ENDEMICITY OF TOXOPLASMOSIS IN COSTA RICA

TRANSMISSION BETWEEN CATS, SOIL, INTERMEDIATE HOSTS AND HUMANS

J. K. FRENKEL and A. RUIZ


Toxoplasma infection can be transmitted to humans either by ingestion of tissue cysts in meat or by ingestion of oocysts in cat feces or fecally contaminated soil. Since meat is traditionally well cooked in Costa Rica and the authors were able to exclude meat and eggs from playing a role in transmission, they postulated that ingestion of oocysts should entirely explain transmission. To test this hypothesis, they studied seven populations in Costa Rica and serologically tested 883 people between 15 and 26 years of age. By interviews and surveys, they determined mode of living, soil contact, cat contact, and cat density. The infection rate in cats was studied by stool and serologic examination, and soil availability for defecation was determined from aerial photographs. Antibody was taken as indicating infection and was found to be acquired most actively in children and young cats. In a retrospective analysis, antibody prevalence in humans correlated highly with individual cat contact (both owned and stray), with cat density, with living in houses with wood floors and a crawl space underneath, and with living with cats on a cement floor. Antibody prevalence in humans correlated negatively with soil availability to cats for defecation and with soil contact by humans, suggesting that oocysts may be transmitted optimally near houses in areas where most terrain is covered by asphalt and concrete and little soil is available for defecation or for human soil contact. Cats acquire infection by eating intermediate hosts; 16% of sparrows, 3.5% of mice and 12.5% of rats have been found infected. Favoring transmission are young and non-immune cat populations, shade and moisture, older intermediate hosts, a high percentage of which have become infected, and concentration of houses in city blocks. Transmission is diminished when houses are dispersed or arranged along a road in an open rural setting, permitting dispersal of cat fecal deposits.

Carnivora; cats; disease reservoirs; feces; soil; toxoplasmosis; zoonoses

Several seroepidemiologic studies of Toxoplasma infection in humans were conducted in Central America before the role of cats as shedders of oocysts was appreciated. In Costa Rica (1, 2), El Salvador (3), Panama (4), and, in fact, all of the Central American countries, high prevalence and incidence rates of anti-

1 Department of Pathology and Oncology, University of Kansas Medical Center, Kansas City, KS 66103 (reprint requests to Dr. Frenkel).
2 Catedra de Patologia, Hospital Mexico, Facultad de Medicina, Universidad de Costa Rica, San Jose, Costa Rica.

Supported by a grant from the Ford Foundation to the University of Kansas for Cooperative Research in Central America, by Grant 02-07-05-001 from the University of Costa Rica, Grant 75-78 from the Consejo Nacional de Investigaciones Científicas y Tecnológicas, Grant INT74-04263 and INT78-21378 from the National Science Foundation, and in part by Grant AI-07489 from the National Institute of Allergy and Infectious Diseases.

The authors thank Drs. John Davis and Curt Conley of the Kansas Geologic Survey for image analysis of aerial photographs, Laura Willhite for...
ENDEMICITY OF TOXOPLASMOSIS IN COSTA RICA

body were found in the human population (2). After cats were recognized in the laboratory as definitive hosts of Toxoplasma (5–12), their epidemiologic role in nature had to be assessed under several circumstances. Wallace (13–15) had already demonstrated on Pacific atolls that without cats, antibody to Toxoplasma was essentially absent in resident humans, rats and domestic animals. Since antibody prevalence rates in humans were several times higher than in the United States, Costa Rica appeared to be a good area in which to examine the role and importance of cats in transmission. Consequently, we studied wild Central American felines as possible vectors (16), the persistence of oocyst infectivity in soil (17), and the infection in domestic cats, intermediate hosts, and humans (18–20). Recovery of Toxoplasma from naturally contaminated soil (21) and from attics of houses (22) was reported.

The other principal mode of transmission, the ingestion of meat containing tissue cysts of Toxoplasma (23) was found to play no role in Costa Rica (20).

The present paper attempts to integrate environmental factors, rural and urban living, and especially the soil reservoir, in an attempt to explain the transmission chain in Costa Rica. As we are studying the endemcity of Toxoplasma infection by means of cumulative seropositivity, it should be recalled that actively acquired IgG antibody measured by dye and IFA tests generally persists in humans, cats and rodents for months or years and possibly a lifetime. In birds, where measurement of antibody may be difficult, the generally persistent infection was determined by isolation of Toxoplasma in mice.

