

SEROEPIDEMIOLOGY OF *TOXOCARA CANIS* INFECTION AMONG MOUNTAIN ABORIGINAL ADULTS IN TAIWAN

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Abstract. Seroepidemiology of *Toxocara canis* infection among adults of one ethnic Han and five aboriginal populations residing in mountainous areas of Taiwan was conducted by detecting serum IgG ($\geq 1:64$) using a *T. canis* larval excretory-secretory antigen-based enzyme-linked immunosorbent assay. A short questionnaire interview was conducted to obtain data concerning their age, sex, occupation, consumption of raw pig liver, and possession of dogs. The overall seroprevalence (46.0%, 247 of 537) in the five aboriginal populations was significantly higher than that of ethnic Han population (30.2%, 13 of 43) ($P = 0.04$). Age, but not sex, seemed to be a factor related to positive serology. Aboriginal adults who had histories of eating raw pig liver (odds ratio [OR] = 1.65, $P < 0.01$), raising dogs (OR = 1.76, $P < 0.01$), or whose occupation was a laborer (OR = 1.78, $P = 0.01$) seemed to be more apt to be infected by *T. canis* than those without such histories and unemployed persons.

INTRODUCTION

Toxocariasis is a zoonosis caused by the ascarids of cats and dogs, the main representative of which is *Toxocara canis*. The eggs of *T. canis* are unembryonated when passed in the feces of dogs into the environment. Under optimal temperatures and humidity, these eggs develop into embryonated eggs that are infectious to both final and paratenic hosts. Infective eggs are reported to survive under optimal circumstances for at least one year. Humans may acquire the infection by ingesting the embryonated eggs by contact with larvae contaminating the teats of bitches that have recently given birth or the muzzle of puppies, or by means of the paratenic hosts of the parasite.^{1,2}

The major clinical consequences of prolonged migration of *T. canis* larvae in humans are visceral larva migrans and ocular toxocariasis.¹ Young children are the main population at risk for *T. canis* infection due to geophagia, poor hygiene, or frequent contact with dogs.¹ Nevertheless, adults who are hunters and dog breeders or have the habit of eating raw viscera, especially the liver, are also at high risk.³ Although most infected people are asymptomatic, adult toxocariasis cases associated with severe asthma, granulomatous hepatitis, pyomyositis, skin manifestations, vasculitis, and neurotoxocariasis have been widely reported over the past 10 years.^{4–12}

Since the larvae migrate in tissue and do not develop further in humans, a definitive diagnosis by tissue biopsy or stool examination is difficult. Diagnosis of toxocariasis relies mainly on a *T. canis* larval excretory-secretory (TcES) antigen-based enzyme-linked immunosorbent assay (ELISA), which reportedly shows a sensitivity of 78.3% and a specificity of 92.3%.¹³ Seroprevalences of toxocariasis among adult populations determined by the TcES-ELISA vary with geographic regions. The seroprevalences of adults were low in developed countries, 7.5% (50 of 660) in Australia and 3.1% (399 of 12,677) in Spain.^{14,15} However, a higher seroprevalence was observed in adults in tropical and developing countries, 20.5% (15 of 73) in Brazil and 30.4% (7 of 23) in Nigeria.^{16,17} In general, reports concerning the seroprevalence of *T. canis* infection in aborigines living in mountainous areas are rather rare.^{18,19}

In this study, the seroprevalence of *T. canis* infection among aborigines and ethnic Han people residing in mountainous areas of Taiwan was evaluated by a TcES-based ELISA from January 1998 to June 2000. Risk factors for the infection were also analyzed by a short questionnaire interview.

MATERIALS AND METHODS

Study population and subject selection. The aborigines of Taiwan represent the indigenous inhabitants of the island at the time of the arrival of the Dutch and Spanish in 1624 and 1626, respectively. Large number of Chinese emigrated from mainland China (mainly ethnic Han people) to Taiwan during from 1661 to 1895 and in 1949. Until 1895, the tribes occupying the mountains and eastern plains were still in control of their aboriginal areas.²⁰ In 1993, there were 10 aboriginal tribes with the Ames (~140,000 people), Atayal (~90,000 people), Paiwan (~60,000 people), Bunun (~41,000 people), and Saisiat (~7,000 people) being the first, second, third, fourth, and tenth largest aboriginal populations in Taiwan, respectively. Most Atayal and Saisiat aborigines live at elevations of 300–500 meters in central and northern Taiwan, while the other three aboriginal populations live at elevations of 100–600 meters in eastern Taiwan. Although these aboriginal populations differ in their traditional culture and food habits,²¹ these five different aboriginal groups still have the habit of hunting wild boar with the help of dogs, and they also frequently eat raw viscera, especially the liver of the wild boar.^{22,23}

