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TOXOPLASMOSIS IN PANAMA: A 10-YEAR STUDY

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Abstract. We studied the prevalence of *Toxoplasma* antibody over a 10-year period in a rural population of 326 people in Chorrera Province of Panama using the dye test. Fifty-five seroconversions were found in 108 people at risk, and 48 (87%) in children between 2 and 13 years with a mean incidence rate of 8.6% per year. Antibody prevalence rose from 25% at 5 years to 50% at 10 years of age, and increased gradually, reaching 90% by 60 years. Mean antibody levels after seroconversion were 1:6,000 in the dye test; they fell to 1:1,000 after 1 year, 1:800 after 2 years, 1:200 after 3 years, and 1:333 after 7–9 years. About 10% of antibody titers ranged between 1:4 and 1:32. *Toxoplasma* antibody prevalence was also studied in the metropolitan Panama City population using 590 sera collected in the fall of 1981. Age-specific incidence rates were similar in the urban and rural setting (correlation coefficient 0.71). The number of cats observed in the rural area and in the city and the degree of soil contact appeared compatible with a hypothesis of transmission by oocysts.

High prevalence rates of antibody to *Toxoplasma gondii* in Central America and Panama have been known from the population studies by Walton,[1] Remington,[2] and Gibson[3] conducted during the 1950s and 1960s. However, the mode of transmission was unknown. In 1970, the oocyst, a resistant stage in the *Toxoplasma* cycle shed by cats, was identified.[4] Study of the transmission of *Toxoplasma* in Costa Rica[5] included the role of cats,[6] contact of humans with soil,[7] and the role of intermediate hosts.[8] No temporal study existed in this setting. Two prior prospective studies had been carried out before transmission by oocysts and tissue cysts was understood.[9,10]

Because effective control of infection depends on an accurate knowledge of the transmission, we felt that a study over a long period of time would give us useful information. We therefore added a study of *Toxoplasma* antibody to an ongoing prospective study of Chagas' infection in a relatively stable population in rural Panama. This could give us information on transmission in relation to age, antibody titer development and stability, the association of seroconversion with morbidity, clustering of infection in families, and the relationship of seroconversion in humans with cats and soil contact. Such information was also necessary to design a prospective study on the transmission of toxoplasmosis and the effects of hygienic measures and a vaccine on morbidity. Because the number of subjects necessary for such a study could only be obtained in Panama City, we included a prevalence survey from this location.

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MATERIALS AND METHODS

Study populations

Altos del Jobo is a rural community 30 km west of Panama City and 14 km WNW of Chorrera. About 370 individuals in 40 families live in as many houses loosely grouped along two roads forming a T. Most houses have dirt floors, board walls, and thatched roofs; only a few structures, such as the school, are of concrete or masonry construction. The area is hilly and used mainly as pasture. There are small stands of trees and shrubbery; coffee, bananas, oranges, pineapple, mango, and avocado are grown along with cultivated plots of beans. The climate is wet tropical; the annual rainfall, as measured on nearby Barro Colorado Island, is between 75 and 140 (mean 107) inches, and occurs mostly between May and December.

People live from agricultural products grown on hillside plots outside of the town which the men tend during the week. Pigs, chickens, and pigeons are raised for food, and dogs and cats are kept as pets and for rodent control. Some men are employed tending cattle on neighboring farms, others work in a chicken hatchery in Chorrera, and commute daily or weekly to their homes. Fifty-one percent of the population is under 16 years of age; those 7–13 years old attend grade school.

Because of the presence of triatomids (*Rhodnius pallescens*) and the typical home construction, a survey of Chagas' infection was started in 1973. A team from Gorgas Memorial Laboratory (GML) visited the town every 2–3 months offering free medical services, some medicines, and, when necessary, referrals to hospitals. Those who came to the clinic because of medical problems and those volunteering for the survey were given a physical examination, and a venous blood specimen was taken for trypanosome culture and serologic tests. All were tested xenodiagnostically for the presence of *Trypanosoma cruzi* and *T. rangeli*. Between 1973 and 1983, 326 people, or 88% of the population of 370 were seen at the dispensary. Between 1 and 8 specimens (mean 2.5) were taken for *Toxoplasma* antibody. Children <9 months were excluded to avoid false positives from passively transmitted antibody.