Materials and methods

General design. Seven human populations were studied from two rural and three urban centers (table 1). The locations of the towns studied are shown in figure 1. Toxoplasma infection was assessed by serum antibody measurements. Sera were obtained from students, blood donors, job applicants, and patients who came to clinic for various reasons. Selection was by feeling well enough to answer questions, and by age. Since we knew from Walton's study (2) that the most active period of seroconversion was the first 15 years of life, we elected to restrict our sample to 883 people between 15 and 26 years of age. Figure 2 shows the antibody prevalence rates we obtained in comparison with those observed by Walton (2).

Questions were asked about present and past residences, number of years lived at each, mode of living at each residence, occupation, preparation of meat and eggs, history of illness, and contact with animals and soil.

Soil contact was graded from 0 to 4 as perceived by the interviewer, based on occupation, sex, activity in field and gardens, and on answer to questions. "No soil contact" indicates that the person does not usually touch soil; "1" indicates occasional or light contact as with flower pots; "2" is equivalent to flower gardening; "3" is equivalent to vegetable gardening; and "4" that the person regularly works as a farmer.

"Owned" cats that were given food and that entered the house were recorded for each residence, and cat contact for the lifetime of the individual was assessed. In addition, the presence was noted of stray cats that were not fed and that found refuge in the crawl space under the house or in the false attics. Details of the human studies were published elsewhere (20).

Cats were enumerated by house for each person as described above, and also in special cat surveys, to assess the density of cats. Owned and feral cats, where
the cats lived, their source of food, and defecation sites were noted. The roaming patterns of distinctively marked cats were studied in selected localities. A total of 237 cats were purchased from seven towns, weighed, measured, and tested for antibody to *Toxoplasma*. The colonic contents of each cat were subjected to a flotation procedure to concentrate oocysts if present; the concentrate was examined microscopically, was freed of bacterial contaminants in 2 per cent sulfuric acid to aid sporulation, and, after neutralization, injected into mice. The diagnosis of *Toxoplasma* oocysts was based on the development of *Toxoplasma* antibody in these mice and in passage mice. Details of the cat studies were published elsewhere (18).

Of intermediate hosts, 202 *Mus musculus*, 120 *Rattus norvegicus*, 106 sparrows (*Zonotrichia capensis*), and 50 chickens from the suburbs of San Jose, Costa Rica, were examined for *Toxoplasma* infection, both serologically and by passage of tissue into laboratory mice. Of possible transport hosts, 120 earthworms, 600

---

**FIGURE 1. Map of Costa Rica showing towns studied.**
Musca domestica, and 322 roaches of four species were examined by injection into mice. Details are described elsewhere (19).

Antibody determination of numerically coded sera of humans, cats, intermediate hosts, and the inoculated laboratory mice was by the dye test of Sabin and Feldman, slightly modified, as described (24, 25). The tests of human and cat sera were done in duplicate in both Costa Rica and Kansas using two-fold dilutions, starting with 1:2 (final dilution). We regarded a titer of 1:2 or greater as positive. Discrepancies were clarified by repeat testing until agreement was achieved; alternately, the human or cat was excluded. Sera of intermediate hosts and laboratory mice were tested only in Costa Rica; diagnosis of infection depended on presence of antibody in original and passage mice, supplemented by finding Toxoplasma organisms in the brains of surviving mice, or the tissue imprints of animals that died. Details of the procedures were given elsewhere (18–20).

Evaluation. Data were transferred to IBM cards and analyzed for relationships by means of computer programs written specifically for this purpose. To determine the significance of differences, Chi square tests were performed with Yates correction for small numbers. Data from the various populations were analyzed by multiple regression (BMDP2R) and univariate correlations (BMDP9R) (Health Sciences Computing Facility, University of California, Los Angeles, CA, revised November, 1979).

Topographic characteristics of localities. Aerial photographs were purchased from the Instituto Geografico Nacional de Costa Rica. The amounts of open land, wooded areas, and areas occupied by houses were determined by means of a VP-8 image analyzer (Interpretation Systems, Inc., Overland Park, KS). The popu-
lation density, number of houses in each locality, and the type of flooring prevalent were derived from the Censo Nacional de Viviendas, Costa Rica (26). Altitudes were obtained from maps and bulletins of the Instituto Geográfico Nacional de Costa Rica. As mentioned, the locations of the towns studied are shown in figure 1.