Populations of Atayal and Saisiat as well as Ames, Bunun, and Paiwan aborigines living in mountainous areas of Hsinchu and Taitung Counties, respectively, were chosen as the study population (Figure 1). The total aboriginal population in these areas is approximately 110,000. A total of 21 aboriginal villages were selected as suggested by district authority of medical affairs because they were near the mountains. In addition, some ethnic Han people living in these districts were also included in the present study. Serum samples were obtained by venipuncture of 580 randomly selected healthy

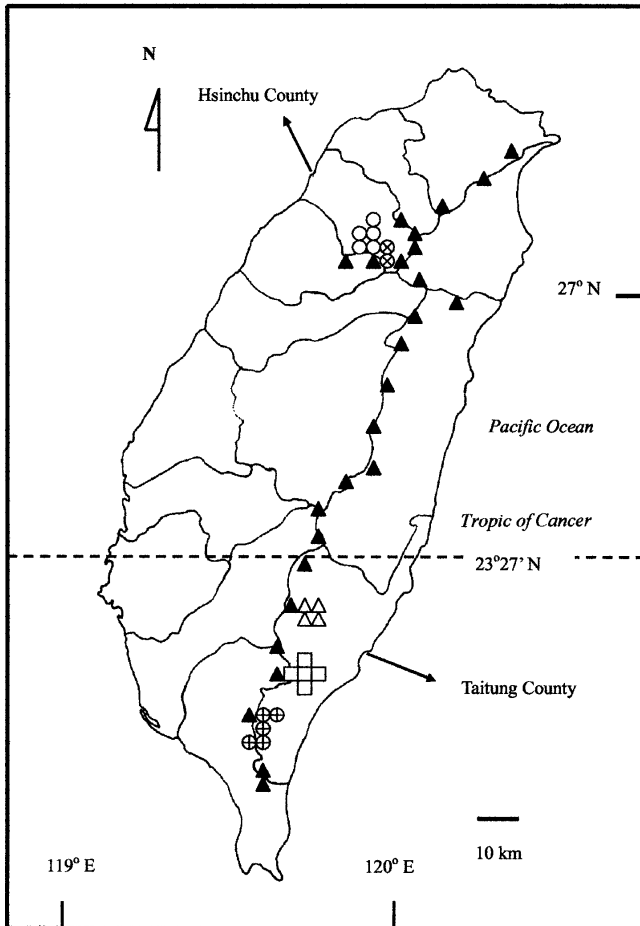


FIGURE 1. Map of Taiwan showing selected study areas. Δ = Ames; \circ = Atayal; \oplus = Bunun; \square = Paiwan; \otimes = Saisiat; \blacktriangle = mountainous areas.

adults who went to the basic medical units to participate in the present study. This included 90 serum samples (33 from males and 57 from females) from Ames, 208 (105 from males and 103 from females) from Atayal, 63 (32 from males and 31 from females) from Bunun, 137 (62 from males and 75 from females) from Paiwan, 39 (14 from males and 25 from females) from Saisiat, and 43 (24 from males and 19 from females) from ethnic Han populations. All serum specimens were kept at -70°C until use.

Questionnaire interview on risk factors. A short questionnaire interview was conducted to obtain information on risk factors for *T. canis* infection. This information included age, sex, weight, height, occupation, residential district, and ethnicity. In addition, items regarding whether the subjects had histories of eating raw wild boar liver and raising dogs were also included in the questionnaire. Oral informed consent was obtained from each subject before participation in the study. The study was reviewed and approved by the Taipei Medical University Ethical Committee.

Egg culture. Adult *T. canis* were collected from the intestines of necropsied stray dogs.²⁴ Infective embryonated eggs were cultured according to the method of Bowman and others²⁵ with slight modifications. Briefly, female worms were dissected, and the anterior one-third of the uterus was stirred in 10 mL of a 1% sodium hypochlorite solution and incubated

for five minutes at room temperature; thereafter, distilled water was added to bring the volume up to 20 mL. The mixture was then filtered through two layers of gauze to remove large tissue debris; the resulting material was centrifuged for five minutes at $2,000 \times g$. The pellet was washed twice with distilled water and once with 2% formalin. The eggs were resuspended in 2% formalin and placed in a 250-mL Erlenmeyer flask into which additional 2% formalin was added to bring the liquid level to a depth of approximately 1 cm. The flask was covered with Parafilm® (American Can Company, Chicago, IL) and incubated at room temperature for 8–9 weeks with gentle weekly agitation. They were then stored at 4°C until use.

