Influx across the apical membrane of absorptive cells occurs via a Na^+/glucose cotransporter (SGLT1) as well as by a fructose transporter GLUT5, and efflux of hexose across the basolateral membrane occurs via GLUT2 (Cheeseman, 1993; Wright, 1993; Reuss, 2000). It is known that a significant degree of peptide absorption occurs through the role of proton-coupled, oligopeptide transporter family members including peptide/histidine transporter 1 (PepT1) (Herrera-Ruiz and Knipp, 2003). For the import and export of amino acids, many types of heterodimeric transporters have been reported, including light chain glycoprotein-associated amino acid transporter LAT1 or LAT2 (Wagner et al., 2001; Verrey, 2003). In the present study, we examined whether *N. brasiliensis* infection induces any alterations in hexose, peptide and amino acid transporter expression in the small intestine of rats.

2. Materials and methods

2.1. Animals and nematode infection

Specific pathogen-free male Brown Norway/Sea rats were purchased from SLC Inc. (Shizuoka, Japan). Animals at 8 weeks of age were injected subcutaneously with 2000 *N. brasiliensis* L3 larvae as described elsewhere (Hyoh et al., 1999). The animals were allowed to feed ad libitum throughout the experiment.

2.2. Preparation of intestinal epithelial cells (IEC)

The animals were sacrificed with an overdose of ether after overnight fasting with free access to water. The separation of IEC was carried out at 4°C in EDTA-Hanks' solution (Ca^{2+}, Mg^{2+}-free Hanks' balanced salt solution supplemented with 10 mM HEPES, pH 7.3, and 1.0 mM EDTA) as described elsewhere (Hyoh et al., 2002) with slight modification. In brief, a piece of jejunum 15–23 cm from the pyloric ring and a piece of ileum 10–18 cm from ileocecal junction were removed, opened longitudinally, and cut into segments 1 cm in length. After a brief wash in PBS, four pieces of tissue were put into a 15-ml tube containing 4 ml of 1.0 mM EDTA-Hanks' solution, and debris attached to the mucosal surface was removed by 15 strokes of vigorous shaking of the tubes by hand. The tissues were then transferred into another tube containing 0.5 mM EDTA-Hanks' solution. After 75 min on ice with occasional agitation of the tissues by inverting the tubes, epithelial cells were separated by 60 strokes of vigorous shaking of the tube by hand. After discarding the tissue, detached epithelial cells were collected by centrifugation at 600 × *g* for 3 min at 4°C, and the cell pellets were kept at −80°C until use. Histological examination of the tissue after collection of epithelial cells showed that villus epithelial cells were separated completely, whereas epithelial lining cells in the lower part of the crypts were still attached to the tissue in approximately half of the crypts. The basal lamina of the epithelium was intact and lamina propria cells were retained in the tissue.

2.3. Extraction of total RNA, cDNA synthesis and PCR

Total RNA was extracted using TRIZOL Reagent (Life Technologies, Rockville, MD). Two-microgram aliquots of RNA were reverse transcribed in 20 μl of reverse transcription (RT) buffer containing 5 mM $MgCl_2$, 1 mM dNTP mixture, 1 U/ml RNase inhibitor, 0.25 U/ml AMV reverse transcriptase and 0.125 mM oligo dT-adaptor primer (Takara RNA LA PCR kit, Takara Biomedicals, Osaka, Japan) at 42°C for 50 min. One-microliter aliquots of synthesised cDNA were added to PCR buffer containing 2.5 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.025 U/ml LA Taq DNA polymerase (Takara RNA LA PCR kit), and 0.2 mM sense and antisense primers in a final volume of 10 μl. PCR was carried out with cycles of 30 s at 94°C, 30 s at 62°C and 30 s at 72°C. The sense and antisense primers used were: 5′-AGAAGAGCTATGAGCTGCCTGACG-3′ and 5′-CTTCTGCATCCTGTCAGCGATGC-3′ for β-actin with 236 bp products; 5′-CATCATCCCTGCATCCACTG-3′ and 5′-CAAAGGTGGAGGAATGGGAG-3′ for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) with 272 bp products; 5′-GGGGCAGATCCAGGTGATTC-3′ and 5′-GCCATCGTTTCACAGAAATCCAC-3′ for thymidine kinase with 284 bp products; 5′-GAAGGGTGCATCGGAGAAGG-3′ and 5′-CTTTAGGGTCCTCCTCGACG-3′ for SGLT1 with 518 bp products; 5′-ATCGTCAACACGGCCTTCAC-3′ and 5′-AAGCCGGAAGCGATCTCATC-3′ for GLUT1 with 458 bp products; 5′-TTCTTTGG TGGGTGGCTTGG-3′ and 5′-TGGGGCTTTCTGGACAGAA-3′ for GLUT2 with 394 bp products; 5′-GGTACAACGTGGCTGCTGTC-3′ and 5′-CATGGGGACCA CGTTGGAAG-3′ for GLUT5 with 347 bp products; 5′-TCTATCCTCCTCCACGGCAA-3′ and 5′-ATGTTGAG GCGGGCAGCAAT-3′ for Na^+,K^+-ATPase with 392 bp products; 5′-GGCTGGACTGGGCTAAAGAG-3′ and 5′-TGGGGAAGACTGGAAGAGTT-3′ for PepT1 with 390 bp products; 5′-AGAAGCTCCTAGGGGTCATG-3′ and 5′-GAAGGCCCAGAACAGCAAGT-3′ for LAT2 with 386 bp products.

2.4. Density analyses of PCR products

Eight microliters of the amplified product was electrophoresed on agar and stained with ethidium bromide. The fluorescence images were saved with a charge-coupled device (CCD) camera-image saver (ATTO incorporation, Tokyo, Japan), and the density of each band was analysed with NIH Image software. To determine the optimal numbers of PCR cycles, the densities of the electrophoresed PCR product were analysed at different PCR cycles. For semiquantitative analyses, band densities of hexose

transporters were normalised relative to those of β-actin and GAPDH, as described elsewhere (Kuroda et al., 2002).

2.5. Western blotting

IEC pellets were immersed in SDS-sample buffer containing 1M dithiothreitol and 0.1% NP40. After 30 min on ice, the samples were heated at 95°C for 5 min. Twenty micrograms of cell extracts were loaded in each lane, separated on 4–20% gradient SDS-polyacrylamide gels, and electrotransferred onto Immobilon P membranes (Millipore Corp.). After blocking with 4% non-fat-dry milk dissolved in phosphate buffered saline, immunodetection was carried out using antibodies against GLUT1 or SGLT1 (Chemicon Inc., Temecula, CA), followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG (Santa Cruz Biotechnology Inc.). Immunoblotting for actin was also performed to control for equal loading of protein. Bound antibodies were detected with the ECL + plus Western blotting detection system (Amersham Pharmacia Biotech Ltd.). Images were saved with a CCD camera-image saver and density analysis was performed as described above.

2.6. Immunohistochemistry

Tissues were fixed in 4% buffered formalin overnight and embedded in paraffin. Sections of 4-μm thickness were cut and mounted on poly-L-lysine-coated slides. The dewaxed sections were treated with 0.3% H_2O_2 for 20 min, then immersed in 0.01 M sodium citrate buffer, pH 6.0, and autoclaved at 121°C for 10 min for antigen retrieval according to the method described by Bankfalvi et al. (1994). The sections were then incubated with anti-GLUT1 antibody (Chemicon Inc.) for 1 h, followed by incubation with goat anti-rabbit immunoglobulins conjugated with peroxidase-labeled dextran polymer (Envision + ®, peroxidase, mouse Dako) for 30 min. Final reaction was carried out in 0.05 M phosphate buffer (pH 7.4) containing 0.2 mg/ml 3,3′-diaminobenzidine tetrahydrochloride (Dojin Lab., Kumamoto, Japan) and 0.005% hydrogen peroxide. For negative control, species-matched normal IgG was employed instead of GLUT1-specific antibody. Since GLUT1 immunohistochemical localisation has been well documented in the kidney (Heiling et al., 1995), kidney sections were included as a positive control.

2.7. Statistics

Student's *t*-test was employed for statistical analysis, and a *P* value of less than 0.05 was considered significant. In semi-quantitative RT-PCR studies, each group consisted of four animals.

3. Results

To determine whether there is any alteration in hexose transporter expression in the absorptive cells of the small intestine after *N. brasiliensis* infection, epithelial cells were separated from the jejunum as described in Section 2.2, and the mRNA expression of apical surface transporters SGLT1 and GLUT5, the basolateral transporter GLUT2 and the ubiquitously expressed GLUT1 was examined. RT-PCR studies showed an up-regulation of GLUT1 transcription in the jejunal epithelial cells 7 and 14 days after infection, and a decreased transcription of GLUT5 after 14 days, while those alterations returned to the preinfection levels after 21 days (Fig. 1). The thymidine kinase mRNA expression levels were also increased 7 days after infection, consistent with previous reports that *N. brasiliensis* infection induced crypt hyperplasia together with significant increases in thymidine kinase activities (Perdue et al., 1989; Hyoh et al., 1999).