The urban Panama City sample was derived from a case-control study of aseptic meningitis; this was based on 10% of children <15 years residing in Panama City who were admitted to either the Children's Hospital or the Social Security Hospital in the fall of 1981 as fully described by Reeves et al.[11] Sera were collected in November 1981 from volunteer family members of meningitis patients and from control families (both neighboring and distant). Five hundred ninety were available for this study, selected by the availability of excess serum. At the time of the study Panama City had about 610,000 residents, which constitutes 30% of the population of the Republic of Panama. The population density in Panama City ranged from 185 to 3,062 people per km^2. About 40% of the metropolitan population was <15 years.[12] People live in single or multiple family dwellings mostly of concrete or masonry, surrounded by yards with a variety of vegetation, edible fruit-bearing trees, ornamental or shade-producing plants, or grass-like weeds. Cats are common and were enumerated with other pets. Both metopolitan and suburban areas were included.

Laboratory methods

The 10-year rural study is based on 824 specimens from 323 people who presented themselves at the dispensary. Initially 228 people were censused. During the study, 95 people were added; of these, 54 were under 10 and born during the study and 41 had moved to the village. Three people died. The yearly number of persons sampled was: 79, 101, 143, 91, 82, 18, 0, 110, 75, 0, and 125. Nine persons originally negative were not retested and 17 of those were born subsequently. Serum samples identified only by numbers were inactivated at 56°C for 30 min and examined using the dye test at the University of Kansas laboratory, employing twofold dilutions starting at 1:2. A standard positive serum was used to assure comparability of results from year to year. Twelve results (1.46%) were disregarded: 5 because of inconsistencies and 7 because titers of 1:2 preceded and followed by titers of <1:2 were considered false positives, and because they occurred when sera were shipped in heat-sealed ampules, a number of which heat-aggregated and failed to dissolve completely.

The 590 sera available from Panama City, in the form of aliquant samples were identified only by code numbers. They were examined for *Toxoplasma* antibody by the indirect fluorescent antibody test (IFAT) at the GML, using fourfold dilutions starting at 1:2 and going to 1:4,096. The specimens read as positive at 1:2 to 1:8 were checked by the dye test; 97 sera were negative and 1 had a titer of 1:2 which was regarded as positive. Fifteen sera with a titer beyond 1:4,000 in IFAT were also tested by the dye test to determine end titers.

Statistical methods

Prevalence data from our cross-sectional survey were transformed into incidence estimates for each age group, by removing those positive at an earlier age (Table 1). The array of incidence rates from the rural (Altos) and the urban (Panama) samples was compared by linear regression, and the SD, variance, and correlation coefficients were calculated. The incidence estimates per age group and for income were subjected to Z tests for linear trends. Antibody prevalence percent-

TABLE 1

Toxoplasma *antibody prevalence, incidence, and geometric mean titers (GMT) in relation to age in Altos del Jobo (A) and Panama City (P), Panama*

Age group	Seroreactivity at initial census (+/−) A only	No. sera examined A	No. sera examined P	Age-related prevalence A	Age-related prevalence P	Incidence rate/year A	Incidence rate/year P	GMT A	GMT P
1–5	10/53	109	93	25.9	37.6	6.3	9.3	4,636	455
6–10	11/27	161	139	49.1	47.5	9.8	9.6	2,760	284
11–15	14/12	143	79	56.6	65.8	11.4	13.2	1,106	236
16–20	12/10	72	59	59.7	52.5	11.9	9.5	472	114
21–25	8/8	75	43	57.3	72.1	11.5	14.4	396	140
26–30	9/3	49	48	55.1	66.7	11.0	13.3	386	128
31–35	7/4	37	27	86.5	74.1	17.5	14.8	159	163
36–45	12/6	70	40	77.1	67.5	7.7	6.8	240	153
46–55	11/5	42	35	73.8	74.3	7.4	7.4	105	172
56–65	3/2	45	16	88.9	87.5	8.9	8.6	201	181
66–75	1/0	8	10	100.0	80.0	10.0	8.0	70	117
76+	—	1	1	100.0	100.0	—	—	(8)	(64)
Total	98/130	812	590	57.5	58.6	10.2*	10.3†	—	—

* Represents mean: SD = 3.2; var. = 9.2; r = 0.71.
† Represents mean: SD = 3.07; var. = 8.4; r = 0.71.

ages in several groups were tested for significance by χ^2 tests.