Meteorologic data. Precipitation per year, and, in some instances, averages per 10 years, hours of sunlight, relative humidity, and temperatures were obtained from the Instituto Meteorologico, San Jose, Costa Rica.

RESULTS

Density of cat populations. The estimated average number of cats per house varied from 1.5 in San Ignacio to 3.8 in San Jose (table 1). This value can be multiplied by the number of houses in the population area. When the number of houses per km² is taken into account (26), one can calculate the approximate number of cats per km² in each population center. Because each cat visits an average of six houses (18), we divided these figures by six for all of the towns except San Ignacio, which has an essentially linear distribution of houses. Accordingly, the population density of cats was estimated to be from 13–31/km² in San Ignacio and 350/km² in Atenas, two rural centers, to 1750–3330/km² in San Jose and Limon. Thus, there may exist a 10- to 250-fold greater population density of cats in some urban than some rural areas.

Concentration of shade. The survival of oocysts is aided by shade (17, 27). Hence, the areas covered by trees or houses were estimated from the aerial photographs and compared with the open areas (table 1). In the rural sites, shade areas varied between 17 and 70 per cent. The largely open areas, since exposed to the sun, would be less suitable for survival of oocysts. In the cities, open areas were under 10 per cent, but shade area under trees was about 40 per cent. In addition, houses and streets accounted for 40–60 per cent of the area, with shade available under all houses with wooden floors, and some of the houses with cement floors.

Opportunities for cats to defecate. Analysis of the aerial maps showed that at least 90 per cent of the rural terrain is available for defecation, either as open agricultural land or wooded areas. However, in towns most of the surface is covered by houses or streets and only 10–40 per cent of the area is covered by trees or shrubbery. Even some of the patios of houses with shade trees are covered in part by cement, further restricting the areas of loose soil where cats prefer to defecate. For statistical analysis, the areas covered by trees and shrubs and the "open" areas were combined, but in San Jose only 10 per cent of the surface was considered soil available for defecation. Crawl spaces, where cats can defecate, exist under all houses with wooden floors. Such houses occur in both rural and urban areas, and are enumerated separately (table 1). Spaces also exist under houses with cement floors; however, they are usually not accessible to cats.

Moisture and rainfall. The survival of oocysts was also found in other studies (17, 27) to be aided by moisture. Yearly precipitation ranged from 180 cm in Atenas to 399 cm in Quesada (table 1). Relative humidity varied between 80 and 100 per cent during the rainy season, April to November. The daytime air humidity varies daily between 55 and 90 per cent during the dry months in most of the country, and dew is formed on the ground almost every night.

Intermediate host density. No determination of intermediate host density was undertaken since we did not develop an adequate method to do so. However, it was our impression that, due to the greater concentration of domestic rodents in urban areas, more food was available for cats in urban than in rural areas, and that this in part supported the greater
### Table 1
Quantitative relationships between physical circumstances, cats and humans relevant to the transmission of Toxoplasma in nine Costa Rican localities

<table>
<thead>
<tr>
<th>Quantitative relationship</th>
<th>Rural centers</th>
<th>Urban centers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>San Ignacio</td>
<td>Atenas</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>1100</td>
<td>700</td>
</tr>
<tr>
<td>Precipitation (m) per year*</td>
<td>2.55</td>
<td>1.81</td>
</tr>
<tr>
<td>Surface area (%) Trees, shrubs</td>
<td>70</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>&lt;1</td>
<td>10</td>
</tr>
<tr>
<td>Houses, streets</td>
<td>83</td>
<td>30</td>
</tr>
<tr>
<td>Present kitchen floor (%)</td>
<td>70</td>
<td>45</td>
</tr>
<tr>
<td>Cats</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td>Per house</td>
<td>1.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Per km²</td>
<td>13–31</td>
<td>350</td>
</tr>
<tr>
<td>Number tested for Toxoplasma</td>
<td>31</td>
<td>41</td>
</tr>
<tr>
<td>Weight (mean) (kg)</td>
<td>0.82</td>
<td>0.86</td>
</tr>
<tr>
<td>% seropositive</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>% shedding</td>
<td>58</td>
<td>20</td>
</tr>
<tr>
<td>Human population</td>
<td>4900</td>
<td>3700</td>
</tr>
<tr>
<td>% with cat contact</td>
<td>70</td>
<td>85</td>
</tr>
<tr>
<td>Human survey</td>
<td>81</td>
<td>64</td>
</tr>
<tr>
<td>Cat survey</td>
<td>105</td>
<td>115</td>
</tr>
<tr>
<td>People bled and questioned</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Soil contact index</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Seropositivity (%)</td>
<td>51</td>
<td>56</td>
</tr>
<tr>
<td>All (y₁)</td>
<td>47</td>
<td>29</td>
</tr>
</tbody>
</table>

