PREVALENCE OF
Toxocara canis
IN BABIES AND IN
ADULTS AS DETERMINED
BY THE ELISA TEST

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INTRODUCTION

IN SPITE OF MANY ADVANCES IN THE CLINICAL AND PATHOLOGIC RECOGNITION of human involvement by the parasite Toxocara canis, the true prevalence of this disease remains relatively undetermined. Until quite recently investigators lacked reliable serologic tests, the diagnosis depending instead on histopathologic confirmation.

Rather specific and sensitive serologic testing techniques have been developed in recent years. The introduction of the enzyme-linked immunosorbent assay (ELISA) test, given its high specificity and sensitivity for T canis, makes possible reliable estimates of the prevalence of the disease in a sample population. This paper presents the estimated prevalence of T canis in Puerto Rico based on a sample of 671 subjects. Given this relatively large sample, it was possible to determine the prevalence in different age groups, particularly in children. In addition, we have tried to determine the earliest instance of infection and to relate relevant factors such as exposure to puppies or dogs and cats to a positive assay. This paper represents the initial step in the broader effort to obtain more information on the early stages of the disease.

LIFE CYCLE OF TOXOCARA CANIS

TRANSMISSION IN DOGS

Toxocara canis, a nematode, is distributed worldwide.1 The parasite is commonly found in dogs, particularly puppies, where the adult worm lives in the small intestine (Fig 1). Dogs can acquire the infection from embryonated eggs in the soil, infective larvae from intermediate hosts, or through
prenatal transplacental infection of advanced-stage larvae from the feces of suckling pups. Somatic distribution of second-stage larvae occurs in the female dog. Due to hormonal changes during pregnancy, the granulomatous lesions release larvae that become active, cross the placenta, and migrate to the lungs of puppies, where they mature to the third stage by birth.\(^2\) By the third day after birth the larvae have migrated through the trachea and esophagus. After the puppies swallow the larvae, the fourth stage emerges in the intestine.\(^3\)

Approximately 21 days after birth, the worms mature sexually, and large numbers of unembryonated eggs are deposited daily in the feces until the puppy is 6 months old. At this time, for unknown reasons, the worms are voided spontaneously and the animal becomes significantly less heavily infected thereafter. For the cycle to begin again the eggs must mature on the outside for about two weeks before ingestion by another animal would lead to its contamination.\(^4\)

There are several implications of transplacental migration of *Toxocara* larvae in dogs. First, a single infected dog can have numerous litters of infected puppies. This may help to explain why *T canis* is a prominent nematode in ocular endophthalmitis. Second, since the various deworming agents act only in the gut, it is possible to deworm a dog and to subsequently find that the dog produces infected litters. Therefore, the chain of infection cannot be broken by deworming agents alone. Third, it helps to explain the clinical observation that it is young puppies, generally under two years of age that most frequently transmit nematode infection.\(^5\)

**TRANSMISSION TO MAN**

After a short incubation period in soil, embryonization of the eggs released in the puppy’s feces takes place. At this time the eggs harbor infective second-stage larvae. Eggs of *T canis* are viable for years under suitable conditions of soil, temperature, and moisture. They are susceptible to sunlight and to dessication but resistant to low temperature and common household disinfectants. A female adult worm may produce 200,000 eggs per day, and, throughout her lifetime of four to five months, she produces several million.

Children aged 1 to 3 years are highly susceptible to infection since a child in that age group may put anything in his or her mouth as part of the learning process (Fig 1). The ova may be picked up from anything contaminated for some time with dog or cat feces as the child plays around the house. Infection is generally associated with dirt-eating or contact with either dogs or cats. The preponderance of *T canis* as opposed to *Toxocara cati* in humans probably results from the toilet habits of cats. Feces of
infected puppies may contain up to 15,000 eggs per gram, which are infectious upon embryonization (within two weeks). A kennel that was hosed down daily was found to contain a total of 31,000 residual eggs (harboring second-stage larvae). Eggs are sticky and adhere readily to the dog's fur or to toys, clothing, and other household articles.

Upon ingestion, the ova hatch to release motile second-stage larvae in the human intestine. The larvae invade the liver and, later, the lungs. Subsequently, they enter the blood and lymphatic systems, from where they circulate to the various organs.

Man is not a well-adapted host for *Toxocara*, and the life cycle typically is not completed. Instead, the larvae are deposited, and later encapsulated, in various tissues where they are surrounded by eosinophilic granulomas, causing a persistent infection. Since completion of the *Toxocara* life cycle with development of a mature worm capable of producing ova is a rare occurrence in man, it is generally unrewarding to examine specimens of feces for nematodes or eggs. Examination of feces is useful only because it might confirm previous geophagia by demonstrating other parasites or ova.
In an intermediate host such as man, the larvae may remain free in the brain while becoming encapsulated in other organs. The larvae may remain viable for one year or more in human tissue. In monkey tissue, the larvae have been found alive after ten years. Invasion of the eye by larvae is well documented but the mechanism of larvae migration into the eye, as well as the route of entrance, remains unknown. A discussion of the ocular manifestations of this migration and other systemic manifestations of *T. canis* follows.

**SYSTEMIC AND OCULAR MANIFESTATIONS OF TOXOCARA CANIS IN MAN: A HISTORICAL REVIEW**

**SYSTEMIC INVOLVEMENT**

In 1952, Beaver and his associates demonstrated through liver biopsy that a syndrome of fever, hepatomegaly, pulmonary infiltration, hyperglobulinemia, and eosinophilia in children was caused by tissue infection with *T*
canis larvae. He labeled this condition visceral larvae migrans (VLM), since all the organs involved were internal. Autopsy studies have revealed that the liver, brain, and skeletal muscles were the organs most severely affected by T canis infection, with significant eosinophilia in most patients.

Fortunately, the disease usually runs a benign course. This syndrome is observed most frequently in children aged one to five who have a history of pica. According to Beaver, the brain is frequently invaded by the larvae, surpassed in frequency by involvement only by the liver and skeletal muscles. Convulsions have been noted in the absence of fever. Epilepsy due to Toxocara encephalitis has been reported, and larvae have been demonstrated in the human brain. It appears possible, therefore, that Toxocara may be responsible for some cases of epilepsy of undetermined origin. A few cases of myocarditis also have been attributed to Toxocara.

In Puerto Rico there is one known case of a patient who recovered dramatically from severe myocarditis by using a combination of thiabendazole and systemic corticosteroids. Sprent, in 1955, suggested that Toxocara may carry other diseases, especially into the central nervous system, thereby transporting viruses and other microorganisms across the blood-brain barrier. In 1965, Hutchinson demonstrated that Toxoplasma gondii may be fecally transmitted inside the ova of Toxocara. This was confirmed in 1967 by Jacobs and his co-workers who have shown also that Toxocara nematodes obtained from cats are naturally infected with T gondii.
OCULAR MANIFESTATIONS

In 1950, Wilder\textsuperscript{13} reported the histopathologic findings in 46 eyes selected from the files of the Armed Forces Institute of Pathology (AFIP). These eyes had been enucleated for suspected retinoblastoma and were found to have eosinophilic abscesses (Fig 2 and 3). These pathologic findings were similar to those associated with nematode infections in other parts of the body.\textsuperscript{13} Using serial sections (Fig 4), Wilder\textsuperscript{13} was able to find nematode larvae or their residual hyaline capsules in 24 of these eyes. Tissue reactions characteristic of nematode endophthalmitis were identified in the remaining eyes.