Preparation of larval ES antigens. Excretory-secretory antigens of *T. canis* infective larvae were prepared by the method of de Savigny²⁶ with modifications. Briefly, *T. canis* embryonated eggs were washed and centrifuged for five minutes min at $2,000 \times g$. The egg pellet was washed twice with sterile phosphate-buffered saline (PBS) and resuspended in 1% sodium hypochlorite before incubation under sterile conditions in a CO_2 incubator in an atmosphere containing 5% CO_2 at 37°C for 30 minutes. After washing several times with sterile PBS containing antibiotics (100 IU/mL of penicillin, 250 $\mu\text{g}/\text{mL}$ of streptomycin, and 25 $\mu\text{g}/\text{mL}$ of nystatin; Biochrom KG, Berlin, Germany), the pellet was resuspended in 100 mL of sterile RPMI 1640 medium (JRH Biosciences, Lenexa, KS) containing the same antibiotics, and placed in a sterile modified Baermann apparatus made up of two layers of cotton cloth in a steel sieve.²⁷ It was then incubated in a CO_2 incubator at 37°C for 12 hours. Motile larvae that had migrated through the cotton cloth were collected and centrifuged. They were transferred to new RPMI 1640 medium containing the same antibiotics in 50-mL tissue culture flasks (BD Biosciences, Franklin Lakes, NJ). Each flask contained 10 mL of medium with a larval concentration of $1 \times 10^4/\text{mL}$. Supernatants containing ES antigens were collected weekly, pooled, and centrifuged to precipitate all debris. The resulting supernatant was sterilized by membrane filtration (0.2- μm Supor Acrodisc 32 syringe filter; Gelman Sciences, Ann Arbor, MI) into a dialysis tube (cutoff molecular weight = 6,000–8,000 kD; Spectrum Medical Industries, Houston, TX). The ES antigen was dialyzed against sterile PBS for 12 hours at 4°C until the added phenol red disappeared, the protein content was estimated by the method of Bradford,²⁸ and the antigen was then stored at -70°C until use.

Enzyme-linked immunosorbent assay. Serum TcES-specific IgG antibodies were detected by an ELISA according to the method of Jimenez and others¹⁵ with our modifications.²⁹ Briefly, wells of microtiter plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with TcES at a protein concentration of 10 $\mu\text{g}/\text{mL}$ in 0.1 M carbonate buffer, pH 9.6. The plates were then washed twice with 0.05% Tween 20 (Wako, Tokyo, Japan) in PBS, pH 7.4, and blocked with 2% skim milk in PBS for 30 minutes at 37°C to avoid non-specific binding. Test serum diluted 1:64 was added to each individual well and incubated for 90 minutes at 37°C . The optimal dilutions were determined by a checkerboard titration and further verified by immunoblotting analysis. Positive control sera were from patients with toxocarasis with proven clinical and laboratory diagnosis (kindly provided by Dr. B. Gottstein, Institute of Parasitology, Berne University, Berne, Switzerland). Duplicate tests were run on each test serum.

Horseradish peroxidase-conjugated goat anti-human IgG (heavy and light chain) (Amersham, Piscataway, NJ), diluted 1:1,000 in PBS, was used as the secondary antibody and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) solution (Calbiochem, Darmstadt, Germany) was used as the substrate. The reaction was stopped by the addition of 1% sodium dodecyl sulfate (SDS) to each well. The absorbance at 405 nm was determined in individual wells with an automated spectrophotometer (EIA reader model EL312e; Bio-Tec, Virginia Beach, VA). Positive and negative control sera were included in each plate. Tested serum whose mean optical density (OD) value was equal to or higher than the mean OD value minus two standard deviations (SD) of the positive control serum was considered positive.