* 10-year annual average.
† One year.
‡ People from different areas of San Jose.
§ p < 0.005.

population of urban cats. Ground-feeding birds such as the rufous-collared sparrow (Zonotrichia capensis) were numerous in cities, suburbs and rural areas. The wild bird and rodent fauna in Costa Rica is known to differ according to elevation. The infection rate among intermediate hosts was 3.5 per cent in mice, 12.5 per cent in rats, and 16 per cent in sparrows (19), and suggests that a cat may eat an infected animal every 7–33 days, assuming that either one mouse, rat or sparrow was consumed per day.

Infection rate of cats. As illustrated in table 1, the seropositivity varied from 29–64 per cent (mean 37 per cent) in rural areas, and from 45–75 per cent (mean 47.5 per cent) in urban areas. In most localities, a good correlation is found between seropositivity and oocyst shedding as expected from the average weight of cats (figure 3).

Association of people with cats. This was evaluated in both the human and the cat surveys, and varied from 48 per cent in the population from the Plasmapheresis Center in San Jose (human survey) to 87 per cent in metropolitan San Jose (cat survey) (table 1). Such differences may not be statistically significant since they reflect both the prevalence of cats and the awareness by people. This contact or awareness appears to be greater in rural than urban areas.

Type of floor in kitchen. The type of floor in the kitchen of the last residence was enumerated for each locality. As shown in table 1, dirt floors are more common in rural areas, and wood or cement floors in urban areas. The presence of a wooden floor in the kitchen is associated with a wooden floor under the remainder of the house in most instances, and with a crawl space below, which provides a good refuge for cats and a place for them to defecate. The correlation coefficient (r) between wooden floor and cats/km² was 0.79.

Soil contact of people. The rural population shows more soil contact than the urban population (table 1). However, this
ENDEMICITY OF TOXOPLASMOSIS IN COSTA RICA

TABLE 2
Univariate correlation coefficients (r) between environmental factors and infection of humans by Toxoplasma in Costa Rica

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Human antibody prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (y)</td>
</tr>
<tr>
<td>Dirt floor</td>
<td>-0.75*</td>
</tr>
<tr>
<td>Wood floor</td>
<td>0.91**</td>
</tr>
<tr>
<td>Soil contact (human)</td>
<td>-0.64</td>
</tr>
<tr>
<td>Cats per house</td>
<td>0.50</td>
</tr>
<tr>
<td>Cats per km²</td>
<td>0.91**</td>
</tr>
<tr>
<td>Soil availability to cats</td>
<td>-0.63</td>
</tr>
</tbody>
</table>

* >0.75 significant at 5%.
** >0.87 significant at 1%.

contact correlates negatively with the infection rate (table 2).

Human antibody prevalence

In San Jose, Limon and Atenas, cat contact could be correlated positively with increased antibody prevalence in humans, which in turn could be positively correlated with the amount of shade and the proximity of defecation by cats to human habitation. However, in San Ramon and San Ignacio, human antibody prevalence was unusual. These towns showed a low to intermediate (50–60 per cent) prevalence rate of Toxoplasma antibody in humans, with a rate not different in those with or without contact with cats. Although the differences were not statistically significant, San Ramon and San Ignacio were the only human groups surveyed where cat contact did not increase antibody rates by at least 10 per cent.

San Ramon. San Ramon is a town of 10,000 with a population density of 7500/km². The cat density per house and per km² was high. Cement and wooden floors predominated, and 20 per cent of households reported defecation by cats inside the house (18). Here, the highest antibody prevalence in cats of all localities sampled (75 per cent) was associated with the lowest oocyst shedding rate observed (8.3 per cent). The mean weight of cats was 2.1 kg, the highest of all groups. We regard the 24 cats examined to be a valid sample, with few kittens present. This suggested a saturation of the area with oocysts and little difference in human exposure whether cats were present or not.

