

# Studies on Medical and Veterinary Students Skin Tested for Toxoplasmosis

WILLIAM F. McCULLOCH, D.V.M., M.P.H., JOHN L. BRAUN, M.S., DARROL W. HEGGEN, M.S.,  
and FRANKLIN H. TOP, M.D., M.P.H.

**M**ORE THAN 25 years have passed since *Toxoplasma gondii* was first established as causing clinical disease in man (1). Nearly every discipline that deals with the health of man and animals has conducted research on toxoplasmosis. The number of articles published in the world literature increases each year, and thus keeping abreast of new developments has become increasingly difficult. However, several publications review recent advances in toxoplasma research (2-5). The success of this ubiquitous parasite is exemplified by (a) its dubious role as a protozoan parasite—the fact that it is not host-specific; (b) its wide distribution in nature, both zoologically and geographically; (c) its ability to parasitize nearly every body cell type with the possible exception of nonnucleated red blood cells, thus causing protean clinical manifestations in man and animals; (d) the inability to define

completely its true life cycle; and, (e) the lack of success in establishing definitive proof of the modes of transmission in man, with the exception of the congenital route.

Toxoplasma is an obligate intracellular parasite. In addition to reproduction by longitudinal binary fission (6, 7), recent studies have described two additional methods. Goldman and co-workers (8) have described the process of internal budding which they call "endodyogeny" (meaning birth of two from within). Gavin and co-workers (9) have described a third method whereby at least four progenies are delineated within a rosette-shaped multinucleated toxoplasma cell, following which they eventually separate to become individual parasites. Morphologically and immunologically, the toxoplasma organisms found in various hosts are considered to be of the same species, the main difference being in the virulence of the strain of organism for different animal species (10). In general, the young of animal species are more severely affected than the old. Infection appears to be the rule; disease the exception.

Toxoplasma occurs in two forms, the crescent-shaped free proliferative form (fig. 1) and the cyst form (fig. 2). Presently, the modes of transmission must be considered in light of knowledge of these two forms. The proliferative form is considered to be sensitive to changes in osmotic pressure, to drying, and to exposure to artificial gastric juices. This form apparently dies rapidly once it leaves the animal host. The cyst form appears to be more resistant and is considered by many to play a

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*All the authors are with the Institute of Agricultural Medicine, State University of Iowa College of Medicine, Iowa City. Dr. McCulloch is assistant professor and public health veterinarian; Mr. Braun is medical field epidemiologist; Mr. Heggen is statistician; and Dr. Top is professor and head of the department of hygiene and preventive medicine and director of the institute.*

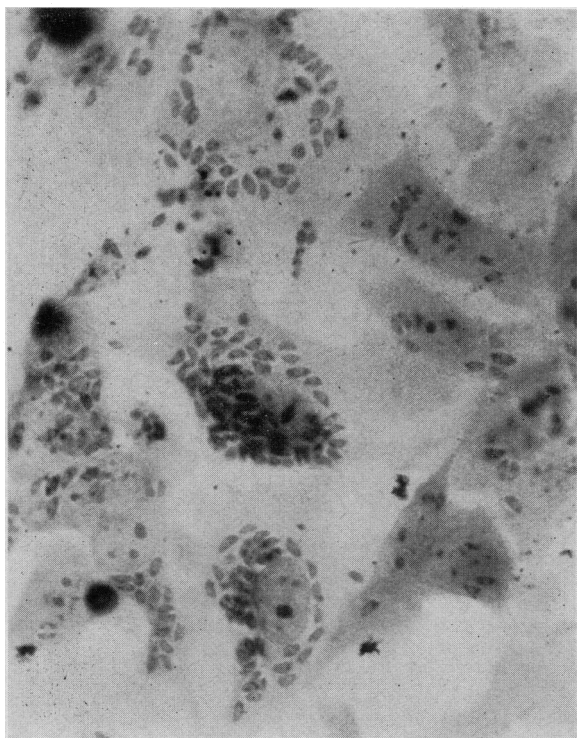
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major role in the transmission of infection, especially in the omnivorous and carnivorous species (11). The cyst form is characteristically found in chronic or latent infections and is considered to be more resistant to environmental influences. Although cysts have been found in various tissues, the heaviest concentration appears to be in the tissues of the central nervous system and in the skeletal muscles (12). When one considers the various methods of direct and indirect disease transmission, these two areas hardly provide an efficient exit from the host.

