

眼トキソカラ症の診断におけるトキソカラチェックの有用性

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Utility of ToxocaraCHECK in Diagnosis of Ocular Toxocariasis

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眼トキソカラ症は人畜共通感染症の一つで比較的診断が困難な疾患である。今回眼トキソカラ症が臨床的に疑われた3症例の血清および硝子体液に対して、トキソカラチェック・ELISA (enzyme-linked immunosorbent assay) (イヌ回虫幼虫排泄物抗原を使用) と multiple-dot ELISA を施行し、その有用性を比較検討した。トキソカラチェック・ELISA では3例ともに血清および硝子体液においてイヌ回虫幼虫排泄物抗体が疑陽性もしくは陽性を示したが、multiple-dot ELISA では2例においてイヌ回虫成虫抗体が陰性であった。イヌ回虫幼虫排泄物抗体を簡便に検出するトキソカラチェックは眼トキソカラ症の迅速診断に有用であった。

Ocular toxocariasis is a zoonosis whose diagnosis is difficult. In 3 cases diagnosed as ocular toxocariasis, we performed ToxocaraCHECK and enzyme-linked immunosorbent assay (ELISA) with the excretory-secretory (ES) antigen of *Toxocara* larva and multiple-dot ELISA with the antigen of *Toxocara canis* against serums and vitreous fluids. Results of ToxocaraCHECK and ELISA were positive in all cases, whereas the results of multiple-dot ELISA were negative in two cases. The immunology tests using ES antigen of *Toxocara* larva are useful for diagnosing ocular toxocariasis, ToxocaraCHECK being especially efficient for rapid diagnosis.

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Key words : 眼トキソカラ症, トキソカラチェック, multiple-dot ELISA, ocular toxocariasis, ToxocaraCHECK, multiple-dot ELISA.

はじめに

近年のグルメブームによる生肉の摂取やペットブームによる仔イヌとの接触などにより、眼トキソカラ症が増加している^{1,2)}。眼トキソカラ症の確定診断には病理学的に幼虫を証明することが必要であるが、実際には不可能なことが多い。したがって、腫瘍性病変や硝子体索状物形成などの特徴的な眼所見と血清における陽性反応から本症を疑い、最終的に眼内液に対する免疫学的検査にて診断することとなる³⁾。しかし、これらの免疫学的検査は大学の寄生虫学教室などの限られた機関でしか実施できない点が大きな問題であった。

近年、トキソカラの抗体を簡便に検出できるトキソカラチェックが開発され⁴⁾、その有用性が眼科領域においても報告

されている⁵⁾。一方、外注検査においても寄生虫抗体のスクリーニング検査としての multiple-dot ELISA (enzyme-linked immunosorbent assay)⁶⁾ が、利用可能な検査として期待されている。しかしながら、両者では検出する抗体が異なっており、診断への有用性については不明である。そこで、筆者らは眼トキソカラ症が疑われた3例から得られた血清もしくは硝子体液を対して multiple-dot ELISA およびトキソカラチェックを行い、対照として実施した ELISA との相同性を検討したので報告する。

I 対象および症例

対象は、2000年4月から2001年5月の間に、愛媛大学眼

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科外来において眼トキソカラ症を疑われた3例3眼である。男性2例2眼，女性1例1眼で，年齢は44～52歳（平均48歳）であった。

以下，各症例について簡単に解説する。

〔症例1〕 52歳，男性。

主訴：左眼の霧視および充血。

生活歴：ウシの生肝臓の摂取歴あり。

現病歴：左眼の霧視および充血を自覚し来院した。

初診時眼科所見：矯正視力は右眼（1.5），左眼（1.2）であった。右眼に特に異常所見は認められなかった。左眼には前房と硝子体中に軽度炎症細胞を認め，眼底周辺部（耳側上方赤道部）の網膜表層に隆起する白色腫瘍を大小数個認めた（図1）。

全身検査所見：白血球は正常範囲内であったが，好酸球は20.8%と増加していた。IgE（免疫グロブリンE）は4,173 mg/dlと著明に上昇していた。

〔症例2〕 48歳，女性。

生活歴：ウシの生肝臓の摂取歴あり。

現病歴：左眼霧視を自覚し来院した。

初診時所見：矯正視力は右眼（1.5），左眼（1.2）であった。右眼に特に異常所見は認められなかった。左眼には前房と硝子体中に軽度炎症細胞を認め，眼底周辺部（耳側）にはテント状の硝子体索状物を認めた（図2）。

全身検査所見：異常は認めなかった。

〔症例3〕 44歳，男性。

生活歴：ウシの生肝臓の摂取歴あり。

現病歴：平成3年より右眼の原因不明の中間部ぶどう膜炎に対して近医にて治療（ステロイド全身・局所）を受け，再発と軽快をくり返していた。平成13年1月再度右眼のぶどう膜炎を認め，治療開始するも4月には硝子体混濁の増強と

