SCHISTOSOMA MANSONI: KINETICS OF GLOMERULONEPHRITIS IN MONGOLIAN GERBILS AND ITS CORRELATION WITH INTENSITY AND DURATION OF INFECTION

CHISTY M.M.*, NARGIS M.*, SATO H.*, INABA T.*, TAKAHASHI G.** & KAMIYA H.*

Summary:

The frequent occurrence of glomerular lesions in schistosomiasis patients has been reported, although appropriate animal models for the study of schistosomal glomerulonephritis have not been developed. To analyze the relationship between glomerulonephritis and *Schistosoma mansoni* infection, gerbils, *Meriones unguiculatus*, were infected with different number of cercariae and sacrificed at different weeks of post infection. Fifty cercariae were the optimum dose to produce the disease, glomerulonephritis, without early death of the animal. Infected gerbils showed heterogeneous types of glomerular lesions with increased serum creatinine level. Immune complex deposition was not detected at glomeruli of infected gerbils even by means of immunuofluorescence and also by transmission electron microscopy. However, infiltration of mononuclear cells in and around some of the altered glomeruli was observed. Immunohistochemical staining, using monoclonal antibody (HUSM-M.g.15) specific to gerbil's T-cells, revealed significant infiltration of T-cells. These findings suggest that T-cells might be involved in the development of glomerulonephritis. Gerbil could be a useful model to clarify the role of T-cells in the development of glomerulonephritis of schistosomiasis.

KEY WORDS : glomerulonephritis, *Schistosoma mansoni*, Mongolian gerbils, T-cells, immunohistochemistry.

Résumé : *SCHISTOSOMA MANSONI* : DÉVELOPPEMENT D'UNE GLOMÉRULONÉPHRITE CHEZ LA GERBILLE DE MONGOLIE ET CORRÉLATION AVEC L'INTENSITÉ ET LA DURÉE DE L'INFECTION

Les lésions glomérulaires des patients atteints de schistosomiase ont été souvent rapportées, cependant, il n'a pas été développé de modèle animal permettant d'étudier les lésions de glomérulonéphrites. Afin d'analyser les relations entre glomérulonéphrite et infection à Schistosoma mansoni, *des gerbilles,* Meriones unguiculatus, *ont été infectées avec des quantités variables de cercaires et sacrifiées à des dates différentes après cette infestation. 50 cercaires sont la quantité optimale pour provoquer une glomérulonéphrite sans entraîner la mort de l'animal. Les gerbilles infectées montrent des lésions glomérulaires hétérogènes avec une élévation de la créatinine sérique. Il n'a pas été observé de dépots de complexes immuns au niveau des glomérules, ni en immunofluorescence, ni en microscopie électronique à transmission. Cependant, un infiltrat de cellules mononuclées dans et autour certains glomérules a été observé. Une étude immunohistochimique, utilisant un anticorps monoclonal (HUSM-M.g.15) spécifique de cellules T de gerbilles, a révélé une infiltration de ces cellules. Cette découverte suggère que les cellules T pourraient intervenir dans le développement de la glomérulonéphrite. Les gerbilles pourraient être un bon modèle afin de clarifier le rôle des cellules T dans le développement des glomérulonéphrites schistosomiennes.*

MOTS CLÉS : *glomérulonéphrite,* Schistosoma mansoni, *gerbille, cellules T, immunohistochimie.*

INTRODUCTION

Infection of humans with schistosomes causes schistosomiasis, affects approximately 300 million peoples, is the most important cause of glomerulonephritis among parasitic infections in Africa, and Latin America (reviewed by Barsoum, 1993). Glomerulonephritis may be defined as a pathological process, characterized by focal or diffuse proliferation, infiltration or destruction of the glomerulus with or without involvement of the tubules or interstitial tissues. The incidence of such glomerulonephritis among patients with *S. mansoni* hepatosplenic disease was variably reported from 15 to 40 % (Andrade *et al.*, 1971; Rocha *et al.*, 1976). It was thought that schistosomal glomerulonephritis might be a typical example of immune complex (IC) glomerulonephritis (GN) because of their presence of schistosomal worm antigen (De Brito *et al.*, 1998) and IC in the glomeruli (Sobh *et al.*, 1991). However, treatment did not show any improvement rather progression to chronic renal failure (Sobh *et al.*, 1988), and polyclonal B-cells activation alone was not enough to induce GN in mice (Fujiwara *et al.*, 1988). Thus besides IC, host related factors, such as T-cells or macrophage function, seem to be involved (Van Velthuysen, 1996). Several laboratory animal species from mouse to chimpanzee have been used for the study of *S. mansoni* infection (De Brito *et al.*, 1971; Brack *et al.*, 1972; Andrade & Susin, 1974; Sobh *et al.*, 1991). However as a model of the disease, none of these hosts was considered as ideal. For example, the lesions in chimpanzees closely resemble those in humans (Sadun *et al.*, 1975),

* Department of Parasitology, ** Second Department of Anatomy, Hirosaki University School of Medicine, Hirosaki 036-8562, Aomori, Japan.
Correspondence: H. Kamiya.
Tel.: +81-172-39-5043 – Fax: +81-172-39-5045.
E-mail: hkamiya@cc.hirosaki-u.ac.jp

but these animals are not widely accepted to use for animal experiments. On the other hand, hamsters and mice spontaneously develop renal pathology with age (Robinson *et al.*, 1982). An animal model should provide a normal worm development and the lesions comparable to those of human. In addition, it should be easily maintained. Worm development and liver lesions recorded in gerbils infected with *S. japonicum* more closely resembled to those of humans than did the lesions observed in mice or rabbits (Yingrui *et al.*, 1983). But the literature does not contain any information regarding schistosomal glomerulonephritis in gerbils.
The objective of this experiment was to study the glomerulonephritis in gerbils infected with *S. mansoni* and its correlation with intensity and duration of infection.

MATERIALS AND METHODS

PARASITES

A Puerto Rican strain of *Schistosoma mansoni* maintained in *Biomphalaria glabrata* snails and Mongolian gerbils, *Meriones unguiculatus*, was used through out the experiments. Cercariae were used within one hour of being shed.

ANIMALS

In the present study 180 gerbils, 6-8 weeks old of either sex were used. Among these, 144 were infected with different doses of cercariae (25, 50, 100 and 150 cercariae) of *S. mansoni* and the remaining 36 animals served as controls. These animals were bred at the Institute for Animal Experiments of our university. Animals were fed food pellets and water *ad libitum*. All animal experiments were performed according to the Guidelines on Animal Experimentation as set out by Hirosaki University.

INFECTIONS

The animals were anaesthetized by intraperitoneal injection of 30 mg/kg Nembutal® (Pentobarbital sodium; Abbot Laboratories, North Chicago, USA). The infections with cercariae were carried out by the ring method of Smithers and Terry (1965). The mean number of cercariae used in each animal was calculated from six random aliquots of the cercarial suspension. For cercarial penetration, one-hour was allowed after which the water in the ring was examined for non-penetrating cercariae.

LABORATORY EVALUATIONS

S. mansoni infected gerbils and controls matched for age and sex were subjected to the following measurements: 1) Serum creatinine concentration (mg/dl); 2) blood urea nitrogen (BUN) concentration (mg/dl); 3) Serum albumin, globulin and total protein concentration (mg/dl), and serum cholesterol concentration (mg/dl). Automatic Biochemical Analyzer (Olympus AU 600) was used for biochemical evaluations.

HISTOPATHOLOGICAL EVALUATIONS

Animals were killed by an anesthetic over dose of ether, at various weeks (wks) post infection (p.i). Kidney tissues of the sacrificed animals were subjected to the following examinations:

A) Light microscopic examination

All collected kidney samples were fixed in 10 % neutral phosphate buffered formalin, routinely processed, embedded in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin (H/E), Periodic Acid Schiff (PAS), Periodic acid silver methanamine (PASM) and Congo red stains. In average, four sections were made from the hilar region of each kidney and were examined microscopically. In each animal, 30 glomeruli were randomly selected from four sections. The mean number of cells per glomerular cross-section (c/gcs) was counted by using high power objectives. The mean glomerular diameter was measured by means of an ocular micrometer. Glomerular abnormalities, especially mesangial cell proliferation, alteration of the mesangial matrix, thickening of the glomerular basement membrane (GBM), hemorrhage and necrosis along with tubulo-interstitial changes were recorded.