Although animals are considered the reservoir for human as well as other animal infections, the widespread nature of the disease in the animal kingdom has made it difficult to incriminate a specific species as related to individually diagnosed human cases. The usual summary comment made at the end of many studies is that very possibly both man and animals acquired the infection from the same source. However, nobody seems to know what the "same" source

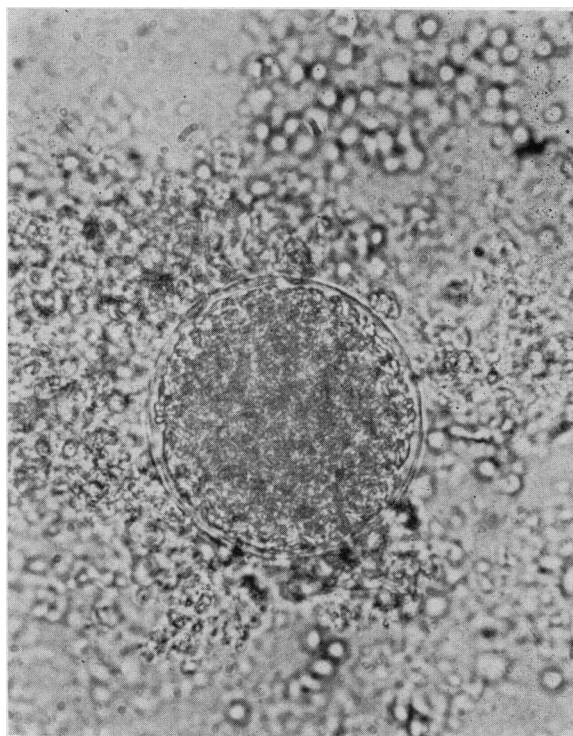
is. It becomes a problem to know who is getting the disease from whom and whether man himself may or may not be his own worst enemy.

Several serologic studies have shown that a higher percentage of persons are serologically positive in the older age groups than in the younger age groups (13, 14). As a result of the difficulty in the diagnosis of acquired cases of acute toxoplasmosis, various serologic and skin-test surveys have been conducted to give insight into the prevalence of the disease in various population groups. Epidemiologic factors such as age, sex, residence background, and animal contact have been related to those positive and to those not positive for possible significant associations. Kimball and co-workers (15) in Minnesota studied the serologic evidence of toxoplasmosis in obstetrical patients. They found that contact with farm animals in general and with cattle, chickens, ducks, and geese were significantly associated with being dye-test or serologically positive. Additionally, they found that of the patients who had always lived on



Courtesy of Dr. M. P. Verma,  
Institute of Agricultural Medicine

**Figure 1.** Geimsa-stained liver cells (Chang) infected with *Toxoplasma gondii*. Crescent-shaped intracellular parasites are in a perinuclear position. Magnification about 720  $\times$ .



**Figure 2.** *Toxoplasma* cyst in a wet brain-smear of a chronically infected mouse. Cyst wall surrounds numerous parasites. Magnification about 500  $\times$ .

**Table 1. Students in medical and veterinary schools participating in the toxoplasmosis skin test study**

Group	University of Minnesota (medical)	University of Minnesota (veterinary)	University of Illinois (veterinary)	State University of Iowa (medical)	Iowa State University (veterinary)	Total
Total.....	141	164	149	231	250	935
Tested.....	74	144	126	202	229	775
Not tested <sup>1</sup> .....	59	17	19	26	17	138
Refused.....	8	3	4	3	4	22
Percent refusal.....	9.8	2.0	3.1	1.5	1.7	2.8

<sup>1</sup> Absent during scheduled test periods because of illness or special school assignments.

farms, 44 percent were dye-test positive, whereas, of those patients who had never lived on farms, 21 percent were dye-test positive. In a study of comparable urban and rural Negro high school students in Tennessee, Gibson (16) found no significant difference between the proportions of positive titers in the urban and rural students.

