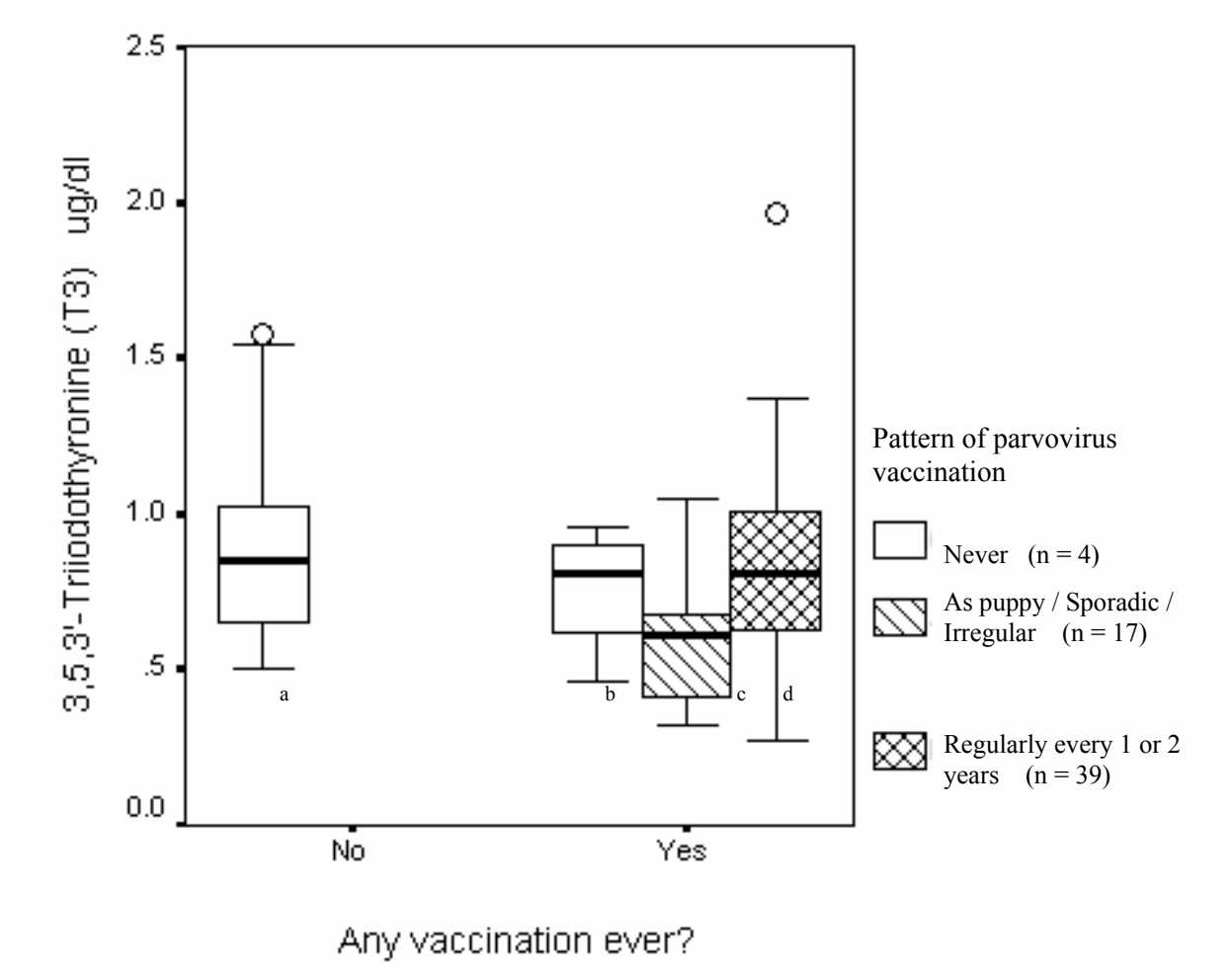

Figure 15B—Boxplots of 3,5,3'-Triiodothyronine (T3) (µg/dl) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers are represented as open circles. Means were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *c*, *c* and *d*.

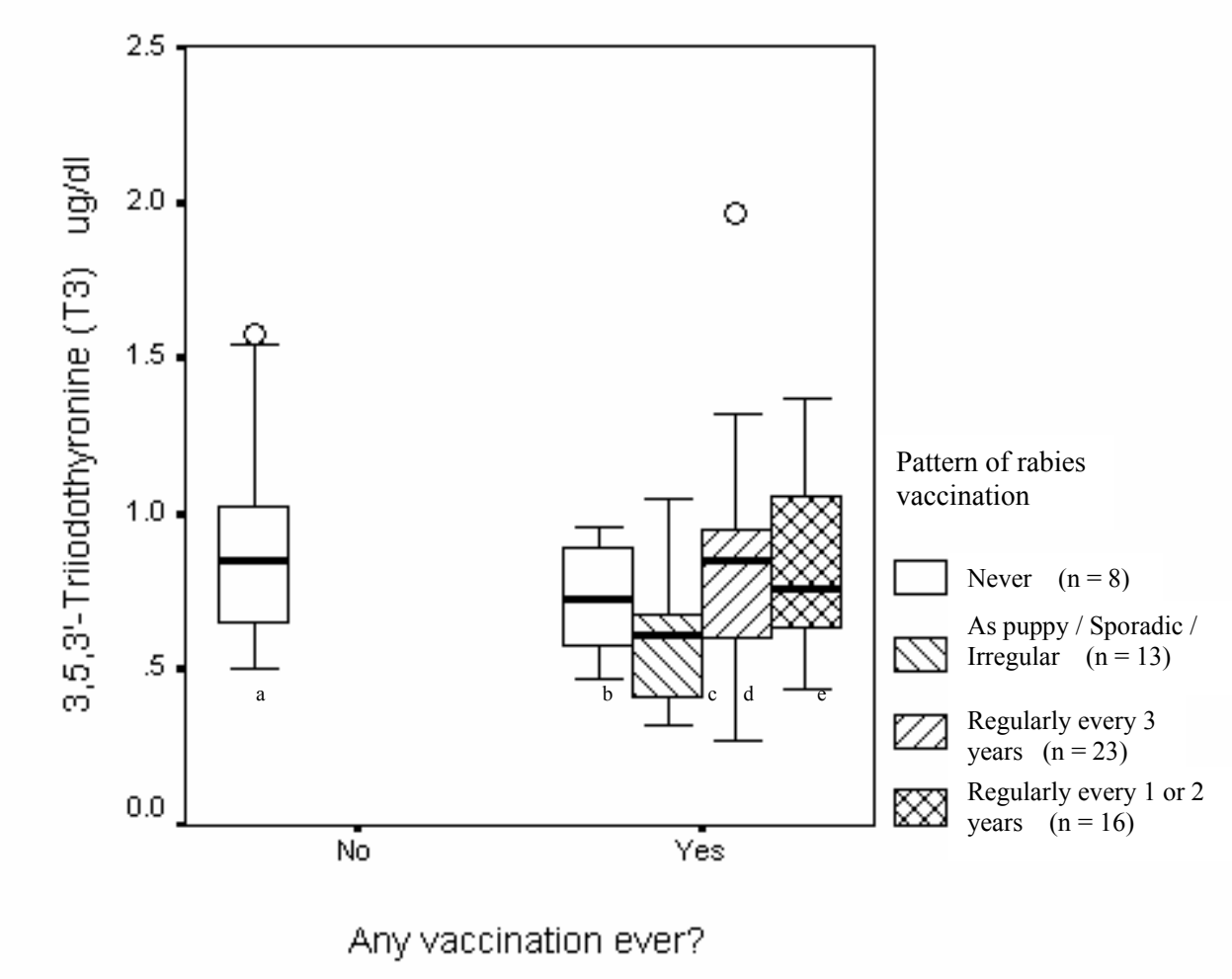

Figure 15C—Boxplots of 3,5,3'-Triiodothyronine (T3) (µg/dl) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers are represented as open circles. Means were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. The following pairs of means were found to be significantly ($p<0.05$) different from each other: *a* and *c*.