Since the up-regulation of GLUT1 transcription as well as the down-regulation of GLUT5 expression was observed in the jejunal epithelium, in the next experiment we examined the hexose, peptide and amino acid transporter expression in both the jejunal and ileal epithelial cells 7 and 14 days after infection. The worm burden in the small intestine was 481.3 ± 96.0 after 7 days of infection, while it was 76.5 ± 14.4 (mean ± SE of four animals) after 14 days, consistent with a previous observation that the majority of the worms were rejected from the intestine after around 10–14 days of infection by a Th2 type cell-dependent mechanism (Uchikawa et al., 1996). Gross inspection of the small intestinal lumen after 7 days showed that the worm population density was the highest in the upper part of the jejunum, while few worms were found in the distal part of the small intestine. Semi-quantitative reverse transriptase-PCR analyses of jejunal epithelial cells, in which the transporter transcription levels were normalised to those of β-actin and GAPDH, also revealed significant increases in the transcription of thymidine kinase on day 7 and GLUT1

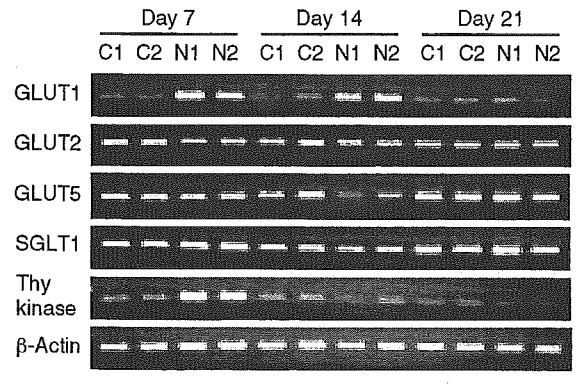

Fig. 1. mRNA transcription of hexose transporters, thymidine kinase and β-actin in the jejunal epithelial cells after *N. brasiliensis* infection. Total RNA was isolated from separated epithelial cells and RT-PCR was performed. C1, C2: age-matched control. N1, N2: nematode-infected.

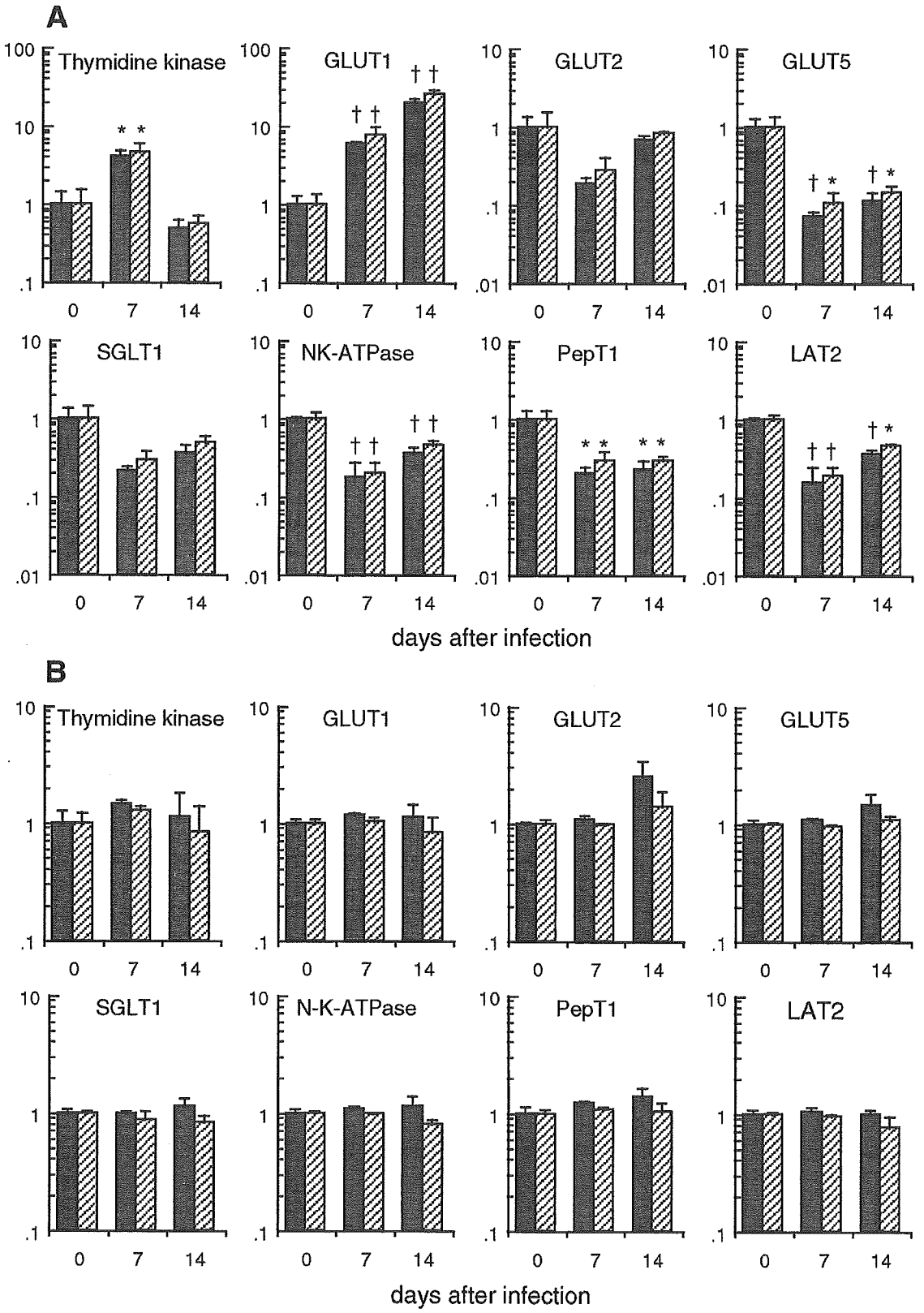

Fig. 2. The mRNA transcription levels of thymidine kinase, hexose transporters, Na^+-K^+-ATPase, oligopeptide transporter and amino acid transporter in jejunal (panel A) and ileal (panel B) epithelial cells after *N. brasiliensis* infection. Total RNA was isolated from separated epithelial cells, RT-PCR was performed and the densities of each PCR product were normalised to that of β-actin (closed columns) or glyceraldehyde-3-phosphate-dehydrogenase (shaded columns). The data are means + SE of four animals. Vertical axes show the expression levels in which day 0 average levels were expressed as 1.0. Significantly different from day 0 level (*$P < 0.05$, †$P < 0.01$).

on days 7 and 14. In contrast, the transcription levels of the fructose/glucose transporter GLUT5, a peptide transporter PepT1 and an amino acid transporter subunit LAT2 showed significant decreases 7 and 14 days after infection (Fig. 2A). Na^+,K^+-ATPase, which has also been reported to be involved in glucose transport across the intestinal absorptive cells (Wild and Murray, 1989), showed a significant decrease after 7 and 14 days. GLUT2 and SGLT1 transcription, on the other hand, did not change significantly after infection. Contrary to the alterations in transporter transcription in the jejunum, ileal epithelial cells showed no significant changes in the levels of thymidine kinase, GLUT1, GLUT2, GLUT5, SGLT1, Na^+,K^+-ATPase, PepT1 and LAT2 after *N. brasiliensis* infection (Fig. 2B), indicating that alterations in transporter transcription occurred in the epithelium where many worms parasitise in close proximity.

SGLT1 is the most important glucose transporter on the apical surface of intestinal absorptive cells (Elsas and Longo, 1992). Although there was no significant decrease in the SGLT1 mRNA transcription levels in the epithelial cells, Western blotting analyses of jejunal epithelial cell lysate showed a decreased expression of SGLT1 protein 7 days after infection (Fig. 3). Contrary to SGLT1, the expression of GLUT1 has been reported to be low in the human and rat adult intestine, while its expression in the fetal gut tube was high (Davidson et al., 1992; Matsumoto et al., 1993). Western blotting analyses showed that the GLUT1 expression in non-infected adult rat jejunal epithelial cell lysate was low, but its expression was increased 7 and 14 days after infection in accordance with the up-regulation of mRNA (Fig. 3). By immunohistochemical staining, GLUT1-specific immunoreactivity was undetectable in the jejunal epithelium of uninfected animals. After 7 days of infection, however, GLUT1 immunoreactivity was detectable in the basolateral membrane of the jejunal epithelial cells (Fig. 4), although the immunoreactive intensity was lower than in the kidney tubules, where GLUT1 is constitutively expressed (Heiling et al., 1995). Negative control incubations for the jejunum of infected rats resulted in no staining in the epithelial cell membrane or cytoplasm.