黄斑上膜の形成を認めたため，手術目的にて来院。

初診時所見：矯正視力は右眼（0.5）であった。前房・硝子体中に炎症細胞を認め，下方網膜周辺部の広範囲に snow-bank 形成，黄斑部に黄斑上膜を認めた。

全身検査所見：異常は認めなかった。

II 方 法

各症例から採取された血清・硝子体液を用いて，下記の免疫学的検査を施行した。

<各種免疫学的検査の原理と方法>

1. トキソカラチェック

トキソカラチェックは，イヌ回虫幼虫排泄物抗原の抗原抗体反応を利用したイヌ回虫幼虫排泄物抗体の検出法である。ニトロセルロース膜にイヌ回虫幼虫排泄物抗原を吸着させてあり，被検血清中の抗体が抗原と結合し，抗原と結合した抗体が protein A と結合すれば赤いスポットとして肉眼視できる。検体を10倍と50倍に希釈し，それぞれキットに滴下するが，50倍希釈で反応がある場合は陽性，50倍希釈で反応がなく，10倍希釈で反応がある場合は疑陽性，両方とも反応がない場合は陰性と判定する。

2. ELISA

酵素により抗体を標識し，酵素反応の結果生ずる色調の変化を分光光度計にて測定し，抗原に結合した抗体量を測定する。今回は，抗原としてイヌ回虫幼虫排泄物抗原を用い，特異抗体を検出した。

3. Multiple-dot ELISA（寄生虫抗体スクリーニング検査）

12種類の寄生虫（ウエステルマン肺吸虫，宮崎肺吸虫，肝蛭・肝吸虫，マンソン孤虫，有鉤囊虫，イヌフィラリア，イヌ回虫成虫，ブタ回虫成虫，アニサキス，顎口虫，糞線虫）

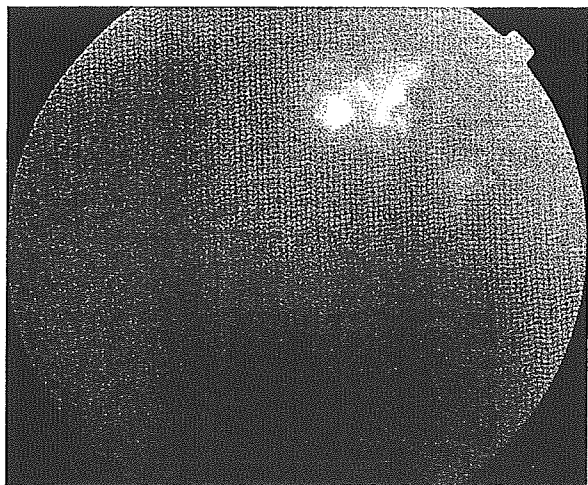


図1 症例1の初診時眼底写真
網膜周辺部に白色腫瘍を数個認める。

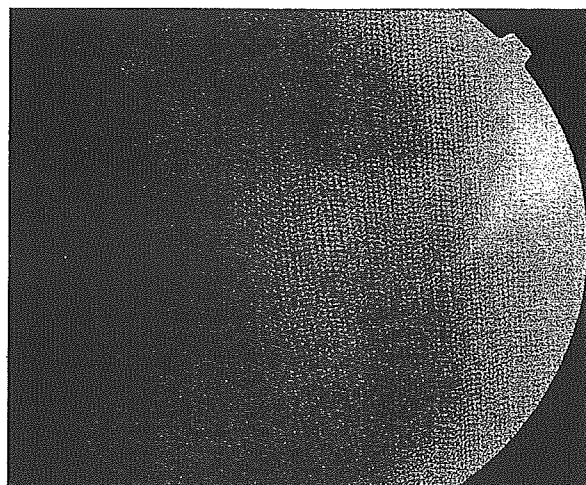


図2 症例2の初診時眼底写真
網膜周辺部に硝子体索状物を認める。

表 1 各症例の血清に対する免疫学的検査の結果

免疫学的検査	トキソカラチェック	ELISA	Multiple-dot ELISA	
			イヌ回虫成虫抗体	他の寄生虫抗体
症例 1	陽 性	強陽性	陰 性	ブタ回虫成虫抗体陽性
症例 2	疑陽性	陽 性	陰 性	イヌ糸状虫, ブタ回虫成虫, アニサキス, 糞線虫, 肝蛭の各抗体疑陽性
症例 3	疑陽性	陽 性	未施行	未施行