Table 14—Distribution of Thyroxine (T4) in vaccinated and unvaccinated Great Danes

	N	Thyroxine (T4) µg/dl					
		Mean	± (SD)	Median	Min, Max	Mann-Whitney U	P-value
Any vaccination ever?							
No	15	2.00	0.86	1.80	0.82, 3.70	376.5	0.33
Yes	60	1.70	0.61	1.70	0.15, 2.90		
Any distemper / measles vaccination ever?							
No	18	1.90	0.92	1.65	0.26, 3.70	469.0	0.58
Yes	57	1.70	0.58	1.70	0.15, 2.90		
Any parvovirus vaccination ever?							
No	19	2.00	0.81	1.80	0.82, 3.70	432.0	0.22
Yes	56	1.66	0.61	1.70	0.15, 2.90		
Any rabies vaccination ever?							
No	23	1.83	0.84	1.80	0.26, 3.70	559.0	0.65
Yes	52	1.70	0.60	1.70	0.15, 2.90		

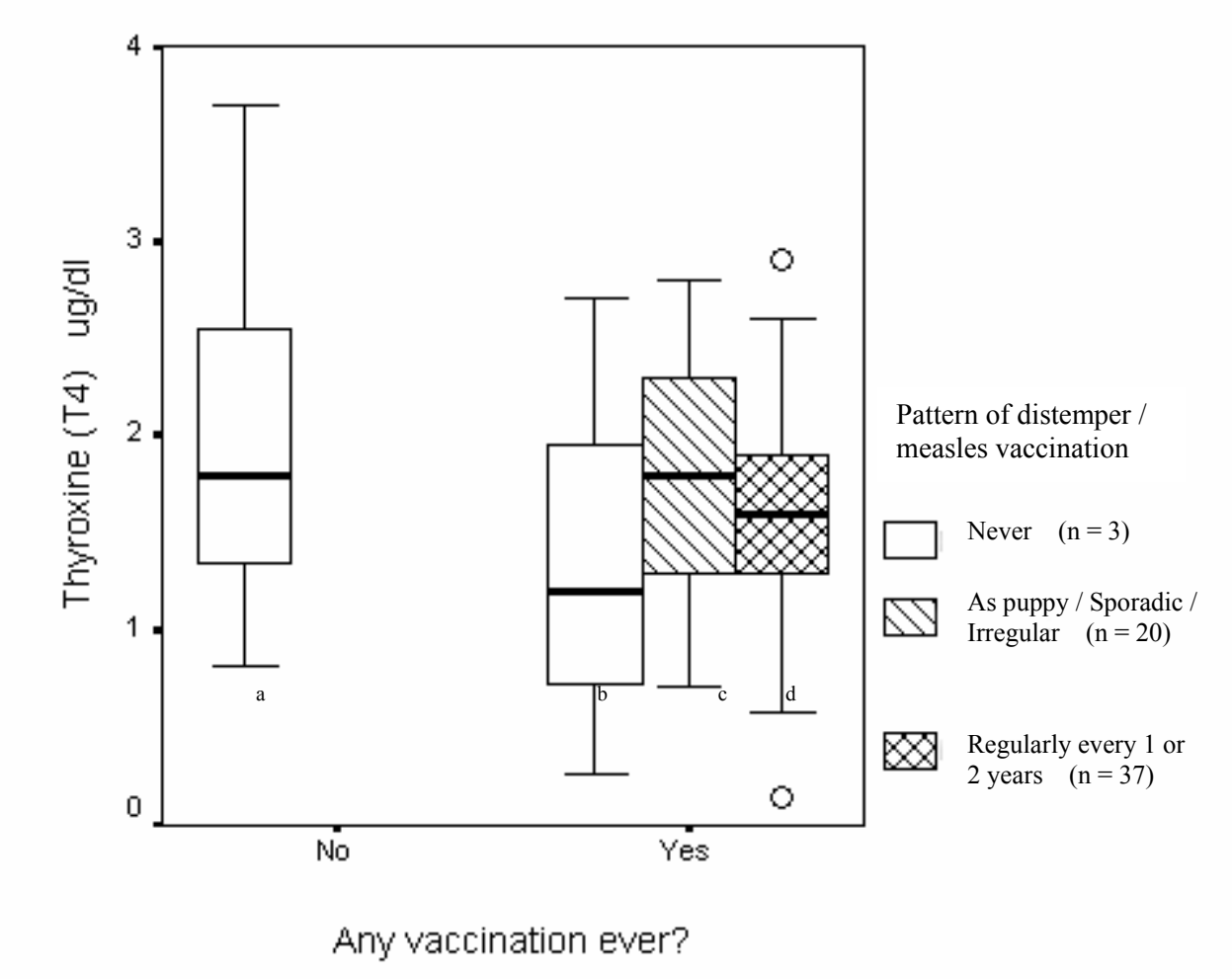

Figure 16A—Boxplots of Thyroxine (T4) (µg/dl) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers are represented as open circles. Means were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. None of the pairs of means were found to be significantly ($p<0.05$) different from each other.