RESULTS

Altos del Jobo (rural)

Antibody prevalence rose from 16% to 29% between 5 and 10 years of age in the original census of 228, and from 25% to 50% in the total sample of 812. Prevalence remained below 60% in the 10–30 year groups but rose to between 70% and 90% in the 30–75 year groups (Table 1, Fig. 1).

Antibody titers were highest in the youngest groups, falling with increasing age as shown by frequency distributions (Fig. 2) and mean geometric titers (Fig. 1). The highest titer of 1:130,000 was found in 6 (1.3%) of positive specimens; the lowest titer of 1:2 was found in 0.65%. Titers of 1:4, 8, 16, and 32 accounted for 1.3%, 1.7%, 2.6%, and 3.4% of test results, respectively. Seven sera with titers of 1:2 were excluded as they were preceded and followed by negative titers; 6 of the 7 occurred in specimens taken in 1976. Three titers of 1:2 were considered valid because

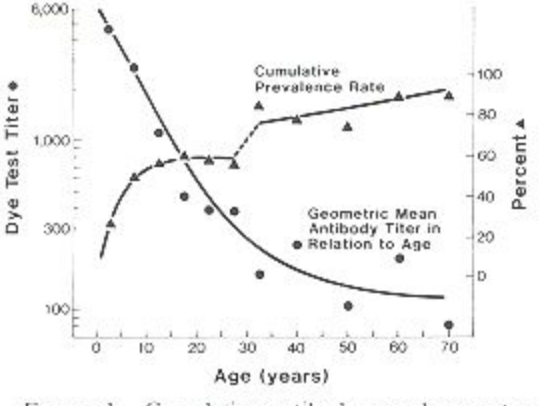

FIGURE 1. Cumulative antibody prevalence rates and mean antibody titers to *Toxoplasma* in Altos del Jobo, Panama, determined by dye test. The break in the cumulative prevalence curve between 30 and 35 years is attributed to two cohorts; the village was founded about 1950 and the younger group was born there, whereas the older group was born elsewhere.

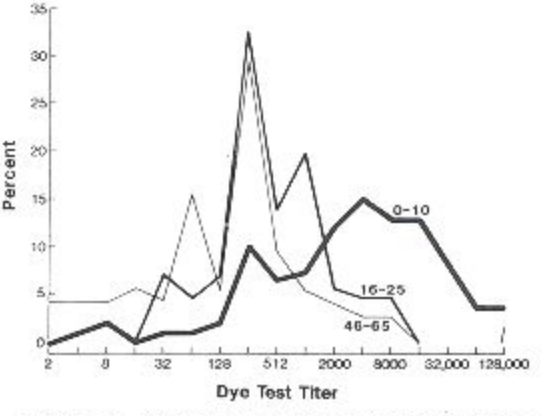

FIGURE 2. *Toxoplasma* antibody titer distribution by age group in Altos del Jobo, Panama, determined in dye test.

TABLE 2

Dye test titers in the year of seroconversion and in given later years (16 children), and in 9 2–4-year-old children from whom no prior specimen was available from Altos del Jobo, Panama

	Year of seroconversion	Years after seroconversion				Age
		+1	+2	3–5	7–9	2–4 (3.1)
	n = 16	n = 9	n = 6	n = 7	n = 12	n = 9
Titer						
Range	512–130,000	8–32,000	64–4,000	64–1,000	64–1,000	2,000–130,000
Mean	6,049*	1,195	813	172	333	8,848

* With the one titer of 1:4 omitted: 1:8,969.

they were accompanied by other low titers in people between 46 and 65 years of age.

A titer of 1:256 was most common (23%) in the entire sample, and in the 16–25 and 46–75 age groups (33% and 30%, respectively). In the 0–10 year age group, however, the most common titer was 1:4,000 (15%, Fig. 2).