Wende and Dienst (17) skin tested 703 patients at a Georgia training school for mental defectives and found that in 2 of the 12 cottages, 51 percent of 86 patients were positive but only 15 percent of 617 patients in the remaining cottages were positive. Dye test results on two large animal herds maintained on the premises revealed 33 of 48 swine and 20 of 24 cattle positive at a 1:16 dilution. The two cottages with the highest proportion of skin test positives housed the patients who were responsible for the farmwork and care of the farm animals. The authors suggest that the high human prevalence of skin test positives was related to association with infected farm animals.

This report describes the results of skin testing veterinary and medical students at four midwestern universities in Iowa, Illinois, and Minnesota.

#### Methods and Materials

*Schools tested.* During 1960-61, a total of 775 veterinary and medical students were skin tested for evidence of previous infection with *Toxoplasma gondii*. Freshman, sophomore, junior, and senior veterinary students were skin tested at the schools of veterinary medicine at Iowa State University, the University of Illi-

nois, and the University of Minnesota. Freshman and sophomore medical students at the State University of Iowa and freshman medical students at the University of Minnesota were also skin tested.

*Skin test materials.* Toxoplasma skin test antigen and control were procured from the Eli Lilly Co. The antigen was prepared as described by Frenkel (18). An intradermal injection of 0.1 ml. of antigen and control material was made on the flexor surface of the left and right arms, respectively. Readings were obtained 48 hours after the skin test was administered. Erythema and induration of more than 10 mm. with a negative control was taken as evidence of past or persistent infection.

*Method of data collection.* A questionnaire was filled out by each student. Epidemiologic items were age, sex, current and past residence, degree and type of animal contact, and degree of soil contact. Each respondent was given four choices as to the various animal contacts experienced. The categories were "never," "rare," "moderate," or "marked." These choices refer to animal contact from age 6 years to skin test date. Students were asked to interpret the term "marked" to apply to situations such as (a) extensive contact for a short period of time—for example, one summer spent assisting in a small animal practice situation would represent marked dog or cat contact; or (b) brief contacts over a long period of time—for example, an hour a week at a riding academy for 2 or 3 years would represent marked horse contact. The term "moderate" was used to represent lesser variations of the sample situations as

outlined for the term "marked." If they were unable to differentiate between moderate and marked, both were to be checked.

The skin test positive rate for those having moderate or marked contact was then compared with the skin test positive rate for those having never or rare contact with the same animal species. In addition, students were

asked to check whether they had worked with farm animals on the following basis: "never," "sometimes," or "frequently." Categories concerning marked soil contact and a history of being bitten by an animal were answered by yes or no. Each student was also asked if he had had a pet dog in the home as a child prior to age 6. This was answered by yes or no.

**Table 2. Comparative prevalence of toxoplasma (skin test) positives among veterinary and medical students, by age group**

Age (years)	Veterinary			Medical			Total		
	Number tested	Positive		Number tested	Positive		Number tested	Positive	
		Number	Percent		Number	Percent		Number	Percent
Total.....	499	97	19.4	276	41	14.9	775	138	17.8
20-22.....	157	22	14.0	129	13	10.1	286	35	12.2
23-25.....	154	38	24.7	106	20	18.9	260	58	22.3
26-28.....	106	21	19.8	21	3	14.3	127	24	18.9
29-31.....	61	12	19.7	16	3	18.8	77	15	19.5
32-34.....	11	0	0	1	0	0	12	0	0
35-37.....	8	4	50.0	3	2	66.7	11	6	54.5
38-40.....	2	0	0	-----	-----	-----	2	0	0
Age range.....	20-38			20-36			20-38		
Mean age.....	24.8			23.5			24.4		
Median age.....	24			23			23		

**Table 3. Comparative prevalence of toxoplasma (skin test) positives among veterinary and medical students, by age group and school**