B) Transmission Electron Microscopy (TEM)

TEM was performed in three animals from each group at each time points. Removed kidneys were immediately sliced at 0.5 mm thickness, and prefixed in cold 2.5 % glutaraldehyde solution in 0.1 M phosphate buffer (PB), pH 7.4, at 4° C for more than two hours. They were washed in two changes of cold PB for 10 min, and post-fixed in cold 1 % osmium tetra-oxide in PB for two hours. Specimens were then washed in three changes of cold distilled water, stained *en block* with 1 % uranyl acetate, dehydrated in a series of alcohol, and embedded in epoxy resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and observed with an electron microscope (JEOL, Japan).

C) Immunohistochemical examination

a) Immunofluorescent microscopy for detection of immune complex-related immunoglobulins (IgG, IgM, and IgA): Kidney cryostat sections (5 µm thick) were air-dried and fixed in acetone for 10 min. The sections were washed with phosphate buffered saline (PBS), pH 7.3 and incubated with PBS containing 10 % normal goat sera to block non-specific binding sites. Indirect immunofluorescence techniques were applied using a panel of antibodies cross-reactive with gerbils immu-

noglobulins, directed against IgG (rabbit antibody to rat IgG (H + L) (Chemicon International Inc., Temecula, CA, USA); IgM (goat $F(ab')_2$ fragment to mouse IgM) (American Qualex, La Mirada, CA, USA), rabbit IgG to goat IgG (H + L) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and IgA (rabbit antibody to mouse IgA) (ZYMED Laboratories, Inc., San Francisco, CA, USA). Antibodies were applied in a working dilution 1:100 as first layers. FITC-conjugated affinity purified goat anti-rabbit IgG (E.Y Laboratories, Inc., San Mateo, CA, USA) were used as second layer in a working dilution 1:200. For control sections the primary antibody was omitted to assess non-specific staining. Kidney cryostat sections from normal gerbils were used as negative control.

b) Immunohistochemical staining for visualization of gerbil T-cells: Cryostat sections were air dried and fixed in cold acetone for 10 min. The immunohistochemical staining was performed using a novel mouse monoclonal antibody (HUSM-M.g.15 of IgG2b isotype) specific to gerbil T-cells (Sato *et al.*, 2000). Undiluted culture supernatant was applied as first layer. Peroxidase conjugated goat $F(ab')_2$ fragment to mouse IgG (Fc) (Organon Teknika Corp., Durham, NC, USA) was used as a second layer in a working dilution of 1:200. Bound antibody was detected using color development by 3, 3'-diaminobenzidine, followed by light counter staining with hematoxylin. Intraglomerular and interstitial T-cells infiltration were estimated in 30 glomerular cross-section (gcs) and 50 high power fields (HPF) for each animal, respectively.

STATISTICAL ANALYSIS

Statistical significance of the results was determined using Student's *t*-test. Data were expressed as mean ± SD and a *P* value of less than 0.05 was taken as the minimum level of significance.

RESULTS

Gerbils showed glomerulonephritis, 17 % (25/144) of the total infected, earliest at 20 wk post infection (p.i). However, the prevalence of such glomerulonephritis became more than 80 % in the group infected with 50 cercariae or more at 30 wk p.i (Fig. 1A). In this study animals were defined positive when over 40 % of the glomeruli present in three non-consecutive kidney sections showed histological and immunopathological lesions. Fifty cercariae were the optimum dose to produce glomerulonephritis without early death of the animals. It was confirmed by repeated experiment for its reproducibility (unpublished data). Groups infected with a higher dose of cercariae showed a higher prevalence of glomerulonephritis but a shorter period of survival (Fig. 1A, B). None of the control animals revealed any glomerulonephritis.

Gerbils infected with 50 cercariae showed gradual and consistent elevations of serum creatinine level (Fig. 2A). However, their serum cholesterol and BUN (Fig. 2B) levels were mild and irregular, did not correlate well with the intensity and duration of infection. The increase in total proteins was considerably greater in those infected gerbils while proportional decrease in serum albumin levels was observed in these animals, but the absolute amount of serum albumin did not diminish (Fig. 3A, B).

Mean glomerular diameters gradually increased in the infected groups and became significantly different from those of controls at 20 wk p.i (Table I). Glomerular cell counts gradually increased in all the infected gerbils. The increased cellularity became significant from 20 wk p.i in all the groups infected with 50 cercariae and more (Table II). Glomerular hypercellularity was due to infiltration of inflammatory cells and endocapillary cellular

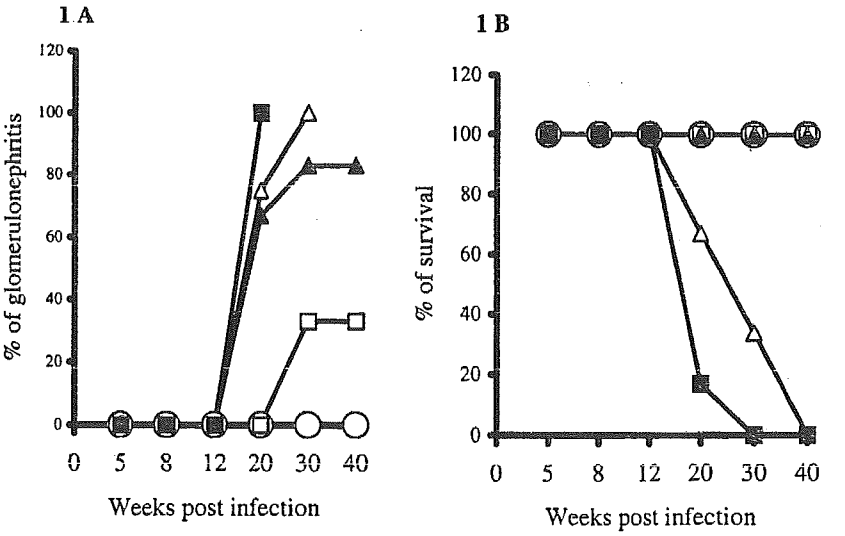

Fig. 1. – Effect of intensity and duration of infection with *Schistosoma mansoni* cercariae on the prevalence of glomerulonephritis (A) and survival rate of gerbils (B). Gr-1: 0 cercaria (○); Gr-2: 25 cercariae (□); Gr-3: 50 cercariae (▲); Gr-4: 100 cercariae (△) and Gr-5: 150 cercariae infection (■). The number of gerbils at the beginning of the experiment was six at each time points.

No. of cercariae infected	No. of cells/glomerulus at different weeks p.i.					
	5 wk	8 wk	12 wk	20 wk	30 wk	40 wk
0	34 ± 1	33 ± 4	34 ± 5	33 ± 3	32 ± 2	36 ± 3
25	37 ± 5	38 ± 6	37 ± 3	38 ± 8	49 ± 4***	57 ± 7***
50	36 ± 4	40 ± 8	40 ± 6	44 ± 5***	54 ± 5***	72 ± 9***
100	34 ± 3	39 ± 8	41 ± 6	47 ± 6[a] **	53 ± 2[b] ***	NA
150	35 ± 3	40 ± 8	40 ± 5	54[c]	NA	NA

Each point represents no. of cells/glomerular cross-section (mean ± SD). Total 30 glomeruli/animal were examined and their contained cells were counted using high power objectives. Cellularity was evaluated by Student's *t*-test and found significant, where $P < 0.05$, comparing with the control (0 cercariae). NA: Animal not available for examination. Six gerbils, at each group except three, where [a]: 4; [b]: 2 and [c]: 1 gerbil. **: $P < 0.01$; ***: $P < 0.001$.

Table II. – Glomerular cellularity of gerbils infected with different number of *Schistosoma mansoni* cercariae at different weeks p.i.