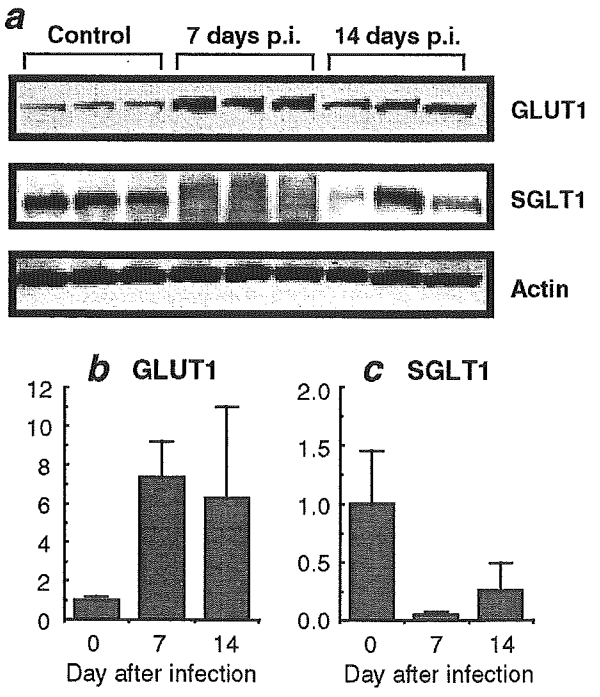

Fig. 3. Western blot analyses of glucose transporter GLUT1 and Na^+/glucose cotransporter SGLT1 in jejunal epithelial cell lysate. (a) Jejunal epithelial cell extracts from uninfected control, 7 days after infection and 14 days after infection were subjected for Western blot analyses. (b, c) Densities of Western blot bands of GLUT1 and SGLT1, which were normalised to that of β-actin. Vertical axes show the expression levels in which day 0 average levels were expressed as 1.0.

4. Discussion

It has been reported that *N. brasiliensis* infection induced decreases in the activities of Na^+,K^+-ATPase and the intestinal brush border enzymes alkaline phosphatase, sucrase and isomaltase together with the development of villus atrophy (Carter et al., 1981; Perdue et al., 1989; Wild and Murray, 1989; Hyoh et al., 1999). The present results further showed that the mRNA transcription of hexose, peptide and amino acid transporters GLUT5, PepT1 and LAT2, respectively, was also decreased in jejunal epithelial cells after nematode infection, although GLUT1 mRNA showed a significant up-regulation. SGLT1 has an important physiological role in glucose absorption from the apical surface of intestinal absorptive cells (Elsas and Longo, 1992). Although the SGLT1 mRNA transcription level did not change significantly, Western blotting analyses showed that the SGLT1 protein expression was decreased after infection. This might be partly due to degradation of SGLT1 protein before and/or after it was transported to the plasma membrane. In fact, it has been reported that SGLT1 protein expression is controlled by mRNA abundance, as well as by posttranscriptional processes including protein trafficking (Thomson et al., 2001). The fall in SGLT1 protein observed in the present study is consistent with reports that glucose uptake from the intestinal mucosa was significantly suppressed 4–19 days after *N. brasiliensis* infection in flux studies using rat intestinal sacs (Carter et al., 1981; Nolla et al., 1985).

An unexpected finding in the present study was that up-regulation of GLUT1 was observed in the jejunal epithelial cells in association with the decreased expression of SGLT1 and other transporters. It has been reported that GLUT1 was detected in the basolateral plasma membrane of differentiated Caco-2 cells, which are a model system for intestinal epithelia (Harris et al., 1992). In addition, GLUT1 was detectable at a low level on the basolateral membrane of normal adult rat, although diabetic rat showed increased

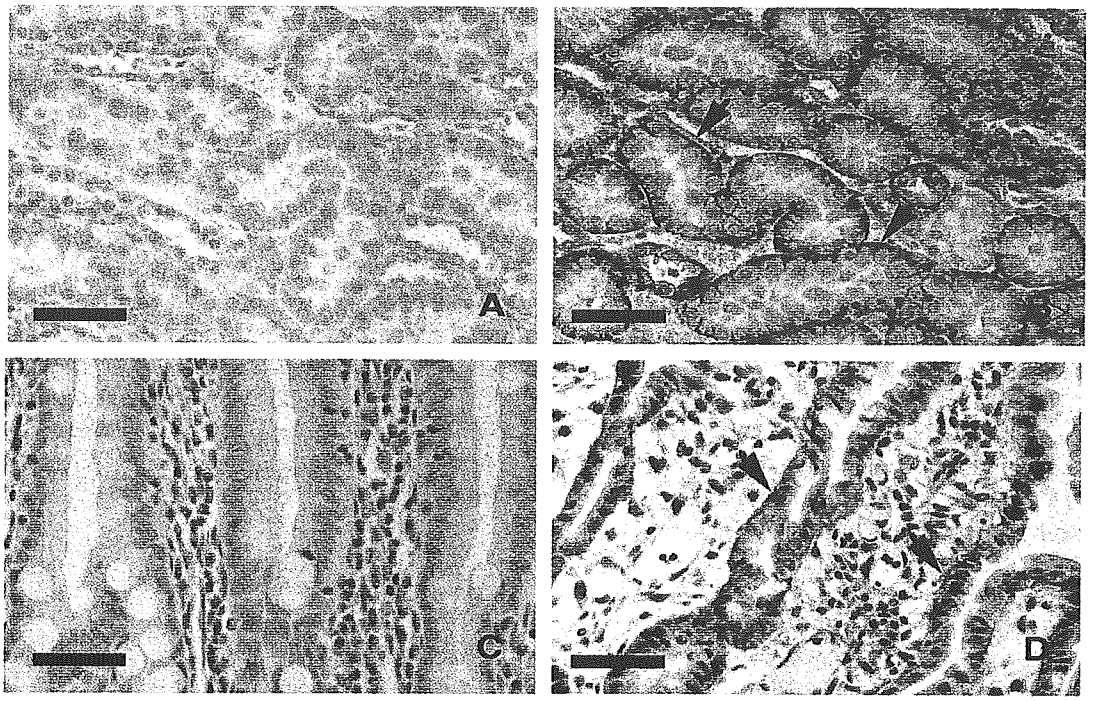

Fig. 4. Immunohistochemical demonstration of glucose transporter GLUT1 in the kidney and jejunum. (A, B) Sections of the kidney from normal rat stained using normal rabbit IgG (A); or GLUT1-specific antibody (B). In B, GLUT1 immunoreactivity (arrows) is observed in the basolateral membrane of tubular epithelial cells. (C, D) Sections of the jejunum obtained from uninfected (C) or infected animals after 7 days (D). In C and D, sections were stained with GLUT1-specific antibody. GLUT1 immunoreactivity (arrows) is observed in the basolateral membrane of villus epithelial cells in D, but not in C. In all sections, nuclei are lightly counterstained with Hematoxylin. Bars indicate 50 μm.

expression of GLUT1 not only at the basolateral membrane but also at the brush border membrane (Boyer et al., 1996). In the present immunohistochemical studies, GLUT1 was not detectable in either the basolateral or apical membrane of intestinal epithelia of normal adult rat, but after 7 days of infection GLUT1 immunoreactivity was found to be localised to the basolateral membrane of the jejunal epithelium.

GLUT1 is essential in maintaining the high rates of glucose influx demanded by glycolysis. GLUT1 gene activation has been reported to develop in renal, muscle, liver and intestinal cells as well as in monocytes and fibroblasts under various stress conditions such as Ca^{2+} influx, hypoxia, and exposure to endotoxin and inhibition of oxidative phosphorylation by azide (Wertheimer et al., 1991; Bashan et al., 1992; Shetty et al., 1993; Dominguez et al., 1996; Spolarics, 1996; Carriere et al., 1998). Wertheimer et al. (1991) and Dominguez et al. (1996) suggested that GLUT1 may be one of the stress response genes, and when oxidative phosphorylation is not possible due to cell injury or hypoxia, glycolysis becomes the main metabolic pathway and is partly conducted by the up-regulation of high affinity GLUT1. In fact, it was reported that the overexpression of GLUT1 attenuated early, energy-dependent neuron death induced by kainic acid in vivo or in vitro (McLaughlin et al., 2000), or inhibited apoptosis in vascular smooth muscle cells (Hall et al., 2001). Although the molecular events that triggered GLUT1 up-regulation in the jejunal epithelium were not clarified in the present study, previous reports suggest that *N. brasiliensis* infection induces changes that indicate the progression of cell injury in the jejunal epithelium: sloughing of enterocytes, progression of apoptosis at villus tips, decreased expression of the epithelial cell adhesion molecule E-cadherin, and decreases in the activities of Na^+,K^+-ATPase and the intestinal brush border enzymes alkaline phosphatase, sucrase and isomaltase (Carter et al., 1981; Perdue et al., 1989; D'Inca et al., 1990; Hyoh et al., 1999, 2002). In addition, the up-regulation of thymidine kinase may reflect an increased turnover of villus epithelial cells to remove damaged or apoptotic cells. Thus, it seems that GLUT-1 up-regulation might be a stress or compensatory response to maintain intestinal epithelial cell viability. Similar alterations have been reported in glucose transporter proteins in alcoholic liver disease in the rat, in which the depletion of liver glycogen was accompanied by decreased GLUT2 expression and increased GLUT1 expression (Nanji et al., 1995).