Age (years)	University of Illinois (veterinary)			Iowa State University (veterinary)			State University of Iowa (medical)			University of Minnesota					
	Number tested	Positive		Number tested	Positive		Number tested	Positive		Medical			Veterinary		
		Number	Percent		Number	Percent		Number tested	Number	Percent	Number tested	Number	Percent		
Total.....	126	29	23.0	229	39	17.0	202	36	17.8	74	5	6.8	144	29	20.1
20-22.....	46	9	19.6	69	6	8.7	86	11	12.8	43	2	4.7	42	7	16.7
23-25.....	45	10	22.2	73	19	26.0	84	17	20.2	22	3	13.6	36	9	25.0
26-28.....	21	6	28.6	51	9	17.6	15	3	20.0	6	0	0	34	6	17.6
29-31.....	9	2	22.2	29	3	10.3	13	3	23.1	3	0	0	23	7	30.4
32-34.....	2	0	0	4	0	0	1	0	0	-----	-----	-----	5	0	0
35-37.....	3	2	66.7	3	2	66.7	3	2	66.7	-----	-----	-----	2	0	0
38-40.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	2	0	0
Age range.....	20-37			20-36			20-36			21-30			20-38		
Mean age.....	24.3			24.8			23.7			22.8			25.4		
Median age.....	23			24			23			22			25		

**Table 4. Comparative prevalence of toxoplasma (skin test) positives, by year in school among veterinary students**

Class of veterinary students	University of Illinois			Iowa State University			University of Minnesota			Total		
	Number tested	Positive		Number tested	Positive		Number tested	Positive		Number tested	Positive	
		Number	Percent		Number	Percent		Number	Percent		Number	Percent
Total.....	126	29	23.0	229	39	17.0	144	29	20.1	499	97	19.4
Freshman.....	42	7	16.7	63	10	15.9	34	6	17.6	139	23	16.5
Sophomore.....	30	8	26.7	55	12	21.8	38	6	15.8	123	26	21.1
Junior.....	24	6	25.0	58	7	12.1	33	10	30.3	115	23	20.0
Senior.....	30	8	26.7	53	10	18.9	39	7	17.9	122	25	20.5

Associations between questionnaire data and skin test results were tested for significance by the chi-square test.

**Results**

*Test group characteristics.* Only 22 students (2.8 percent) refused to take part in the test program (table 1). A relatively large number were not tested at the University of Minnesota medical school primarily because of scheduling difficulties associated with split laboratory sections. Other students absent during the scheduled test periods had special school assignments or were absent because of illness. The testing program was accomplished during laboratory and clinic hours.

Table 2 summarizes the skin test results of the students tested by 3-year age groups. The age groups were decided in advance of the

study with the idea that a 3-year grouping would reveal age trends within the age range of the group tested. Of the 499 veterinary students tested, 97 (19.4 percent) were skin test positive. Of the 276 medical students tested, 41 (14.9 percent) were skin test positive. There is a difference, not readily explainable, in the percentage positive between the ages of 20 and 22 and 23 and 25. With this exception, there seems to be no consistent age trend. This might be expected in view of the narrow age range of the students. Beyond the age group 29-31, numbers are too small to speculate on possible differences.

Table 3 gives the skin test results by the same 3-year age groups in the three veterinary and two medical schools. The highest skin test positive rate (23.0 percent) occurred among the veterinary students at the University of Illinois, while the lowest rate (6.8 percent) was encountered in the medical students at the University of Minnesota. At Iowa State University the proportion of positive subjects among veterinary students was 17.0 percent; at the State University of Iowa, among medical students it was 17.8 percent; and, at the University of Minnesota among veterinary students, 20.1 percent.