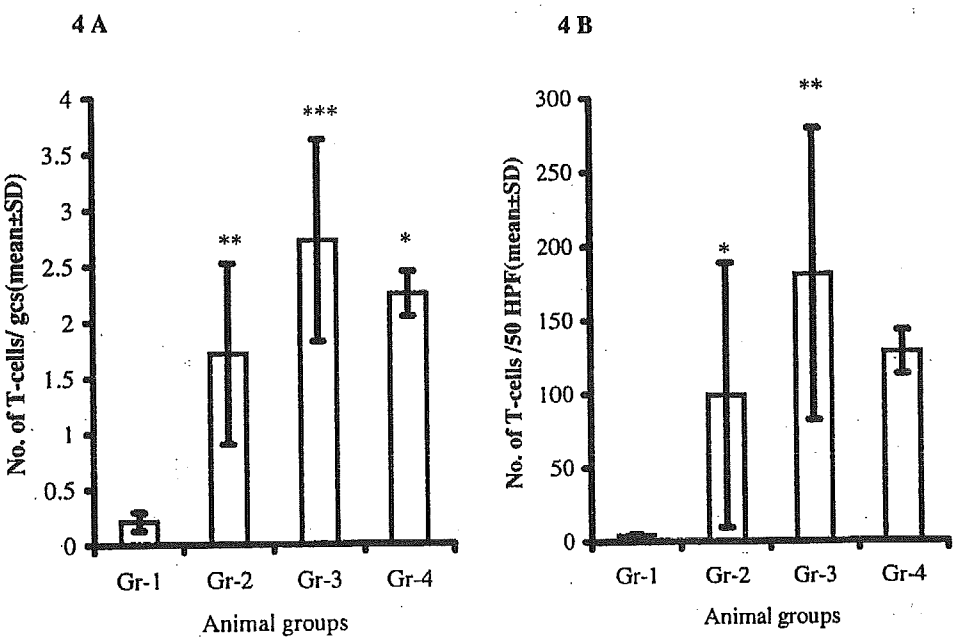

Fig. 4. – T-cell infiltration, intraglomerular (A) and interstitial (B), in gerbils infected with *S. mansoni* at 30-wk p.i. Gr-1: Control, n = 6; Gr-2: infected with 25 cercariae, n = 6; Gr-3: infected with 50 cercariae, n = 6 and Gr-4: infected with 100 cercariae, n = 2. (A) No. of T-cells/glomerular cross-section (c/gcs), total 30 glomeruli/animal were examined; (B) interstitial infiltration of T-cells/50 HPF. The values were evaluated by *t*-test and found significant, where $P < 0.05$, comparing with control group. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

assumed hyaline like appearance in H/E, which was PAS and PASM positive. Some of the tubules showed cellular and hyaline cast. Accumulations of MNCs in and around some of the altered glomeruli (Fig. 5E) were observed. TEM revealed renal abnormalities earliest at 20 wk p.i in the 50 cercariea and more infected gerbils. The abnormalities were wrinkling and irregular thickening of the glomerular basement membrane (GBM) with variable degree of severity (Fig. 6A, B), increased mesangial area and cellularity (Fig. 6C) in more than 40 % of the infected gerbils. Peritubular accumulation of MNCs and tubular basement membrane (TBM) abnormalities were also observed (Fig. 6D). Out of 144 infected gerbils only two showed granuloma with renal egg deposition. Five (3.5 %) of the infected gerbils exhibited amyloid deposition earliest at 30 wk p.i in the renal glomeruli and interstitium. Electron microscopy showed that the glomerular amyloid deposits were mainly subendothelial (Fig. 6E, F). None of the control gerbils showed any amyloid deposits.

DISCUSSION

Schistosomal glomerulonephritis is considered a late complication of hepatosplenic schistosomiasis with collateral circulation, where eggs bypass the hepatic filter and are carried to the lungs and then to the systemic circulation (Andrade *et al.*, 1971). This will permit the diversion of the immune complex (IC) away from the liver and its Kupffer' cells. Thus the complexes will reach the kidney and other organs by the systemic circulation.

In our present study, 50 cercariae were the optimum dose of infection at which majority of gerbils showed glomerulonephritis (Fig. 1A) at 30-wk p.i. This dose was well tolerated up to 40-wk p.i and glomerular changes were almost similar to higher dose groups. None of the control animals showed any glomerulonephritis. The serum biochemical findings in infected gerbils contrasted in some important respects (cholesterol and BUN) with the biochemical observations

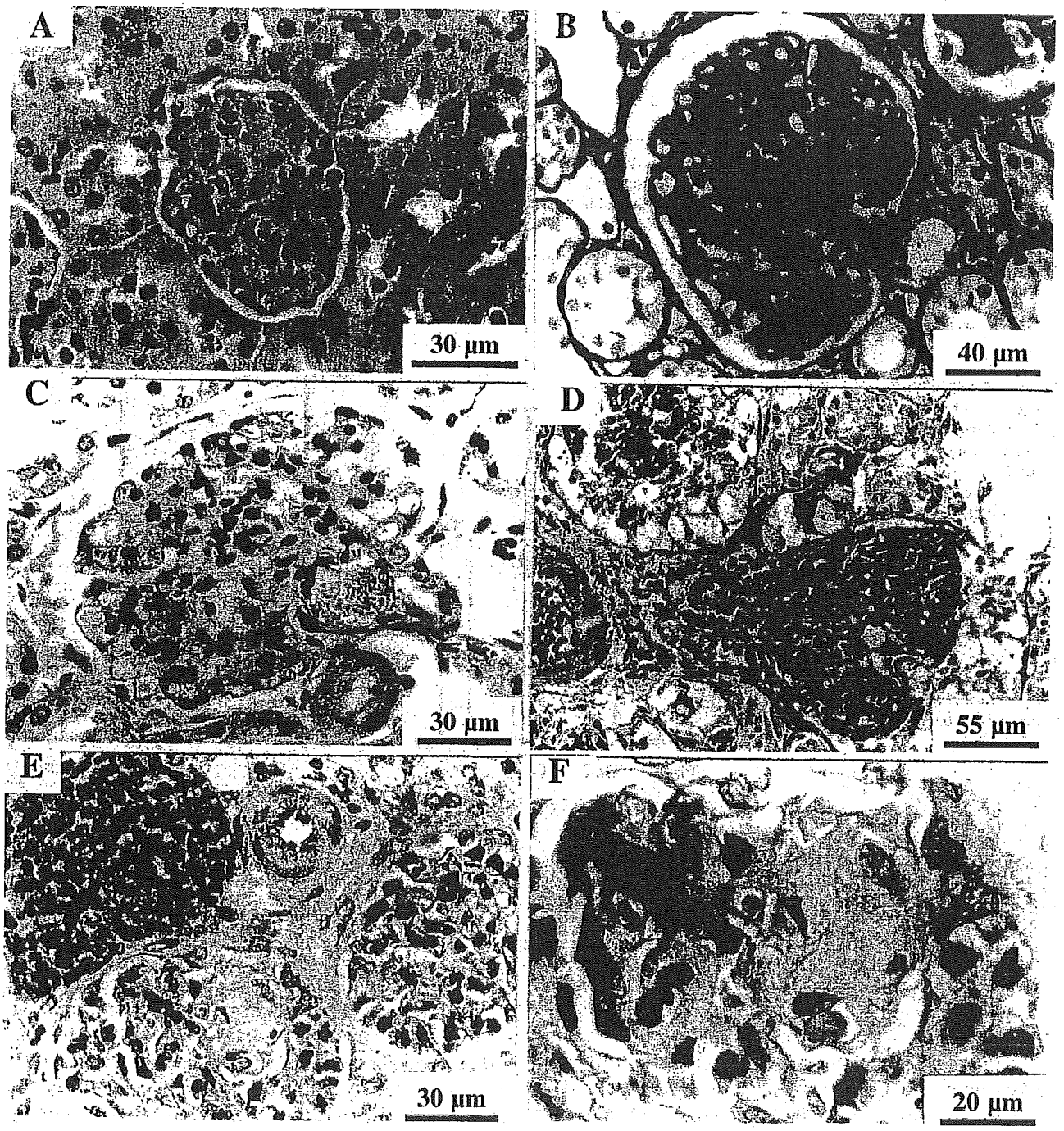

Fig. 5. – Pathological changes in the kidney of gerbils exposed to 50 cercariae at 30 wk p.i. (A) morphologically normal (control, H/E); (B) mesangioproliferative GN (PASM); (C) proliferative GN (H/E); (D) necrotizing GN (H/E); (E) Segmental glomerulosclerosis with periglomerular and interstitial mononuclear cells infiltration (H/E); (F) Glomerulosclerosis where sclerosing capillary loops assumed hyaline appearance (H/E).