San Ignacio. San Ignacio is a rural district seat, with a population density of 1100/km² in the center and 50/km² in the periphery, where 85 per cent of houses are located. The cat densities per house and per km² were low (table 1), and oocysts were presumably widely dispersed. This resulted in the lowest antibody prevalence in cats and humans. The unusually high oocyst shedding rate suggested a temporary epidemic, which was accidentally observed when 23 of 30 young kittens were obtained within a three-week period in June–July, 1974.

Comparison of seven populations. Antibody prevalence rates for the seven localities from which human data are available were correlated by stepwise multiple regression with many of the independent measurements discussed above and noted in table 1. Antibody prevalence, the dependent variable y, was calculated for the total population (y1) and for those with (y2) and without (y3) cat contact. In the following equations, the correlation coefficient is given by multiple $R^2$, and the significance levels (F) at the 1 per cent level by **, and the 5 per cent level by *:
\( y_1 = 53 + 0.23 \text{ wood floor} - 0.15 \text{ oocysts shed} + 0.003 \text{ cats/km}^2 \)
\( (R^2 = 0.99; F = 48^{**} \text{ for wood floor}; 25^{*} \text{ for oocysts shed}; 13^{*} \text{ for cats/km}^2) \)

\( y_2 = 61 + 0.007 \text{ cats/km}^2 - 0.089 \text{ soil availability to cats} \)
\( (R^2 = 0.97; F = 46^{**} \text{ for cats/km}^2; 8.6 \text{ for soil availability}) \)

\( y_3 = 46 + 1.0 \text{ wood floor} - 6.7 \text{ soil contact} + 1.6 \text{ cats/house} - 0.015 \text{ cats/km}^2 \)
\( (R^2 = 0.99; F = 1837^{*} \text{ for wood floor}; 241^{*} \text{ for soil}; 15 \text{ for cats/house}; 558^{*} \text{ for cats/km}^2) \)

Correlations between the measurements listed in table 1 and human antibody prevalence were determined, the more significant of which are shown in table 2.

**WORKING HYPOTHESIS ON THE TRANSMISSION OF TOXOPLASMA IN COSTA RICA**

Based on the findings of this study and our previous studies (18–20), we can postulate that there are three main reservoirs of Toxoplasma in the transmission chain: cats, soil and intermediate hosts (figure 4). Man and other carnivores are shown in side-spurs as biologically non-functional intermediate hosts since they are rarely eaten. Each reservoir is influenced by several modifiers.

**Cats.** The cat population density per house and per km\(^2\) determines in general the concentration of cat feces and the potential availability of the infectious agent. Most of the cats were strays that visited up to a dozen neighboring houses while hunting and foraging. After primary infection, cats are capable of shedding millions of oocysts in their feces, which are usually deposited in loose soil and covered lightly (23). The age composition of the cat population and its character, whether stray or owned, and the type of supplementary food given all participate in determining the rates of oocyst

\[\text{TISSUE CYSTS} \quad \text{BIROS MICE} \quad \text{SHEEP} \quad \text{MAN} \]
\[\text{RATS} \quad \text{PIGS} \quad \text{LAROE, CONCENTRATED} \]
\[\text{YOUNG NON-IMMUNE POPULATION} \quad \text{OCCYSTS} \]
\[\text{MOISTURE, SHADE} \quad \text{DESISSICATION, HEAT} \]

**FIGURE 4.** Working hypothesis on the transmission of Toxoplasma in Costa Rica. There are three important reservoirs of Toxoplasma: cats, soil and intermediate hosts. Fecal or soil contamination transmits the oocysts from cat feces, and carnivorism the tissue cysts from the intermediate hosts. Man and other carnivores are essentially dead-end hosts. (The interrupted arrow linking carnivores and man refers to conditions thought to exist in the United States and Europe.)
shedding. Cats shed oocysts mainly after the first infection, which occurs in Costa Rica after kittens start to eat solid food at about four weeks of age. A cat population with an average age of three months would be comprised of many cats that shed oocysts, whereas in a 1–2-year-old population, most cats would be immune and little oocyst shedding would be found (figure 3). It should be noted that Costa Rican cats are more precocious than those in Hawaii, which start to acquire infection mainly after six months (28).