Fifty-five seroconversions were found over a 10-year period in the sample population of 326. Because only 108 were at risk (negative either initially or born during study), the yearly incidence rates varied between 6.3% and 11.9%, with a mean rate of 10.2 ± 3.2 (Table 1). In the year of seroconversion the titers varied from 1:4 to 1:130,000 with a mean geometric titer of 1:8,969. This calculation excluded one titer of 1:4 found apparently in the early stage of infection because a titer of 1:8,000 was measured the following year (Table 2). Similar titers were found in 2–4-year-old children without prior negative titers (Table 2). Following seroconversion, the mean antibody titer in 16 children aged 2–15 years was 1:11,094, and in 6 adults between 16 and 30 years, 1:4,598. Antibody titers declined following seroconversion, with a mean of 1:1,195 after 1 year, 1:813 after 2 years, 1:172 after 3 to 5 years, and 1:333 after 7 to 9 years (Table 2).

In those in whom seroconversion was not observed the general trend was also toward lower titers (Fig. 1). However, persistently high titers were found in 8 people between 10 and 21 years. In 1, a titer of 1:32,000 persisted for 10 years. In 6, titers of 1:8,000 were followed by 1:4,000 after 8 to 10 years, and in 1, a titer of 1:2,000 persisted for 11 years. Low titers of 1:2 to 1:32 persisted in 6 people aged between 46 and 56 years. No single individual titer dropped to <1:2 during the 10-year period. Absence of antibody over a period of 5 to 10 years was found in 32 people whose average age was 25 years at the end of the study.

Thirty-three family units consisted of >4 people; in 58% of these, more than two-thirds of the children had antibody, in 18% between one- and two-thirds of children had antibody, in 18% between one-third and one-tenth had antibody. In two families none of the children had antibody; their houses had concrete floors, and one a concrete apron. In one family the father and 4 of the 5 children developed antibody in the same year; the mother had a stable titer of 1:128–1:256 throughout the 10-year period.

No symptoms were elicited that could reasonably be linked to primary infection during the year of seroconversion or before. No instances of retinochoroiditis, or of scars, were detected in routine fundus examinations during clinic visits (without dilation). In addition, 50 of the general population and 12 with titers >1:1,000 were examined by indirect ophthalmoscopy in the tenth year of the study without finding lesions.

No accurate past history of dog or cat ownership or adoption could be established. Between 50 and 200 cats and 71 to 200 dogs were present in town in the tenth year. Based on data from 66 people in 15 families in the tenth year, *Toxoplasma* antibody was found in 77% with cats and in 53% people without cats, in 70% with dogs and in 62% without dogs.

Panama City (urban and suburban)

Antibody prevalence rose sharply from 38% to 48% between 5 and 10 years and thereafter increased gradually to about 80% (Table 1, Fig. 3). Antibody titers were highest in the young and fell to lower levels in the older groups. In the youngest group, the mean geometric titer was 1:455.

Antibody prevalence rates were higher in the poor, in those with cats as pets, in people who did not have private bathrooms, who had a storm

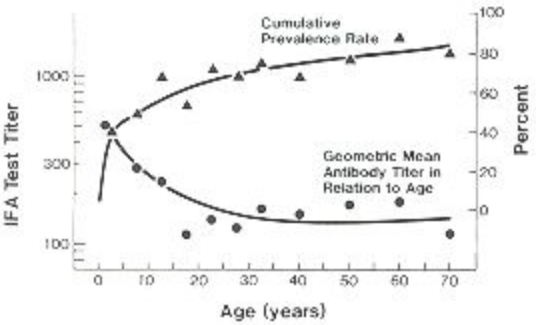

FIGURE 3. Cumulative antibody prevalence rates and mean antibody titer to *Toxoplasma* in Panama City determined by indirect fluorescent antibody test, and dye test check of low and high titers.

sewer on their property, who lived in a shanty town, and whose house was constructed of both wood and cement (Table 3). No significant difference in antibody prevalence was found by race, occupation, or number of people per home or sleeping room, or in relation to flooding, numbers of rooms per house, or where food was purchased.

DISCUSSION

This study of *Toxoplasma* antibody in an area of high prevalence complements a 10-year study in Cleveland, Ohio,[9] and a 4-year study in Syracuse, New York.[10] In Cleveland,[9] 35% of adults between 31 and 40 years of age were seropositive and in Syracuse, 41%;[10] in contrast, about 80% of similarly aged adults were positive in Panama. The difference in antibody prevalence of 10-year-old children was striking; about 5% in Cleveland and in Syracuse, and 49% in Panama. High early seroconversion rates also have been reported from Colombia,[12] Guadeloupe,[13] San Salvador,[2] Costa Rica,[5] and other Central American countries.[1]

The age-specific antibody prevalence in Altos del Jobo was derived from the total number of sera collected because the number of those initially censused was small. The prevalences in Altos and Panama City were similar to the pattern in the other Latin countries mentioned.