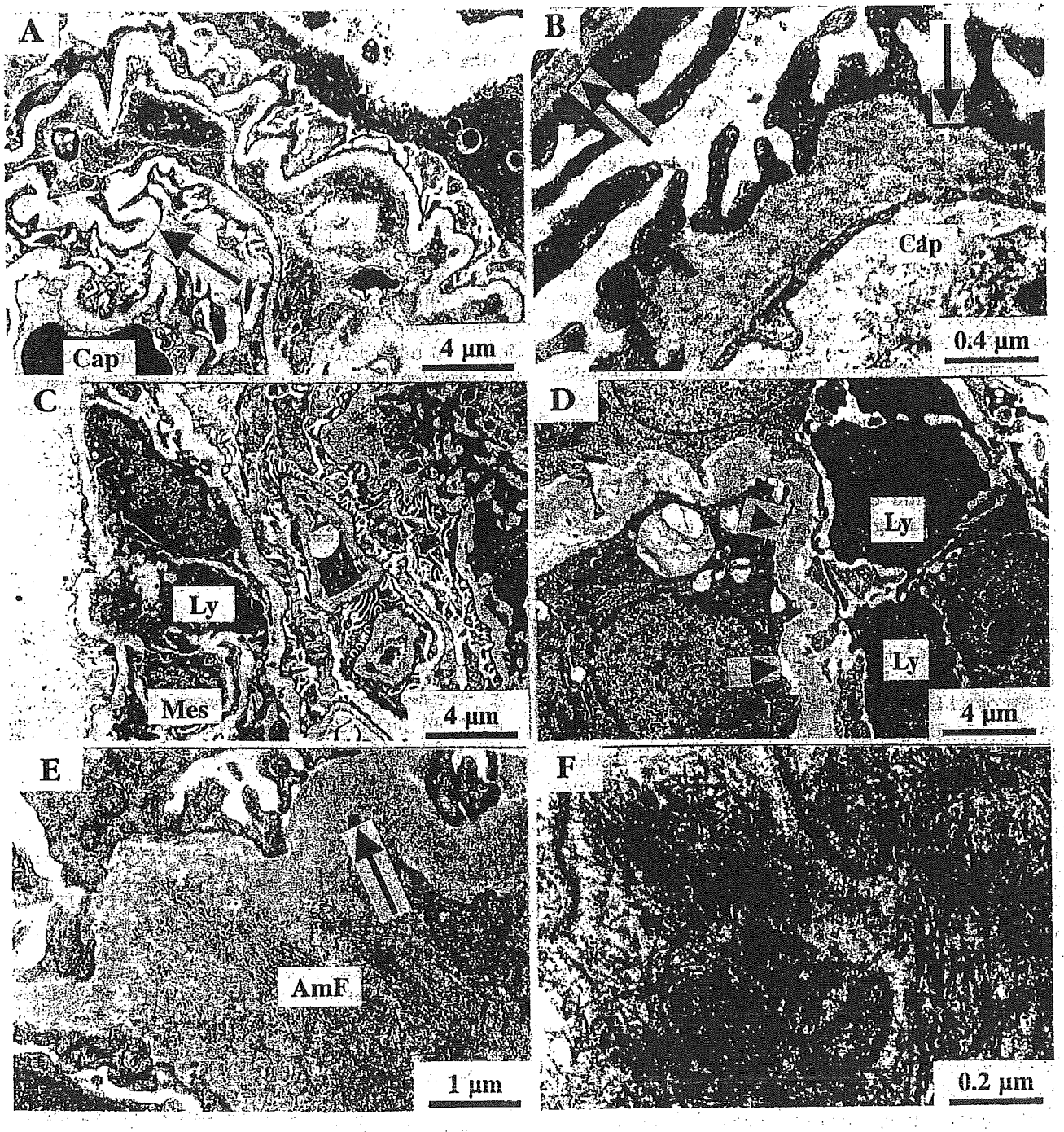

Fig. 6. – Electron micrograph of a glomerulus of gerbils exposed to 50 cercariae of *S. mansoni* is showing wrinkling and irregular thickening of the glomerular basement membrane (A & B) with increased no. of cells in the mesangial area (C), along with alteration of tubular basement membrane (D) at 30 wk p.i., subendothelial amyloid deposition (E) with randomly oriented fibrils of higher magnification (F) at 40 wk p.i. Note: arrow, indicate glomerular basement membrane; arrow head, tubular basement membrane; Mes, mesangial cell; Cap, capillary lumen; Ly, lymphocyte; AmF, amyloid fibril.

reported for mice with schistosomiasis (Sadun & Williams, 1966). On the other hand, the significant and consistent increases in creatinine, total protein and globulin concentration recorded in hamsters (Sobh *et al.*, 1991), mice (Sadun & Williams, 1966) and chimpanzees (Sadun *et al.*, 1970) were also observed in infected gerbils. The increase in globulin concentration in the absence of corresponding increases in albumin produced striking reduced albumin: globulin ratio. These were more evident in the animals with heavier infections and became more marked with time (Sobh *et al.* 1991; Sadun *et al.*, 1970).

Heterogeneous types of glomerular lesions (Fig. 5) along with tubulo-interstitial changes were observed in gerbils. Similar morphological changes were also observed in humans (Cheever, 1968; Andrade *et al.*, 1971; Sobh *et al.*, 1989) and other animals (Sadun *et al.*, 1975; Sobh *et al.*, 1991). Several investigators demonstrated mesangial hypercellularity accompanied by IgG glomerular deposits (Sobh *et al.*, 1991; Hilleyer & Lewert, 1974) without abnormality of the GBM (Hilleyer & Lewert, 1974) in hamsters. Mesangial hypercellularity was seen in our experiment but did not affect all the glomeruli of the infected gerbils. However, wrinkling and irregular thickening of the GBM were observed in more than 40 % of the infected gerbils. But the mechanism of these pathological changes remained unknown. It cannot be explain by renal egg deposition since renal egg depositions were sporadic (2/144) but glomerular lesions were much more prevalent.

Amyloidosis may be one of the pathogenetic mechanisms of schistosomal glomerulonephritis, where 3.5 % of the infected gerbils revealed amyloidosis (Fig. 6E, F). None of the control gerbils showed any amyloid deposits. Amyloidosis secondary to schistosomal infections has also been reported in 8% infected hamsters (Sobh *et al.*, 1991) and 16 % of humans schistosomiasis (Barsoum *et al.*, 1979).

In schistosomiasis, IC mediated glomerulonephritis have been reported in mice (Natali & Cioli, 1976; Fujiwara *et al.*, 1988), hamsters (Sobh *et al.*, 1991), monkeys (Tada *et al.*, 1975) and humans (Sobh *et al.*, 1987). Recently immunoelectron microscopic localization of schistosomal antigen in the glomerulus of hamsters, where mesangial expansion with increased cellularity has been reported (De Brito *et al.*, 1998). It is interesting to note here that GBM, tubules and interstitium were unremarkable in hamsters but in our study, infiltration of MNCs with irregular thickening of the GBM were observed. In our gerbil model the glomerular lesions are most probably not IC mediated, since IF staining and TEM of kidney tissues did not reveal the deposition of IC in the mesangium and capillary walls. In humans IC negative GN with increased number of MNCs in the glomeruli has been reported and subtyping of these MNCs in kidney showed significant increases (0.5 ~ 2 c/gcs) of T-cells (Nolasco *et al.*, 1987; Tipping *et al.*, 1985). There is now evidence that T-cells play a major role in glomerular injury, where $CD4^+$ T-cells responsible for the induction of autoimmune syndrome and glomerular infiltrations of $CD8^+$ T-cells are directly involved in the onset of proteinuria (Van Velthuysen, 1996). Glomerular hypercellularity, due to influx of $CD8^+$ T-cells, was reported in murine malaria (Lloyd *et al.*, 1993).