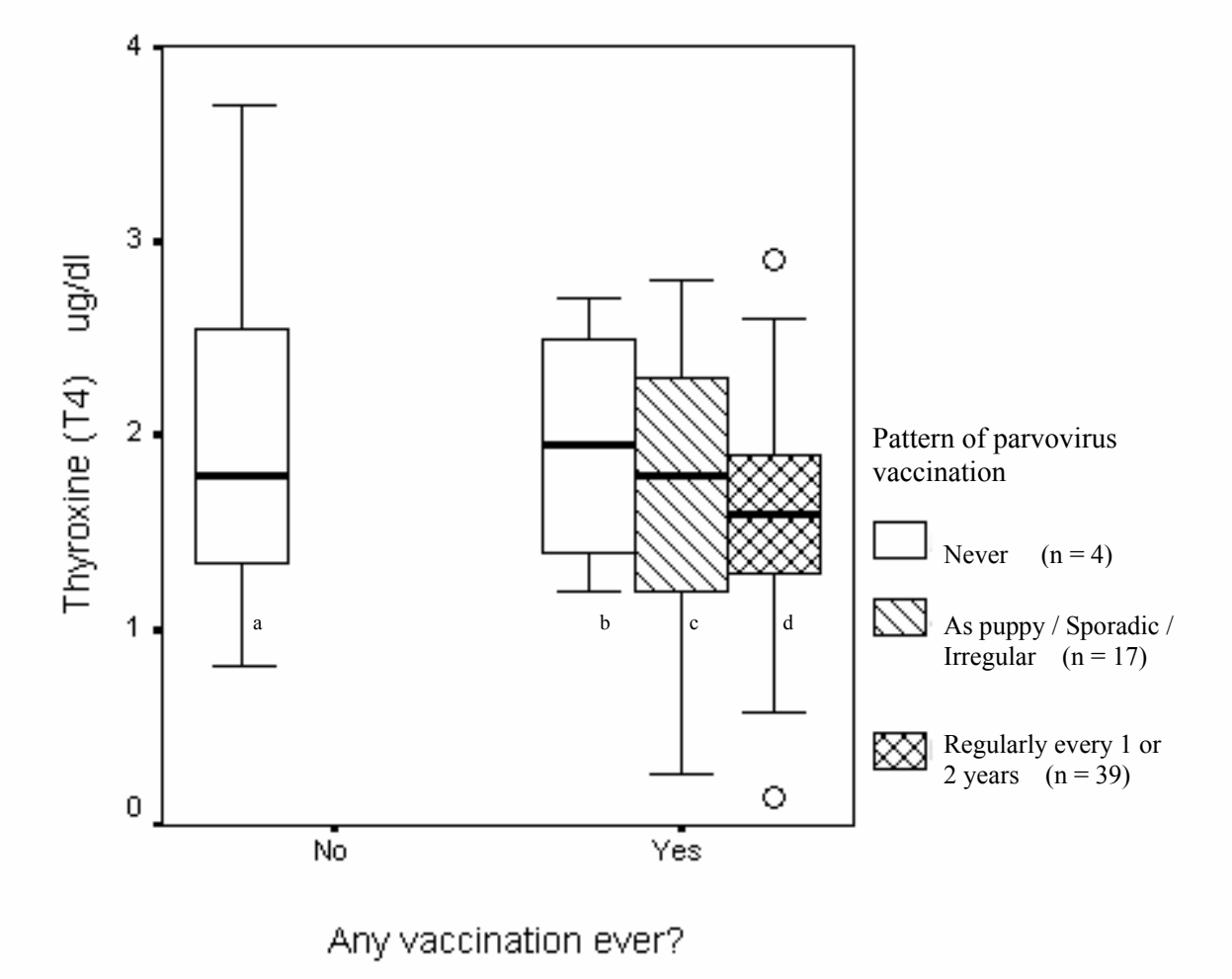

Figure 16B—Boxplots of Thyroxine (T4) (µg/dl) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers are represented as open circles. Means were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. None of the pairs of means were found to be significantly ($p<0.05$) different from each other.

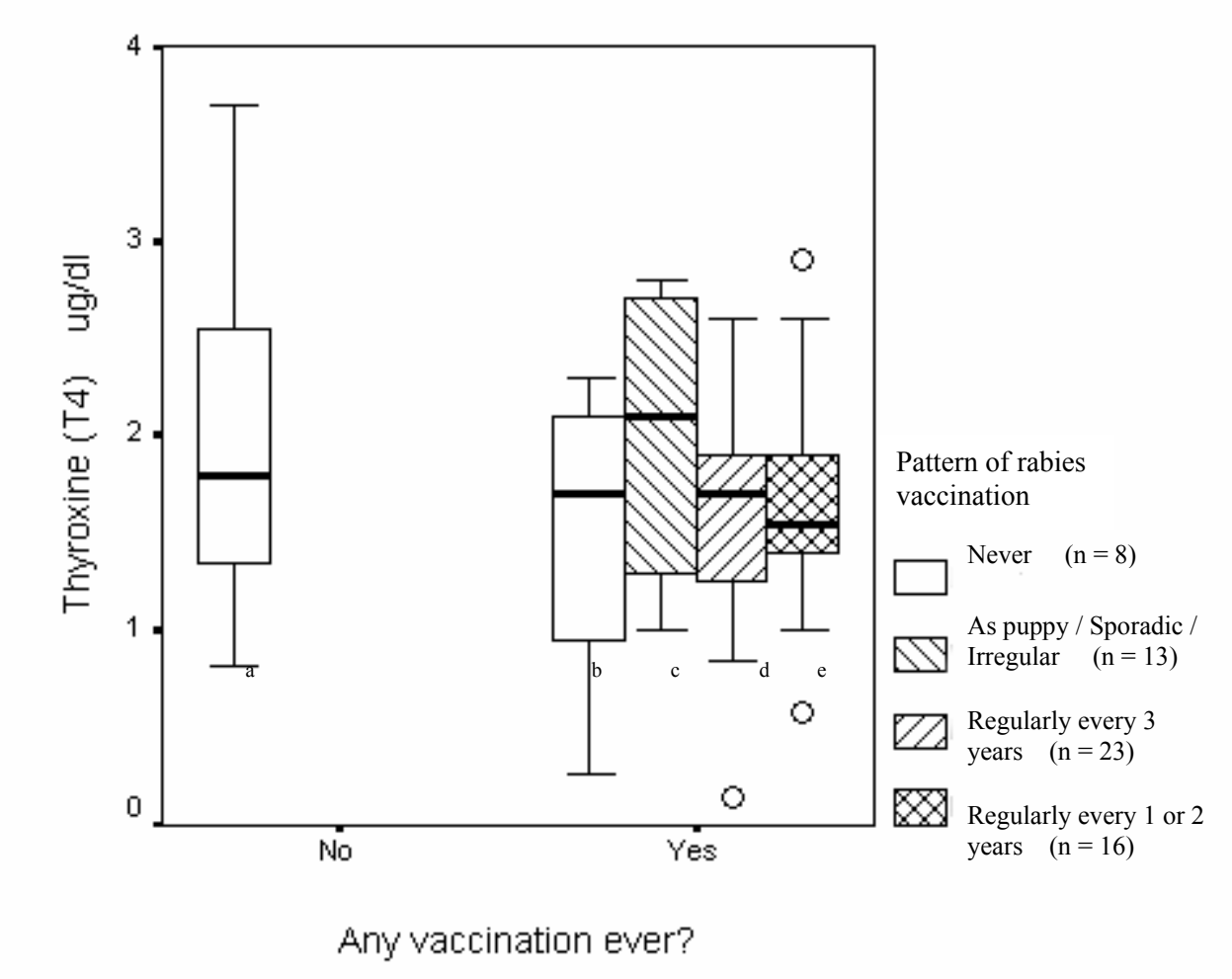

Figure 16C—Boxplots of Thyroxine (T4) (µg/dl) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers are represented as open circles. Means were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. None of the pairs of means were found to be significantly ($p<0.05$) different from each other.

Table 15—Distribution of Thyroid Stimulating Hormone (TSH) in vaccinated and unvaccinated Great Danes

	N	Thyroid Stimulating Hormone (TSH) ng/ml					
		Mean	± (SD)	Median	Min, Max	T-test	P-value
Any vaccination ever?							
No	15	0.44	0.30	0.41	0.05, 1.10	0.80	0.42
Yes	60	0.37	0.34	0.26	0.02, 2.00		
Any distemper / measles vaccination ever?							
No	18	0.45	0.28	0.45	0.05, 1.10	1.02	0.31
Yes	57	0.36	0.35	0.25	0.02, 2.00		
Any parvovirus vaccination ever?							
No	19	0.44	0.27	0.41	0.05, 1.10	0.86	0.40
Yes	56	0.36	0.35	0.25	0.02, 2.00		
Any rabies vaccination ever?							
No	23	0.43	0.27	0.41	0.04, 1.10	0.81	0.42
Yes	52	0.36	0.36	0.25	0.02, 2.00		