表 2 各症例の硝子体液に対する免疫学的検査の結果

免疫学的検査	トキソカラチェック	ELISA
症例 1	陽 性	強陽性
症例 2	陽 性	陽 性
症例 3	陽 性	陽 性

について、抗原をニトロセルロース膜に吸着させ、抗体を検出するスクリーニング検査である(外注にて依頼可能)。

III 結 果

各症例の血清・硝子体に対する免疫学的検査の結果を表 1, 2 に示す。

血清に対するトキソカラチェックにおいて症例 1 は陽性、症例 2, 3 では疑陽性を示し、ELISA においては 3 例とも陽性であった。一方、血清に対する multiple-dot ELISA では施行できた 2 症例ともにイヌ回虫成虫抗体は陰性で、他の寄生虫抗体において陽性または疑陽性を呈した。

硝子体液に対するトキソカラチェックおよび ELISA は 3 例とも陽性であった。

IV 考 察

眼トキソカラ症は幼虫移行症の一つで、仔イヌが排泄した虫卵を摂取した場合や幼虫を含む生肉(肝臓)を食した場合にヒトへ感染する。経口摂取された虫卵や幼虫は第 2 期幼虫まで成育した後、消化管壁を破って血管内に侵入し、血行性に種々の組織に移行する。欧米では、小児に好発するぶどう膜炎の一つとして注目されていたが、わが国での報告例はそれほど多くなかった。しかし、近年のグルメブームやペットブームにより、わが国の報告例は次第に増加しているようである^{1,2)}。しかしながら、診断は、白色腫瘍や硝子体索状物などの特徴的所見に依存することがほとんどであり、また、特徴的な所見を欠く場合には見過ごされてしまう可能性も高い。

客観的な診断としては病理学的に組織中に幼虫を発見するのが理想的であるが、実際にはきわめて困難であり、補助診断として血清、確定診断として眼内液(前房水・硝子体液)に対する免疫学的検査を行うこととなる³⁾。しかしながら、ELISA や二重寒天ゲル法などの特殊な免疫学的検査は専門機関への依頼が必要であり、結果が得られるまでに時間を要するという難点があった。

近年、迅速検査として開発された⁴⁾ トキソカラチェックは、イヌ回虫幼虫排泄物抗体を検出する検査法で、特異性が高いうえ、手技も簡便で、反応時間は 5 分ときわめて短い。

また、12 種類の寄生虫感染症のスクリーニング検査として開発された multiple-dot ELISA は、外注にて依頼できるため利用しやすい⁶⁾ が、イヌ回虫成虫抗体を標的とする点が疑問である。

今回の検討によると、ELISA によってトキソカラ症と診断された 3 症例について、トキソカラチェックは高い相関を示した。すなわち、症例 1 においては血清、硝子体液の両方で陽性であり、また、症例 2, 3 においても血清では疑陽性、硝子体では陽性を呈した。血清においては ELISA よりも陽性率が落ちるものの、硝子体液においては ELISA の結果とよく相関しており、本症の診断に非常に有用と思われる。

一方、イヌ回虫成虫に対する抗体検出を目的とした multiple-dot ELISA では、前述の 3 症例の血清では、全例で陰性であり、逆に、他の寄生虫に対する抗体が陽性を示していた。このことは、眼トキソカラ症ではイヌ回虫成虫抗体の上昇はなく、他の寄生虫に対する抗体と交差反応を起こす可能性を示唆している。実際、酒井らは、ブタ回虫抗原により RAST (radioallergosorbent test) 検査が本症の診断に有用であった⁷⁾ と報告している。硝子体液についての検討は行っていないため断言はできないが、multiple-dot ELISA は眼トキソカラ症の診断にはさほど有用ではない可能性が高い。

今回の検討から、眼トキソカラ症の診断にはイヌ回虫幼虫排泄物抗体の検出が有用で、特にトキソカラチェックは迅速検査として優れていると思われた。今後はさらに症例を増加させ、イヌ回虫成虫抗体、イヌ回虫幼虫抗体、イヌ回虫幼虫排泄物抗体などの各種抗体の陽性率を比較し、最適な診断法について検討していく必要がある。

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period of time, in spite of repeated infection, or it may emerge just after infection for a short period of time.

While many cases appear to be asymptomatic, there may be some background factors for the symptomatic cases, such as a compromised immune system. In mice, the levels of both IL-12 and tumor necrosis factor alpha were significantly lower in the infected group as compared with the controls (Kuroda *et al*, 2001). Although Akao *et al* (2000) and Hayashi *et al* (2003) reported an animal model for ocular toxocariasis, further studies are required to better evaluate the mechanism of toxocariasis. While it is useful to assess the pathogenesis of this disease using such animal models, a more complete understanding will be reached when they are combined with careful population studies.

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Table 5

Number of students of each range of percentage of blood eosinophil counts out of total white blood cells, shown with index value to *T. canis* by plate-ELISA and result of dot-ELISA screening for 7 parasitic diseases.