Significant T-cells infiltrations in and around some of the altered glomeruli were observed in our study (Fig. 4A, B). These infiltrated T-cells were unable to be clarified due to lack of information on T-cell subset of gerbils. This abnormal infiltration of T-cells and macrophages in the glomeruli may indicate participation of cellular immunity (Bolton *et al.*, 1987; Saito & Atkins, 1990; Hooke *et al.*, 1987), although some defects in the macrophage activation system and also in complement have been reported in gerbils (Nasaree *et al.*, 1998; Kamiya *et al.*, 1980). This is in agreement with our previous experiment, where we found lymphocytic myocarditis in gerbils infected with *S. mansoni* (Chisty *et al.*, 1999). To elucidate the role of T-cells in glomerular injury associated with parasitic infections further studies are suggestive.

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どのように検査すればよいか？

特集

呼吸器検査
—喀痰，胸水，気管支鏡—

神谷晴夫　弘前大学医学部寄生虫学講座　教授

SUMMARY

・呼吸器系として，鼻腔，咽頭から肺までを検査対象とし，肺を好占部位とする寄生虫と，一時的に滞在あるいは移行途中の寄生虫とに区分した．
・ニューモチスチス・カリニあるいは肺吸虫感染を考慮して，喀痰・胸水を検査材料とする検査法を記述した．
・赤痢アメーバによる肺膿瘍の検査法について記述した．
・検査時の侵襲の少ない検査法を可能な限り強調した．

呼吸器検査を行う必要性のある寄生虫（症）を表1にまとめた．ここでは呼吸器系を鼻腔，咽頭から肺まで，それに加えて横隔膜と広くとらえ，原虫，吸虫，条虫，線虫それに衛生動物に属する主なものをまとめた．しかも，それらは肺など呼吸器系を好占部位とするものと体内移行途中に一時的に滞在あるいは通過するものと区分することができる．それぞれの寄生虫に関しては別項目で詳細に述べられるので，ここでは主に喀痰，胸水の検査法について記述した．

I　喀痰検査

喀痰は，採取後，短時間のうちに雑菌が繁殖し，検査に好ましい状態でなくなるので，採取後2～3時間以内に検査に供するのが好ましい．しかし，医療機関へ持参するまでそれ以上の時間が経過するのが一般的であり，冷蔵保存しておく必要がある．

持参された喀痰は，その色調，血液は混じっていないか，粘性の程度などに注意する．また蠕虫卵，虫体，好酸球等の細胞成分やCharcot-Leyden結晶の有無などを，新鮮材料をスライドグラス上に取り生理食塩水で薄め鏡検する．時に，剥離した気管粘膜繊毛上皮細胞があたかも活発に運動している原虫のように誤認されることがあるので注意が必要である．

表1　呼吸器系に係わる主な寄生虫

	起因寄生虫種	寄生部位・体内移行	検査材料**	特徴的様相
原　虫	ニューモチスチス・カリニ*	肺	喀痰・組織切片	肺炎症状
	赤痢アメーバ*	肺	穿刺材料・組織切片	肺腫瘍症状
	トキソプラズマ*	肺	組織切片	肺炎症状
	リーシュマニア	肺	組織切片・穿刺材料	鼻腔粘膜潰瘍
	クルーズ・トリパノソーマ	肺	組織切片	肺炎症状
線　虫	回虫*	肺・移行途中	喀痰	Löffler 症候群
	ブタ回虫*	肺・移行途中	喀痰	Löffler 症候群
	イヌ回虫*	肺・移行途中	喀痰	Löffler 症候群
	鉤虫*	肺・移行	喀痰	Löffler 症候群
	イヌ糸状虫*	肺	切除・穿刺組織	肺腫瘍症状
	広東住血線虫*	肺動脈	切除組織	好酸球性髄膜脳炎
	バンクロフト糸状虫	肺・移行途中	喀痰・胸水・血液	熱帯性好酸球症
	糞線虫*	肺・移行途中	喀痰	熱帯性好酸球症
	旋毛虫*	横隔膜・横紋筋	血清抗体検査、時に筋肉	筋肉痛・好酸球増多・呼吸障害
条　虫	マンソン孤虫	肺・異所寄生	虫体	移動性腫脹
	エキノコックス*	肺	切除組織	肺腫瘍性症状・居住歴
	有鉤嚢虫	肺	切除虫体	肺腫瘍性症状・居住歴
	芽殖孤虫	肺	虫体・切除組織	移動性腫脹
吸　虫	ウエステルマン肺吸虫*	肺	喀痰・胸水	血痰・胸膜炎
	宮崎肺吸虫*	肺・胸腔	喀痰・胸水	血痰・胸膜炎
	日本住血吸虫*	肺	穿刺・切除組織	肺性心
	マンソン住血吸虫*	肺	穿刺・切除組織	肺性心
	Clinostoma complanatum	咽頭	虫体	halzoun（寄生虫性咽頭炎）
節足動物	シタムシ類	肺・鼻腔・咽頭・気管粘膜	虫体	
	肺ダニ（ホコリダニ、コナダニ）	肺	喀痰・切除組織	アレルギー症状
	幼蛆	鼻腔	虫体	ハエ幼虫症
その他	ヒル類	鼻腔	虫体	halzoun

＊：国内での重要種
＊＊：免疫学的検査材料は除外

① ニューモチスチス・カリニ肺炎の検査[1～3)]

何らかの**免疫不全状態**が基礎にある患者で肺炎の徴候が見られ，**胸部X線写真**で肺野が広くスリガラス状に淡い陰影となり，**動脈血酸素分圧**が，通例 80mmHg 以下に低下している場合，本症を疑う必要がある．確定診断は，***Pneumocystis carinii*** (Pc) を喀痰，**気管支肺胞洗浄液**（BALF）などより検出することである．

a．検査材料の採取とその処理

通常 Pc 肺炎患者では，喀痰排出量が極端に少ないので，積極的に喀痰の排出に努めさせ，1～2日分貯めた喀痰からの検出を試みる**集シスト法**が有効である．また，口腔を十分洗浄した後に，ネブライザーなどで，3％食塩水を約5～15分間吸引後，数回深呼吸をさせてから，強く咳を促して排出を誘引し採取した痰を用いる方法も第1次選択検査材料としては有効である．

さらに，右第4・第5肋骨間を 20～23G の針で穿刺し，経皮的肺吸引（needle aspiration）によって，**肺浸潤液**を採取しその後の検査に用いる場合もあるが，侵襲の程度を十分に考慮しなければならない．

あるいは，気管支鏡的肺生検（taransbronchial lung biopsy，後述）材料，BALF（後述）あるいは気管吸引痰，さらには剖検肺組織を検査材料として用いることもある．しかし，国内では喀痰を用いた集シスト法が一般的に行われている．採取した喀痰は次のように処理する．

①**真菌の増殖を防止**するために，アムホテリシンB（1.2μg/mL）を加えた喀痰採集容器に，1～2日分の喀痰を患者に積極的にためさせる．

②**喀痰の粘性を除く**ために acetyl-L-cystein を 0.2N NaOH に溶解させた溶液を喀痰の10倍量を加え，良く攪拌する．

③その溶液を1枚のガーゼでろ過し，ろ液を，3,000rpm/5分遠心後，上清をすてる．少量の生食水で沈渣をほぐし，さらに生食水を加え，再度同様に遠心・洗浄を繰り返す．