Taken together, the present results showed that *N. brasiliensis* infection induced significant up-regulation of GLUT1 and significant decreases in mRNA and/or protein levels of membrane transporters SGLT1, GLUT5, PepT1 and LAT2. It is known that *N. brasiliensis* infection induces not only local pathological changes but also a systemic deteriorating status such as hypoglycemia and

hypoalbuminemia (Lunn et al., 1986; Ovington, 1987). At least two mechanisms might be involved in the induction of hypoglycemia and/or hypoalbuminemia. First, a biphasic form of anorexia has been reported to occur after *N. brasiliensis* infection, the first phase coincident with lung invasion, and the second when the worms mature in the intestine (Mercer et al., 2000). Secondly, increases in gastrointestinal leakage of plasma protein have also been suggested to have an important role in the progression of hypoalbuminemia (Lunn et al., 1986). Together with these alterations, decreased expression of some of the hexose, peptide and amino acid transporters in absorptive cells might also be involved at least partly in the development of malnutrition status.

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国内の寄生虫症の動向

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● はじめに

第2次世界大戦直後までのわが国は，回虫症，鉤虫症，日本住血吸虫症，マラリア，フィラリアなどの寄生虫症が高度にまん延する地域であった。しかし，大戦直後60%前後の国民が感染していた回虫や5%前後の感染率のあった鉤虫は，戦後の復興と高度経済成長の過程で，1970年代前半に1%以下，1980年代前半には0.1%以下に激減した（**図1**）。寄生虫症の減少は，農業の人糞から化学肥料使用への転換，公衆衛生基盤の確立や教育の向上などに依存するところが大きい。しかしこれらの役割以上に，日本においては保健所や学校を核として，積極的に寄生虫対策（教育，検査，治療）を行うというわが国独自の方策が，単一民族国家としての日本人の気質とあいまって，このような，まれにみる短期間での古典的寄生虫症の撲滅を達成できたといえるだろう。対策が難しいとされる糸状虫症も1980年代に入ってから新患の発生はなく，日本住血吸虫症も，1980年代に広島県片山地方および筑後川流域で，1990年代には山梨県において安全宣言あるいは流行終息宣言が出された（**図1**）。日本ならではの快挙であったといえる。

しかし，日本が無寄生虫地帯となったわけでは決してない。現在のわが国には，その現状に応じた特有な寄生虫症問題が存在する。日和見寄生虫症，輸入寄生虫症，食生活や生活習慣の

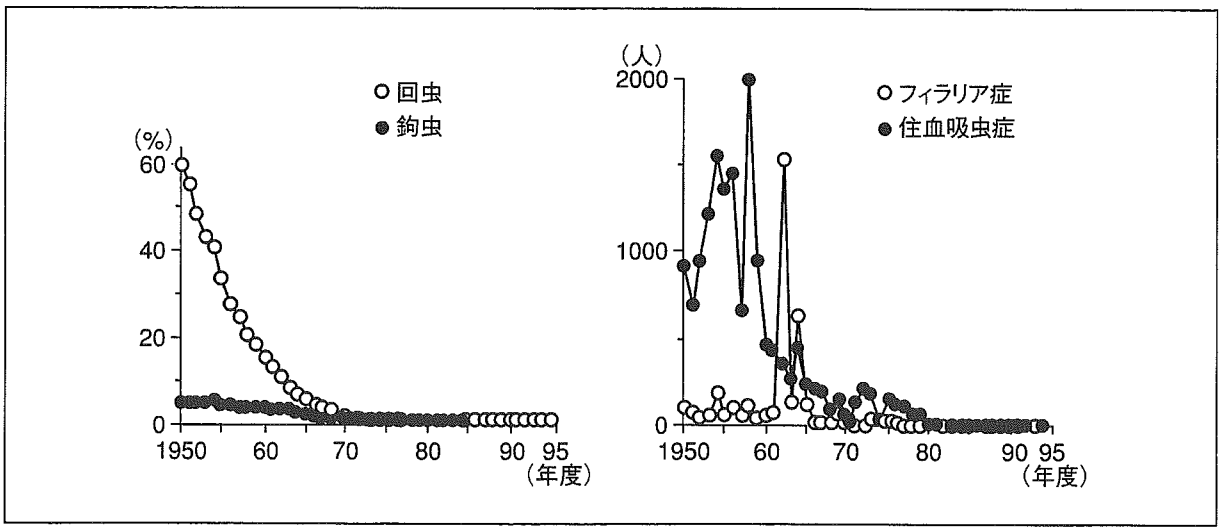

図1　1950年から1995年のわが国における寄生虫症の年次推移
回虫，鉤虫は検便による虫卵保有率，フィラリア症および日本住血吸虫症は患者届け出数を示す。
（厚生省編．国民衛生の動向1995より）

変化や多様化に伴って再び増加してきたり，新たに出現してきた寄生虫症などである。これらの症例の実数は，かつての古典的寄生虫症と比べれば決して多いとはいえないが，診断・治療の困難さ，専門家の不足，監視体制の不備などさまざまな問題を抱えている。

● 原虫疾患の動向

現在の日本の原虫症の中で症例報告数が多いのは，アメーバ症，カリニ肺炎，マラリア，トキソプラズマ症である(**表 1**)。赤痢アメーバは，近年病原性の *Entamoeba histolytica* 以外に非病原性の *E. dispar* の存在が知られるようになり，嚢子形態だけからはただちに赤痢アメーバと同定できないが，腸炎や肝膿瘍などの病態を示す症例は赤痢アメーバといってよい。アメーバ症は従来から輸入例よりも国内感染例が多い。アメーバ症は嚢子に汚染された飲水，食物から感染するが，1970 年代後半から，欧米の男性同性愛者の間に高率に感染していることが報告されるようになった。この傾向はわが国でも同様であり，実際に HIV 感染者に見出される症例の報告が増加している（**表 1**)。

マラリア症例は最近では年間 100〜150 名の間で推移している。原則的にほぼすべてが輸入例と考えられ，輸入寄生虫症のなかでもっとも重要な疾患である。約 4 割が熱帯熱マラリアであり，毎年死亡例も発生していることが憂慮される。薬剤耐性熱帯熱マラリアの激増，診断の遅れなど，問題点が多い。なお症例数は少ないものの，輸入リーシュマニア症も診断が困難で治療に難渋する例が多い。

トキソプラズマで問題となるのは先天性感染と HIV 感染者にみられるトキソプラズマ脳炎である。先天性トキソプラズマ症は一時期わが国には存在しないとされ，多くの施設で妊婦におけるトキソプラズマ抗体検査が検査対象からはずされたが，実際には毎年のように重症例が報告されている（**表 1**)。また，日本における HIV 感染の増加に伴って，カリニ肺炎のほか，トキソプラズマ脳炎の症例報告も増加してきている。

表 1　日本における原虫症例の学会および原著報告数の推移

	1988〜1992 年	1993〜1997 年	1998〜2002 年
アメーバ症	223 (1)	143 (6)	235 (12)
カリニ肺炎*	108 (5)	113 (23)	214 (43)
マラリア	78	99	149
トキソプラズマ症	98 (0) [16]	80 (5) [22]	116 (7) [18]
アカントアメーバ角膜炎	6	29	44
ジアルジア症	19 (1)	13 (0)	22 (0)
クリプトスポリジウム症	4 (0)	6 (0)	9 (2)
リーシュマニア症	10	13	14

( ) は HIV に合併した症例，[ ] は先天性感染に関する報告数。*ニューモシスチス・カリニは現在真菌に分類されているが，ここでは従来からの慣習により原虫に含めた。(本表に示す数値は医学中央雑誌に収載されている寄生虫症例について，会議録，原著の中から検索しその報告数をカウントしたもの。1 会議録または 1 原著を 1 とカウントしており，同一症例が複数の学会で報告されたり，単一の報告で多数の症例が提示されていたりするので，本表に示す数は症例実数を直接反映するものではない)

下痢症を生じるジアルジアやクリプトスポリジウム感染は，水道水汚染による集団発生が報告され，新興・再興感染症として注目されるようになった。厚労省による上水道採水地の調査でも，多くの場所でジアルジアやクリプトスポリジウムの嚢子が検出されており，監視の継続が必要である。なお，近年著しい増加をみるのがアカントアメーバによる角膜炎である。ソフトコンタクトレンズ着用者における発生が多く，診断の遅れによる難治例が多い。

● 吸虫，条虫症の動向

吸虫感染の中で症例の報告が多いのは住血吸虫症と肺吸虫症である(**表 2**)。住血吸虫症は，日本国内での新たな感染はなくなっている。報告の多くは陳旧例で，大腸癌や肝癌内に住血吸虫卵が見出されたとの報告が多い。発癌との関連でさらなる調査と研究が必要である。また，マンソン住血吸虫やビルハルツ住血吸虫の輸入例の報告が増加している。一方，肺吸虫症は大半が新鮮例で，本疾患は決して日本から消失して

表 2　日本における吸虫および条虫症例の学会および原著報告数の推移

	1988～1992 年	1993～1997 年	1998～2002 年
吸　虫			
住血吸虫症	130 (2)[a] [4][b]	74 (7) [6]	88 (8) [20]
肺吸虫症	67	56	54
肝蛭症	44	17	17
肝吸虫症	27	21	15
条　虫			
エキノコックス症	46 (2)[c]	39 (2)	60 (6)
裂頭条虫症	35	44	44
マンソン孤虫症	37	17	36
有鉤条虫/嚢虫症	18	21	24
大複殖門条虫症	14	16	3
無鉤条虫症	8	6	3