Unfortunately, Wilder\textsuperscript{13} erroneously identified them as third-stage hookworm larvae (Fig 4). In performing a biopsy of the liver in one patient with eosinophilic granulomatous lesions in the liver, Beaver and his coworkers\textsuperscript{7} discovered a larval nematode that he identified as \textit{Toxocara}.

Taking advantage of these new findings, Nichols,\textsuperscript{5} in 1956, performed histologic examinations on five enucleated eyes with the clinical diagnosis of suspected retinoblastoma, apparently from Wilder's specimens, at the
AFIP. He was able to recognize the organism as second-stage *T. canis* larvae segments, the roundworm commonly found in dogs throughout the world. Thus Nichols\(^5\) established *T. canis* as the organism responsible for some cases of nematode endophthalmitis (Fig 5). His findings have been confirmed by other investigators.\(^{14,15}\)

Duguid\(^6\) and, later, Ashton\(^7\) have reported the three more frequently encountered ocular manifestations of this disease entity: (1) chronic endophthalmitis (most prevalent), (2) solitary granuloma, and (3) peripheral retinitis (rarely). Ashton believes that these three ocular manifestations
Toxocara Canis in Babies

Maumenee\textsuperscript{18} and, later, Wilkinson and Welch,\textsuperscript{19} reported that a peripheral retinal mass was the most frequent manifestation of ocular toxocariasis. They noticed that elevated retinal folds commonly extended from the peripheral mass to the disc. They concluded that \textit{Toxocara} infection should be suspected in any patient with unilateral pars planitis. In 1972, O'Connor\textsuperscript{20} found additional patients with peripheral lesions and a subretinal tube-like structure running from the area of the disc to the peripheral mass. He was convinced that this retinal fold in the form of a tube-like structure was specific for \textit{Toxocara} infection, and was able to confirm the diagnosis in the only eye obtained for microscopic examination.

In this geographic area, the peripheral inflammatory lesion with vitreous bands and retinal folds running posteriorly has been the most frequently

\textbf{FIGURE 6}

Fundus photograph of posterior granuloma in one of our patients.
encountered ocular manifestation of *Toxocara* infection. We were able to confirm the diagnosis in 85% of our patients, using the enzyme-linked immunosorbent assay (ELISA) (Fig 7).

In 1978, Gass and co-workers\(^1\) reported a peculiar syndrome affecting one eye, characterized initially by visual loss, vitritis, and crops of multiple evanescent, gray-white, deep retinal lesions. Widespread diffuse and focal depigmentation of the pigment epithelium developed over many months, with narrowing of the retinal arteries, optic atrophy, severe visual loss, and electroretinographic (ERG) changes. A motile subretinal roundworm, probably *Toxocara*, was observed in two patients. This syndrome has been

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**FIGURE 7**
Fundus drawing of peripheral retinal mass, with retinal fold attached to the optic disc.
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termed diffuse, unilateral, subacute neuroretinitis (DUSN). Probably more than one etiologic agent can produce DUSN.\textsuperscript{21}

In 1978, Raymond and his associates\textsuperscript{22} reported two patients in whom a motile worm was observed creating tracks on the pigment epithelium of the retina. In both cases the motile worms were destroyed by laser photocoagulation. In each case, the morphologic appearance of the parasite corresponded with that of a nematode. They concluded that migrating retinal nematodes warrant inclusion in the differential diagnosis of unilateral pseudoretinitis pigmentosa.\textsuperscript{22}

Other rare ocular manifestations that have been reported include conditions such as optic neuritis,\textsuperscript{23,24} exudative retinitis, anterior uveitis,\textsuperscript{25} and corneal and lens invasion by the larvae.\textsuperscript{26}

\section*{DISTRIBUTION OF TOXOCARA CANIS}

\subsection*{HUMAN EPIDEMIOLOGY}

Until quite recently the actual prevalence of \textit{T canis} infection in humans was not known.\textsuperscript{8,27} Lacking an accurate serologic test, investigators were forced to rely on nonspecific and indirect testing techniques.\textsuperscript{28,29} Examination of human feces is of no use as a diagnostic technique since the larvae rarely develop to the adult stage in humans. While most patients develop eosinophilia during the migratory stage of the disease, by the time ocular involvement takes place eosinophilia or other systemic symptoms are not observed.\textsuperscript{16} In particular, eosinophilia typically is not detected in children with ocular manifestations of \textit{Toxocara}.

Woodruff\textsuperscript{30} developed a skin test that employed antigens obtained from adult worms to test for \textit{Toxocara} infestation. Numerous surveys employing skin tests have been reported in the literature. Woodruff\textsuperscript{30} in 1964, reported a 2\% prevalence of \textit{Toxocara} infection in the general healthy population.

Unfortunately, subsequent researchers questioned the reliability of the skin test. Collins and Ivey\textsuperscript{32} found that antigens produced from larvae were more sensitive than those produced from adult worms. However, specificity and reliability of even these improved procedures were inadequate. The attempts of Huntley and his co-workers\textsuperscript{28,29} to develop diagnostic tests employing hemagglutination and flocculation tests were unsuccessful, reporting positive results in only 30\% of the cases with visceral larvae migrans (VLM). In other papers, Huntley\textsuperscript{28,29} reported an elevated \textit{\(\gamma\)}-globulin factor (but only in less than one half of the affected patients) and a higher titer of anti-A and anti-B human blood groups among infected
patients as compared with controls. Two surveys conducted in England, using the fluorescent antibody test (FAT), reported an extremely low prevalence of infection among kennel workers,\textsuperscript{32} and a 5.8\% infection rate among healthy blood donors.\textsuperscript{33} Other tests employed include the bentonite flocculation test and the indirect hemagglutination test; however, problems of both sensitivity and specificity make these tests, and the other tests described previously, rather unreliable for the diagnosis of \textit{Toxocara} infection or of VLM.\textsuperscript{34} Apparently the antigens isolated are not specific enough or the antibodies could have receded significantly during the chronic phase of the disease, or both.