The incidence of infection varied by age (Table 1) and was similar in the two locations. The incidence started at 6.3%–9.3% between 1 and 5 years, and rose to 9.7% between 5 and 10 years of age. It ranged close to 11% and 12% between 10 and 30 years in Altos, and between 13% and 15% in Panama City. The 17.5% value between 30 and 35 years in Altos was attributed to a cohort of people born elsewhere who moved to Altos about 1950 when the village was founded. The incidence ranged between 6.8% and 10% in those >40 years of age.

TABLE 3

Toxoplasma *antibody prevalence rates in several subpopulations in Panama City, Panama*

	No.	%	*P*
Monthly income			
$0–$75	53	66.0	
75–150	105	64.7	
151–300	190	58.4	0.06
301–500	103	42.7	
501–1,000	47	46.8	
1,001+	67	56.7	
Pets			
Dog	170	55.3	
Cat	40	65.0	NS
Other	58	70.7	
None known	311	52.7	
Bathrooms			
Individual	311	48.2	
Communal	92	65.2	0.0002
Latrine	122	67.2	
None	35	68.6	
Water supply			
Inside house	373	51.7	
Communal supply	112	61.6	0.006
Private, outside	87	68.9	
Open storm sewer			
On property	151	65.6	0.005
None	386	52.3	
Residence			
One family house	267	53.6	
Multifamily house	191	52.8	0.121
Shanty town	48	68.8	
House construction			
Cement	344	51.2	
Wood	139	58.3	0.012
Wood and cement	75	69.3	

The peak prevalences of 90% were reached in Panama around 60 years (Table 1); however in San Salvador,[2] 80% was reached at 35 years. In Costa Rica[5] and Colombia[12] prevalence rates peaked near 60% at 25 years, but in Guadeloupe[13] had already peaked by 10 years.

The reasons for the different incidence and prevalence levels in Panama and other countries are not clear. The importance of where children play is suggested by the comparison of families in which more than two-thirds or less than one-third of children were infected. In the former

group, all the homes had dirt floors and 64% had cats. Of 5 houses with fewer than a third of children infected, 2 had concrete floors, 1 had a concrete apron or patio on which the children played, and only 40% had cats. It appears that in this rural environment, concrete floors are associated with less infection. However, in the urban environment of Costa Rica, where there is little soil for cats to defecate in, especially in multistory houses, concrete floors were associated with higher prevalence rates.[5]

The most common antibody titers in the 20- to 25-year-old populations are different in different countries.[5] In the United States[10] the most common titer was 1:16, in Colombia[12] 1:64–128, in Costa Rica[5] 1:1,000, in Altos del Jobo 1:256, and in Panama City 1:128. In Altos del Jobo the most frequent titer was 1:4,000 in the 0- to 10-year range, and 1:250 in the 16- to 25-year and the 46- to 65-year-old groups. It is tempting to relate the higher titers to relative recency of primary infection in the younger group and to high reinfection rates, which are indicated by the 5%–17% yearly incidence rates in Panama. Secondary peaks in antibody frequency were found at 1:1,000 in the 16–25-year group, and at 1:64 in the 46–65-year groups (Fig. 2).

A prolonged study lends itself to developing an understanding of serologic behavior, such as patterns of titer decline. The means of 1:6,000 in the year of seroconversion, and that of 1:8,800 in the 2–4-year age groups, were similar (Table 2), followed by a geometric decline. In the Cleveland study,[9] where tests were done by microhemagglutination, the post seroconversion titers were 1:19,000 in 2 children, and 1:2,000 in an adult and a child. In Syracuse,[10] seroconversion titers of 1:512 and 1:1,024 were found in 2 children, and 1:64 in an adolescent. We found higher titers in 16 children (1:11,000) than in 6 adults (1:4,600) after seroconversion.