Eosinophils count (%)	No. of students n=34 (%)	Index value to <i>T. canis</i> by plate-ELISA ^a	Result of dot-ELISA screening
1-5	10 (29.4)	4 to 14	
6-14	19 (55.9)	4 to 14	
15-19	2 (5.9)	6, 13	
20-29	2 (5.9)	9, 14	<i>A. suum</i> +
55	1 (2.9)	17	<i>A. suum</i> + , <i>F.h</i> +

^aIndex value is defined 1 to 2 as negative, 3 as weak positive, more than 4 as positive, more than 10 as strong positive.

Table 6

Number of positive samples of intestinal parasites eggs or cyst by stool examination among village people in district B.

	Number of positive samples in District. B (n = 53)
Hookworm	22 (41.5%)
<i>Ascaris lumbricoides</i>	2 (3.8%)
<i>Trichuris trichuria</i>	6 (11.3%)
<i>Strongyloides stercoralis</i>	1 (1.9%)
<i>Entamoeba coli</i>	15 (28.3%)
<i>Endolimax nana</i>	7 (13.2%)
<i>Iodamoeba buetschlii</i>	1 (1.9%)
<i>Blastocystis hominis</i>	9 (17%)
<i>Giardia lamblia</i>	1 (1.9%)

the junior high school students we studied may have been infected repeatedly, and may have remained infected for several years. These data also suggest that *T. canis* infection is asymptomatic, being a "covert type," in most cases.

The antibody titers we observed indicated the possibility of a past infection, a covert infection, or a cross-reaction. Many previous studies have shown that antibodies persist for long periods of time due to repeated antigen stimulation resulting from the persistence of live larvae in tissues (Beaver, 1969) or from periodic reinfection (Glickman and Shantz, 1981). Lynch *et al* (1988) reported the existence of significant

cross-reactivity of non-homologous parasites with *Toxocara* in regions of endemic helminthiasis. In the present study, we performed dot-ELISA screening to detect the antibodies for other parasitic diseases, and detected *Ascaris suum* infection in 29 out of 71 cases (40.8%). Of these, four cases showed no cross-reactivity with *Toxocara*. Further studies are needed to differentiate these parasites by detecting their antibodies.

Magnaval *et al* (1997) suggested that the migration of *T. canis* larvae in the human brain does not frequently induce a recognizable neurological syndrome but is correlated with several risk factors, including exposure to dogs, a situation possibly responsible for repeated low-dose infections.

Gonzalez *et al* (2000) reported that high resolution ultrasonography revealed hypoechoic areas in the liver in 50% of their cases. Their patients had a mean age of 2 years and 9 months, presented with anemia, long-standing fever, and eosinophilic leukocytosis, and had been diagnosed as having toxocarasis based on a high titer of *Toxocara* antibodies according to an ELISA. Kaushik *et al* (1997) also reported granulomatous hepatitis caused by *Toxocara canis* that showed clinical improvement without specific therapy in all cases. Considering these reports and the present study, hepatitis caused by *T. canis* may occur in infants and may improve within a few years. Liver functional disorder may not persist in its active stage for a long

Table 3

Percentage of the answer of questionnaire about the condition of the rash and visual trouble among junior high school students in 3 districts.

	District A (urban) (n = 39)	District B (rural) (n = 36)	District C (rural) (n = 46)
Do you have ever developed rash?			
Yes	22 (56.4%)	23 (63.9%)	24 (52.2%)
Which is the part of rash?			
Hands or wrist	14	18	5
Forearm	1	2	2
Arm	1	1	0
Head or neck	1	1	1
Body	1	5	7
Thigh	0	0	11
Leg	3	4	3
Ankle or foot	4	2	1
What type of rash?			
Itchiness	12	17	21
Painful	0	0	0
Swelling	2	3	1
Blisters	0	0	0
Discharge / weeping	1	3	12
Crusting	0	1	1
Red spots	8	7	2
Do you have ever developed the visual trouble?			
Yes	23 (20.5%)	28 (35.3%)	8 (15.2%)
What kind of trouble?			
Muscae volitantes	1	7	1
Pain of eyes	3	0	1
Paropsia	4	7	1
Visual field loss	1	0	0
Photophobia	2	15	6
Lacrimation	2	3	2
Feeling of foreign body	3	4	1
Congestion	1	3	1
Itchiness	10	12	3
Blurred vision	3	3	1

dren having high antibody titers but no specific clinical findings led to the concept of "covert toxocarasis."

In the present study, which was performed in a tropical country, we observed a higher seroprevalence of anti-*Toxocara* antibodies in a rural area of Manado (81.7%) than would have been predicted from the literature. We selected the *Toxocara*-CHECK kit for our primary survey due to its speed and ease of use. This screen-

Table 4

Result of dot-ELISA screening for detecting the antibodies for pathogenic parasitic diseases.