④沈渣をスライドに塗布し，風乾させ，**ギムザ染色，トルイジンブルーO染色メテナミン銀染色，蛍光抗体法**[4,5]などでPcを染色する．

b．染色法

1）トルイジンブルーO染色法

①沈渣を塗布した前述のスライドグラスを固定

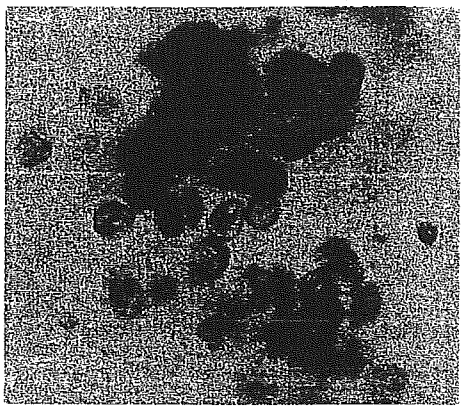

図1　トルイジンブルーO染色で染まった嚢子
嚢子壁が青紫色に染まるが，嚢子内小体は染色されない．（文献2）より引用）

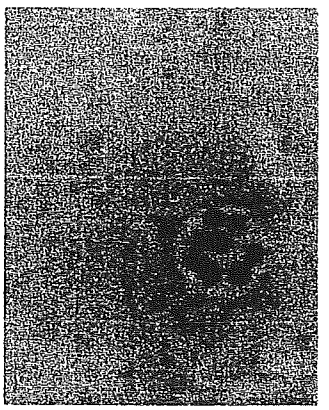

図2　ギムザ染色で染まった8個の嚢子内小体を容れた嚢子
嚢子壁は染まらない．隣接の赤血球より少し小さい．（文献2）より引用）

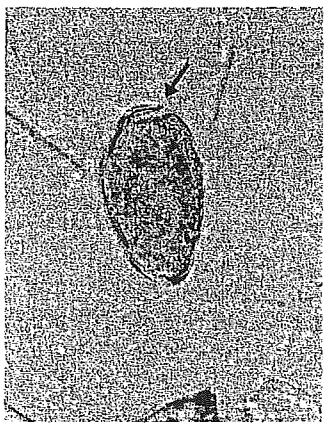

図3　ウエステルマン肺吸虫卵
色調は黄褐色で，左右非相称．卵蓋（↑）側が最大幅を示す．
2.8cm＝100μ

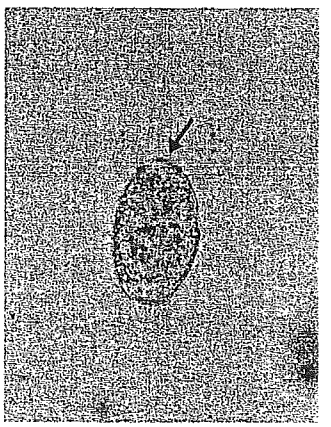

図4　宮崎肺吸虫卵
ウエステルマン肺吸虫卵に比して，大きさは小さく，卵殻は薄く，しかも卵蓋側の幅は大きくない．
2.8cm＝100μ

せずに，10～15分間硫酸エーテルに浸漬し，エステル化する．

②約10回水洗する．

0.15％トルイジンブルーO染色液に3分間つける．

③イソプロピールアルコールを3回通し，脱水する．

④キシレンで透徹後，バルサムなどで封入し，鏡検する．**Pcシスト壁は紫色に染まる（図1）．**

〈参　考〉

トルイジンブルーO染色液の調製法：トルイジンブルーO 300mg，蒸留水60mL，濃塩酸2mL，無水エタノール140mLをこの順に混合して作製する．

2）メテナミン銀染色

本法は，Pcの嚢子壁を染めるのに好適であるが，大多数の真菌類も染め出されるので鑑別が必要である．**壁は黄褐色～黒褐色に染まる．**

3）ギムザ染色

嚢子内小体（図2）の検出には必須である．**嚢子壁は染まらない．**

4）免疫染色法[4,5]

Pcに特異的なモノクロナール抗体を用いて，蛍光抗体法で検査材料中のシストを染め出して，検出する．

② ウエステルマン肺吸虫検査法

本虫は肺に寄生し，虫嚢を作り，基本的には2虫体が一つの虫嚢入る．産卵された虫卵は，通常**チョコレート色の血痰**中に排出される．しかし，飲み込まれて，糞便からも検出される点を考慮しておく必要がある．結核と誤認されることがしばしばであった．

喀痰を，直接にスライドグラス上に採り，カバーグラスを乗せ，鏡検すれば、排出虫卵数が多い場合には，虫卵を検出できる（図3）．しかし，虫卵が少ない場合には，十分量の喀痰を集め，その約5倍量の0.1規定のNaOHを加え，良く攪拌して，数時間放置して十分に溶解させる．その後，2,000～2,500rpmで数分間遠心し，沈渣を鏡検して，虫卵を検出する．

最近，前述のウエステルマン肺吸虫と同様に，宮崎肺吸虫でも虫嚢を形成し，喀痰中に虫卵の排出が認められた症例もあるので，虫卵での鑑別が必要である．

Ⅱ　胸水検査

胸水が検査材料となる場合は，宮崎肺吸虫症，ウエステルマン肺吸虫症がその対象となるが，流行地ではバンクロフト糸状虫のミクロフィラリアが検出されることもある．

① 宮崎肺吸虫症の検査法

本吸虫は，イタチなど野生動物を終宿主とし，ヒトは好適宿主ではない．中間宿主のサワガニなどを食して感染しても，本来，**肺に虫嚢はつくらず，胸腔内で発育する．**したがって血痰を排出することはなく，**気胸や胸水貯留**が認められる．遠心した胸水沈渣中の虫卵（図4）の確認や顕著な**好酸球増多**は有力な診断の根拠となる．

なお，ウエステルマン肺吸虫でも虫嚢を形成せずに，胸腔内を移動する場合があり，その際には本虫と同様に検査時に対応する必要がある．

Ⅲ その他

① 膿瘍の検査[6]

赤痢アメーバの肺膿瘍が検査対象となることがある．その際には，肺膿瘍内容を超音波ガイド下で穿刺またはドレナージして採取する．採取膿瘍内容物より栄養型は赤血球を取り込み活発に運動する．ただし，検査材料は，鏡検まで，37℃位に保温しておかなければならないので材料の輸送時にはその点の注意が必要である．また，エキノコックスが肺に寄生していることもあり，その際，穿刺施行前に，十分な類症鑑別が必要である．赤痢アメーバの具体的な検査法に関しては，アメーバ赤痢―検査・診断マニュアル[6]が有効である．

② 気管支鏡による検査

気管支鏡はその侵襲性から寄生虫学的検査のための使用頻度は多くない．したがって，気管支鏡によって，直接的診断に繋がる検査は極めて限定されているが，前述したPc検査材料の採取上からも利用されることがある．気管支肺胞洗浄液，気管支鏡的肺生検材料などがそれである．

おわりに

診断に呼吸器検査―喀痰，胸水，気管支鏡―が必要な寄生虫症は決して多くない．しかし，表1にまとめたように，呼吸器系を広く解釈すれば，かなりの寄生虫症の関与をあげることができる．しかし，実際には，ここで詳述したカリニ肺炎，肺吸虫症の検査が最も深く関与する．しかし，肺移行途中の寄生虫感染の検査に関しては，その技法が決して十分確立しているわけではなく，その際の臨床症状も考慮して，総合的に把握して検査しなければならない．

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□寄生虫学□ Full paper

Analyses of Regional Environmental Factors on the Prevalence of *Echinococcus multilocularis* in Foxes in Hokkaido, Japan

Yasushi YOKOHATA[1] and Masao KAMIYA[2]

1) Laboratory of Environmental Biology, Faculty of Education, Toyama University, Gofuku 3190, Toyama City, Toyama 930-8555, Japan
2) Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan (Present address:Center for Advanced Science and Technology, Hokkaido University, Sapporo 001-0021, Japan)
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北海道におけるキツネの多包条虫 *Echinococcus multilocularis* 感染率に対する地域的環境因子の分析

横畑泰志[1], 神谷正男[2]

1) 富山大学教育学部環境生物学研究室 〒930-8555 富山県富山市五福 3190
2) 北海道大学大学院獣医学研究科動物疾病制御学講座寄生虫学教室 〒060-0818 北海道札幌市北 18 条西 9 丁目
(現. 北海道大学先端科学技術共同研究センター 〒001-0021 北海道札幌市北区北 21 条西 11 丁目)

ABSTRACT. Relationships between the prevalence of alveolar hydatid (*Echinococcus multilocularis*) in red foxes (*Vulpes vulpes*) captured in 74 regions of Hokkaido, Japan from 1985 to 1990 and some regional environmental factors (including population density of voles, temperature, snowfall depth, mean degree of slope, mean altitude, human population density, etc.) were examined using simple and multiple regression analysis methods. Eight explanatory variables were selected from 15 types of candidate variables belonging to eight respective categories of the regional environmental factors, based on the simple regression analysis. In the multiple regression analysis, only two of these eight explanatory variables were selected with a stepwise process, and the following model was obtained: $Y = 0.00979X_1 - 0.00037X_2 + 0.23833$ (Y: arcsin-root transformed prevalence of *E. multilocularis* in foxes, X_1: captive number of voles in September, X_2: number of days with snowfall deeper than 50 cm, $r=0.32180$, $P=0.0001$). The higher density of the voles is supposed to make the establishment of the life cycle of this cestode species more successful. The negative influence of deeper snowfall on the prevalence is attributable to the lower predation pressure on the voles by the foxes in deeper snowfall, which suppresses the hunting behavior of the foxes.