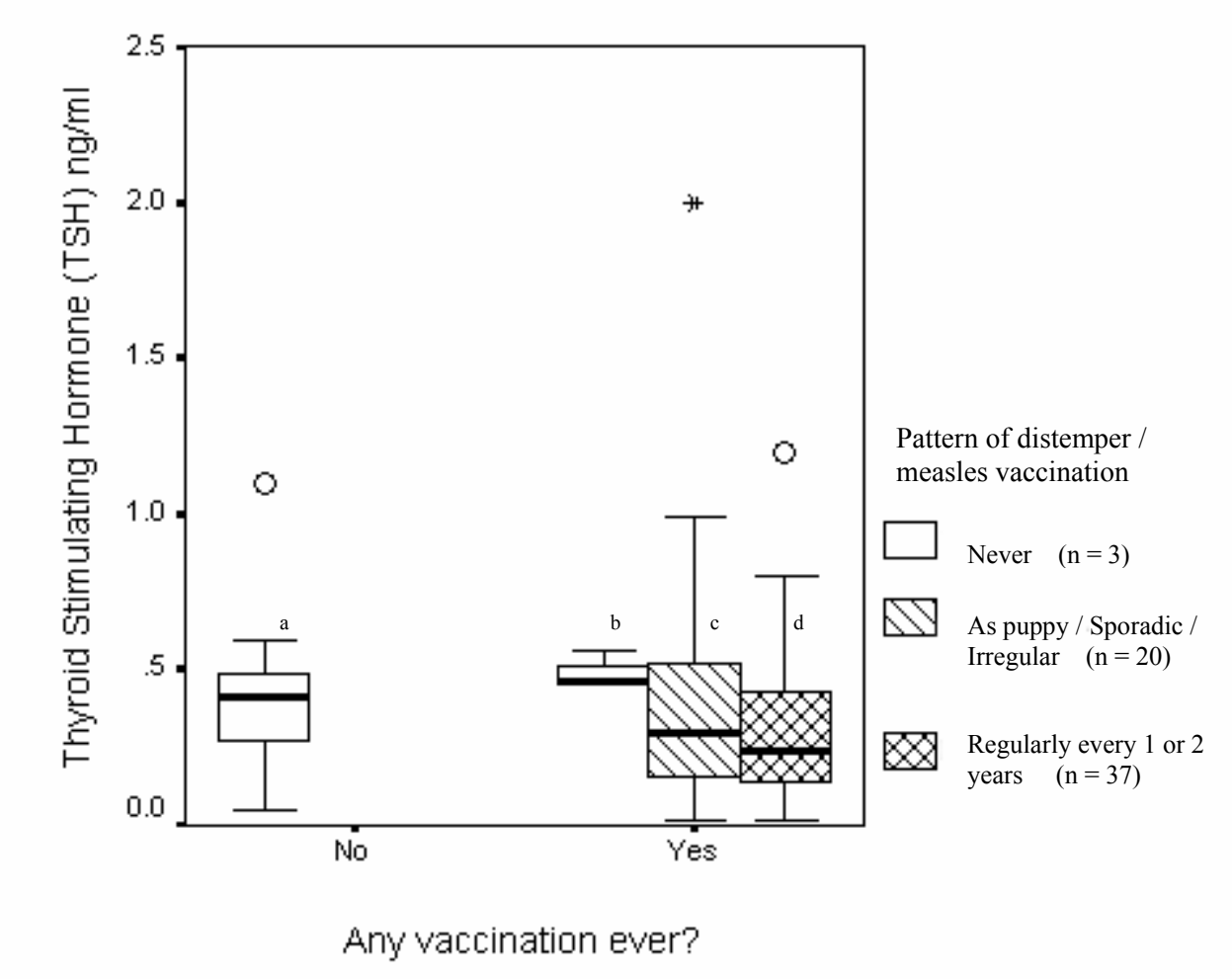

Figure 17A—Boxplots of Thyroid Stimulating Hormone (TSH) (ng/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. None of the pairs of means were found to be significantly ($p<0.05$) different from each other.

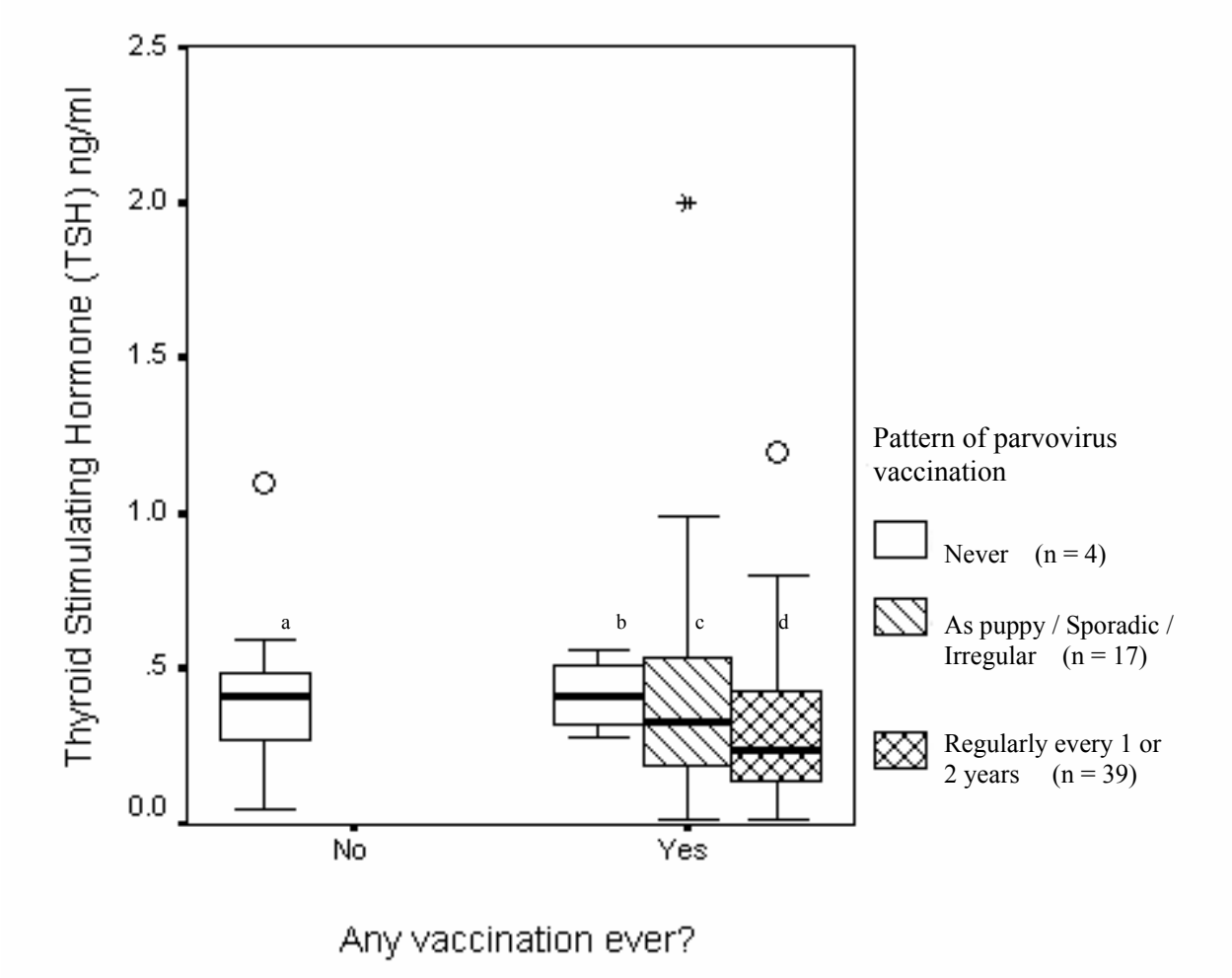

Figure 17B—Boxplots of Thyroid Stimulating Hormone (TSH) (ng/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. None of the pairs of means were found to be significantly ($p<0.05$) different from each other.