Antigens	Number of positive, n = 71 (%)
<i>Entamoeba histolytica</i>	4 (5.6)
<i>Spirometra erinacei</i>	0 (0)
<i>Fasciola hepatica</i>	2 (2.8)
<i>Paragonimus miyazakii</i>	0 (0)
<i>Ascaris suum</i>	29 (40.8)
<i>Anisakis simplex</i>	5 (7.0)
<i>Dirofilaria immitis</i>	2 (2.8)

ing method revealed a high prevalence, and confirmed that the prevalence could be easily assessed. We concluded that the kit was useful for an initial survey in areas with a high rate of environmental contamination with *Toxocara* eggs.

We found a significant difference in seroprevalence between the two rural districts and the urban district, based on our questionnaire findings, this difference may be attributable to differences in the behaviors of feeding animals and in the management of wastes between urban and rural areas. Our findings further suggest that improvements in sanitation would markedly reduce these infections. While hand washing prior to eating was reported in both areas, it did not appear to be adequate to prevent infection in many cases.

In the present study, we found no clinically symptomatic case attributable to *Toxocara* infection, except for one case in which there were problems with the visual field. Of course, self-reports by children regarding symptoms of *Toxocara* infection may be unreliable. Bass *et al* (1987) suggested that longer periods, in excess of seven years, would be necessary to entirely discount the possibility of latent ocular disease, and they found that treatment with thiabendazole did not affect the course of the infection in a controlled year-long evaluation based on eosinophil counts and specific *T. canis* antibody titers. We observed a high prevalence in subjects aged between 10 and 15 years, suggesting that

Table 1
Result of the screening of specific anti-*Toxocara* antibody by Toxocara-CHECK.

	District A (urban) (n = 41)		District B (rural) (n = 71)		District C (rural) (n = 46)	
	1:10	1:50	1:10	1:50	1:10	1:50
Strong positive	1 (2.4%)	0 (0%)	11 (15.5%)	0 (0%)	13 (28.3%)	0 (0%)
Positive	4 (9.8%)	1 (2.4%)	26 (36.6%)	12 (16.9%)	15 (32.6%)	16 (34.8%)
Weak positive	0 (0%)	0 (0%)	34 (47.9%)	25 (35.2%)	0 (0%)	0 (0%)
Negative	36 (87.8%)	40 (97.6%)	0 (0%)	34 (47.9%)	18 (39.1%)	30 (65.2%)

Each sample was diluted 1:10 and 1:50 in dilution buffer (0.5% BSA in PBS, pH 7.2).

Table 2
Percentage of the answer of questionnaire about the junior high school students' life style in the 3 districts.

	District A (urban) (n = 39)	District B (rural) (n = 36)	District C (rural) (n = 46)
Source of water supply for drinking and cooking			
Public water supply	61.5%	0%	6.5%
Well	38.5%	100%	93.5%
Management after visiting the Toilet			
With paper	87.2%	0%	0%
With hand and water	12.8%	100%	100%
Mode of fecal disposal			
River	0%	13.9%	17.4%
Pit latrine	43.5%	47.2%	47.8%
Pour flush	56.5%	38.9%	34.8%
Washing hands before eating			
Yes	97.3%	100%	97.8%
No	2.6%	0%	2.2%
Habit of eating raw vegetables			
Yes	38.5%	45.7%	8.7%
No	61.5%	54.3%	91.3%
Way of eating			
With hands	20.5%	35.3%	15.2%
With spoon and fork	79.5%	64.7%	84.8%
Animals at home			
Yes	64.1%	97.1%	100%
No	35.9%	2.9%	0%
Bathing in the pond or river			
Yes	10.3%	27.3%	45.7%
No	89.7%	72.7%	54.3%

Totally 158 were distributed, and 121 (76.6%) were returned.

is made before the age of 6, in compliance with a governmental public health program, which may account for our findings: in district B, no cysts of *Entameba histolytica*, or eggs of either Trematoda or Cestoda were detected.

DISCUSSION

Toxocariasis is a multisystemic disease of parasitic zoonosis that occurs predominantly in young children, and it is a significant public health problem in both developed and developing countries. Until recently, toxocariasis was a relatively neglected disease in tropical regions; however, overcrowding and cohabitation of the peri-domestic environment by dogs and cats has markedly reinforced the transmission cycle of this disease in many regions. Since people feed dogs freely in rural areas, eggs have been deposited extensively in the soil. Thus, disease exposure in such areas seems inevitable.