Soil. The soil reservoir preserves oocysts well if moist and shaded, whereas exposure to sun and drying shortens survival of oocysts (17, 27). With the unavailability of soil, cats defecate on solid surfaces such as the floors of kitchens, beneath furniture, or in the false attics of houses, as reported by 35 per cent of homeowners (18). The relative humidity in the localities sampled reaches around 90 per cent once every 24 hr, permitting fecal specimens to remain infectious for 24–98 days in simulation experiments (22). Whereas earthworms will mix oocysts with soil, roaches and flies have access to cat feces in soil and on the floors of houses and might serve as transport hosts, as shown experimentally (29–32). However, we have never isolated Toxoplasma from these potential transport hosts. The role of dung beetles, examined by others (33, 34), has not been investigated in Costa Rica.

Intermediate hosts. The intermediate hosts acquire infection by ingestion of sporulated oocysts from soil. We found to be infected 3.5 per cent of mice, 12.5 per cent of rats, and 16 per cent of sparrows (19). Older rodents and bird populations are more likely to be infected than younger ones since most animals, once infected, remain infected, with infectious bradyzoites in cysts persisting until the animal is eaten by cats or another carnivore. Sheep, pigs and chickens are commonly infected and may serve to transmit Toxoplasma to kittens that are fed scraps of meat (19).

Humans. Humans are intermediate hosts outside of the main transmission chain. Children start to become infected when they play in soil and sand, at an age when they are particularly prone to place their (soiled) fingers into their mouths. We observed an antibody prevalence of 51 per cent in 15-year-olds, which rose to 77 per cent in those with first cat contact during the first 15 years (20). Walton (2) and Remington et al. (3) have illustrated infection rates from neighboring Central American countries of up to 6 per cent per year in early childhood. These rates suggest that humans become infected mainly from soil contaminated with cat feces and less from direct contact with the fur of cats. The character of stray cats in Costa Rica does not invite playing or petting by young children. A crawl space under houses with wooden floors provides a refuge for cats, and consequently appears to increase antibody prevalence in humans. In cities, soil contact scores become unimportant in the presence of cats (20). Association with cats increases antibody prevalence markedly, especially when combined with a cement floor in the kitchen. In view of the greater density of the cat population in urban areas and the limited area available for defecation due to paving and houses, the oocyst concentration in soil near urban houses should be much greater than in rural areas, where cat feces are greatly dispersed and not as commonly shaded. Positive values for cats per km² and negative values for soil availability correlate significantly with human infection with cat contact (y) and support this hypothesis (table 2).

Congenital toxoplasmosis in man. Congenital toxoplasmosis in man (and probably in most animals) is a biologically rare, but medically and economically important event; it probably plays little or no role in the endemicity of infection (23). However, in spite of the high rate of
infection-immunity in women, the seroconversion rate in women of childbearing age (15–35 years) is higher in Costa Rica than in the United States (figure 2).

**DISCUSSION**

Our data support the hypothesis that oocyst shedding by susceptible cats, with suitably moist and shaded soil conditions, and in the presence of a sufficient number of intermediate hosts, can explain the infection rate of humans. This is especially true for young children during the crawling and dirt-eating stages. This hypothesis, developed after the identification in the laboratory of cats as the final host of *Toxoplasma*, has not been strongly supported by epidemiologic studies in North America. On several Pacific islands, Wallace (13, 15) demonstrated that the presence and absence of cats is qualitatively related to the presence and absence of antibody to *Toxoplasma*. In the United States, although consistent data were obtained in a survey in Seattle and in a seroepidemiologic study in Alaska (35, 36), no correlation whatsoever was found in a study of the association of cats and *Toxoplasma* antibody in humans (37). There are more studies in which no close relationship between cats and humans with *Toxoplasma* antibody was found than the reverse (20). These must be analyzed for adequacy of investigating *Toxoplasma* infection and contact with cats and for differentiation of the main confounding factor, acquisition of infection by the ingestion of undercooked meat containing tissue cysts of *Toxoplasma* (23).

Serologic evaluation of cumulative *Toxoplasma* infection depends on antibody titers that tend to be low when compared to titers considered in the differential diagnosis of clinical illness. Particularly in the United States where a titer of 1:16 can be the most frequent titer in a survey of a normal population (20), many studies have sacrificed sensitivity by disregarding such low titers that might constitute 30 per cent or more of the sample. Exclusion of low titers has become a habit, probably since they are rarely utilized clinically. To our knowledge, no non-specific titers have been demonstrated in man. Because the available evidence indicates that specificity errors from cross-reactions would be absent, or smaller than the sensitivity error from disregarding low titers, we opted to include all titers.