In table 4 the prevalence of toxoplasma (skin test) positives among veterinary students is compared by year in school. There is no significant trend in the proportion of skin test positives from the freshman year to the senior year in school. This may suggest that conversion from negative to positive from year to

**Table 5. Distribution of toxoplasma (skin test) positives according to time spent on farm or in town or city**

Lifetime residence of all students	Number tested	Number positive	Percent positive
Total.....	775	138	17.8
More than 70 percent on farm.....	226	57	25.2
More than 70 percent in town of less than 2,500.....	61	7	11.5
More than 70 percent in city of more than 2,500.....	269	28	10.4
Other <sup>1</sup> .....	219	46	21.0

<sup>1</sup> Less than 70 percent in town or city or on farm.

year in veterinary school is inconsequential. Similar results with the medical students have not been presented in table form, as only the freshman class was tested at the University of Minnesota, and freshman and sophomore classes at the State University of Iowa.

*Association between residence and test results.*

In the questionnaire each respondent was asked to list his places of residence since birth and to check whether it was on a farm, in a town (less than 2,500 population), or in a city (more than 2,500 population). The distribution of toxoplasma (skin test) positives according to the time spent on a farm, in a town, or in a city

is presented in table 5. This includes both the medical and veterinary students. The "more than 70 percent" category was chosen because all students in the study had spent from 2 to 8 years at colleges or universities in cities of more than 2,500 population. Therefore, no student could report a 100 percent farm background, and very few reported more than an 80 percent farm background. The farm group contained students who had lived most of their lives on farms with the exception of the years spent in college. The "other" category (table 5) contained 219 students who had less than 70 percent lifetime residence in a town or a city or

**Table 6. Distribution of toxoplasma (skin test) positives among medical students according to time spent on farm or in town or city**

Lifetime residence of medical students	State University of Iowa			University of Minnesota			Total		
	Number tested	Positive		Number tested	Positive		Number tested	Positive	
		Number	Percent		Number	Percent		Number	Percent
Total.....	202	36	17.8	74	5	6.8	276	41	14.9
More than 70 percent on farm.....	20	7	35.0	3	1	33.3	23	8	34.8
More than 70 percent in town of less than 2,500.....	26	3	11.5	10	1	10.0	36	4	11.1
More than 70 percent in city of more than 2,500.....	115	16	13.9	42	2	4.8	157	18	11.5
Other <sup>1</sup> .....	41	10	24.4	19	1	5.3	60	11	18.3

<sup>1</sup> Less than 70 percent in town or city or on farm.

**Table 7. Distribution of toxoplasma (skin test) positives among veterinary students according to time spent on farm or in town or city**

Lifetime residence of veterinary students	Iowa State University			University of Minnesota			University of Illinois			Total		
	Number tested	Positive		Number tested	Positive		Number tested	Positive		Number tested	Positive	
		Number	Percent		Number	Percent		Number	Percent		Number	Percent
Total.....	229	39	17.0	144	29	20.1	126	29	23.0	499	97	19.4
More than 70 percent on farm.....	110	24	21.8	59	16	27.1	34	9	26.5	203	49	24.1
More than 70 percent in town of less than 2,500.....	14	1	7.1	4	0	0	7	2	28.6	25	3	12.0
More than 70 percent in city of more than 2,500.....	36	1	2.8	24	2	8.3	52	7	13.5	112	10	8.9
Other <sup>1</sup> .....	69	13	18.8	57	11	19.3	33	11	33.3	159	35	22.0

<sup>1</sup> Less than 70 percent in town or city or on farm.

on a farm. Of these, 46 (21.0 percent) were skin test positive.

Of the 226 students with a more than 70 percent farm lifetime residence, 57, or 25.2 percent, were skin test positive, whereas 28 of 269 (10.4 percent) students with a more than 70 percent lifetime residence in a city of more than 2,500 population were skin test positive. Among the 61 students, each of whom had spent more than 70 percent of his lifetime in a town of less than 2,500 population, 7 (11.5 percent) were skin test positive. The difference in the rate of positives between farm (25.2 percent) and city (10.4 percent) is statistically significant at the 1 percent level.