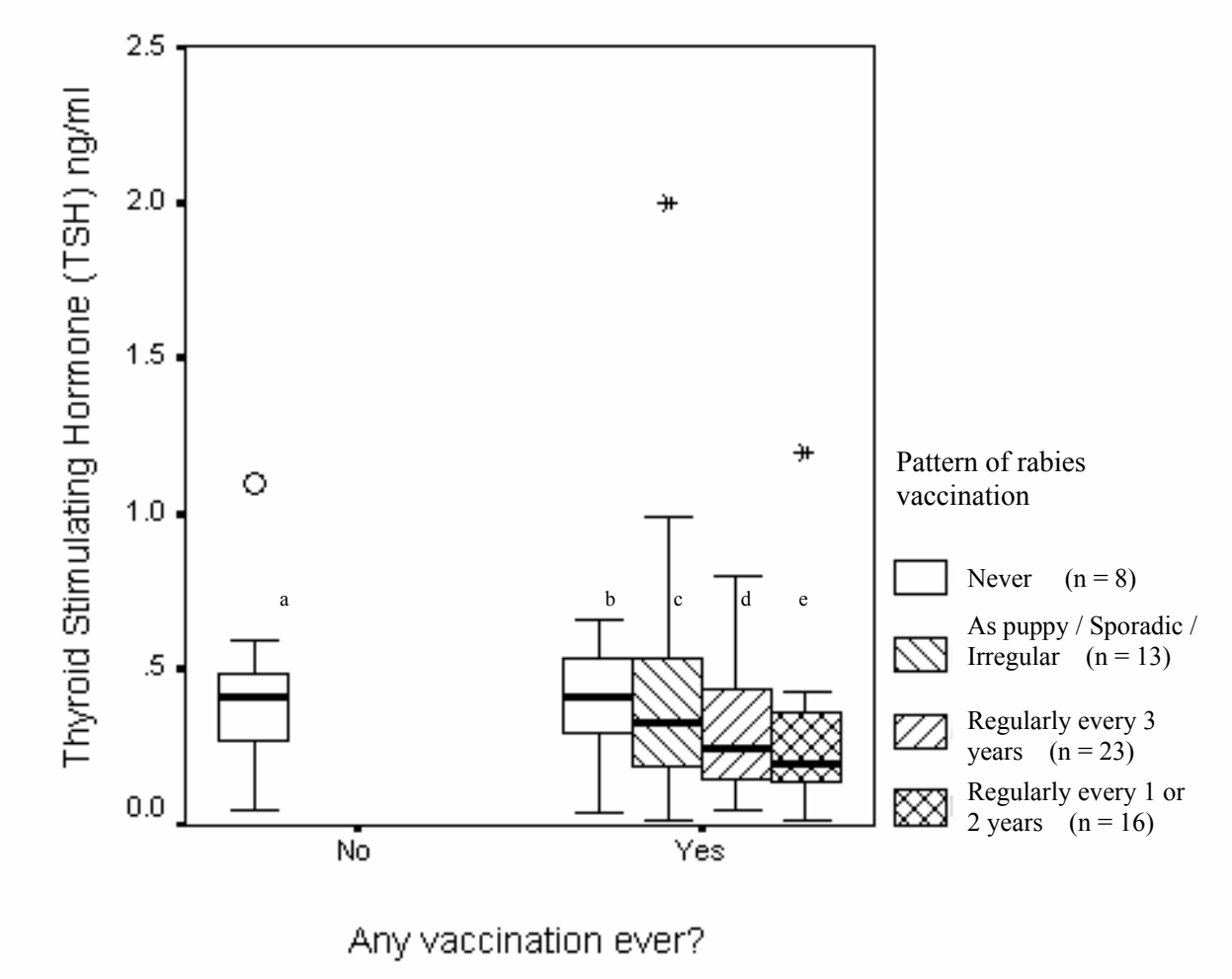

Figure 17C—Boxplots of Thyroid Stimulating Hormone (TSH) (ng/ml) in 15 Great Danes that were never vaccinated and 60 Great Danes that were vaccinated at least once in their lifetime. Outliers (open circles) and extreme values (asterisks) are also represented. Means were compared using one-way ANOVA and Tamhane (or Dunnett's T3, Games-Howell) test for multiple comparisons. None of the pairs of means were found to be significantly ($p<0.05$) different from each other.

Appendix 1

OWNER QUESTIONNAIRE

Please provide the following information that will remain **confidential**. This information is for use only by the study staff at Purdue University.

Name:_____

Street Address:
_____

City/Town, State, Zip Code, Country:
_____

Telephone Number with Area Code:
_____

email Address:
_____

SELECTING DOGS FOR ENTRY INTO THE 2002 GREAT DANE HEALTH AND VACCINE STUDY

Each participant may enter **as many dogs as desired** in this study. Eligible dogs must meet all of the following criteria:

1. The dog is currently alive and at least 2 years old
2. You have a complete lifetime history for the dog
3. You have a complete medical history for the dog
4. You are willing to have a blood sample taken by you or your veterinarian
5. The dog has not been vaccinated in the last 30 days (if it has, you can still participate by waiting 30 days following vaccination)

Please feel free to share this questionnaire with owners of other eligible Great Danes. Remember that a blood sample must be submitted from each participating Great Dane. Please complete a separate questionnaire for each dog entered. Additional questionnaires can be obtained either by contacting Dr. Malathi Raghavan at 765-496-2634 or via email at raghavam@purdue.edu or from the Internet at www.gdhfa.org.

Please return completed questionnaires to: Dr. Malathi Raghavan
Great Dane Health and Vaccine Study
School of Veterinary Medicine
Purdue University
1243 Veterinary Pathology
West Lafayette, IN 47907-9977

I. General Information for your dog:_____

Fill in your dog's name

1. Date of birth: ____ month ____ day ____ year
2. Sex: ___ male ____female
   Neutered: ____ yes ____ no
3. If neutered, date of surgery: ____ month ____ year
4. What is your dog's current weight and height?
   _____ weight (pounds) _____ shoulder height (inches)
5. What is your dog's current body condition (see definitions)?

____ obese ____ overweight ____ average/optimum ____ underweight

Definitions: *Obese: unable to feel ribs, large amounts of subcutaneous fat*
Overweight: rib cage barely palpable, little or no waist
Average: slight abdominal fat, rib cage palpable but not too obvious
Underweight: little body fat, rib cage easily palpable, obvious waist

6. Record the percentage of each food type fed daily, and indicate the brand and variety. For example, if Eukanuba Adult Maintenance dry food is fed daily, the brand should be listed as 'Eukanuba' and the variety as 'Adult Maintenance.' Please make sure that these items total to 100%.

Type of Food	**%**	**Brand**	**Variety**
Dry			
Canned			
Home-prepared or table scraps			
Other (specify): _____			

100%

7. Please **check /fill in** the boxes below to indicate the supplements that you currently give to your dog on a regular basis:

Type of Supplement	**Supplements Given**		
	Brand / Size	Quantity / Total dose	How often?
Vitamins			□ Daily □ Weekly □ Monthly
Minerals			□ Daily □ Weekly □ Monthly
Cartilage supplement (e.g., glucosamine)			□ Daily □ Weekly □ Monthly
Food supplement (e.g., vinegar, garlic)			□ Daily □ Weekly □ Monthly
Other (specify): _____			□ Daily □ Weekly □ Monthly

8. a. Please enter the total number of times your dog has attended the following events as a competitor / participant:

____ conformation shows ____ obedience trials ____ coursing trials

____ other (specify) _____

b. Please enter total number of times your dog has attended the following events as a non-competitor / observer:

____ conformation shows ____ obedience trials ____ coursing trials

____ other (specify) _____

9. Indicate how your dog is primarily housed and the percentage of the time in each environment *(total must equal 100%)*:

Type of Housing	**%**
Crate	
Kennel (indoors)	
Free in the house	
Kennel (indoor/outdoor)	
Fenced yard	
Garage	
Other (specify): _____	