Determination of cat contact depended usually on "presence of cats on property," "ownership," or "having looked after cats" either at the time of the surveys, or at some time, without evaluating cat contact for the entire period at risk. Not evaluated were generally the more important contact with cat feces and fecally contaminated soil, sites of defecation, moisture and shade furthering oocyst survival, and the age stratification, immune status and density of the cat population, including stray cats. Neither have food sources of cats been studied, including the availability of biologically important intermediate hosts (birds, mice and rats) or the supply of canned or dry pet food, as generally used in the United States (37), or of the supply of raw meat, as often used in England (38). The type of meat given should be considered since beef or brooder chickens are rarely infected, while pork, mutton and farm chickens can quite commonly be infected (19, 39).

Carnivorism is an effective mode of transmission for *Toxoplasma* in animals and man (figure 4). The importance of carnivorism was shown by the common finding of *Toxoplasma* in meat (39–41) and its presumed role in the hamburger epidemic (42), and was demonstrated statistically (43) by studying the feeding of raw mutton and horsemeat to French children, a traditional means of augmenting their resistance to tuberculosis. We (20) and others (2, 3, 44) have presented evidence consistent with oocyst trans-
mission in children during the crawling and playing stage, as illustrated by the steep cumulative seroconversion in Central America in the first 10 years of life (figure 2). In contrast to this, surveys in New York (45) and Maryland (37) have shown a much more gradual seroconversion (figure 2), confirmed also by studies in several US city populations by Feldman and Miller (46). Seroconversion in these US adults appears typically to be due to transmission of Toxoplasma by undercooked or raw meat, the eating of undercooked or raw meat being a cultural habit developed during adulthood in the US. Although there is probably a subliminal mixture of transmission by oocysts, its magnitude is unknown and perhaps unknowable with present techniques.

It is difficult to conceive of a human population entirely infected by meat without possible cat contact, whereas we believe infection solely due to cat contact to exist in Costa Rica and probably most of Central America. We consider seroconversion of Latin American humans to be close to an all-oocyst pattern and seroconversion in New York close to an all-meat transmission pattern. It is therefore not surprising that Ganley and Comstock (37) found no correlation between positive Toxoplasma titers and exposure to cats either in their study, or in other US studies which they reviewed. However, we also feel that none of these studies exhausted the state of the art in pursuing the role of cats and their feces in causing toxoplasmosis. We agree with Ganley and Comstock (37) that in Maryland "correlation of positive serology in house cats may be too crude a discriminator to show an association," and that oocysts "may be so ubiquitously distributed in nature and contact with the organisms so limited that risk of acquiring positive antibody is primarily a function of increasing age and nonspecific opportunity." As they suspected, "hobbies, occupation or cleanliness may play a more important role than the possession of cats" (37). Indeed, recently, Stagno et al. (44) correlated geophagia with Toxoplasma infection of children in the small outbreak in Georgia where cats were prevalent. Examination of the role of meat in transmitting Toxoplasma has been selectively neglected in all US studies except those of Peterson et al. (35), Teutsch et al. (47) and Stagno et al. (44), but in these latter studies no significant correlations were found. In the presence of unrecognized transmission by meat and oocysts, accurate histories that permit the exclusive identification of one or the other contact cannot likely be obtained—certainly not in a population spanning the major part of a lifetime.

Being able to exclude meat in the transmission of Toxoplasma to man in Costa Rica (20), we postulate that contact with oocysts must explain all transmission. Since most cats in Costa Rica are not tame pets but are stray to feral, wandering from house to house in a small area, the acquisition of infection by children cannot be attributed to them playing with cats. Instead, we incriminate soil and other surfaces contaminated with cat feces containing oocysts and which constitute a durable reservoir of contamination under prevalent conditions of shade and moisture (17). The degree of correlations positive with cats per km² and negative with soil availability to cats for defecation, in both multivariate and univariate analyses was highly predictive (table 2). The isolation of Toxoplasma from soil (21) and from false attics (22) provides supporting evidence.