The development of rather precise and specific tests, such as the enzyme-linked immunosorbent assay (ELISA) test, has significantly enhanced the researcher's diagnostic capability and has made possible reliable epidemiologic surveys.\textsuperscript{35-39} In the ELISA test used in this research, larval rather than adult worm antigens were employed. This may explain the high specificity (92\%) and sensitivity (78\%) of the technique\textsuperscript{34} (Table I). Glickman and Cypress\textsuperscript{40} reported, in 1977 an 11\% positive ELISA test among animal hospital employees. More recently, de Savigny and his co-workers\textsuperscript{39} used the ELISA test on 922 healthy adults and reported a 2.6\% prevalence of elevated specific antibody levels. All 62 patients with nontoxocarial helminthic infection had antitoxocarial antibody levels within the range of values observed in healthy controls. All 13 patients with clinical toxocariasis had ELISA antibody levels above the 100th percentile of both the healthy population and the helminthic-infected group.\textsuperscript{39}

The only way to make an accurate diagnosis of infection by \textit{T canis} is by observing the larvae in biopsy tissues.\textsuperscript{5,13,14,41} Such identification is difficult because of the small size of the larva and the fact that it might be damaged owing to tissue reaction. Ashton\textsuperscript{17} believes the presumptive diagnosis is possible, based on typical tissue response, without requiring serial sectioning. The typical tissue changes consist of an eosinophilic abscess followed by a granuloma. This granuloma is often surrounded by a dense hyalinized capsule. Fibrinoid necrosis is usually found in the central area where the larva is often identified (Fig 3). In patients with VLM, a liver biopsy performed under direct observation has been recommended to obtain the biopsy material from an area of granulomatous reaction.\textsuperscript{5} These biopsies do require surgical intervention for diagnostic purposes only.

\textbf{DISTRIBUTION IN DOGS}

The reported prevalence of infection in dogs around the world varies widely, depending on the method of sampling and on the age of the dogs.
<table>
<thead>
<tr>
<th>Test</th>
<th>No. tested</th>
<th>Results</th>
<th>VLM cases Score (5-6)</th>
<th>Controls (Score 0-2)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Predictive value</th>
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<tbody>
<tr>
<td>IHA</td>
<td>61†</td>
<td>+</td>
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<td>3</td>
<td>18.2</td>
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<td>Total</td>
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<td>92.3</td>
<td>85.7</td>
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<td>39</td>
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<tr>
<td>Ouchterlony</td>
<td>62</td>
<td>+</td>
<td>15</td>
<td>2</td>
<td>65.2</td>
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<td>37</td>
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<td></td>
<td>Total</td>
<td>23</td>
<td>39</td>
<td></td>
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</tr>
</tbody>
</table>

*Six criteria: leucocytosis (WBC, 10,000/cu mm); eosinophilia (>10%); anti-A titer ≥1:400 or anti-B ≥1:200; IgG >2 SD above age and sex specific normal value; IgM >2 SD above age and sex specific normal value; and hepatomegaly.
†IHA test not available on 1 person.
‡BF test not available on 2 persons.
The prevalence of *Toxocara* infection in different geographic areas, as reported in the literature, is shown in Table II.\textsuperscript{4,42,43}

One study now in progress (Glickman and Schartz, unpublished data) suggests that *T canis* infection is most common in dogs from warmer climates and that children from these same areas have a higher intensity and prevalence of anti-*Toxocara* antibodies.

<table>
<thead>
<tr>
<th>Location</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcutta</td>
<td>83%</td>
</tr>
<tr>
<td>Philippines</td>
<td>77% (puppies only)</td>
</tr>
<tr>
<td>Australia</td>
<td>22%</td>
</tr>
<tr>
<td>Indiana</td>
<td>21%</td>
</tr>
<tr>
<td>Boston</td>
<td>20%</td>
</tr>
<tr>
<td>England</td>
<td>16%</td>
</tr>
<tr>
<td>Marseilles</td>
<td>14%</td>
</tr>
</tbody>
</table>

**Figure 8**

Development of serum immunoglobulin levels in the human being.
DISTRIBUTION IN SOIL

More importantly, soil samples in England indicate that 25% of the samples were positive for ova of *T.canis* and *T.cati*. These findings indicate that direct contact with domestic animals is not necessarily the only means of infection.

The review of the literature conducted previously points to several interesting lines of inquiry. What is the true prevalence of toxocariasis in a given sample population, and how does it vary among different age groups? Is there any correlation between environmental factors such as pica or exposure to puppies to the incidence of the disease? Does transplacental transfer of IgG provoke false-positive results during the first trimester of life? (Fig 9).

Before describing the research procedure used in this study, it is necessary to describe briefly the immunologic mechanism and the ELISA test.

IMMUNOLOGIC MECHANISM

The immune system consists of immunoglobulins complement, macrophages, and lymphocytes. The main role of the immune system is to protect the host from viruses, bacteria, fungi, and parasites. It acts also as a homeostatic mechanism to prevent the development of an autoimmune disease. Immunoglobulins, one of the important primary components of the immune system, are produced by plasma cells and are responsible for the host’s immunity. The association of antibody activity with the γ-globulin fraction of the serum was recognized by Tiselius and Kabat. After obtaining a high concentration of circulating antibody from rabbits, both Tiselius and Kabat used antigens to absorb the hyperimmune serum and observed the effect on the electrophoretic pattern. Surprisingly, after the removal of the antibody, only the γ-globulin fraction was markedly reduced (Fig 8).

It is now customary to use the general term immunoglobulin for those types of molecules that can be subdivided into different classes on the basis of their “backbone” structure rather than on their specificity for given antigens. Five major structural classes can be differentiated in humans: immunoglobulin G, M, A, D, and E, abbreviated IgG, IgM, IgA, IgD, and IgE. Initially, immunologists believed that each antibody molecule had a unique and totally specific antigen-binding site that could react with only one antigen. However, it has been shown recently that the antigen receptor sites in the molecules may combine with more than one antigen, although the binding affinity may vary considerably.

The biologic characteristics of the different immunoglobulins identified in man are briefly reviewed.
IMMUNOGLOBULIN G

Immunoglobulin G is the most prevalent antibody and constitutes approximately 80% of the total serum immunoglobulins. IgG is the most abundant Ig of internal body fluids. It is particularly present in the extravascular fluids where it combats microorganisms by binding to them, enhancing their phagocytosis. It also neutralizes bacterial toxins \(^45,48\) (Fig 9). Since it is able to cross the placenta, IgG provides the major line of defense against infection for the first few weeks of a newborn's life (Fig 8). Its response has a long latency, and remains elevated for long periods of time. When the host is exposed to the same antigen on a subsequent occasion, the immune response that occurs is due almost entirely to IgG. \(^45,48\) Thus, the biologic characteristics of IgG are of primary importance to our epidemiologic study since the ELISA test, as modified by Glickman, measures a specific antibody, namely anti-Toxocara IgG.