[a]マンソン住血吸虫症，[b]ビルハルツ住血吸虫症，[c]単包虫症（データソースは表 1 と同様）

表 3　日本における線虫症症例の学会および原著報告数の推移

	1988～1992 年	1993～1997 年	1998～2002 年
アニサキス症	230	84	137
回虫症	80	75	53
糞線虫症	69 (1)[a]	65 (6)	58 (9)
イヌ糸状虫症	57	42	55
トキソカラ症	44	16	49
顎口虫症	28	22	27
鉤虫症	21 (0)[b]	11 (2)	19 (12)
旋尾線虫症	3	19	25
ブタ回虫症	0	5	26
広東住血線虫症	6	5	15
旋毛虫症	0	0	5

[a]HTLV-1 陽性例，[b]皮膚瘢行症例（データソースは表 1 と同様）

いないことがわかる。

条虫症で現在もっとも憂慮されるのが北海道におけるエキノコックス症である。本症は 1936 年礼文島において最初の患者が見出されて以来，130 名を超す患者が発生したが，対策により 1970 年には終息した。一方，1965 年に根室で見出された本症は，その流行が全道に拡大し，終宿主のキタキツネにおける感染率も 30％以上を示す地域が少なくない。1997 年までに約 250 名の患者が報告されているが，今後さらに患者数が増加していく可能性がある。本症は主に肝臓に多胞性嚢胞を形成し，浸潤性に増大していく悪性寄生虫症であり，発症までに 20 年近くを要すること，化学療法が未確立など，きわめて問題点の多い疾患といえる。

裂頭条虫症やマンソン孤虫症は従来からの発生状況に変化はなく，筆者の経験ではその実数は相当に多いと思われる。有鉤条虫/嚢虫症は神経系への嚢虫の寄生が大きな問題であり，その多くは外科的に摘出されている。日本には沖縄を除いては本症は存在しないと考えられてきた。実際に**表 2** に示した有鉤条虫/嚢虫症の大半は輸入症例であり，流行地域の東南アジアや中国からの来日者の増加に伴い，日本の医療現場で遭遇する機会が今後さらに増えていく可能性があると考えられる。なお無鉤条虫は，いわゆるやせ薬として中国から持ち込まれ，ひそかに高額で売買されていると聞き及んでいる。なんらかの対策が必要なことはいうまでもない。

● 線虫疾患の動向

まず糞線虫症や古典的寄生虫症の代表である回虫症は，決して日本から消失していないということを指摘したい。**表 3** に示す回虫症の報告は，その多くが胆道迷入による閉塞性黄疸の発症など異所寄生に基づくものである。感染源として有機農業野菜や輸入野菜との関連を指摘する声もあり，今後の調査が必要である。糞線虫症は従来から沖縄に多く，対策が取られてきたものの継続してみられる。本症は免疫抑制下で重症化し，HTLV-1 キャリアにおいては糞線虫治療に抵抗を示すなど，問題点が多い。

線虫症の中で診断，治療上問題点の多いのが幼線虫移行症の一群（アニサキス症，イヌ糸状虫症，トキソカラ症，顎口虫症，旋尾線虫症，ブタ回虫症，広東住血線虫症など）である。これらの疾患の多くは比較的最近になって見出されるようになった。たとえば旋尾線虫やブタ回虫幼虫移行症は 1990 年代以降に見出されるようになったものである（**表 3**）。アニサキス症は

ほぼ消化管に限定されるが，顎口虫や旋尾線虫は皮膚跛行症を生じる例が多く，広東住血線虫はクモ膜下腔に侵入し好酸球性髄膜脳炎を，イヌ糸状虫は肺のほか皮下や軟部組織に，トキソカラとブタ回虫は肝臓，肺，網膜などに移行し臓器特異的症状を示す。いずれも幼線虫であるため虫卵を対象とした検査は無効で，虫体自体の確認も困難な例が多く，免疫診断の感度の向上と特異性の確立が大きな課題となっている。また，抗線虫薬の多くは成虫に対して有効でも，幼虫に対しては無効のものが多く，現在のところチアベンダゾール，アルベンダゾールなどのベンズイミダゾール系薬剤が試験的に用いられているにすぎない。

● 国内寄生虫症をめぐる諸問題

さきに述べた日本における寄生虫症の動向の特徴をまとめると，①アメーバ症，糞線虫症，肺吸虫症など，一部の古典的寄生虫症の持続および再興寄生虫症としてのエキノコックス症の拡大，②HIV 感染者の増加に伴う各種日和見寄生虫症の増加，③マラリアのほか各種輸入寄生虫症の増加，④新興寄生虫症の出現，各種幼虫移行症の多発などであろう。その背景には性風俗の変化，旅行者の急増，生鮮食品やペット動物の輸入の急増，あまりにも無防備化してきた現代日本人の食生活など，さまざまな要因の存在が示唆される。

現代日本で遭遇する寄生虫症には致死的な疾患も多く含まれているが，その診断と治療の現状はある意味で混沌といっても過言でない状況にある。医療現場における医師の寄生虫症に対する知識の不足による診断の遅れのみならず，寄生虫症に精通した専門家も著しく減少してきている。さらに，新しい寄生虫症に対する的確な診断法の開発や組織内寄生虫症に対する免疫診断の感度と特異性の向上に関する研究も順調に進んでいるとは言いがたい。一方，抗寄生虫薬は日本で入手困難なものも少なくなく，さらにマラリアにみられるように薬剤耐性の出現や，まだ化学療法自体が確立していない寄生虫症も多い。

このような中で，熱帯病に対するオーファンドラッグ研究班は薬剤提供，情報提供において一定の役割を果たしてきたが，それとともに，寄生虫症に対する調査・監視体制を強化し，その実態のより正確な把握に努めることが当面の緊急課題であると思われる。

広節／日本海裂頭条虫症および無鉤条虫症の疫学的動向

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Key Words：裂頭条虫，無鉤条虫，疫学

はじめに

広節／日本海裂頭条虫や無鉤条虫などによる大型腸管寄生条虫症は，症例報告が散見されるものの，その実数の把握は困難で，疫学的な動向についての報告はほとんどない。今回京都府立医科大学医動物学教室において1980年から2002年までの間に遭遇した大型腸管寄生条虫132例の疫学的特徴を解析したので報告する。

調査対象と方法

京都府立医科大学医動物学教室において1980年から2002年までの間に，治療に関与した，あるいは虫体同定の依頼を受けた大型腸管寄生条虫症132例を対象とした。マンソン孤虫や有鉤嚢虫などの組織内条虫は除外した。132例の中で治療に直接または間接的に関与したのは93例であった。患者現住所は78%が京都府内で残りは近畿各府県であった。住所不明者が8%あったが，その多くは検査会社を通じた同定依頼であり，大半が京都府内在住と推測される。

条虫の種類と年齢分布

条虫の同定は，すべて体節の形態をもとに行った。無鉤条虫と有鉤条虫の鑑別は全例墨汁注入法による子宮分岐数を参考に決定した。132例の内訳は広節／日本海裂頭条虫83%，無鉤条虫17%で（表1），大複殖門条虫やその他の海洋性裂頭条虫は見られなかった。

年齢分布は裂頭条虫症が4-77歳，無鉤条虫症が8-51歳と，幅広い年齢層にみられたが，裂頭条虫症については，小児の感染例が意外に多いことが見出された（図1）。この傾向は医学中央雑誌に見られる全国からの報告例についても同様であった。男女比は裂頭条虫症2.4：1，無鉤条虫症1：1で両条虫症で異なっていたが，この点も医学中央雑誌に見られる報告例と同様であった。

Epidemiological Trend on Diphyllobothriasis Latum/ Nihonkaiense and Taeniasis Saginatus in Kyoto

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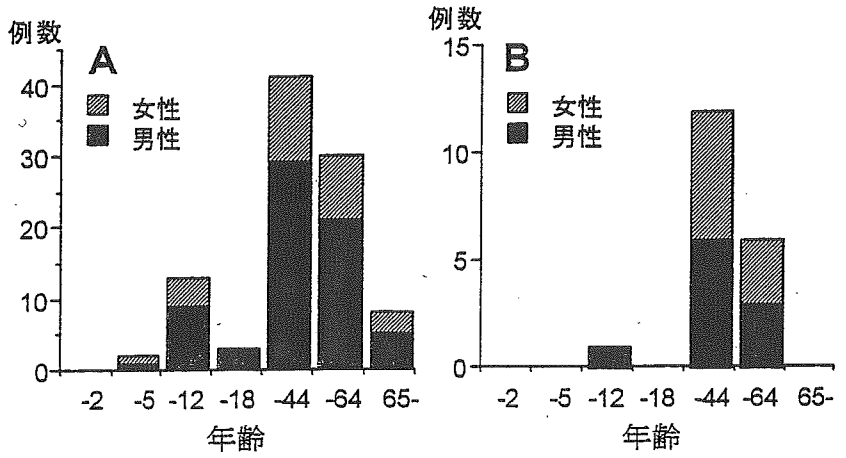