Since humans do not recognize having ingested cat feces, we attempted to use as indicators several circumstances that make such ingestion plausible. As detailed elsewhere (17), cat feces lose their odor, consistency, color and other identifying characteristics within two weeks in moist soil; however, viable oocysts have been recovered for 18 months from such
soil by inoculation into mice. Hence, we attempted to use soil contact as an approximator of possible oocyst ingestion, and cat prevalence as a possible indicator of the contamination of soil by oocysts. However, these two indices tended to diverge. Soil contact was greatest in rural areas and correlated negatively with human infection. Population density of cats was 10–250 times greater in the cities and correlated significantly with human infection by univariate analysis. The resulting greater concentration of cat feces in urban areas was compounded by the restriction of available soil surface for defecation because most of the terrain is covered by houses and streets, as shown by negative correlation by univariate analysis (table 2). Also, shade from trees, shrubs and under houses is more available. Although not quantitated, these factors may also influence infection rates in mice, rats and birds as intermediate hosts in urban as opposed to rural areas. The rapid acquisition rate of infection from oocysts in suburban soil was illustrated in young chickens in which the percentage of infection increased from 23 to 73 per cent while the chickens increased from 500 to 1000 gm in weight (19).

Seropositivity in humans turned out to be inversely related to the index of soil contact by univariate analysis (table 2). The soil contact index is higher in rural areas where cat density is low, where defecation sites are more dispersed and where there is greater exposure to drying in open country (table 1).

Intermediate hosts play an important role in the natural transmission cycle because cats do not contract and disseminate infection as efficiently when infected with oocysts as they do when infected with tissue cysts from intermediate hosts (48). The biological importance of tissue cysts is further indicated by the fact that after their ingestion by cats, the prepatent period to oocyst shedding is reduced to 3–5 days, whereas after the ingestion of oocysts the prepatent period is three weeks (6, 15, 48). The percentage rates of infection in the intermediate hosts sampled, 3.5 per cent in mice, 12.5 per cent in rats, and 16 per cent in sparrows, appear adequate to maintain infectious inocula for cats. Cats are considered the principal predator of these sparrows in Costa Rica (49). Flies and roaches, although shown to be capable of serving as transport hosts in the laboratory, were not found infected in nature. Infection was found associated with earthworms from naturally contaminated soil (19). Earthworms are capable of carrying oocysts deeper to a more favorable, moist environment, and peripherally beyond the soil area contaminated with feces.

A prospective study of seroconversion in humans, together with indices of infection in intermediate hosts and cats from each locality, will be essential in order to adequately analyze opportunities to become infected with Toxoplasma and differences in prevalence rates in humans. Conceivably, the intensity of contact of humans with cat feces in soil and in the house varies with the localities. However, we have not discerned differences in customs or habits to support this possibility. Instead, the prevalence and availability of intermediate hosts to cats may account for some differences between localities. Indeed, they are our prime suspects in modifying prevalence rates according to altitude, as observed by Walton et al. in Panama (4) and Costa Rica (2).

Both the geometric pattern of houses and the population density affect the opportunity for humans to become infected. An essentially linear arrangement of houses in San Ignacio, with 650 of the houses (85 per cent) dispersed and a population density of 50/km², was associated with the lowest human antibody prevalence observed.

City block configurations would give rise to the greatest fecal concentrations with a given number of houses and cats
FIGURE 5. Factors modulating the level of contamination available for the infection of humans, other intermediate hosts and cats.
per house. Smaller aggregates of city blocks made up Atenas, Cot and San Ramon, where a cat population density of about 400/km² and intermediate antibody levels were seen, while large aggregates of city blocks made up Limon and San Jose, where the cat population was estimated to be 1700–3000/km² and the highest human antibody prevalences were found.

From the available data, we have analyzed retrospectively what appear to be the important factors determining the rates of feline and human toxoplasmosis infection. In the absence of additional quantitative data, we cannot construct hierarchies of relative importance, such as of rodents vs. birds as intermediate hosts, or house density vs. cat density in contamination of soil, or the role of precipitation in supporting the survival of oocysts vs. its role in sorting or washing them away. However, the data permit construction of a flow chart characterizing the primary fate of cat feces and oocysts, and indicating secondary modifiers which participate in determining the ultimate level of contamination available for the infection of cates, humans and other intermediate hosts (figure 5). Many of these items can be quantitated and subjected to statistical scrutiny in a prospective study.

REFERENCES