IMMUNOGLOBULIN E

Immunoglobulin E is the antibody responsible for immediate hypersensitivity, such as anaphylaxis. Since an extremely small proportion of all the plasma cells in the body are synthesizing this immunoglobulin, only extremely low concentrations of IgE are present in the serum. This immunoglobulin is cytophilic and binds rapidly to both mast cells and eosin-
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ophils. Contact with the antigen leads to degranulation of the mast cells, with relapse of vasoactive amines (eg, histamines). This process explains the symptoms of hay fever and asthma when patients with atopic allergy come in contact with the allergen (eg, grass pollen). The physiologic rate of IgE is still uncertain, but it has been noted that the serum level rises considerably after infection with certain parasites, particularly helminths. The contact of parasite antigens with mast cell-bound IgE antibody in the gut wall releases histamine, which is believed to promote the expulsion of the intruders.45,48

IMMUNOGLOBULIN M

Immunoglobulin M is produced early in immune response to a new antigen. It is found to be exclusively intravascular. Since IgM does not cross the placenta, it is rewarding to measure IgM antibodies to toxoplasmosis in newborns suspected of carrying the organism. The response of IgM consists of a rapid rise and fall in titer within two weeks. IgM antibodies are most efficient agglutinating and cytolytic agents. The iso-hemoagglutinins (anti-A and anti-B) are usually IgM.45,48

IMMUNOGLOBULIN A

The major immunologic component of the external seromucous secretions such as tears, nasal fluids, saliva, and sweat is IgA. The physiologic role of IgA is to defend the exposed surfaces against microorganisms. It has a relatively short serum half-life of four to six days.45,48

IMMUNOGLOBULIN D

The most recently discovered immunoglobulin is IgD. The serum concentration of this molecule is extremely low. It has a short half-life (two to eight days) in plasma, and it is present on the surface of lymphocytes, acting as cell surface receptors for the recognition and binding of antigens.53,57

IMMUNITY IN THE NEWBORN

While the ability of a newborn to reject grafts and to mount an antibody response is reasonably well developed at birth, the immunoglobulin levels, with the exception of IgG, are rather low. Acquired through placental transfer from the mother, IgG is catabolized with a half-life of 30 days. A fall in IgG titer over the first three months of life is registered,acerbated by the increase in the blood volume of the growing infant. Thereafter, the rate of
synthesis overtakes the rate of breakdown of maternal IgG, and the overall concentration increases steadily\textsuperscript{45} (Fig 8).

Since the other immunoglobulins do not cross the placenta, only traces are present in the circulation of the newborn.\textsuperscript{48} The exception is IgM. Low but significant levels of IgM in cord blood are present in the newborn. These are synthesized by the body, and reach adult levels after nine months.

**ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)**

Immunologic assays, because of their marked specificity and sensitivity, have been utilized in the past to identify antibodies of specific microbial excitors. By identifying antibodies and other microbial products (such as antigens and exoenzymes) in various body fluids, these techniques permit precise diagnosis. Some examples of these new diagnostic techniques are countercurrent immunoelectrophoresis (CIE), radioimmune assay (RIA), and ELISA.

The ELISA technique shares many similarities with both CIE and RIA techniques. Enzyme immunoassay starts from the premise that (1) an antigen or antibody can be fixed to a surface without loss of activity and (2) an enzyme can be attached to an antigen or antibody without loss of either immunologic or catalytic activity. The advantage of this immunochemical assay is that it has a simple colorimetric end-point, and it does not require the use of expensive equipment or radioisotopes. The ELISA technique takes advantage of chromogenic substrates, which are colorless prior to enzymatic stimulation but develop a colored product after enzymatic degradation. The intensity of the liberated chromogen, as determined colormetrically, is directly proportional to the concentration of antigen or antibody present in the test sample.

The ELISA technique has been used both experimentally and clinically for the diagnosis of infectious diseases as well as for noninfectious, autoimmune disease. Detection of hepatitis B surface antigen and the heat-labile enterotoxin of *Vibrio cholerae* and *Escherichia coli* has been performed successfully.\textsuperscript{49} Numerous viral agents have also been assayed by the ELISA technique. Also, antibodies for *Salmonella*, *Brucella*, *Treponema*, *Toxoplasma*, *Aspergillus*, and *Toxocara* organisms have been assayed by this exciting new technique.\textsuperscript{50}

ELISA is performed as follows: (Fig 10)
**ELISA**

**Indirect and Double Antibody Sandwich Method**

1. **Indirect**
   - Antigen adsorbed to vessel surface

2. Add test serum. Specific antibody, if present, will bind to antigen

3. Add enzyme labelled anti-immunoglobulin or anti species specific globulin

4. Add enzyme substrate. If enzyme-antibody added in step 3 bound to reactants of steps 1 and 2, then amount of substrate hydrolyzed (color change) equals amount of antibody in specimen

1. **Double Antibody**
   - Specific antibody adsorbed to vessel surface

2. Add suspension to be tested. If antigen is present, it will combine with antibody

3. Add enzyme labelled specific anti-immunoglobulin.

4. Add enzyme substrate. Amount of hydrolysis equals amount of antigen in test specimen.

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**Key**
- Antigen
- Antigen-antibody complex
- Antigen-enzyme labelled immunoglobulin complex
- Liberated chromogen
- Antibody
- Antibody-antigen complex
- Antibody-antigen-enzyme labelled specific anti-globulin complex
1. Tubes are coated with a known antigen.

The adult *T. canis* organisms were obtained from puppies and the adult *Ascaris suum* organisms from swine. The gravid uteri of the live worms were removed by dissection after having washed the worms repeatedly with 0.15 M NaCl. Eggs were removed from the uteri and placed in 1% formalin at room temperature for 21 days to induce embryonization. Using the Fairbairn method, free second-stage larvae were hatched from aliquots of the embryonated eggs. The embryonated eggs were homogenized separately in 0.05 M borate buffer (pH 8.6), using glass Ten-Broeck homogenizers. This mixture was then centrifuged at 2,000 g for 30 minutes. The supernatant fractions were used as antigens. The ELISA used in this study for the detection of antibodies in the serum of patients is a modification of the method developed by Engvall and Perlman. Microtiter plates were used to perform the assay. The plates were made of polystyrene and the wells coated with the appropriate antigen.

2. The serum to be tested is added, and the reactants are incubated. If the test serum contains the specific antibody to the antigen, it will attach to the fixed antigen.

3. An enzyme-linked anti-immunoglobulin (either whole or species-specific IgG) is added and will become fixed to any antibody that had combined with the antigen.

4. The enzyme substrate (either horseradish peroxidase or alkaline phosphatase) is then added, yielding, through hydrolysis, the chromogenic component that is measured colorimetrically. The intensity of the color reaction generated by the enzyme-anti-immunoglobulin (or antispecific globulin, IgG) will be proportional to the amount of antibody present in the original test serum.

The choice of a serologic diagnostic test for an epidemiologic survey should be based on the following criteria: (1) high sensitivity, to detect patients with the disease; (2) high specificity, to exclude patients without the disease; (3) low incidence of false-positive results; and (4) low incidence of false-negative results.

On the basis of these four criteria, the ELISA test was found to be most reliable. The reasons are as follows: (1) antigens prepared from *T. canis*-embryonated eggs have been found to be stage-specific; cross-reaction between the antigens used and the heterophile antibodies or isohemagglutinins does not occur; and (3) ELISA results have proved to be reproducible in different laboratories.
Toxocara Canis in Babies

The double diffusion in the agar (Ouchterlony) test is a close competitor. It also employs a purified larval antigen, and its specificity and predictive value of positive and negative results were quite similar to those of the ELISA test. Furthermore, the Ouchterlony test is less expensive, easier to perform, and requires minimal skills of interpretation when compared with the ELISA test.

Two other tests currently in use—indirect hemagglutination and bentonite flocculation—lack specificity and fail to distinguish toxocariasis from ascariasis, filariasis, or even trichinellosis34 (Table I).