図1　広節/日本海裂頭条虫症94例（A）および無鉤条虫症19例（B）の年齢階層別分布（京都府立医科大学 1980-2002年）。

表1　京都府立医科大学医動物学教室において1980年から2002年までの間に駆虫または虫体同定に関与した腸管寄生大型条虫132例の内訳

	広節/日本海裂頭条虫	無鉤条虫	計
例　数*	109 (77)	23 (16)	132
性　別			
男	75	12	87
女	31	11	42
不明	3	0	3
年齢分布†	4-77 (38.0)	8-51 (35.1)	

＊（ ）の数字は治療に関与した症例数
†（ ）の数字は平均年齢

表2　無鉤条虫の感染地の推定
（京都府立医科大学 1980～2002年）

	日本人	外国人	計
総　数	20	3	23
感染地を聴取りできた例	9	3	12
国　外＊	4	1	5
国　内	4†	1	5
判定不可	1	1	2

＊タイ，トルコ，エチオピア，ブラジルを含む。†国内感染の内1例は，中国からやせ薬として持ち込まれ，ひそかに販売されていた無鉤嚢虫を購入し服用した例。

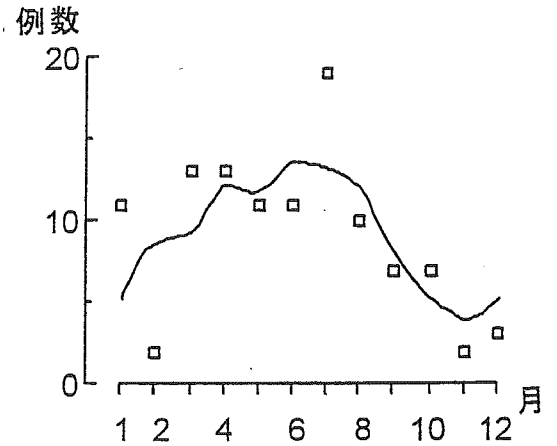

図2　広節/日本海裂頭条虫（109例）の月別発生頻度（京都府立医科大学 1980-2002年）。図中のラインは，前後3ヵ月の平均値をとりスムージングしたもの。

広節/日本海裂頭条虫症の月別発生状況と年次変動

広節/日本海裂頭条虫の月別発生状況を図2に示した。発生数が0の月は存在せず通年性に見出されたが，春から夏にかけて患者数が増加し，秋以降に減少する傾向が見られた。日本における重要な中間宿主であるサクラマスの漁獲盛期が春季であることと関連があるのかもしれない。

広節/日本海裂頭条虫症は1970-80年代に全国的にピークが見られ，90年代以降減少傾向にあると報告されている[1)2)]。今回の成績では，1990年代後半から漸減傾向が見られた（図3）。京都卸売市

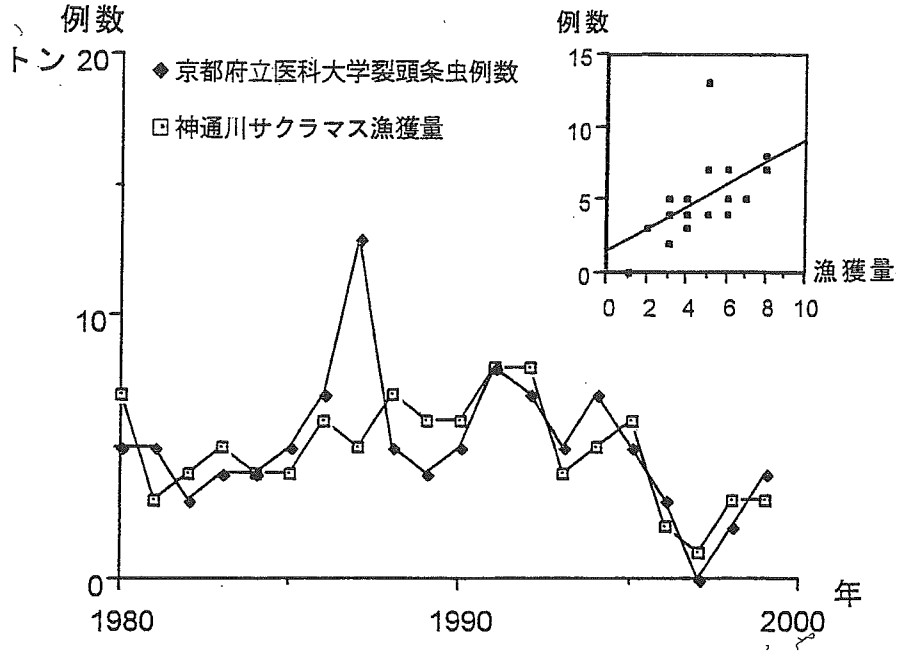

図3　広節/日本海裂頭条虫症例（京都府立医科大学）の年次変動と神通川サクラマス漁獲量（トン）。インセット：漁獲量（横軸）と症例数（縦軸）の相関。r＝0.553　P＜0.01

場に入るサクラマスは富山県産のものが多いと聞いている。図3に示したように，京都府立医科大学で遭遇した裂頭条虫例数の年次変動は，神通川サクラマス漁獲量と強い相関を示すことが明らかになった。一方，裂頭条虫症の全国の発生状況と京都における発生状況はかならずしも強く相関していないことから（データ示さず），産地とその漁獲量，および流通経路が各地域における発生に大きく関与するものと思われた。

無鉤条虫症の感染地

無鉤条虫症の月別発生状況は，例数が少なく評価できなかった。年次変動では近年やや減少傾向にある（データ示さず）。今回の調査対象23人の中に3名の外国人が含まれていた（表2）。無鉤条虫は牛肉を生で食することによって感染するが，何処で生肉を食したかを聴き取りできた12人中5名が外国での摂取と答えており，輸入感染症としての色彩も濃厚に存在することが明らかになった。また日本人の1名は，やせ薬と称してカプセルに入れられた中国産無鉤嚢虫を摂取している。

まとめ

京都府立医科大学医動物学教室において1980年から2002年までの間に遭遇した広節/日本海裂頭条虫症109例，無鉤条虫症23例について解析し以下の結果を得た。

1）裂頭条虫症，無鉤条虫症ともに小児から青壮年に幅広く分布していたが，男女比は裂頭条虫症2.4：1，無鉤条虫症1：1と異なる傾向を示した。

2）広節/日本海裂頭条虫症は近年若干減少の傾向が見られ，その変動はサクラマス漁獲量の変動と関連している可能性が示唆された。

3）無鉤条虫症は国内感染例のほか，外国での感染例が相当数存在した。

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Immunological characteristics of patients infected with common intestinal helminths: results of a study based on reverse-transcriptase PCR

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To determine whether common helminth infections could modify the intestinal immunopathological status of the host, the expression in the human duodenal mucosa of cytokines, eosinophil- and mast-cell-specific molecules and monosaccharide transporters of the glucose-transporter (GLUT) family was explored. The 31 subjects were all patients at the gastro-intestinal disease unit of Nongkhai Hospital, Thailand. Four of the 10 patients who presented with eosinophilia ($\geq$6.0% of their leucocytes were eosinophils), and five of the other 21 patients, had intestinal infections with helminths when they presented or within the previous 3 months. Studies based on semi-quantitative, reverse-transcriptase PCR revealed that the interleukin-5/interferon-γ ratio was significantly higher in the non-eosinophilic, helminth-infected patients than in the non-eosinophilic, uninfected patients, whereas the IgE receptor type I (FcεRI)/mast-cell tryptase ratio was significantly higher in the eosinophilic, helminth-infected patients than in the eosinophilic, uninfected patients. Expression of Charcot–Leyden-crystal protein, GLUT-1 and GLUT-5, however, showed no significant inter-group differences. Principal-components analysis of the data on eosinophils, interleukin-5, interferon-γ, FcεRI and mast-cell tryptase revealed that one principal component could discriminate the patients who had helminth infection from the non-eosinophilic, uninfected patients, but not from the eosinophilic, uninfected patients. These results indicate that, whatever the intestinal pathology, patients infected with common intestinal helminths tend to develop a mucosal immunological response of the Th2 type.

High prevalences of infection with intestinal helminths were revealed when, in 2000 and 2002, stool samples from humans living in Nongkhai province in north-eastern Thailand were examined. Data collected by the provincial health office indicated that, of the general public (and schoolchildren) checked in each of the two study years, 22.0%–33.3% (19.3%–28.2%) were infected, and 5.1%–7.2% (3.5%–11.4%) and 3.8%–9.0% (0.8%–2.6%) were excreting hookworm and *Opisthorchis* eggs, respectively (unpubl. obs.). *Ascaris lumbricoides*, *Strongyloides stercoralis* and *Taenia* accounted for most of the other infections detected (unpubl. obs.). The rather high variation seen between the results in 2000 and those recorded in 2002 may reflect differences in the local communities examined in each year, and/or temporal differences in anthelminthic use. The traditional agricultural and food-handling practices of the region probably account for most of the infections with food-borne or soil-transmitted helminths. Although infection with common intestinal helminths does not necessarily cause overt clinical signs, long-term infections may induce malnutrition and result in retardation

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of the intellectual and physical growth of children (Oberhelman *et al.*, 1998).