Low titers are often disregarded; however, their validity is shown in our 10-year rural study, where 6 individuals had titers of 1:2 to 1:32 over 7–10 years. Three values of 1:2 in older people, preceded and followed by other low titers were regarded as true positives. Thirty-three people were consistently negative over a period of 5–11 years (mean 8.1) as shown by 108 specimens negative at 1:2 (or 1:4 when only small volumes were available). There were no spurious positives and the titers of individual patients were consistent with a sharp rise followed by gradual fall, similar to the mean geometric titer curves (Figs. 1, 3). We found no reason to disregard low titers in the dye test. Its reading based on the number of lysed or staining organisms is clear and unambiguous. As discussed previously,[4] the dye test is highly specific. It is misleading and unwise in epidemiologic studies to arbitrarily reduce the sensitivity of the dye test by starting a dilution series at 1:16 or 1:64. Had we used 1:64 as lowest dilution, 45 sera, or 9.6% of measurable titer would have been falsely labeled negative.

The specificity of other serologic techniques must be considered separately. In the IFAT test, titers between 1:2 and 1:8 were based on what seemed to be faint fluorescence of tachyzoites in one dilution only; all 98 such sera were retested by the dye test and were negative except one.

Since the early studies in the U.S.,[9, 10] the life cycle of *Toxoplasma* appears to have been clarified and transmission by cat feces-contaminated soil has been postulated.[4] The present study was undertaken in part to test this postulate. Fifty percent of children are infected by the age of 10 years. Because interviews indicated that meat was well cooked, transmission by tissue cysts could be excluded. There was no evidence of congenital transmission. How can the observed transmission be explained by the ingestion of oocysts?

At the end of the tenth year of our study there were between 50 and 200 cats in the village (the lower figure is our count and the higher one the estimate of a group of informants, who estimated the number of dogs at 200, whereas we counted 71). The number of litters born was estimated between 7 and 30 per year from which between 30 and 120 nonimmune kittens were added to the population. Each of these after its first infection was expected to shed between 10^5 and 10^7 oocysts. As mentioned, Altos del Jobo is a collection of houses strung along a T-shaped configuration of roads. If the average living area is 20 m from each side of the road we can calculate the actual living space from an aerial photograph to be about 77,538 m^2. If we assume, conservatively, 10^6 oocysts to have been shed after the first infection in the first year of life and that (because of the cats' forays) only one half of these oocysts are deposited in the mainly inhabited area (near houses where the cats are fed), from 193 to 774 oocysts would have been deposited per m^2.

Actually, around areas of cat fecal deposits concentrations will be much higher, interspaced

with areas of lighter contamination. These appear to be credible orders of magnitude and compatible with observed infection rates. However, the rates of oocyst attrition during the 4 months of dry season and due to washing during the 8 months of rainy season, as studied in Costa Rica and Kansas,[14] have not been evaluated in Panama.

Subgroups of people showed differences in antibody prevalence, probably because the manner of living is more diverse in Panama City than in Altos del Jobo. Higher prevalence rates were found in Panama City related to poverty, contact with earth, and low standards of hygiene. Although feeding a pet cat was associated with a 65% prevalence rate, and feeding a pet dog with only a 55% rate, this difference was not statistically significant because of the small number of people feeding pet cats.

The risk of becoming infected was considered to be "identical for each year of life from birth to death" in a study from France[15] based on data from women between 15 and 44 years of age; the risk of infection varied narrowly between 3.4% and 5.1% and averaged 3.7% per year. This may be attributed to the ingestion of raw and undercooked meat which is probably the main vehicle for the transmission of *Toxoplasma* in Paris, and which may be consumed at a relatively uniform rate during adulthood. However, in Latin American countries meat is generally eaten well cooked, and *Toxoplasma* is transmitted via cat feces. The present data, those from Costa Rica[5-7] and elsewhere,[1, 2, 3, 12, 13, 16] clearly show that seroconversion in childhood differs from that in adult life, apparently because of the environment or changing habits. In the two Panamanian sites, age-specific incidence rates were 6.3%–9.8% per year between 1 and 10 and 11%–15% per year between 11 and 35 years; thereafter they declined to 7%–9% per year, probably due to lesser contact with soil contaminated by cats. In the Colombian study[12] the annual risk of infection which was 3.2% in the first decade and fell to 0.7% in the fourth.

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