The main disadvantage of the ELISA test is that it is not practical for small-scale, routine diagnostic procedures. However, the minimum amount of antigen required and the large number of tests that can be performed in a given time, makes it ideal for epidemiologic surveys.39 It is possible to perform 60,000 tests with the antigen extracted from one month’s culture of 106 larvae39 (Fig 11, A and B).

MATERIALS AND METHODS

Serum specimens were obtained from 671 healthy patients from low- and low-middle-class socioeconomic groups attending health centers in this geographic area. Specimens were obtained from 312 healthy infants aged 4 weeks to 2 years, and from 303 healthy adults aged 18 years to 80 years. An additional 56 samples were obtained from healthy children aged 25 months to 18 years.

Information about the patient’s socioeconomic background, the presence of pets in the household, age, sex, pica history, blood count, and other relevant data were prospectively collected and recorded in special questionnaires designed for this purpose. The adult patients and parents of the infants were interviewed.

In each case the blood was collected in a vacutainer tube, centrifuged twice, and separated immediately. The serum was frozen in a refrigerator-freezer. The samples were placed in a special fiberglass refrigerator and covered with dry ice to prevent thawing while being delivered to the central laboratory. The blood samples were analyzed by the Cornell University Veterinary Research Laboratories under the supervision of Lawrence Glickman, MD, assistant professor of epidemiology and preventive medicine.

The 312 infants aged 1 month to 2 years were examined at the Healthy Baby Clinic. The following tests were performed at the initial visit: (1) external evaluation of the conjunctiva, cornea, and pupils; (2) motility examination, and (3) a complete pediatric evaluation. The baseline labora-
Indirect ELISA for measuring antibody.
Microtitration plate method.
tory studies that were done at the first visit included the following: (1) hematology—hematocrit, WBC and differential count, and circulating eosinophil count; (2) serology—ELISA test; (3) parasitology—examination of feces for ova and parasites, using both direct and concentration methods and evaluation of blood smear for parasites.

In those cases with an ELISA test of over 1:2 dilution, some of the laboratory studies were repeated. The hematologic and parasitologic tests, as well as the blood smears for parasites, were performed in the clinical laboratories of our hospital. The hematocrit, WBC, and differential count were done by a technician. The WBC count was accomplished in a hemocytometer-counting chamber using a 1:10 dilution with 4% acetic acid. Hematocrit studies were performed using two heparinized microcapillary tubes, sealed in the bottom and centrifuged in a microcentrifuge for four minutes. The packed cells were measured, and the determinations of the two hematocrit tubes were averaged. To obtain the differential counts, the blood smears were stained with Wright stain and 100 cells were counted. The eosinophil counts were performed in a hemocytometer, using phloxine B as the diluent in propylene glycol. Examination of feces for ova and parasites was done by the technician in the parasitology section of the clinical laboratories. A direct smear was examined. A concentration method using formalin and ther (centrifuged for three minutes) was also employed. The supernatant fluid was discarded, and the sediment was examined for ova and parasites. The serologic studies were performed at the New York State College of Veterinary Medicine at Cornell University, under Dr Glickman. The ELISA test technique used in the study for the detection of antibodies in the serum of patients is a modification of the method developed by Engvall and Perlman. In some instances both the ELISA and the agar gel immunodiffusion tests were performed. The Cornell group interpreted the test titers as follows: (1) ELISA, any titer higher than 1:2 as consistent with past or present *T canis* infection; (2) a titer greater than 1:4 as diagnostic in a child with clinical ocular toxocariasis; and (3) a titer greater than 1:16 as diagnostic of visceral toxocariasis.

A sample of the more interesting positive results is provided in an appendix, which gives laboratory results, together with a short case history.

RESULTS AND CONCLUSIONS

The prevalence of positive serology (≥ 1:32) in infants up to age 6 months is 0%, while in infants aged 7 months to 12 months it is 1% (Table III).

The prevalence increases sharply to 6% among infants from 1 to 2 years of age, and in children from 2 years to 9 years of age, it increases even
### TABLE III: PREVALENCE OF POSITIVE SEROLOGY—AGE DISTRIBUTION

<table>
<thead>
<tr>
<th>Age, yr*</th>
<th>0-½</th>
<th>½-1</th>
<th>1-2</th>
<th>2-9</th>
<th>10-19</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>60-69</th>
<th>70-79</th>
<th>All ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive serology, % (≥ 1:32)</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>8.3</td>
<td>17</td>
<td>5.1</td>
<td>7.8</td>
<td>15</td>
<td>10.7</td>
<td>13.9</td>
<td>15</td>
<td>6.5</td>
</tr>
<tr>
<td>Number of positive points</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>43</td>
</tr>
<tr>
<td>Total number of points</td>
<td>74</td>
<td>119</td>
<td>115</td>
<td>72</td>
<td>24</td>
<td>55</td>
<td>51</td>
<td>40</td>
<td>28</td>
<td>43</td>
<td>20</td>
<td>641</td>
</tr>
</tbody>
</table>

*In 80-90 yr age group, there was one positive case in five patients = ½ or 20%.
further to 8.3%. Finally, in those from 10 years to 80 years of age, the prevalence is a surprising 10.1%.

In the total sample population used in this study the prevalence of positive serology (≥ 1:32) was found to be 6.5% (Table III).

The high prevalence of positive serology in this sample population (6.5%) could be explained on the basis of the tropical climate with its higher temperatures and high humidity. These factors favor the preservation of the eggs in the soil. Also, a higher prevalence of infected dogs has been found in warmer and more humid geographic areas (Glickman and Schantz, unpublished data).

The high prevalence and the intensity of positive serology titer in the adult population sampled suggest that these patients have been exposed to the *T. canis* organism on more than one occasion, giving an amnestic response.

Six out of eight (75%) of the infants with positive serology (≥ 1:32) had a definite history of pica, while only 7.0% (22/312) of the infants with negative serology had a definite history of pica. These findings are conclusive in establishing pica as an important mechanism in acquiring this disease (Table IV).

Seven out of eight (88%) of the infants with positive serology had a definite history of exposure to puppies, while only 21% (65/312) of the infants with negative serology had a definite history of exposure to puppies.

These findings are conclusive in establishing that exposure to puppies is an important mechanism in the epidemiology of this disease (Table IV).

Clinical and laboratory findings (Table IV) are presented for infants whose serum specimens were submitted for *Toxocara* serology.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>0</th>
<th>1-4</th>
<th>≥ 5</th>
<th>Probability, 0-4 vs -5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pica</td>
<td>3.2</td>
<td>4.2</td>
<td>75</td>
<td>6</td>
</tr>
<tr>
<td>Puppies</td>
<td>14</td>
<td>75</td>
<td>88</td>
<td>6</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0</td>
<td>11</td>
<td>88</td>
<td>7</td>
</tr>
<tr>
<td>WBC (&gt; 10,000 cu mm)</td>
<td>0</td>
<td>5</td>
<td>50</td>
<td>4</td>
</tr>
</tbody>
</table>

These data indicate that serum of some infants (less than 6 months of age, 2.8%) revealed a positive but low titer of anti-*Toxocara* IgG. In all probability these infants had been exposed to the embryonated eggs, since they had not yet started to crawl. The most logical explanation must be the transplacental penetration of IgG (Fig 8).
In each instance of low but positive titer, the mother's serum was examined, and in each one anti-Toxocara IgG was identified in titers higher than in their respective infants but less than 1:32. These findings in the serum of the mothers give additional support to the transplacental penetration hypothesis.