100%

II. Health Related Conditions

Use these codes for the **Malignant Neoplasms** (Cancer) question on the next page. First select a code for the ***Tumor Type*** and then select a code for its ***Location***. **Write these two codes in the chart on next page.**

Codes for <u>Tumor Type</u>	**Codes for <u>Location</u>**
1. Adenocarcinoma	A. Bladder
2. Chondrosarcoma (cartilage)	B. Bone
3. Fibrosarcoma	C. Brain
4. Hemangiosarcoma	D. Digits
5. Interstitial cell tumor	E. Eye
6. Liposarcoma	F. Heart
7. Lymphoma (Lymphosarcoma)	G. Intestine
8. Malignant giant cell tumor	H. Kidney
9. Mast cell tumor	I. Liver
10. Melanoma	J. Lung
11. Mesothelioma	K. Lymph nodes
12. Myeloma	L. Mouth
13. Neuroblastoma	M. Muscle
14. Neurofibrosarcoma	N. Nasal cavity
15. Osteosarcoma	O. Nerve
16. Seminoma	P. Ovary
17. Sertoli cell tumor	Q. Pancreas
18. Squamous cell carcinoma	R. Prostate
19. Transitional cell carcinoma	S. Skin
20. Transmissible venereal tumor	T. Spleen
21. Carcinoma, unspecified	U. Testes
22. Sarcoma, unspecified	V. Uterus
23. Other (specify): _____	W. Abdomen
24. Unknown type	X. Legs
	Y. Mammary / breast
	Z. Other (specify) _____

1. For each of the conditions listed below, please indicate those that affected your dog, the **age** at **first diagnosis and** whether a veterinarian confirmed that diagnosis. Room is provided for you to list additional conditions. If you are not sure about these items, please check with your veterinarian.

For **malignant neoplasms**, please use both the **tumor type codes** and **location codes** from the table on the preceding page. For **non-malignant neoplasms**, only use the **location codes** from the table on the preceding page.

Condition	**Age at Onset**	**Diagnosed by Veterinarian**	
	Years	**Yes**	**No**
MALIGNANT NEOPLASMS (Cancer) ***Use codes from page 4***			
Tumor Type Code ____ Location Code ____			
Tumor Type Code ____ Location Code ____			
Tumor Type Code ____ Location Code ____			
Tumor Type Code ____ Location Code ____			
Tumor Type Code ____ Location Code ____			
Tumor Type Code ____ Location Code ____			
NON-MALIGNANT NEOPLASMS			
Lipoma Location Code ____			
Papilloma (wart) Location Code ____			
Histiocytoma Location Code ____			
Adenoma Location Code ____			
Polyps Location Code ____			

Condition	Age at Onset	Diagnosed by Veterinarian	
	Years	Yes	No
NON-MALIGNANT NEOPLASMS (continued)			
Cysts Location Code ____			
Hemangioma Location Code ____			
Melanocytoma Location Code ____			
Other Non-malignant: _____ Location Code ____			
CARDIOVASCULAR			
Heart failure-unknown cause			
Cardiomyopathy			
Heartworm infection			
Heart arrhythmia			
Heart murmur			
Pulmonic stenosis			
Subaortic stenosis			
Valve dysfunction			
Ventricular septal defect			
Other:_____			
ALLERGIES			
Allergic dermatitis due to:			
Fleas			
Food			
Inhaled allergens			
Flea dip/insecticide			
Atopic rhinitis			
Insect bites			
Anesthesia			

Condition	Age at Onset	Diagnosed by Veterinarian	
	Years	Yes	No
Other allergy:_____			
ENDOCRINE			
Hypothyroid			
Hyperthyroid			
Cushing's (hyperadrenal)			
Addison's (hypoadrenal)			
Diabetes mellitus			
Other: _____			
AUTOIMMUNE			
Autoimmune hemolytic anemia (AIHA)			
Thrombocytopenia (ITP) (or platelet dysfunction)			
Systemic lupus erythematosis (SLE)			
Arthritis (immune-mediated)			
Thyroiditis (autoimmune)			
Other:_____			
URINARY TRACT/RENAL			
Kidney infection			
Kidney failure			
Kidney stones			
Bladder infection			
Bladder stones			
Cystinuria			
Urinary incontinence			
Glomerulonephritis			
Other: _____			

Condition	Age at Onset	Diagnosed by Veterinarian	
	Years	Yes	No
NEUROLOGICAL			
Seizures of unknown origin (epilepsy)			
Seizures of known origin			
Wobbler syndrome			
Dementia (senility)			
Nerve degeneration			
Tremors - generalized			
Cerebellar abiotrophy			
Progressive ataxia			
Necrotizing myelopathy			
Cerebellar ataxia			
Other:_____			
MUSCULOSKELETAL			
Eosinophilic panosteitis (PANO)			
Osteochondritis dissecans (OCD)			
Hip dysplasia (CHD)			
Elbow dysplasia			
Spondylosis			
Degenerative disc disease			
Anterior cruciate ligament (ACL) tear			
Arthritis			
Patellar luxation			
Hypertrophic osteodystrophy (HOD)			
Other: _____			

Condition	Age at Onset Years	Diagnosed by Veterinarian Yes	No
GENETIC DISEASES			
Microphthalmia			
Eversion of third eyelid			
Entropion			
Ectropion			
Cataract			
Glaucoma			
Progressive retinal atrophy			
Heterochromia iridis ("Walleye")			
Deafness			
Dermoid cyst			
Calcinosis circumscripta			
Color dilution alopecia			
Demodicosis			
Persistent right aortic arch			
Patent ductus arteriosus			
Bloat / gastric torsion			
von Willebrand's disease			
Pancreatic insufficiency			
Other: _____			
Other: _____			

2. If your dog has ever been vaccinated, please check the appropriate boxes below to describe the lifetime vaccination history. *If your dog has never been vaccinated, please check here □ and skip to question 6.*

Type of Vaccination	**Frequency of Vaccination**					
	Yearly	**Every 2 years**	**Every 3 years**	**Sporadically** (based on titers)	**Only as a Puppy**	**Never**
Rabies						
Distemper						
Parvovirus						
Adenovirus						
Leptospirosis						
Parainfluenza						
Hepatitis						
Corona virus						
Lyme disease						
Kennel cough						
Giardia						
List of Initials						
Other:_____						

3. Please indicate below the vaccination types and brands your dog received in the last 30 days and in the past year (not including those in the last 30 days) as well as the dates they were given. If more than one vaccine was given on a particular date, please list the details (type, manufacturer, name) for each vaccine separated by commas. Please send a copy of the most recent vaccination certificate(s) for your dog. If you have this information for earlier vaccinations, please send copies of these as well. Please check with your veterinarian if you do not have this information.

Vaccines in the last 30 days:

a. Type (for example, rabies):_____

b. Manufacturer (for example, Ft. Dodge):

_____

c. Name of Vaccine (for example, Duramune®):_____

d. Date:_____

Vaccines in the past year (exclude those in the last 30 days):

e. Type (for example, rabies):_____

f. Manufacturer (for example, Ft. Dodge):

_____

g. Name of Vaccine (for example, Duramune®):_____

h. Date:_____

4. Who routinely vaccinates your dog against diseases other than rabies?

___ I do it ___ breeder ___ veterinarian

___ other:_____

5. Has your dog ever had an adverse reaction to a vaccination?
_____ yes _____no (*If no, skip to question 6*)

If yes, describe last 3 reactions:

Most recent vaccine reaction:

a. If yes, what specific vaccine was involved (type / company):

_____

b. How old was your dog when this adverse reaction occurred? ___ years ___ months

c. Was this vaccine reaction seen by a veterinarian? ____ yes ____ no

d. Was this vaccine reaction treated by a veterinarian? ____ yes ____ no

e. How was this reaction treated?