Immunoblotting verification. Serum at a 1:64 dilution selected by an ELISA titration study was further verified by immunoblotting analysis using negative and positive control sera. Briefly, TcES antigen preparations (9 µg per slab) were separated by electrophoresis on a 12.5% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Amersham) in a semi-blotter (Hoffer, Fremont, CA). The strips were then incubated with serum samples diluted 1:64. A Western Lightning® kit (Perkin Elmer Life Sciences, Boston, MA) was used to detect the immunoreactions, and positive reactions were ascertained by the presence of low-molecular-weight bands of either 24, 28, 30, or 35 kD that specifically correlated with *T. canis* infection.³⁰ Some ELISA-positive and -negative tested sera were also subject to confirmation by immunoblotting.

Statistical analysis. In the present study, subjects were categorized into five age groups (20–29, 30–39, 40–49, 50–59, and ≥ 60 years old). Statistical analysis was performed using the SAS software system (SAS Institute, Cary, NC). The increasing trend of age-specific seropositive rates was tested for

statistical significance using the chi-square test for trends. Multivariate adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were estimated by means of multiple logistic regression analysis. The statistical significance of differences in age-, sex-, risk factor-, occupation-, and ethnicity-adjusted seropositive rates among comparison groups was examined by testing the statistical significance of the regression coefficients. *P* values < 0.05 were considered to be statistically significant.

RESULTS

The ELISA study showed that the mean ± SD value calculated from positive control sera from each plate was 0.574 ± 0.067 ; thus, the cut-off OD value was 0.440 (mean – 2 SD of positive control sera OD values). Randomly selected ELISA-positive tested sera in immunoblotting could recognize both specific low-molecular-weight bands of 30 and 35 kD, whereas ELISA-negative tested serum did not react with any of these bands (Figure 2).

Of the total 580 serum samples studied, 46.0% (247 of 537) aborigines and 30.2% (13 of 43) ethnic Han people were positive for IgG antibody to *Toxocara* as determined by TcES-ELISA (Table 1). Seroprevalence of toxocarasis among different aboriginal tribes ranged from 38.9% (35 of 90) for the Ames to 59.0% (23 of 39) for the Saisiat. The seroprevalence in the Saisiat and Paiwan tribes were significantly higher than that of the other ethnic groups. No significant difference in seroprevalence was detected between the ethnic Han people and the remaining tribes (Table 1).

Seroprevalence tended to increase with age. It was 31.8% for people in their 20s, fluctuated between 43.9% and 47.5% in those 30–59 years old, and reached a high of 51.5% in those

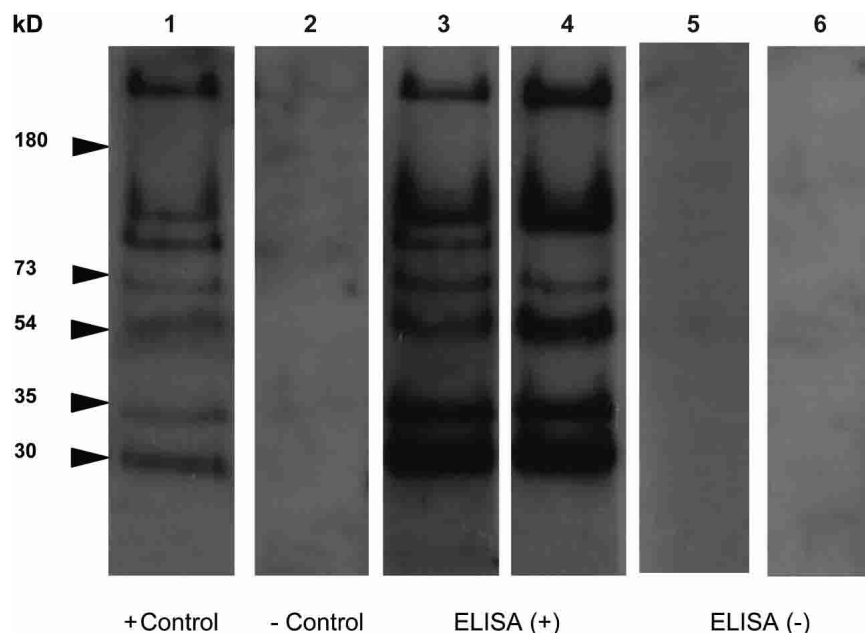


FIGURE 2. Immunoblotting analysis of clinically proven positive control sera and randomly selected enzyme-linked immunosorbent assay (ELISA)-positive sera showing reactive low-molecular-weight bands at 30–35-kD specifically related to toxocarasis. Lane 1, positive control serum; lane 2, negative control serum; lane 3, ELISA-positive serum 1 (mean ± SD = 1.848 ± 0.036); lane 4, ELISA-positive serum 2 (mean ± SD = 1.492 ± 0.055); lane 5, ELISA-negative serum 1 (mean ± SD = 0.346 ± 0.078); lane 6, ELISA-negative serum 2 (mean ± SD = 0.292 ± 0.017).