On the other hand, additional data obtained in this study do not seem to support transplacental penetration of anti-Toxocara IgG antibodies. This contention is based on the following factors:

1. In two instances, the mothers had positive serology tests of 1:32 and 1:128, respectively (diagnostic for having the systemic disease). In their respective infants, however, each age 2 months, no antibodies were found in the blood.

2. Infants with titers of 1:32 revealed other parasites in the feces and significant eosinophilia. Also, these were older infants (over 1 year), suggesting that the high titers were the result of primary infestation with *T. canis*.

Theoretically, one method to establish without question whether there has been transplacental penetration is by also preparing anti-Toxocara IgM antibodies. Since these IgM antibodies do not cross the placental barrier, if they were to be found in the infants it would indicate that they were infected after birth.

Unfortunately, it has been technically most difficult to obtain an anti-Toxocara IgM antibody bioassay. Nevertheless, this remains an important aspect to be elucidated fully in the future. At present, we can state, in regard to these low titers of anti-Toxocara IgG antibodies detected in these infants of less than 6 months of age, that there is no proved explanation available. We do not understand the true significance of these low titers in these infants. To the best of my knowledge, a study of this nature, correlating the titers of both infants and their respective mothers, has never before been performed.

The prevalence and intensity of positive ELISA titers increase significantly among the children who have started to crawl (from 0% before crawling to 5.2% after starting to crawl). This meaningful finding is no surprise, since these children have been exposed to the parasite in their own environment once they have started to crawl.

This study leads to the following important findings and conclusions:

1. The prevalence of toxocariasis in this geographic area varies in different age groups.

2. No conclusion could be drawn as to whether or not transplacental penetration of IgG antibodies occur and, if so, whether this penetration provokes a false-positive serologic
titer during the first trimester of life\textsuperscript{35,36} (Table V and VI).

3. Environmental factors such as pica and exposure to puppies are significantly related to the prevalence of the disease.

As the accuracy and reliability of the ELISA test and its greater applicability are established, the natural course of the disease could be studied more fully.

When is it advisable to perform an ELISA test for \textit{Toxocara canis}\textsuperscript{2}

1. To confirm the clinical diagnosis of ocular or systemic toxocariasis\textsuperscript{52}

2. To help exclude nematode endophthalmitis when suspecting a retinoblastoma\textsuperscript{53}

3. In unilateral pars planitis, especially in children\textsuperscript{19}

4. In unilateral uveitis of unknown causes in children\textsuperscript{54}

\begin{table}[h]
\centering
\caption{Mothers with Infants with ELISA Titer Over 1:2}
\begin{tabular}{lcc}
\hline
Mothers, & Age, & TEE ELISA & Infants, & Age, & TEE ELISA \\
patient no. & TEE ELISA & & patient no. & (mo) & titer \\
\hline
1 & 26 & 1:16 & 1 & 4 & 1:2 \\
2 & 19 & 1:16 & 2 & 16 & 1:4 \\
3 & 21 & 1:8 & 3 & 15 & 1:2 \\
4 & 23 & 1:16 & 4 & 4 & 1:2 \\
5 & 20 & 1:16 & 5 & 1/2 & 1:4 \\
6 & 23 & 1:4 & 6 & 22 & 1:32 \\
7 & 24 & 1:8 & 7 & 20 & 1:32 \\
8 & 22 & 1:16 & 8 & 30 & 1:128 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Mothers with Infants with Negative ELISA Titer: Control Mothers}
\begin{tabular}{lcc}
\hline
Mothers, & Age, & TEE ELISA & Infants, & Age, & TEE ELISA \\
patient no. & TEE ELISA & & patient no. & (mo) & titer \\
\hline
1 & 19 & 0 & 1 & 4 & 0 \\
2 & 22 & 1:2 & 2 & 3 & 0 \\
3 & 30 & 1:8 & 3 & 5 & 0 \\
4 & 27 & 1:2 & 4 & 2 & 0 \\
5 & 20 & 0 & 5 & 3 & 0 \\
6 & 24 & 0 & 6 & 4 & 0 \\
7 & 18 & 1:128 & 7 & 2 & 0 \\
8 & 21 & 1:4 & 8 & 3 & 0 \\
9 & 23 & 1:8 & 9 & 4 & 0 \\
10 & 29 & 1:32 & 10 & 2 & 0 \\
11 & 21 & 1:8 & 11 & 4 & 0 \\
\hline
\end{tabular}
\end{table}

It must be realized that the ELISA test, although a useful diagnostic adjunct, does not replace sound clinical judgment and experience.
Toxocara Canis in Babies

PRACTICAL IMPORTANCE OF USING A RELIABLE SEROLOGIC TEST FOR TOXOCARA CANIS CLINICALLY

If a physician finds a positive result in any given case and is confident that it is not a false-positive result, he or she will be more inclined to use potentially toxic larvicidal drugs.

In the situation of a suspected retinoblastoma, a false-positive test might result in failure to promptly enucleate an eye with a potentially fatal retinoblastoma. The ELISA test must be interpreted with extreme care because retinoblastoma and toxocariasis are not mutually exclusive: both conditions can be present simultaneously. However, a negative result is strong evidence against the child having been infected with T canis. In all of the three more frequently occurring ocular manifestations of Toxocara, ie, chronic endophthalmitis, posterior granuloma, and peripheral mass, confusion may arise in regard to differentiating such lesions from retinoblastoma. Even in the peripheral retinal mass category it must be remembered that Howard and Ellsworth reported that in 28% of their cases of retinoblastoma, the major growth of the tumor was localized in the periphery.

However, the specificity of the ELISA test for ocular toxocariasis has been increased with the use of vitreous sampling (Table VII). Biglan and co-workers found equal or higher anti-T canis antibodies titer in the vitreous than in the serum in five eyes with nematode endophthalmitis (Table VIII). These findings suggest the possibility of either a local intraocular antibody production or concentration in the vitreous.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>ELISA titer</th>
<th>Serum</th>
<th>Vitreous</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1:8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1:2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>1:4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE VIII: TOXOCARA ANTIBODY IN THE SERUM AND VITREOUS OF PATIENTS WITH PRESUMED OCULAR TOXOCARIASIS

<table>
<thead>
<tr>
<th>Patient no., age (yr), sex</th>
<th>Clinical observation</th>
<th>Duration of signs, mo</th>
<th>ELISA titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 6 M</td>
<td>Endophthalmitis</td>
<td>1</td>
<td>Serum 1:8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vitreous 1:64</td>
</tr>
<tr>
<td>2 7 M</td>
<td>Peripheral mass, retinal detachment</td>
<td>1</td>
<td>Serum 1:1,024</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vitreous 1:1,024</td>
</tr>
<tr>
<td>3 7 M</td>
<td>Endophthalmitis</td>
<td>1</td>
<td>Serum 1:16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vitreous 1:256</td>
</tr>
<tr>
<td>4 7 M</td>
<td>Peripheral granuloma, retinal detachment</td>
<td>2</td>
<td>Serum 1:16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vitreous 1:64</td>
</tr>
<tr>
<td>5 5 F</td>
<td>Vitritis, retinal detachment</td>
<td>1</td>
<td>Serum 1:64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vitreous 1:1,024</td>
</tr>
</tbody>
</table>

CASE REPORTS

CASE 1
A 16-month-old female infant was born following a nine-month gestation and normal delivery. This was the mother’s third pregnancy. No allergies or apparent eye diseases were present. At age 5 months the infant started crawling, and at age 9 months she began to walk without help. There were no pets either in her house or in the neighborhood. In her grandparents’ house, however, where she was cared for during the day, there were two dogs and ten cats. She played with them.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 38%; WBC, 9,900/cu mm; differential count (100 cells), normal; and eosinophils, 17%.