___ epinephrine ___ Benadryl® ___ transfusion ___ IV fluids ___ other:_____

f. What were your dog's signs? Check all that apply

___ face swelling ___ couldn't breathe ___ turned blue ___ fainting/collapse

___ vomiting/diarrhea ___ hypersalivating ___ weakness ___ turning pale

___ turning yellow ___ bloody stool ___ blood in urine ___ bloody nose

___ other: _____

g. How long after vaccination did these signs appear? Indicate a number (for example, 5 hours)

___ minutes ___ hours ___ days ___ weeks ___ months

h. Since this reaction, has your dog been vaccinated again? ____ yes ____ no

i. If yes, were any precautions taken to prevent another reaction? ____ yes ____ no

j. If so, what precautions were taken?

___ Benadryl® ___ prednisone ___ stay longer at vet hospital for observation
___ vaccinate less often ___ other: _____

k. Were there any long term / permanent effects of the adverse reaction: _____ yes _____ no

If yes, please describe:_____

Second most recent vaccine reaction:

l. If yes, what specific vaccine was involved (type / company):

_____

m. How old was your dog when this adverse reaction occurred? ___ years ___ months

n. Was this vaccine reaction seen by a veterinarian? ____ yes ____ no

o. Was this vaccine reaction treated by a veterinarian? ____ yes ____ no

p. How was this reaction treated?

___ epinephrine ___ Benadryl® ___ transfusion ___ IV fluids ___ other:_____

q. What were your dog's signs? Check all that apply

___ face swelling ___ couldn't breathe ___ turned blue ___ fainting/collapse
___ vomiting/diarrhea ___ hypersalivating ___ weakness ___ turning pale
___ turning yellow ___ bloody stool ___ blood in urine ___ bloody nose
___ other: _____

r. How long after vaccination did these signs appear? Indicate a number (for example, 5 hours)

___ minutes ___ hours ___ days ___ weeks ___ months

s. Since this reaction, were any precautions taken to prevent another reaction?

____ yes ____ no

t. If so, what precautions were taken?

___ Benadryl® ___ prednisone ___ stay longer at vet hospital for observation
___ vaccinate less often ___ other: _____

u. Were there any long term / permanent effects of the adverse reaction: ___ yes ___ no

If yes, please describe:_____

Third most recent vaccine reaction:

v. If yes, what specific vaccine was involved (type / company): _____

w. How old was your dog when this adverse reaction occurred? ___ years ___ months

x. Was this vaccine reaction seen by a veterinarian? ____ yes ____ no

y. Was this vaccine reaction treated by a veterinarian? ____ yes ____ no

z. How was this reaction treated?

___ epinephrine ___ Benadryl® ___ transfusion ___ IV fluids ___ other:_____

aa. What were your dog's signs? Check all that apply

___ face swelling ___ couldn't breathe ___ turned blue ___ fainting/collapse

___ vomiting/diarrhea ___ hypersalivating ___ weakness ___ turning pale

___ turning yellow ___ bloody stool ___ blood in urine ___ bloody nose

___ other: _____

bb. How long after vaccination did these signs appear? Indicate a number (i.e., 5 hours)

___ minutes ___ hours ___ days ___ weeks ___ months

cc. Since this reaction, were any precautions taken to prevent another reaction?

____ yes ____ no

dd. If so, what precautions were taken?

___ Benadryl® ___ prednisone ___ stay longer at vet hospital for observation
___ vaccinate less often ___ other: _____

ee. Were there any long term / permanent effects of the adverse reaction: ___ yes ___ no

If yes, please describe:_____

6. Frequency of routine worming:

___ Yearly ___ Every 2 years ___ Every 3 years

___ Sporadically based on fecal exam ___ As puppy only ___ Never

Frequency of heartworm preventative:

___ Daily ___ Monthly ___ Seasonally ___ Sporadically ___ Never

7. Please check and fill in the appropriate boxes based on your dog's exposure to flea and tick products:

Type of insecticide	Brand or chemical name	Pattern of Use				Times Per Year	Years Used
		Never	Sporadic	Seasonal	Regular		
Flea/tick shampoo							
Flea/tick dip							
Flea/tick powder							
Flea/tick collar							
Flea/tick sprays							
Flea/tick skin application(spot-on)							
Flea/tick pill							
Other insecticide: _____							
Other grooming product: _____							

8. Has your dog ever had an adverse reaction to a drug? ____ yes ____ no
   a. If yes, what was the specific drug involved: _____
   b. Was this drug reaction seen by a veterinarian ? ____ yes ____ no
   c. Was this drug reaction treated by a veterinarian ? ____ yes ____ no
   d. How was this reaction treated?

___ epinephrine ___ Benadryl® ___ transfusion ___ IV fluids ___ other:_____

   e. What were your dog's signs? Check all that apply

___ face swelling ___ couldn't breathe ___ turned blue ___ fainting/collapse

___ vomiting/diarrhea ___ hypersalivating ___ weakness ___ turning pale

___ turning yellow ___ bloody stool ___ blood in urine ___ bloody nose

___ other: _____

   f. How long after drug administration did these signs appear? Indicate a number (for example, 5 hours)

___ minutes ___ hours ___ days ___ weeks ___ months

   g. How old was your dog when this adverse drug reaction occurred? ___ years ___ months

   h. Were there any long term / permanent effects of the adverse reaction: ___ yes ___ no

      If yes, please describe:_____

III. Additional Comments

Please indicate whether you would like us to send you a check for $25 to defray the cost of your veterinary visit (we'll send this after your dog's blood sample is received). Alternatively, would you like us to use this to recruit more Great Danes into our study?

_____ send it to me _____use it to recruit more dogs

Please use the bottom of this page to tell us anything else about the health of your dog that was not covered in the questionnaire.

After the data is analyzed at Purdue University, a summary report will be sent to all participants. It will also be posted on both the Purdue and Great Dane Health Foundation of America web sites. **Thank you** for your participation in this health and vaccine study of Great Danes!

ID# _____

Appendix 2

Veterinarian Questionnaire for _____ belonging to _____

___ / ___ / ___

Dear Doctor,

Your client, _____, is participating in the Purdue University Great Dane Health and Vaccine Study. A question frequently asked is whether repeated vaccination could be responsible for the increasing prevalence of autoimmune diseases, cancer, and other health problems in this breed. Therefore, we are conducting a survey of Great Dane owners whose dogs have different vaccination histories. Information about the dog's diet and life experiences is being collected directly from owners.

Your client has completed the owner questionnaire and has made an appointment with you for a physical examination and for collection of blood to be sent to Purdue University. Please use the enclosed pre-addressed mailer to return the medical history form and serum to our laboratory. For each eligible dog, a new medical history form and blood sample kit should be returned. **Please collect blood before giving a vaccination**, if vaccination is part of today's appointment**.**

Please complete the information below so that we can contact you if we have further questions. If you have questions, please contact us at the address below. Thank you for your participation and assistance. [Your client is being offered $25 to help defray the costs of this veterinary visit.]