Tables 6 and 7 present the distribution of toxoplasma (skin test) positives according to the

**Table 8. Distribution of toxoplasma (skin test) positives according to degree of animal and soil contact among medical and veterinary students**

Animal and soil contact	Number tested	Number positive	Percent positive
Total.....	775	138	17. 8
Pet dog prior to age 6....	518	110	<sup>1</sup> 21. 2
No pet dog prior to age 6..	257	28	10. 9
Cat contact:			
Moderate or marked....	576	117	<sup>1</sup> 20. 3
Rare or never.....	199	21	10. 6
Horse contact:			
Moderate or marked....	505	110	<sup>1</sup> 21. 8
Rare or never.....	270	28	10. 4
Sheep contact:			
Moderate or marked....	313	69	<sup>2</sup> 22. 0
Rare or never.....	462	69	14. 9
Cattle contact:			
Moderate or marked....	566	116	<sup>1</sup> 20. 5
Rare or never.....	209	22	10. 5
Chicken contact:			
Moderate or marked....	508	108	<sup>1</sup> 21. 3
Rare or never.....	267	30	11. 2
Swine contact:			
Moderate or marked....	504	109	<sup>1</sup> 21. 6
Rare or never.....	271	29	10. 7
Turkey contact:			
Moderate or marked....	111	38	<sup>1</sup> 34. 2
Rare or never.....	664	100	15. 1
Worked with farm animals:			
Never.....	130	9	6. 9
Sometimes.....	185	30	16. 2
Frequently.....	460	99	<sup>1</sup> 21. 5
Marked soil contact.....	548	114	<sup>2</sup> 20. 8
No marked soil contact....	227	24	10. 6

<sup>1</sup> Significant at 0.01 level.

<sup>2</sup> Significant at 0.05 level.

time spent on a farm, in a town, or in a city, for medical and veterinary students. The same difference noted in table 5 is consistently seen in the total veterinary and total medical student tabulations as well as in each individual school. The numbers in these categories by school are too small for proper statistical evaluation.

Table 8 shows the distribution of toxoplasma (skin test) positives according to the degree of animal and soil contact among the students tested. The animal contacts are those in the questionnaire which were demonstrated to be statistically significant. The authors are aware that the significance tests which were used in this study are not independent. In general, it was consistently seen that those students having moderate or marked contact with swine, for instance, also had moderate or marked contact with horses, chickens and the other animals. Thus, if the significance is a spurious one, it will appear in each test of each animal contact by the very nature of the associations. Nevertheless it is interesting to note which animal contacts were associated with positive skin tests and those which were not. Positive skin tests were significantly associated with contact with the following mammalian and avian species: swine, horses, sheep, cattle, cats, chickens, and turkeys. Items showing no significant association were dogs, wild rabbits, geese, domestic ducks, pigeons, and a history of animal bites.

Of the 130 students who stated that they had "never" worked with farm animals, 9, or 6.9 percent, were skin test positive, but 99, or 21.5 percent, of the 460 students who stated that they had worked "frequently" with farm animals were positive. This difference is significant at the 1 percent level. The students were also asked whether or not they had had a pet dog in the home as a child (prior to age 6). Of the 518 students who replied affirmatively, 110, or 21.2 percent, were skin test positive compared with 28 of 257 (10.9 percent) in the group of students who had had no dog. Students who indicated marked soil contact had a significantly higher proportion of skin test positives than those who did not.

#### Discussion

*Skin test validity.* Although the skin test is of limited value in the diagnosis of acute cases

of toxoplasmosis, it is of value as an epidemiologic tool for studying large population groups (19). Jirovec and Jira (20) state that the toxoplasma skin test, in addition to being easy to perform, is well suited for estimating the amount of toxoplasma infection in a community. Feldman (21), however, states that standardizing the skin test is a major problem and feels that if the test has any real value, it is for survey work. Several investigators (22-24) have found 90 percent or higher correlation between skin test and serologic results using the Sabin-Feldman dye test.

*Age and toxoplasma positives.* Although the age range for those tested was from 20 to 38 years, the majority (546 of 775) were 20-25 years of age. From the data it was not possible to detect factors responsible for the increased proportion of persons positive in the 23-25 age group (22.3 percent) compared with the 20-22 year age group (12.2 percent). Significant factors associated with being skin test positive (farm background or animal contact) did not account for this difference. Although the 3-year age increments were decided in advance of this survey, it is possible that this age breakdown produced a spurious difference. However, this phenomenon was seen even when each school was characterized separately.