Serology.—ELISA test was positive up to 1:16 dilution.

Parasitology.—Examination of feces for ova and parasites revealed Trichiurus trichiura present in slight amount, using both the direct method and the concentration method.

CASE 2
A 4-month-old male infant had been born following a nine-month normal gestation and normal delivery. This was the mother’s third pregnancy and delivery. No allergies or apparent eye diseases were present. The infant did not crawl or walk as yet and was not placed on the floor. He was the brother of the infant in case 1. There were no pets in the home. In his grandparent’s house, however, there were two dogs and ten cats, but he visited there only occasionally.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 39%; WBC, 8,500/cu mm; differential count (100 cells), normal; and eosinophils, 1%.

Serology.—ELISA test was negative.

Parasitology.—Examination of feces for ova and parasites was negative, using both the direct method and the concentration method.
CASE 3
A 20-month-old female infant was born following a nine-month normal gestation and normal delivery. This was the mother's second pregnancy and delivery. No allergies or apparent eye diseases were present. At age 4 months she started to crawl, and at age 9 months she began to walk without help. While no dogs or cats were present around her house, there were three dogs and four cats in her grandparents' house where she spent the day. She did play with these pets.
Baseline laboratory studies performed disclosed the following values:
Hematology.—hematocrit, 32%; WBC, 9,400/cu mm; differential count (100 cells), normal; and eosinophils, 11%.
Serology.—ELISA test was positive up to 1:32 dilution.
Parasitology.—Examination of feces for ova and parasites revealed *T. trichiura* in moderate amount, using both the direct method and the concentration method.

CASE 4
A 22-month-old male infant had been born following a nine-month normal gestation and normal delivery. This was the mother's first pregnancy and delivery. He was allergic to heat and wool clothing. At age 3 months he was dehydrated as the result of a high fever, cold, and diarrhea. No apparent eye diseases were present. At age 6 months he started to crawl, and at age 12 months he began to walk.
Two pets, one cat and one dog, lived inside the house. There were also dogs and cats in his neighborhood. While the boy did not play with the neighborhood animals he did come in contact with them.
Baseline laboratory studies disclosed the following values:
Hematology.—hematocrit, 34%; WBC, 13,500/cu mm; differential count (100 cells), normal; and eosinophils, 14%.
Serology.—ELISA test was positive up to 1:32 dilution.
Parasitology.—Examination of feces for ova and parasites revealed *Necator americanus* ova present (only one ovum seen), using both the direct and the concentration method.

CASE 5
A 22-month-old female infant was born prematurely after seven months of gestation. She weighed 5 lb 4 oz. She was jaundiced at birth, but no Rh factor was involved. She was placed in an incubator for three days, and stayed in the hospital for one month until the jaundice resolved. This was the mother's eighth pregnancy and delivery. She had a history of frequent coughing and fever. No apparent eye diseases were present. A age 5 months she started to crawl, and at age 12 months she began to walk.
One dog lived inside the house, and the girl played with it.
Baseline laboratory studies disclosed the following values:
Hematology.—hematocrit, 37%; WBC, 8,900/cu mm; differential count (100 cells), normal; and eosinophils, 4%.
Serology.—ELISA test was positive up to 1:4 dilution.
Parasitology.—Examination of feces did not reveal any ova or parasites, using both the direct and the concentration method.
CASE 6
A 4-month-old female infant had been born following a nine-month normal gestation and normal delivery. This was the mother's first pregnancy and delivery. There was no history of allergies, and no apparent eye diseases were present. While the baby had not yet started to crawl, she was placed on the floor during the day while her mother did her household chores.

One puppy lived inside the house. In the neighborhood there were many dogs and cats, but they did not get inside the house.

Baseline laboratory studies disclosed the following values:
Hematology.—hematocrit, 40%; WBC, 9,200/cu mm; differential count (100 cells), normal; and eosinophils, 5%.
Serology.—ELISA test was positive up to 1:4 dilution.
Parasitology.—Examination of feces did not show any ova or parasites, using both the direct method and the concentration method.

CASE 7
A 6-week-old male infant had been born following a nine-month normal gestation and normal delivery. This was the mother's second pregnancy and delivery. There was no history of allergies, and no apparent eye diseases were present. The baby has not yet crawled. During his first six weeks of life there were two dogs, two puppies, and one cat in the house. In the neighborhood there were another two dogs and four cats, and these got inside the house.

Baseline laboratory studies disclosed the following values:
Hematology.—hematocrit, 33%; WBC, 7,900/cu mm; differential count (100 cells), normal; and eosinophils, 6%.
Serology.—ELISA test was positive up to 1:4 dilution.
Parasitology.—Examination of feces did not show any ova or parasites, using both the direct method and the concentration method.

CASE 8
A 16-month-old female infant had been born following a nine-month normal gestation and delivery by Caesarean section. This was the mother's second pregnancy and delivery. There was no history of allergies, and no apparent eye diseases were present. The infant never crawled on the floor; she was always confined to her play-pen. She first walked when she became 10 months of age. There were no dogs, puppies, or cats in the house. However, there were a few dogs and cats in her neighborhood, and she played with them on the patio.

Baseline laboratory studies disclosed the following values:
Hematology.—hematocrit, 36%; WBC, 7,900/cu mm; differential count (100 cells), normal; and eosinophils, 8%.
Serology.—ELISA test was positive up to 1:4 dilution.
Parasitology.—Examination of feces did not show any ova or parasites, using both the direct and the concentration method.
CASE 9
A 30-month-old male infant had been born following a nine-month normal gestation and normal delivery. This was the mother’s second pregnancy and delivery. There was no history of allergies. No apparent eye diseases were present. He began to crawl on the floor at age 3 months. He first stood up at age 6 months, and started walking without help at age 11 months. There were no dogs or cats present in the house. In his neighborhood there were six dogs and 12 cats, but they did not get inside the house. He did play outside on the patio, however, and came in contact with the animals.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 34%; WBC, 12,200/cu mm; differential count (100 cells), normal; and eosinophils, 18%.

Serology.—ELISA test was positive up to 1:128 dilution. Sera were positive as determined by agar gel immunodiffusion.