TABLE 1

Multivariate-adjusted odds ratios for various risk factors associated with seropositivity of antibodies to *Toxocara canis* among aboriginal adults in Taiwan

Variable Group	No. tested	No. (%)	Multivariate-adjusted odds ratio (95% confidence interval)	P
Sex				
Male	246	118 (48.0)	1.00* (referent)	
Female	291	129 (44.3)	1.16 (0.82–1.63)	0.40
Age group (years)				
20–29	63	20 (31.8)	1.00† (referent)	
30–39	71	33 (46.5)	1.87 (0.92–3.78)	0.08
40–49	114	50 (43.9)	1.68 (0.88–3.21)	0.11
50–59	118	56 (47.5)	1.94 (1.02–3.69)	0.04
≥60	171	88 (51.5)	2.28 (1.24–4.19)	<0.01
Risk factors				
Eating raw liver				
No	301	122 (40.5)	1.00‡ (referent)	
Yes	236	125 (53.0)	1.65 (1.17–2.33)	<0.01
Raising dogs				
No	132	47 (35.6)	1.00‡ (referent)	
Yes	405	200 (49.4)	1.76 (1.18–2.65)	<0.01
Occupation				
Unemployed	129	52 (40.3)	1.00§ (referent)	
Farmer	201	82 (40.8)	1.02 (0.65–1.60)	0.93
Laborer	207	113 (54.6)	1.78 (1.14–2.78)	0.01
Ethnicity				
Han	43	13 (30.2)	1.00¶ (referent)	
Ames	90	35 (38.9)	1.47 (0.68–3.19)	0.33
Atayal	208	92 (44.2)	1.83 (0.90–3.17)	0.09
Paiwan	137	66 (48.2)	2.15 (1.03–4.46)	0.04
Bunun	63	31 (49.2)	2.24 (0.99–5.06)	0.05
Saisiat	39	23 (59.0)	3.32 (1.33–8.25)	<0.01
Aboriginal populations	537	247 (46.0)	1.97 (1.00–3.85)	0.04

* Adjusted variables include age, risks factors, occupation, and ethnicity.

† Adjusted variables include sex, risk factors, occupation, and ethnicity.

‡ Adjusted variables include sex, age, occupation, and ethnicity.

§ Adjusted variables include sex, age, risk factors, and ethnicity.

¶ Adjusted variables include sex, age, risk factors, and occupation.

greater than 60 years of age. The seropositivity for the IgG antibody against TcES in those who had histories of eating raw pig liver or raising dogs was 53.0% (125 of 236) or 49.4% (200 of 405) in the aboriginal population. With respect to occupation, seroprevalences in laborers, farmers, and persons who were unemployed were 54.6% (113 of 207), 40.8% (82/201), and 40.3% (52 of 129) in aborigines, respectively. Multiple logistic regression analysis showed that persons ≥ 60 years old had a higher risk for infection with *T. canis* compared with those 20–29 years old (adjusted OR = 2.28, 95% CI = 1.24, 4.19, $P < 0.01$). This was also true in those who had histories of eating raw pig liver or raising dogs compared with those without such a history (adjusted OR = 1.65, 95% CI = 1.17, 2.33, $P < 0.01$ and 1.76, 95% CI = 1.18, 2.65, $P < 0.01$), respectively, and in laborers compared with persons who were unemployed (adjusted OR = 1.78, 95% CI = 1.14, 2.78, $P = 0.01$). Aborigines as a group had a higher risk for infection with *T. canis* than the ethnic Hans (adjusted OR = 1.97, 95% CI, 1.00, 3.85, $P = 0.04$) (Table 1).

DISCUSSION

Serologic tests are of considerable importance in the detection of human infection with *T. canis* because the clinical symptoms of toxocariasis are of limited value in differential diagnosis of toxocariasis.³¹ The TcES-ELISA is a specific diagnosis test for toxocariasis³² and shows no cross-reactivity

between TcES and sera from individuals infected with *Ascaris lumbricoides*, hookworm, *Entamoeba coli*, or *Giardia lamblia*.¹⁹ Gueglio and others³³ also reported that TcES showed no cross-reactivity with sera from patients with trichinosis or ascariasis as analyzed by the TcES-ELISA and Western blotting.