*Residence background.* Gibson (16) found no significant difference in the proportions of persons serologically positive in a comparable urban and rural population of junior and senior high school students in Tennessee. The studies by Kimball and co-workers (15) in Minnesota revealed that dye test positivity in obstetrical patients did not vary according to current residence (urban or rural) in each of the three groups tested, but did find that among patients who had never lived on a farm, 21 percent were dye test positive, whereas 44 percent of the patients who had always lived on farms were positive.

The comparisons of this study are based upon lifetime residence. Prior to undertaking this project, it was hypothesized that those persons with more than 70 percent lifetime residence in a town of less than 2,500 population would have a proportion of skin test positives similar to the more than 70 percent farm background group. The data do not support this hypothesis. The

less than 2,500 population town group was similar to the more than 2,500 population city group (11.5 percent and 10.4 percent respectively). The rate for students of the farm group was 25.2 percent.

The "other" group contained persons who had less than 70 percent lifetime residence on a farm or in a town or a city. Of the 219 students in the "other" group (table 5), 46, or 21.0 percent, were skin test positive. This is comparable to the more than 70 percent farm group. Of the 46 positives in the "other" group, 61 percent had 50 to 69 percent lifetime residence on a farm. Of the 173 negatives in the "other" group, only 41 percent had 50 to 69 percent lifetime residence on a farm. These facts lend additional support to the influence of farm residence on skin test positive status.

*Animal and soil contacts.* It is difficult to assess fully the significance of the association with farm animals and skin test positive status. Evidence of toxoplasma infection has been found in avian (25) and mammalian species, especially domestic ones (14). The animals listed as being significant are species quite commonly found on many farms in the midwestern United States. Nevertheless, the association with animals may play a role in transmission, as only 6.9 percent (9 of 130) of the students who had never worked with farm animals were skin test positive as opposed to 21.5 percent (99 of 460) who had frequently worked with farm animals.

It was hypothesized that a student who had had a dog in the home prior to age 6 would have had opportunities for direct and indirect contact with the pet. Young children crawl on the floor, are licked on the face, and have ample opportunity for intimate contact with the pet. The skin test positive status of the student, if infected prior to age 6, must have remained positive since that time to be measured in the present study.

In light of the resistance of the presently known proliferative and cyst forms of toxoplasma, the role of marked soil contact in relation to being skin test positive also remains to be determined, but gives indirect support to Jacobs' hypothesis (26) that an intermediate stage of toxoplasma may possibly occur in the soil.



The data presented in this paper attest that students with a principal farm background, marked contact with the soil, and frequent contact with farm animals had a greater chance of being skin test positive for toxoplasma.

### Summary and Conclusions

During 1960-61, 775 veterinary and medical students were skin tested for evidence of previous infection with *Toxoplasma gondii*. The students who reported a more than 70 percent farm background had a significantly greater proportion of skin test positives than did the group with more than 70 percent of lifetime residence in a city of more than 2,500 population. No significant differences were found in the proportion of skin test positives among the four academic classes of veterinary students.

Skin test positive status was significantly associated with moderate or marked contact with swine, horses, sheep, cattle, cats, chickens, and turkeys. Other statistically significant items included marked soil contact, frequent work with farm animals, and a recollection of having had a pet dog in the home prior to age 6. Items showing no significant association were moderate or marked contact with dogs, wild rabbits, geese, domestic ducks, and pigeons and a history of animal bites.

With an infection as widespread as toxoplasmosis, additional studies of the relationship of animal (avian and mammalian) and human toxoplasmosis should be undertaken if insight into possible modes of transmission is to be gained. It is likely that this organism has a most efficient mode of transmission and the mode or modes may differ from one area to another. Only through intensified studies in specific geographic areas can a full evaluation of the epidemiology of toxoplasmosis be made.

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