Intestinal infections with helminths such as *Ascaris* and hookworm induce expansion of the Th2 lymphocyte subset that regulates IgE antibody production, eosinophilia and mastocytosis (Pritchard *et al.*, 1995; Cooper *et al.*, 2001). Responses of the Th2 type may play an important role in protection against helminth infections, and may counterbalance any potentially damaging Th1 responses (Pritchard and Brown, 2001). Helminth infections may, however, impair immune responses to viruses, bacteria or protozoa, and even those to oral vaccines, through the antagonistic effects of Th2 cytokines on the expansion of the Th1 lymphocyte subset (Cooper *et al.*, 2001). In rodents, infection with intestinal nematodes such as *Nippostrongylus brasiliensis* and *Trichinella spiralis* not only causes the induction of Th2 cytokines, such as interleukin-4 (IL-4), IL-5 and IL-13, but also induces IgE antibody responses, eosinophilia and intestinal mastocytosis (Matsuda *et al.*, 1995; Garside *et al.*, 2000). In such experimental nematode infections, various pathological changes occur in the intestinal mucosa of the hosts, including partial villus atrophy and crypt hyperplasia, together with decreases in the activities of the brush-border enzymes sucrase and alkaline phosphatase, alterations in the expression of hexose, amino-acid and peptide transporters, and deterioration in epithelial permeability and barrier function (Perdue *et al.*, 1989; Hyoh *et al.*, 1999, 2002; Sekikawa *et al.*, 2003). In human intestinal parasite infections, however, it remains unclear whether Th2-associated responses dominate in the intestinal mucosa of the host. In the present study, in order to determine the immunopathological status of the infected human intestinal mucosa, the expression of the cytokines interferon-γ (IFN-γ), IL-5 and IL-13, as well as marker molecules of mast cells and eosinophils in the duodenal mucosa, was investigated in patients from Nongkhai, where intestinal helminth infection is clearly not uncommon. Two members of the glucose-transporter (GLUT) family, GLUT-1 and GLUT-5, were also investigated, to evaluate the functional status of the absorptive cells. GLUT-5 is located in the brush border of intestinal absorptive cells and has an important role in the absorption of fructose from the intestinal lumen (Gould and Holman, 1993). GLUT-1, on the other hand, is expressed in almost every tissue, and its expression has been reported to have a strong association with cellular glycolysis, which is accelerated, with the up-regulation of GLUT-1, after various cellular stresses, including hypoxia (Wertheimer *et al.*, 1991)

PATIENTS AND METHODS

Patients

Patients who visited the gastro-intestinal disease unit at Nongkhai Hospital, because of symptoms such as abdominal discomfort, abdominal pain and vomiting (of various severities and durations) were enrolled in the study if they (1) did not have a dysenteric disease; (2) required fibre-optic examinations of their upper gastro-intestinal tract, for the diagnostic evaluation of their diseases; and (3) gave their written, informed consent for the collection, at the time of their diagnostic endoscopies, of duodenal mucosal biopsies (for the investigation of cytokine and inflammatory-mediator expression in the duodenal mucosa). Overall, 31 patients met the inclusion criteria and were enrolled. A stool sample was collected from each subject, in the week prior to the fibre-optic examination, and used to make thick and thin smears that were checked for protozoa and the ova and larvae of helminths.

Tissue Processing

A piece of the duodenal mucosa 3 cm distal to Vater's papilla was obtained from each patient. Each of these biopsies was immediately immersed in 1 ml of RNA-preservation solution (RNAlater®; Ambion, Austin, TX)

and kept in a refrigerator for 4–50 days (mean = 22.6 days) until the RNA could be extracted. Total RNA was extracted using TRIzol® reagent (Life Technologies, Rockville, MD), and 5-μg aliquots of RNA were then reverse-transcribed, at 42°C for 50 min, in 20 μl reverse-transcription buffer containing 5 mM $MgCl_2$, 1 mM deoxynucleotide-triphosphate (dNTP) mixture, 1 U RNase inhibitor/ml, 250 U AMV (avian myeloblastosis virus) reverse transcriptase/ml, and 0.125 mM oligo dT primer (Takara RNA LA PCR kit; Takara Biomedicals, Osaka, Japan).

PCR

For the PCR, 1-μl aliquots of synthesised complimentary DNA were added to PCR buffer containing 2.5 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.025 U LA Taq DNA polymerase (Takara RNA LA PCR kit)/ml, and 0.2 mM of each sense and antisense primer (see Table 1) in a final volume of 25 μl. The thermocycler was set to give cycles of 30 s at 94°C, 30 s at 62°C and 30 s at 72°C. To determine the optimal numbers of PCR cycles, the densities of the bands produced, from one representative sample, when the products from different numbers of PCR cycles were subjected to electrophoresis, were compared. This allowed the number of PCR cycles that allowed the best comparison of the levels of gene expression to be determined, for each molecule of interest.

DENSITY ANALYSES OF PCR PRODUCTS

To separate the products of each PCR, 8-μl aliquots of the amplified products were subjected to electrophoresis on agar, stained with ethidium bromide, and visualized with ultra-violet trans-illumination. The banding patterns were recorded using a charge-coupled-device (CCD) camera (ATTO, Tokyo, Japan), and the density of each band was evaluated using NIH Image software (National Institutes of Health, Bethesda, MD). The band densities were normalized relative to those of β-actin.

RESULTS

The baseline characteristics of the 31 patients enrolled in the study are summarized in

TABLE 1. *The primer pairs used for the PCR amplifications*

	Primers	Size of expected product (bp)
Interferon-γ	5'-GGGTTCTCTTGGCTGTTACTG-3' 5'-GACAGTTCAGCCATCACTTGGA-3'	384
Interleukin-5	5'-GAAATTCCCACAAGTGCATTGG-3' 5'-CTTTCTATTATCCACTCGGTGTTC-3'	335
Interleukin-13	5'-AGGAGCTGGTCAACATCACC-3' 5'-GTTGAACCGTCCCTCGCGAA-3'	296
IgE receptor type I (FcεRI, α chain)	5'-TCAGTGACTGGCTGCTCCTT-3' 5'-GGGGTTTGGCTTAGGATGTG-3'	437
Mast-cell tryptase β1 (McTr)	5'-TCAGCAGGATCATCGTGCAC-3' 5'-TGGGGACATAGTGGTGGATC-3'	507
Charcot–Leyden-crystal protein	5'-TACCCGTGCCATACACAGAG-3' 5'-CTCTCCACACTTGCACCATC-3'	375
Glucose transporter 1 (GLUT-1)	5'-ATCGTCAACACGGCCTTCAC-3' 5'-AAGCCGGAAGCGATCTCATC-3'	458
Glucose transporter 5 (GLUT-5)	5'-GGTACAACGTGGCTGCTGTC-3' 5'-CATGGGGACCACGTTGGAAG-3'	347
β-Actin	5'-TCAGAAGGATTCCTATGTGGGC-3' 5'-CCATCACGATGCCAGTGGTA-3'	317

TABLE 2. *The baseline demographic and laboratory data for the 31 subjects*

	Total	Helminth-infected	Uninfected	With peptic ulcer	Without ulcer
No. of subjects	31	9*	22	6†	25
No. of males/females	22/9	9/0	13/9	5/1	17/8
MEAN VALUE, (S.D.) AND [no. of subjects for whom data were available]					
Age (years)	45.8 (13.5) [31]	43.3 (10.8) [9]	46.8 (14.5) [22]	47.8 (8.9) [6]	45.3 (14.6) [25]
Albumin (g/dl)	3.9 (0.7) [23]	4.1 (0.6) [8]	3.8 (0.7) [15]	3.5 (1.0) [5]	4.0 (0.6) [18]
Haemoglobin (g/dl)	13.3 (2.8) [29]	13.5 (2.2) [8]	13.0 (3.0) [21]	12.3 (4.1) [6]	13.4 (2.4) [23]
Eosinophils (% of leucocytes)	4.8 (4.6) [31]	5.3 (4.6) [9]	4.6 (4.7) [22]	3.1 (3.2) [6]	5.2 (4.9) [25]

*With intestinal helminth infection (two with *Strongyloides*, six with *Taenia* and one with *Ascaris*) on presentation or within previous 3 months.
†One with ulcer in the duodenum and five with stomach ulcers.