Parasitology.—Examination of feces for ova and parasites revealed T trichiura present in moderate amount, using both the direct and the concentration method.

CASE 10
A 13-month-old male infant had been born following a nine-month normal gestation and normal delivery. He was the brother of the infant in case 8. This was the mother’s second pregnancy and delivery. There was no history of allergies. No apparent eye diseases were present. He had been crawling on the floor since age 4 months, and he started walking when he became 12 months old. There were no dogs or cats present in the house, although there were six dogs and 12 cats in the neighborhood. However, they did not get inside the house and he did not play outdoors.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 36%; WBC, 8,100/cu mm; differential count (100 cells), normal; and eosinophils, 1%.

Serology.—ELISA test was negative.

Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.

CASE 11
A 4-month-old male infant had been born following a nine-month normal gestation and normal delivery. This was the mother’s third pregnancy and delivery. There was no history of allergies. No apparent eye diseases were present. The infant did not yet crawl, though he was placed on the floor to play. Three cats lived in the house. In his grandparents’ house, where he stayed during the weekends, there were four cats and one dog.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 31%; WBC, 9,100/cu mm; differential count (100 cells), normal except for some hypochromia; and eosinophils, 5%.

Serology.—ELISA test was positive up to a 1:2 dilution.

Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.
CASE 12
A 2-month-old male infant had been born prematurely after seven months of gestation. At birth he weighed 4 lb 6 oz. He was in the incubator for one week and stayed in the hospital for another week before going home. This was the mother’s third pregnancy and delivery. The infant did not have any allergies or apparent eye diseases. He did not crawl, and was not placed on the floor. There were no dogs or cats present in the home. There were dogs and cats in his neighborhood, however, and they did get inside the house.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 29%; WBC, 7,500/cu mm; differential count (100 cells), normal except for some anisocytosis, hypochromia, and polychromatophilia; and eosinophils, 2%.

Serology.—ELISA test was positive up to a 1:2 dilution.

Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.

CASE 13
A 6-month-old, male infant had been born following a nine-month normal gestation and normal delivery. This was the mother’s third pregnancy and delivery. There was no history of allergies. No apparent eye diseases were present. The baby started to crawl at age 4 months and was allowed to play on the floor. No dogs or cats were present in the home, nor were there any animals in the neighborhood.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 34%; WBC, 7,200/cu mm; differential count (100 cells), normal; and eosinophils, 1%.

Serology.—ELISA test was positive up to a 1:2 dilution.

Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.

CASE 14
A 15-month old, female infant had been born following a nine-month normal gestation and normal delivery. There was no history of allergies. No apparent eye diseases were present. The baby never crawled, but was always confined to her play-pen. She started to walk without help when she became 15 months of age. There were no pets in her household. However, there were dogs and cats in her neighborhood that did get into the house, although confined to the balcony. The mother reported that the infant did not play with them.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 36%; WBC, 6,500/cu mm; differential count (100 cells), normal; and eosinophils, 2%.

Serology.—ELISA test was positive up to a 1:2 dilution.

Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.
CASE 15
A 13-month-old, male infant had been born following a nine-month normal gestation and normal delivery. This was the mother’s first pregnancy and delivery. No allergies or apparent eye diseases were present. The baby crawled at age 7 months. There were no pets in his house. In his grandparents’ house, however, there were two dogs, he visited there only occasionally and played with the dogs.

Baseline laboratory studies disclosed the following values:

- Hematology.—hematocrit, 37%; WBC, 9,900/cu mm; differential count (100 cells), normal; and eosinophils, 9%.
- Serology.—ELISA test was positive up to a 1:4 dilution.
- Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.

CASE 16
A 12-month-old, male infant had been born following a nine-month gestation and normal delivery, except for a hip fracture during delivery. This was the mother’s third pregnancy and delivery. No allergies or apparent eye diseases were present. The infant did not crawl as a result of his hip fracture. There was a puppy in his house, and he played with it.

Baseline laboratory studies disclosed the following values:

- Hematology.—hematocrit, 36%; WBC, 12,800/cu mm; differential count (100 cells), normal; and eosinophils, 13%.
- Serology.—ELISA test was positive up to a 1:32 dilution.
- Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.

CASE 17
A 13-month-old, female infant had been born following an eight-month normal gestation and normal delivery. This was the mother’s first pregnancy and delivery. No allergies or apparent eye diseases were present. The baby crawled at age 12 months and had started to walk. There were two puppies in a neighbor’s house, and they came to this boy’s house. The boy played with them.

Baseline laboratory studies disclosed the following values:

- Hematology.—hematocrit, 36%; WBC, 8,500/cu mm; differential count (100 cells), normal; and eosinophils, 4%.
- Serology.—ELISA test was positive up to a 1:4 dilution.
- Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.

CASE 18
An 18-month-old, male infant had been born following a nine-month normal gestation and normal delivery. This was the mother’s fourth pregnancy and delivery. No allergies or apparent eye diseases were present. The baby crawled at age 5 months and walked at age 12 months. The infant played with dirt outside and had two puppies in his home.
Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 38%; WBC, 12,500/cu mm; differential count (100 cells), normal; and eosinophils, 13%.

Serology.—ELISA test was positive up to a 1:32 dilution.

Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.

CASE 19
A 4½-month-old, female infant had been born following a nine-month gestation and had been delivered by Caesarean section. This was the mother's first pregnancy and delivery. No allergies or apparent eye diseases were present. The infant did not crawl or walk and was not placed on the floor. There were no pets in the house.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 30%; WBC, 8,800/cu mm; differential count (100 cells), normal except for slight anisocytosis, hypochromia, and poikilocytosis.

Serology.—ELISA test was positive in a 1:2 dilution.

Parasitology.—Examination of feces for ova and parasites was negative, using both the direct and the concentration method.

CASE 20
A 22-month-old, female infant had been born following a nine-month normal gestation and normal delivery. This was the mother's third pregnancy and delivery. No allergies or apparent eye diseases were present. The infant crawled at age 5 months and started to walk at age 10 months. There was one puppy in the house, and the infant played with it.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 32%; WBC, 9,900/cu mm; differential count (100 cells), normal, with some hypochromia and anisocytosis present; and eosinophils, 7%.

Serology.—ELISA test was positive in a 1:16 dilution.

Parasitology.—Examination of feces for ova and parasites was negative, using both the direct method and the concentration method.

CASE 21
A 17-month-old, male infant had been born following a nine-month normal gestation and normal delivery. This was the mother's first pregnancy and delivery. No allergies or apparent eye diseases were present. The infant crawled at age 3 months. He had walked without help since age 9 months. There were no pets in his house. He played outside with dirt, and when smaller he ate dirt.

Baseline laboratory studies disclosed the following values:

Hematology.—hematocrit, 35%; WBC, 9,500/cu mm; differential count (100 cells), normal; and eosinophils, 9%.

Serology.—ELISA test was positive in a 1:16 dilution.

Parasitology.—Examination of feces for ova and parasites revealed the presence in slight amount of *T trichiura*, using both the direct method and the concentration method.
Toxocara Canis in Babies

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REFERENCES