Epidemiological surveys in industrialized countries of the northern hemisphere have revealed that the seroprevalence of helminthiasis ranges between 2.5 and 5% in urban adults (Glickman and Schantz, 1981; Glickman *et al*, 1985; Cauchie *et al*, 1990). In Europe, however, a higher rate has been reported among rural populations, ranging from 17% among Swedish farmers (Ljungstrom and Van Knapen, 1989) to over 40% in Irish children (Holland *et al*, 1995). A case-control study of Irish children reported that 27% of those who had fever, headache, and so forth, along with a high anti-*Toxocara* antibody titer still had normal blood eosinophil counts (Taylor *et al*, 1988). The existence of chil-

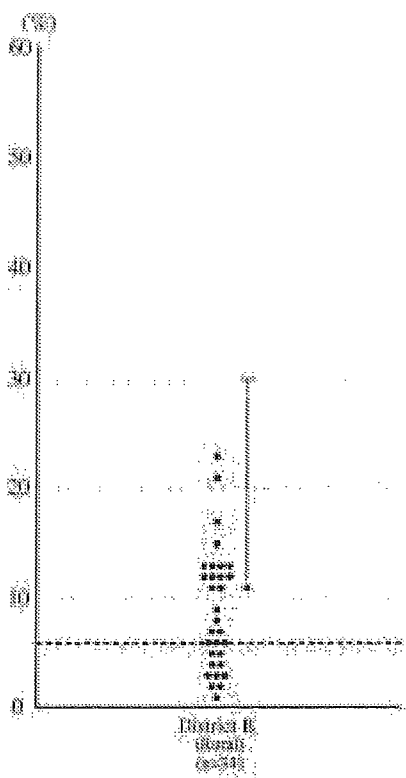


Fig 2-Distribution of the percentage of eosinophils in the total white blood cells among 34 subjects having a high antibody titer against the second stage larval ES antigen of *Toxocara* in district B. The eosinophil counts were performed by a thin-smear stained with May- Grunwald-Giemsa staining. The right side point represents the mean ± 2 SD.

Clinical evaluation

No hemorrhagic lesions or granulomatous lesions were detected in any of the 34 students examined by ophthalmoscopic examination (data not shown).

Our count of the eosinophil rate relative to the total white blood cell count in the peripheral venous blood showed that 24 of 34 subjects (70.6%) had hypereosinophilia, with five of these showing extremely high levels (Fig 2).

No significant abnormality of serum transaminase (AST, ALT) or gamma-GPT was detected in district B or district C (Fig 3). In one case, a moderate abnormality was detected, although this case was negative for *Toxocara* antibody.

Dot-ELISA detection of other important pathogenic parasitic diseases

Table 4 shows the prevalence of each parasite. Table 5 shows the relationship, in five subjects, between an extremely high eosinophil rate ($>14\%$ eosinophil count) and the results of the dot-ELISA screening. One subject who had a 55% hypereosinophilia rate showed positive for both *A. suum* and *F. hepatica* antigen.

Stool examination

Table 6 shows the results of the stool examinations of the general public. In Indonesia, a mass treatment of elementary school students

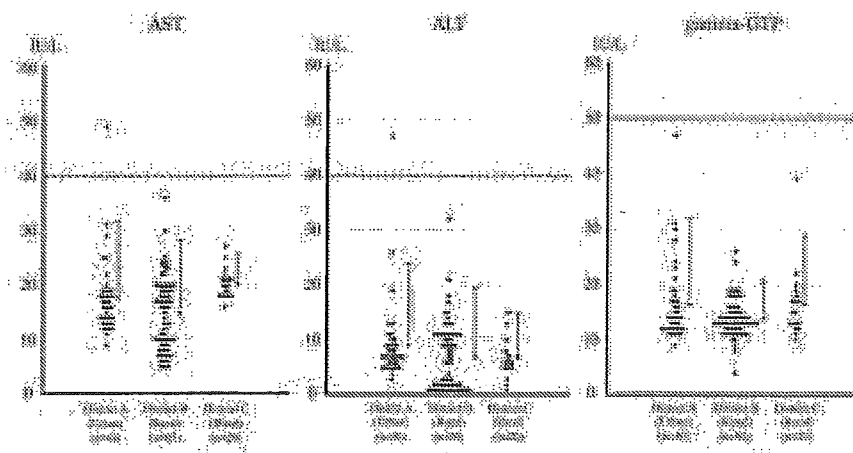


Fig 3-Distribution of the value of serum aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and gamma-glutamyltranspeptidase (gamma-GTP) among the subjects from three districts. The right side point represents the mean ± 2 S.D..

Dirofilaria immitis, *Anisakis simplex*, and *Spirometra erinacei*. All but *A. simplex* and *S. erinacei* were derived from adult-stage antigens.

Stool examination

To evaluate the intestinal parasitic infection of the local area, stool samples were collected in district A. A total of 53 samples were collected from village residents of various ages who lived near a rice field and a lake. Samples were fixed with sodium acetate-acetic acid-formaldehyde (SAF) solution (sodium acetate 1.5 g, acetic acid 2.0 ml, formaldehyde 4.0 ml, distilled water 92.0 ml), and the eggs of helminths and the cysts of protozoa were detected using the formalin-ethyl acetate sedimentation technique.