Key words : *Clethrionomys* spp., *Echinococcus multilocularis*, environmental factor, multiple regression analysis, *Vulpes vulpes*

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INTRODUCTION

The alveolar hydatid (*Echinococcus multilocularis* (Leuckart 1863)) has a very wide host range and worldwide distribution, and the alveolar hydatid disease (AHD), which is caused by this cestode, causes substantial public health problems in many areas in the world [1]. In Japan, this disease is the most important zoonosis on the main island of Hokkaido, where the major intermediate and final hosts of *E. multilocularis* are red-backed voles of genus *Clethrionomys* and red foxes (*Vulpes vulpes* ; Linnaeus 1758), respectively. More than 200 patients and numerous infected host animals have been found in most areas of Hokkaido since 1937 [2-4]. Intensive monitoring has been performed on the prevalence of *E. multilocularis* on wild, feral and domestic animals in many regions by Hokkaido's Prefectural Government, but this data has never been used for epidemiological study on the AHD, except for Takahashi [5] and Saitoh and Takahashi [6]. Especially, Saitoh and Takahashi [6] examined annual fluctuation of the prevalence of this cestode species in the fox in three areas in eastern Hokkaido by means of multivariate statistical comparison with density of the voles and with that of the cestode in the preceding years with the aspect of delayed density-dependence.

In the present study, an attempt has been made to analyze

these monitoring data for critical factors that determine the regional degree of endemicity of AHD from many candidate regional emvironmental factors, and to clarify relationships between the incidence and these factors, based on single and multiple regression analyses.

DATABASE AND METHODS

The regional incidence of AHD in Hokkaido has been monitored yearly in the prevalence of *E. multilocularis* in red foxes, racoon dogs (*Nyctereutes procyonoides* ; Gray 1834), rodents, feral cats, feral dogs, and domestic animals including horses and pigs [5]. However, datasets on these animals other than the red foxes were available only in small parts of Hokkaido and their sample sizes were often very small or unknown. Hence, in this study, the prevalence of this cestode in the foxes examined from 1985 to 1990 was used as the criterion variable in 74 regions (Cities, Towns and Villages; see Appendix) where more than 40 foxes were examined in this period.

In general, the degree of endemicity of parasitosis in humans and animals and the prevalence of their causal parasites are often dependent on various environmental factors, such as host density [7-11], climate [6, 10, 12-13] and topography [12]. In this study, 15 types of candidate explanatory variables belonging to eight categories of environmental factors, i.e., population density of the voles, temperature, depth of snowfall, mean degree of slope, mean altitude, human population density, number of milch cows per area and percentage of forest area, were collected from various sources. The datasets of the population density of voles were accumulated every July, August and September at hundreds of census points in national, prefectural and private forests in Hokkaido by the Forestry Agency of the Japanese National Government and the Hokkaido Prefectural Government, based on the number of voles captured with each 50 snap trap set in an area of 0.5 ha for serial three nights [14-15]. The density data of the voles used in the present study was collected from 1980 to 1989 in private forests, which are more frequently located near human activity and probably more important on the epidemiology of AHD than the national and prefectural forests. Most of these data are on gray red-backed voles (*Clethrionomys rufocanus* ; Sundevall 1846), and very small numbers of *C. rutilus* (Pallas 1779) and *C. rex* Imaizumi 1971 are included in them [15]. The data on climatic factor were obtained from datasets by Sapporo District Meteorological Observatory from 1985 to 1989, from which six types of variables were used for the analyses, including total mean and cumulative mean temperatures, cumulative snowfall depth and number of days with snowfall deeper than 10, 20 and 50 cm (The detasets are shown in the homepage of Japan Meteorological Agency; http://www.data.kishou.go.jp/index.htm). The total mean and cumulative mean temperatures were from the monthly mean of respective values for the five years. The cumulative snowfall depth was the sum of snowfall depth for each day of the snowfall in each month from January to April and in December. The mean degree of slope in an inland region was obtained from the altitudinal difference divided by the horizontal distance between the highest and lowest points in the region. In seaside regions, the alternative mean degrees of slope were calculated according to the following formula:

$$M.D.S.=2A\,/\,(N+F)\ ,$$

where M.D.S. and A are the mean degree of slope and the altitude of the highest point, respectively. N and F are the respective horizontal distance from the highest point to the nearest and farthest points on the coastline of the region. The mean altitude is the mean of the altitudes of the highest and lowest points in each region. These altitudes and distances were measured on 1/200,000 topographical maps of Hokkaido by Geographical Survey Institute of Japan. Data of the human population density and the number of milch cows were based on the National Population Census of the 1985 (shown in the homepage of Hokkaido Prefectural Government; http://www.pref.hokkaido.jp/skikaku/sk-kctki/index.html) and 1980 World Census of Agriculture, respectively. The area of forest was based on the datasets of public information by the Hokkaido Prefectural Government in 1990. Some regions lacked data on the density of the voles, temperature or snowfall depth, where the means of variables of surrounding regions were used instead.

The prevalence of *E. multilocularis* in the foxes and the percentage of forest area were used after arcsin-root transformation. Simple correlation coefficients were calculated between this prevalence and all 15 types of candidate explanatory variables of the eight categories of regional environmental factors (Table 1) to determine any apparent relationships between the prevalence of the cestode in foxes and these environmental factors (Table 1). In three categories with multiple types of variables, i.e., population density of voles, temperature and depth of snow, each type of variable showing the highest simple correlation coefficient in this analysis was sellected for adoption in later analyses. A correlation matrix among the eight variables of respective categories was constructed to examine the relationships between each pair of the variables. Multiple regression analysis using the prevalence of *E. multilocularis* in the foxes and these eight variables as criterion and explanatory variables, respectively, with a selection process of these explanatory variables by stepwise method was performed with the REG and STEPWISE Procedures in SAS Program Version 5 [16].

RESULTS

Simple correlation coefficients between the prevalence of *E. multilocularis* in foxes and each of the 15 types of variables were shown in "r" of the Table 1. The population density of voles, temperature, snowfall depth, number of milch cows per area and percentage of forest area significantly correlated with the prevalence. The captive number of voles in September, the

Table 1. Simple correlation coefficients (r) between the prevalence of *Echinococcus multilocularis* in foxes *Vulpes vulpes* and each of the 15 variables on eight (1 ~ 8) candidate environmental factors

Categories (units) and/or their variables	r
1. Population density of voles (/0.5 ha/3 nights)	
Number of voles captured in July	0.116
Number of voles captured in August	0.384**
Number of voles captured in September	0.417**
Mean number among three month	0.397**
2. Temperature (℃)	
Total mean temperature	−0.268*
Cumulative mean temperature	−0.371**
3. Snowfall depth (total snow depth: cm; others: days)	
Cumulative snowfall depth	−0.371**
Number of days with snowfall deeper than 10 cm	−0.341**
Number of days with snowfall deeper than 20 cm	−0.390**
Number of days with snowfall deeper than 50 cm	−0.391**
4. Mean degree of slope (m/km)	−0.199
5. Mean altitude (m)	−0.227
6. Human population density (/km)	0.191
7. Number of milch cow per area (/km)	0.287*
8. Percentage of area of forest (transformed%)	−0.314**

*$P < 0.05$, **$P < 0.01$.

cumulative temperature and the number of days with snowfall deeper than 50 cm showed the highest correlation coefficients for the prevalence among the types of variables in respective categories. The correlation matrix among these eight types of variables on the respective categories indicated some significant correlations of pairs of the types of variables (Table 2). The smallest correlation coefficient was calculated between the variables on the population density of voles and the snowfall depth ($r = -0.005$, $P = 0.968$). Multiple regression analysis using these eight types of variables was performed to explain the prevalence of *E. multilocularis* in the foxes, and the following regression model was obtained:

$$y = 0.00979X_1 - 0.00037X_2 + 0.23832$$

$$(r = 0.32180, F = 16.84, P < 0.01)$$

In this model, Y, X_1 and X_2 mean the arcsin transformed prevalence, the captive number of voles in September and the number of days with snowfall deeper than 50 cm, respectively (see Appendix). The other variables were selected out from this model with the stepwise process. The standard error, F value (and P) and standardized partial regression coefficient of X_1 were 0.00232, 17.71 ($P = 0.0001$) and 0.41141, and those of X_2 were 0.00010, 15.50 ($P = 0.0002$) and −0.38485, respectively.