Table 2. All the patients investigated were from Nongkhai province, where agriculture is the main industry. The oesophago-gastro-duodenal fibre-optic examinations carried out for the diagnostic evaluations revealed 17 cases of chronic gastritis, six of peptic ulcer, four of oesophagitis, and three of erosive gastritis; the other three cases showed no particular findings. The stool examinations revealed two cases excreting *Strongyloides stercoralis* larvae and two others excreting the ova and/or proglottids of *Taenia*. The intensities of both *S. stercoralis* infections detected were low, with only one or two rhabditoid larvae detected in each thin faecal smear, and neither patient found excreting the larvae had eosinophilia (0.5% and 2.1% of their leucocytes were eosinophils). The intensity of *Taenia* infection could not be determined but it is known that infection with *Taenia* usually consists of a single worm (Beaver *et al.*, 1984). The medical records of the patients who were found stool-negative for helminths revealed that five had had intestinal infections with helminths in the 3 months before their presentation: one had had an *Ascaris* infection (scored ++ for the number of *Ascaris* ova seen on a thin faecal smear), and another four had each had a *Taenia* infection (with ova or proglottids in their stools). Overall, therefore, nine of the patients investigated were considered 'infected', having being found positive for intestinal helminths either at their enrolment or in the previous 3 months.

None of the 31 patients appeared to have an enteric infection with parasitic protozoa such as *Entamoeba histolytica* or *Giardia lamblia*, either at enrolment or whenever checked in the previous 3 months. The results of urea tests for *Helicobacter pylori* infection, carried out on all 31 subjects at enrolment, were positive for seven patients, all of whom appeared smear-negative for helminths at enrolment and had no obvious history of helminth infection in the previous 3 months. Only one of the 31 subjects (an asthmatic with mild dyspnea, as well as *S. stercoralis* infection) had a history or the signs and symptoms of an allergic disease. In terms of age, gender and the results of the laboratory tests, the helminth-infected subjects were similar to the uninfected, and the patients with peptic ulcers were similar to those without such ulcers (Table 2).

To determine the mucosal immunological status of each subject, the levels of expression of IL-13, IL-5, IFN-γ, Charcot-Leyden-crystal protein (CLC), IgE receptor type I (FcεRI), mast-cell tryptase (McTr), GLUT-1 and GLUT-5 in the duodenal mucosa were evaluated by reverse-transcriptase PCR (RT-PCR). Expression of the Th2 cytokines IL-5 and IL-13 was detectable in the biopsies from 20 (64.5%) and eight (25.8%) of the patients, respectively, whereas expression of the Th1 cytokine IFN-γ and of all the other molecules investigated was detectable in all of the biopsies examined (Fig. 1). Expression

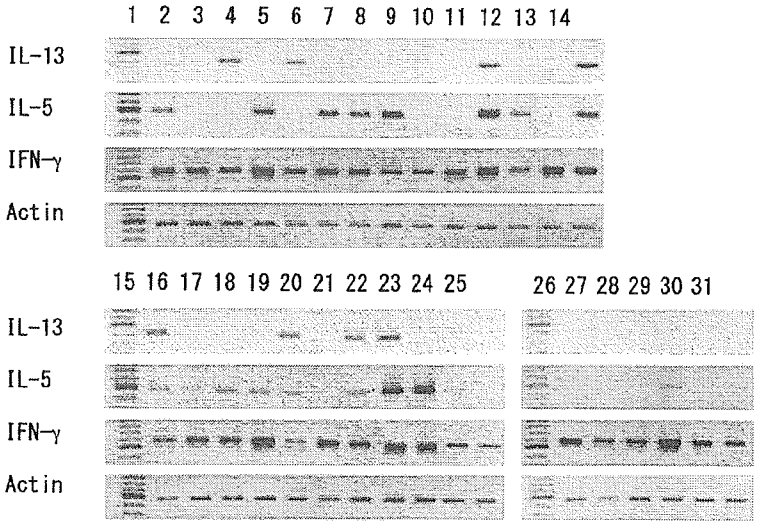

FIG. 1. Some of the PCR results, showing the expression of interleukin-13 (IL-13), interleukin-5 (IL-5), interferon-γ (IFN-γ) and, for reference, β-actin (Actin) in the duodenal mucosa of the 31 patients. Samples from patients with helminth infection on presentation or in the previous 3 months were run in lanes 15–23.

of IL-5 showed no relationship with subject age, the period of sample preservation, the gender of the subject, the presence of a peptic ulcer, or helminth infection (Fig. 2). This cytokine was, however, expressed by a significantly higher proportion of the subjects with peripheral-blood eosinophilia (defined here as those in whom at least 6% of leucocytes were eosinophils) than of the other subjects (Fig. 2). The expression of IL-13 did not show significant association with eosinophilia or helminth infection.

For some of the data analysis, the subjects were split into four groups: those with eosinophilia, with and without helminth infection, and those without eosinophilia, with and without helminth infection. Five of the 21 patients who were not eosinophilic and four of the 10 with eosinophilia were considered infected with intestinal helminths. Although the overall levels of IL-5 expression did not differ between the eosinophilic and non-eosinophilic subjects, the levels of IFN-γ expression were significantly higher in the eosinophilic subjects (Table 3). Among the subjects who did not have eosinophilia, the IL-5/IFN-γ ratio, which might reflect one aspect of the Th1/Th2 balance in each individual, was significantly higher in the helminth-infected subjects than in the uninfected; no such difference was observed among the eosinophilic patients (Table 3).

The expression levels of CLC, FcεRI, McTr, GLUT-1 and GLUT-5 showed no significant differences between the subjects groups (Table 3). Although the expression levels of FcεRI and McTr might be directly related to mast-cell density in the mucosa, it has been reported that nematode infection or exposure of mast cells to a large amount of IgE antibodies results in a striking up-regulation of FcεRI expression on mast cells (Chen and Enerback, 1996; Shaikh *et al.*, 1997; Yamaguchi *et al.*, 1997). Among the eosinophilic subjects of the present study, the FcεRI/McTr ratio, which might represent the FcεRI expression level/mast cell in each individual, was found to be significantly higher in the helminth-infected than in the uninfected; no such difference was observed in the patients who were not eosinophilic (Table 3).

To examine whether a component composed of eosinophils, IL-5, IFN-γ, FcεRI

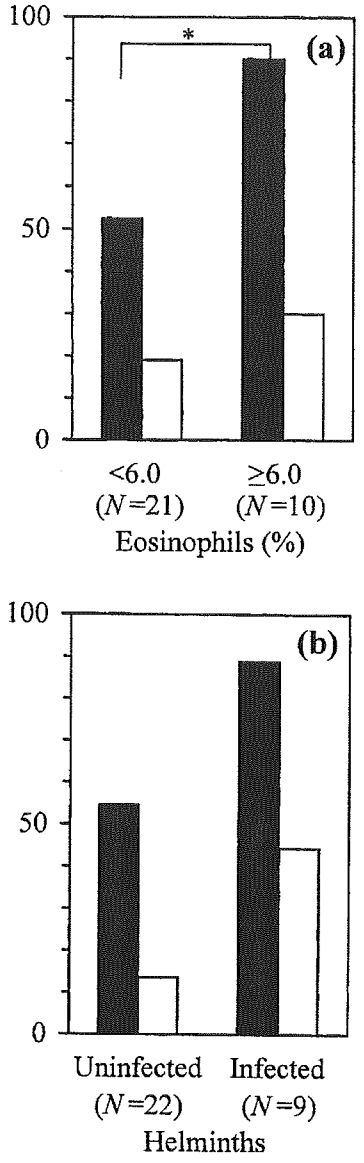

FIG. 2. The frequencies of expression of interleukin-5 (■) and interleukin-13 (□) in the duodenal mucosa — of the 10 patients with eosinophilia and the 21 without (a), and in the nine patients with helminth infection (on presentation or in the previous 3 months) and the 22 without (b). The frequencies of positivity for interleukin-5 expression were significantly higher for the eosinophilics than for the non-eosinophilics ($P=0.041$).

and McTr could be a predictor of helminth-infection status, principal-component analysis was carried out. This is a method to replace the original set of variables (in which one variable might not be independent of another) by one or a few variables (principal components) that account for the bulk of the variation observed, making the results easier to understand. As shown in Table 4, although the principal component identified gave a score for the helminth-infected patients that was significantly different to that for the uninfected patients without eosinophilia, it could not discriminate between the helminth-infected patients and the uninfected patients with eosinophilia.

DISCUSSION

In rodent models, *Trichinella spiralis* infection has been reported to induce a predominantly Th2-type cytokine response in the intestinal lymph (Ramaswamy *et al.*, 1996). In addition, comparative studies of mucosal and peripheral cytokine responses in *Trichuris muris*-infected mice indicated that the dominant cytokine responses of the mesenteric lymph nodes can be detected by sampling peripheral-blood lymphocytes (Taylor *et al.*, 2000). Although intestinal helminth infections such as ascariasis and strongyloidiasis have been reported to induce the production of type-2 cytokines by peripheral-blood mononuclear cells (Cooper *et al.*, 2001; Porto *et al.*, 2001), the effect of helminth infections on the expression of these cytokines in the intestinal mucosa has not been elucidated. Besides cases of strongyloidiasis and ascariasis patients, the present study included six cases of taeniasis. Cases of human taeniasis are not uncommon in Thailand. Most are attributed to *Ta. saginata*, on the bases of the parasite's unarmed scolex and the uterus-branching pattern of the proglottids. None of the subjects of the present study showed any clinical sign of cysticercosis (caused by *Ta. solium*). Recently, it has been suggested that at least some cases of human taeniasis in Thailand are caused by *Taenia saginata asiatica*, a subspecies that is distributed throughout many other Asian countries,