RESULTS

Toxocara-CHECK screening

In the on-site screening in district B, all samples diluted 1:10 were positive, and 11 (15.5%) showed strongly positive results. Thirty-seven out of 71 (52.1%) serum samples diluted 1:50 were positive ("weak positive" and "positive") according to the Toxocara-CHECK (Table 1). In the other districts, positive results were found in 5 out of 41 (12.2%) samples from district A and in 28 out of 46 (60.9%) samples from district C. The differences between the findings of the urban district (A) and of the two rural districts (B and C) were considered to be statistically significant according to a chi-square test.

Plate-ELISA for *T. canis* second stage larval ES antigen

Fifty-eight of 71 (81.7%) serum samples showed positive by plate-ELISA in district B, and 23 of these positive samples showed strongly positive results (Fig 1). The index values ranged from 1 to 21.

Questionnaire about life style

The findings from the questionnaire regarding the life styles of the subjects from the three districts are shown in Table 2. Of the 158 questionnaires distributed, 121 (76.6%) were returned with complete answers. Differences were found between the urban area (district A) and the rural areas (districts B and C) regarding the source of water supply for drinking and cooking, manage-

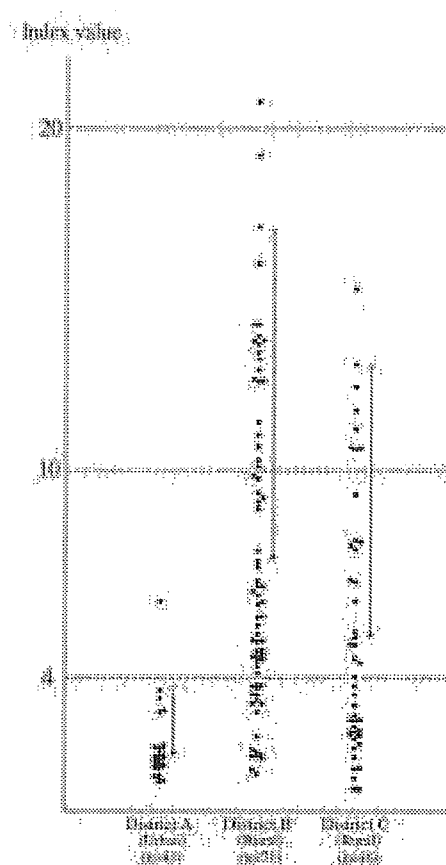


Fig 1—Distribution of index values of the samples collected from subjects from each district calculated by optical density (OD) measured by a plate-ELISA using a second stage larval excretory-secretory (ES) antigen of *T. canis*. The right side point represents the mean \pm 2 SD.

ment after visiting toilets, and the mode of fecal disposal. No significant differences were found regarding hand washing before eating or in the feeding of animals at home.

Table 3 presents the questionnaire data regarding the rash condition and visual trouble among our subjects. Between 52.2% and 63.9% of students reported a past history of an appearance of a skin rash. The most common report was one of a red spot on the skin. Between 15.2% and 35.3% of students reported a past history of ophthalmic trouble, with the most common complaint being itchiness. Only one subject reported problems of the visual field.

tween the seropositive rate and the clinically symptomatic rate. The purpose of the present study was to evaluate the factors contributing to high serological prevalence of toxocariasis and to investigate the relationship between these pathogens and clinical symptoms. We performed a serological survey of junior high school students in two rural districts and in one urban district, and found a very high prevalence rate in the rural district, with most cases being of the covert type.

MATERIALS AND METHODS

Specific antibody detection

Serum specimens were obtained from 158 junior high school students, aged between 10 and 15 years, from three distinct geographical areas in northern Sulawesi, Indonesia. These areas consisted of an urban area of Manado city (district A); an agricultural village (district B), which is located 32 km southwest from Manado city; and a fishing village (district C), which is located 120 km west from Manado city. Serum samples were collected from 41 individuals from district A, 71 individuals from district B, and 46 individuals from district C. Informed consent was obtained before the blood drawing in all cases. The samples were tested in the field by Toxocara-CHECK, a rapid-test kit for *Toxocara* infection, to screen for the specific anti-*Toxocara* antibody (Akao *et al*, 1997; Dubinsky *et al*, 2000). Each sample was diluted 1:10 and 1:50 in a dilution buffer (0.5% BSA in PBS, pH 7.2) and tested simultaneously. According to the test kit instructions, samples having a positive result at a serum dilution of 1:10 and a negative result at 1:50 were considered to be weakly positive, those having a positive result at 1:50 were considered to be strongly positive, and those having a negative result even at 1:10 were considered to be non-significant for toxocariasis. In addition to the on-site examination, we carried out a plate-ELISA with 158 serum samples from the three districts using the larval excretory-secretory (ES) antigen of *T. canis*. We objectively assessed the measurement of the optical density (OD) of the results of the plate-ELISA and used this as an index value for comparison with

the three control samples: strongly positive, weakly positive, and negative results. We assigned an index value of 10 to the strongly positive control serum, 3 to the weakly positive control serum, and 1 to the negative control serum. Samples registering above 4 on the index value were considered to be positive.