According to the U.S. Centers for Disease Control and Prevention, a cut-off titer in the *Toxocara* ELISA in the diagnosis of infection with *T. canis* was set at 1:32, with a sensitivity of 78% and specificity of 92%.³⁴ When the cut-off titer was decreased to 1:8, the sensitivity increased to 90%.³⁵ In the present study, we set a higher serum dilution of 1:64 for the TcES-ELISA and immunoblotting analysis. This may be beneficial in increasing the specificity in the detection of “true” *T. canis* infection among Taiwanese aborigines, and was supported by immunoblotting analysis that showed ELISA-positive but not ELISA-negative tested sera showed positive reactions to specific bands of 30 and 35 kD related to toxocariasis.

The overall seroprevalence for infection with *T. canis* among healthy adults of the five aboriginal populations was not low (46.0%, 247 of 537). The seroprevalence reportedly varies with differences in geography, ethnicity, and cut-off titer used. Thus, results from different studies may not be directly comparable. However, lower prevalence rates in healthy adults of Amazon Indians in Venezuela (34.9%),¹⁷ Orang Asli aborigines in Malaysia (29.3%),¹⁸ and natives in

Nigeria (30.4%) were observed,¹⁹ whereas higher rates were found in healthy adults in Indonesia (68.0%) and Nepal (81.0%).^{36,37} Sex did not seem to be a major factor related to infection with *T. canis* among healthy Taiwanese aboriginal adults due to a lack of a significant association between sex and frequency of *Toxocara* seropositivity in the present study. Similar findings have been reported in Malaysia, Venezuela, Nigeria, and Spain.^{15,17–19} Conversely, mean seroprevalence of infection with *T. canis* in aboriginal populations was found to increase with age. A similar result was also found in people in La Reunion.³⁸ A possible explanation for higher seroprevalence in older aboriginal adults might be due to their longer exposure to various risk factors related to infection with *T. canis*.

Although many studies have indicated that the predominant mode of transmission to humans is by ingestion of embryonated eggs in soil, ingestion of uncooked internal organs or meat of paratenic hosts is another possible source for human infections that cannot be ignored.³⁴ In the present study, eating raw wild-boar liver seemed to play an important role in acquisition of infection with *T. canis* in Taiwanese aborigines. This may be the reason for the high seroprevalence of infection with *T. canis*. Not surprisingly, a previous report indicated that infection with *Taenia saginata asiatica* (Taiwan taenia) from eating raw viscera of wild boar, especially the livers, was common in many different aboriginal groups in Taiwan.²² In Japan, infection of three adults with *T. canis* possibly occurred through the consumption of raw cow liver has also been reported.³⁹ Moreover, a significant association was observed between ownership of dogs and infection with *Toxocara* in aboriginal adults. It was also possible that aboriginal adults acquired the infection through inadvertent consumption of eggs contaminating the dog's body when they came in direct contact with their dogs; this is supported indirectly by our previous finding that the prevalence of *T. canis* eggs in dog stools deposited in soils was not low (18%) as surveyed in a Bunun community in eastern Taiwan.⁴⁰ A recent study also indicated that dogs infected with *T. canis* might infect people by direct contact because of the high density of embryonating and embryonated eggs found in dog fir.⁴¹

Aboriginal adults whose occupation was a laborer also seemed to have greater opportunity for exposure to infection with *T. canis*. However, a single risk factor alone is not sufficient to produce infection or disease. The risk of infection may be related to the intensity and duration of exposure and the behavior of people.³⁴ Thus, it could be postulated that they acquire the infection by eating raw liver or raising dogs, or that both factors might have contributed to this infection because these individuals seemed to have higher frequency than other occupational people in hunting wild pigs with the help of their dogs in the remote mountains, especially during hunting season (Fan CK, unpublished data). Adult aboriginal Taiwanese seemed more apt to be infected with *T. canis* than ethnic Han Taiwanese who lived in the same mountainous areas. This may be highly ascribed to the frequency of eating raw liver, contact with dogs, or both behaviors of the former individuals. It was observed during the course of this study that there was a generally high level of ignorance about the public health hazards posed by *T. canis*. We therefore conclude that exposure to *T. canis* infection is not uncommon among Taiwan aborigines, and it affects both sexes and all age groups.

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