Veterinarian's Name _____
Clinic Name _____
Street Address _____
City/Town, State, Zip Code, Country: _____
Telephone Number with Area Code: _____
email Address: _____

Name of Person completing this form: _____
Date: _____

For questions about this study, please contact:

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Physical Examination / Medical History Form:

Dog's Name: _____Client's Name: _____

1. What is this dog's body condition (see definitions)?

_____obese _____overweight _____average/optimum

_____underweight

Definitions: *Obese: unable to feel ribs, large amounts of subcutaneous fat*
Overweight: rib cage barely palpable, little or no waist
Average: slight abdominal fat, rib cage palpable but not too obvious
Underweight: little body fat, rib cage easily palpable, obvious waist

2. Have you vaccinated this dog in the last 30 days? _____yes
_____no

If yes, please wait to obtain this dog***'s blood sample for at least 30 days following vaccination.***

3. What vaccinations has this dog received at your clinic? Please list all if possible, with the most recent vaccinations listed first.

Type of Vaccination	Date Administered (month/day/year)	Manufacturer's Name	Brand Name

4. Has this dog had any adverse vaccine reactions?

_____ yes _____ no (*If no, please skip to question 5)*

If yes, please describe 3 most recent reactions:

Most recent vaccine reaction:

ff. If yes, what was the specific vaccine involved: _____

gg. How old was this dog when this adverse reaction occurred? __ years __ months

hh. How was this reaction treated?

___ epinephrine ___ Benadryl® ___ transfusion ___ IV fluids ___ other:_____

ii. What were this dog's signs?

___ face swelling ___ couldn't breathe ___ turned blue ___ fainting/collapse
___ vomiting/diarrhea ___ hypersalivating ___ weakness ___ turning pale
___ turning yellow ___ bloody stool ___ blood in urine ___ bloody nose
___ other: _____

jj. How long after vaccination did these signs appear? Indicate a number (i.e., 5 hrs)

___ minutes ___ hours ___ days ___ weeks ___ months

kk. Since this reaction, has this dog been vaccinated again? ___ yes ___ no

ll. If yes, were any precautions been taken to prevent another reaction? ___ yes__ no

mm. If so, what precautions were taken?

___ Benadryl® ___ prednisone ___ stay longer at vet hospital for observation
___ vaccinate less often ___ other: _____

nn. Were there any long term / permanent effects of the adverse reaction:__ yes__ no

oo. If yes, please describe:_____

Prior vaccine reaction:

pp. If yes, what was the specific vaccine involved: _____

qq. How old was this dog when this adverse reaction occurred? __ years __ months

rr. How was this reaction treated?

___ epinephrine ___ Benadryl® ___ transfusion ___ IV fluids ___ other:_____

ss. What were this dog's signs?

___ face swelling ___ couldn't breathe ___ turned blue ___ fainting/collapse
___ vomiting/diarrhea ___ hypersalivating ___ weakness ___ turning pale
___ turning yellow ___ bloody stool ___ blood in urine ___ bloody nose
___ other: _____

tt. How long after vaccination did these signs appear? Indicate a number (i.e, 5 hrs)

___ minutes ___ hours ___ days ___ weeks ___ months

uu. Since this reaction, have any precautions been taken to prevent another reaction?
____ yes ____ no

vv. If so, what precautions were taken?

___ Benadryl® ___ prednisone ___ stay longer at vet hospital for observation
___ vaccinate less often ___ other: _____

ww. Were there any long term / permanent effects of the adverse reaction:

_____ yes _____ no

If yes, please describe:_____

Earlier vaccine reaction:

xx. If yes, what was the specific vaccine involved: _____

yy. How old was this dog when this adverse reaction occurred? __ years __ months

zz. How was this reaction treated?

___ epinephrine ___ Benadryl® ___ transfusion ___ IV fluids ___ other:_____

aaa. What were this dog's signs?

___ face swelling ___ couldn't breathe ___ turned blue ___ fainting/collapse
___ vomiting/diarrhea ___ hypersalivating ___ weakness ___ turning pale
___ turning yellow ___ bloody stool ___ blood in urine ___ bloody nose
___ other: _____

bbb. How long after vaccination did these signs appear? Indicate a number.

___ minutes ___ hours ___ days ___ weeks ___ months

ccc. Since this reaction, were any precautions been taken to prevent another reaction? _____ yes_____ no

ddd. If so, what precautions were taken?

___ Benadryl® ___ prednisone ___ stay longer at vet hospital for observation
___ vaccinate less often ___ other: _____

eee. Were there any long term / permanent effects of the adverse reaction:

_____ yes _____ no

If yes, please describe:_____

5. Has this dog ever had an adverse reaction to a drug? ____ yes ____ no

a. If yes, what was the specific drug involved: _____

b. How old was this dog when this adverse drug reaction occurred? _ years _months

c. How was this reaction treated?

___ epinephrine ___ Benadryl® ___ transfusion ___ IV fluids ___ other:_____

d. What were this dog's signs?

___ face swelling ___ couldn't breathe ___ turned blue ___ fainting/collapse
___ vomiting/diarrhea ___ hypersalivating ___ weakness ___ turning pale
___ turning yellow ___ bloody stool ___ blood in urine ___ bloody nose
___ other: _____

e. How long after drug administration did these signs appear? Indicate a number (for example, 5 hours)

___ minutes ___ hours ___ days ___ weeks ___ months

f. Were there any long term / permanent effects of the adverse reaction: __ yes__ no

If yes, please describe:_____

6. Please fill in the following table for this dog. *If 'yes' to any condition, please indicate the age at onset.*

Condition	Yes	No	Age at Onset (Months/Years)
AUTOIMMUNE			
Autoimmune hemolytic anemia (AIHA)			
Thrombocytopenia (ITP)			
Systemic lupus erythematosis (SLE)			
Arthritis (immune-mediated)			
Thyroiditis (autoimmune)			
Other:_____			
ENDOCRINE			
Hypothyroid			
Cushing's (hyperadrenal)			
Addison's (hypoadrenal)			
Diabetes mellitus			
Other: _____			

7. Has this dog been treated for any other medical condition(s)? ____yes ____no

a. If so, what is the diagnosis? _____

b. When was this diagnosis made? _____

c. How has this condition been managed? _____

d. Is this condition present at this time? _____yes _____no

If more room is required, please use the back of this page!

Instructions for Blood Collection

Important: If the dog is to be vaccinated at this appointment, blood should be collected first! If the dog has been vaccinated within the past 30 days, blood collection should be delayed for 30 days following vaccination.

1. Collect 20 ml blood into two 10 ml red-top tubes. Do not use serum separator tubes!
2. Allow the blood to clot (at least 10 minutes).
3. Centrifuge / spin down at least 10 minutes.
4. Withdraw the serum and place it into the serum tubes provided (approximately 5 ml serum per tube) using the enclosed plastic pipettes.
5. Replace the serum tube screw tops so that the tubes are closed and will not spill or leak.
6. Write today's date on the tubes provided.
7. Please freeze the serum tubes for at least one day before shipment.
8. Please freeze the gel packs for at least one day before use in shipping.

Instructions for Shipping Blood Sample

1. Please use the enclosed shipping materials to return the serum tubes and enclosed physical examination / medical history form to Purdue University.
2. Place the serum collection tubes in the foam packing material inside the Styrofoam box. Place the frozen gel pack in the other side of the box.
3. Place the physical examination/medical history form in the plastic bag between the gel pack and the serum tubes and close the box.
4. Insert the Styrofoam box into the pre-addressed mailing sleeve.
5. On the shipping day, store the complete package in the refrigerator until shipment.
6. Complete the prepaid shipping label as needed and contact FedEx to arrange for package pick-up or drop into any FedEx box that will be picked up the same day.
7. Please do not prepare the box until the day of shipping. Please do not ship on Fridays, Saturdays, or the days before holidays.

Thanks very much in advance! If you have any questions, please contact Dr. Malathi Raghavan at
765-496-2634 or via email at raghavam@purdue.edu.