Questionnaire survey

All subjects were given a questionnaire about their lifestyle, water supply, eating habits, method of defecation, feeding of animals, and so forth. To evaluate the clinical symptoms caused by VML or OLM, questions about the condition of the skin rash and visual trouble were also included.

Ophthalmoscopic examination

The ocular fundi of 34 subjects that had a high antibody titer were carefully observed using a portable ophthalmoscope (Hand-Held Fundus Camera GENESIS, Kowa Corporation, Japan) after the pupils were dilated with tropicamide (one drop for each eye; Mydrin-P, Santen Pharmaceutical, Japan). The images of the fundi were recorded photographically. No complications were reported after the examinations.

Eosinophil counts and assessment of liver function

Eosinophil counts were performed using a thin smear stained with May-Grunwald-Giemsa staining for 34 students having a high antibody titer. To evaluate the liver functional disorder caused by VLM, we measured serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltranspeptidase (gamma-GTP) in 125 students from the three districts. An eosinophil rate of over 6% was considered to indicate hypereosinophilia; while an AST and ALT over 40 IU/l, and a gamma-GTP over 50 IU/l were considered to indicate a significant abnormality.

Dot-ELISA for detecting the antibodies for pathogenic parasitic diseases

For 71 students in district B, we also conducted a screening test by dot-ELISA to detect pathogenic parasitic diseases such as those caused by *Entamoeba histolytica*, *Fasciola hepatica*, *Paragonimus miyazaki*, *Ascaris suum*,

THE HIGH PREVALENCE OF ASYMPTOMATIC *TOXOCARA* INFECTION AMONG SCHOOLCHILDREN IN MANADO, INDONESIA

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Abstract. We performed a serological survey of *Toxocara canis* infection in junior high school students from three districts in northern Sulawesi. Almost all of the 117 subjects from two rural districts near Manado allowed dogs in their houses, and there was an 84.6% prevalence of *T. canis* infection in this group. Fifty-three subjects (45.3%) had serum samples with a high titer of specific anti-*Toxocara* antibody. By contrast, 41 students tested in one urban district showed a 12.2% prevalence. To confirm the clinical symptoms of visceral larva migrans (VLM) and ocular larva migrans (OLM) caused by *Toxocara*, we administered a questionnaire survey, serological liver function tests, and an ophthalmoscopic examination in 34 subjects having high anti-*Toxocara* antibodies. One rural district showed a high prevalence; 58 out of 71 subjects (81.7%) had a high titer of anti-*Toxocara* antibodies according to a plate-ELISA test, although none showed clinical signs. Five of these subjects exhibited hypereosinophilia. These results indicated that *T. canis* infection in northern Sulawesi is latent in many more cases than previously estimated, and suggest that people living in environments polluted by *Toxocara* eggs become easily infected with *T. canis* and show a high prevalence of infection.

INTRODUCTION

Toxocara canis and *T. cati* are ubiquitous gastrointestinal parasites in dogs and cats, respectively. Humans, especially young children, can be infected with these parasites following accidental ingestion of eggs containing infective-stage larvae. These parasites cause visceral larva migrans (VLM), which is characterized by fever, eosinophilia, hepatomegaly, cough, and wheezing. They also cause ocular larva migrans (OLM), which is characterized by a severe vision defect and neurologic larva migrans showing seizures and meningoencephalitis (Mok, 1968; Schantz and Glickman, 1978; Taylor *et al*, 1988). The definitive diagnosis of toxocariasis is based on the identification of larvae in tissue biopsies,

while a presumptive diagnosis may be based on clinical and laboratory findings and on the presence of significant anti-*Toxocara* antibody titer (Glickman *et al*, 1978). In developed countries, there have been numerous serological surveys on toxocariasis in young children, but few such studies have been done in developing countries. We assumed that this was partly due to a lack of suitable tools for easy on-site testing. While the introduction of the enzyme-linked immunosorbent assay (ELISA), which is based on the use of excretory-secretory (ES) antigen, has resulted in greatly increased specificity, it is still difficult to perform this test in developing countries. However, since toxocariasis remains a pressing public health problem in developing countries, serological epidemiological surveys are essential to provide detailed information regarding the prevalence of *T. canis* infection and its clinical features if the transmission of *T. canis* infection to humans is to be prevented.

Although these diseases can cause a severe vision defect or liver function disorder, there are few reports on the precise relationship be-

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