DISCUSSION

The present simple and multiple regression analyses showed that the population density of the red-backed voles is one of the important regional environmental factors on the prevalence of *E. multilocularis* in foxes in Hokkaido. Saitoh and Takahashi [6] performed similar epidemiological analyses on the prevalence of *E. multilocularis* in foxes in Hokkaido. However, their previous analyses treated only three categories of environmental factors i.e., density of voles, temperature and snowfall, and the regions adopted in the work were limited to three Districts in eastern Hokkaido.

Many works have addressed quantitative relationships between both abundance (or density) of parasites and their hosts from theoretical [7, 9] and empirical [6, 8, 10] aspects. These studies show that the higher abundance (or density) of hosts makes a higher prevalence and larger abundance of parasites possible. This result is demonsrated mainly with the more successful establishment of the life cycles of parasites in the higher abundance of hosts [7, 9], which is consistent with the present findings. As above mentioned, Saitoh and Takahashi [6] examined annual fluctuation of the prevalence of this cestode species in the fox in three districts in eastern Hokkaido by means of statistical comparison with density of the voles, and high correlations were shown between the prevalence of cestode and the density of voles of the respective and/or preceding years. Additionally, the functional response of predators (change of exploitation pattern related to the amount of their food resources) may play an important role in the relationships in the case of heteroxenous parasites depending on the prey-predator relationships of intermediate and final hosts [6, 11]. On the other hand, the population density of the foxes in Hokkaido has been measured in only a

Table 2. Correlation matrix among explanatory variables used in multiple regression analysis

	X_1	X_2	X_3	X_4	X_5	X_6	X_7
X_2	− 0.492**						
X_3	− 0.005	0.136					
X_4	− 0.287*	0.287*	0.457**				
X_5	− 0.263*	0.251*	0.344**	0.623**			
X_6	0.087	0.176	− 0.134	− 0.057	− 0.102		
X_7	0.593**	− 0.409**	− 0.216	− 0.255*	− 0.183	− 0.107	
X_8	− 0.357**	0.248*	0.292*	0.352**	0.392**	− 0.295*	− 0.383**

*P < 0.05,**P < 0.01, X_1: number of voles captured in September; X_2: cumulative mean temperature; X_3: number of days with snowfall deeper than 50 cm; X_4: mean degree of slope; X_5: mean altitude; X_6: human population density; X_7: number of milch cow per area; X_8: percentage of area of forest.

few areas (for example, Morishima et al. [4]), so that this data is unfortunately unavailable in this study.

For the prevalence of some heteroxenous parasites dependng on prey-predator relationships between their intermediate and final hosts, predation pressure on the prey by the predator is assumed to be an important factor as well as the population densities of these hosts. The foxes are the important predators of voles in Hokkaido, so that many studies have been performed on their relationships [17-23]. In an area of eastern Hokkaido, it was shown that the hunting pressure on wild rodents, mainly composed of voles, by foxes changes seasonally, with the pressure becoming high in spring and autumn and low in summer and winter remarkably [20]. This phenomenon is attributable to a seasonal change in the amount of ground surface cover with grass and snowfall, which suppresses the hunting behavior of the foxes to the voles. This aspect can explain the function of the snowfall depth as another important environmental factor on the prevalence of *E. multilocularis* in foxes in Hokkaido, i.e, the snow is a barrier to infection of the foxes by this cestode species (also see Lindström [24]). Some reports show or discuss such relationships between the prevalence of *E. multilocularis* and the snowfall depth [4, 6]. Especially, Saito and Takahashi [6] obtained similar result from the present one based on the comparison among three districts with varied amount of snowfalls in Hokkaido. Some programs to control AHD in Hokkaido may be more effective if they are more intensively performed in years with a lower population density of voles and deeper snowfall.

Other than the two factors discussed above, three environmental factors showed significant positive or negative correlations in the single regression analysis, but they were not included in the present multiple regression model. The negative correlation between the prevalence and temperature may be attributable to the higher mortality of eggs of *E. multilocularis* in warmer environments [25]. On the other hand, the number of milch cows per area and the percentage of forest area correlated to the prevalence of *E. multilocularis* in foxes positively and negatively, respectively. It is known that foxes in Hokkaido frequently utilize livestock garbage such as milch cow placentae in winter as a compensative food resource in this season [17] and that the red foxes prefer complex habitats including various types of landscape and avoid uniform habitats simply composed of closed forests [26-27]. Additionally, Yamamoto [28] showed that red foxes in Japan tend to avoid severely sloped habitats, using radiotracking data. The mean degree of slope showed an inverse tendency to the prevalence of this cestode in the foxes, although it was statistically insignificant. As mentioned above, the population density data of the foxes in Hokkaido were unavailable; however, these results may suggest that these two (or three) factors influence the prevalence via the population density of the foxes. These factors showed positive or negative significant correlations with the vole density and/or snow depth (Table 2), so that it is impossible to exclude the possibility that their significant correlations in the simple regreesion analysis may be spurious correlations.

The distribution of *E. multilocularis* and AHD is changing in various areas in the world, including Japan [1-2]. Especially in this country, there are three recent problems on the temporal change on the worm and disease: distribution expanding in western Hokkaido, remarkable increase of the prevalence in the foxes in most of Hokkaido, and possibility to colonize in Honshu [2, 29] (also see the Homepage of Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University; URL: http://vpcserv.vetmed.hokudai.ac.jp/). The present analyses can explain at least partly the contemporary spatial difference of the prevalence of *E. multilocularis* in the foxes in many regions in the Hokkaido with a few environmental factors. In the near future, more available models must be developed to explain both spatial and temporal difference of the distribution and prevalence of the worm in not only Hokkaido, but also other areas in Japan and other counties.

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要　約

北海道の各地で 1985 年から 1990 年にかけて捕獲されたアカギツネ *Vulpes vulpes* における多包条虫(タホウジョウチュウ) *Echinococcus multilocularis* の感染率と，地域ごとの環境因子の関係を単回帰および重回帰分析法を用いて分析した。8 つのカテゴリーに属する 15 種類の説明変数のうちから，単回帰分析によってカテゴリーごとに 1 つ，計 8 つの変数を選択した。それらを用いたステップワイズ重回帰分析において，以下の重回帰モデルが得られた:$Y = 0.00979X_1 - 0.00037X_2 + 0.23833$ (Y：平方根ー逆正弦変換を行ったキツネにおける多包条虫の感染率，X_1：9 月におけるヤチネズミ類 *Clethrionomys* spp. の捕獲数，X_2：50 cm 以上の積雪のあった日数，$r = 0.32180$，$P = 0.0001$)。ヤチネズミ類の密度が高いほど多包条虫の生活環が成立しやすくなり，積雪による本条虫の感染率への負の効果は，積雪がキツネの捕獲行動を妨げるためであると考えられた。

キーワード:ヤチネズミ類，多包条虫，環境因子，重回帰分析，アカギツネ

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Appendix. Two significant variables of environmental factors in the present analysis (number of voles captured in September (X_1) and number of days with snowfall deeper than 50 cm (X_2)) in 74 regions in Hokkaido, Japan.

Regions	X_1	X_2	Regions	X_1	X_2	Regions	X_1	X_2
Chitose	9.0	252	Asahi	18.8	596	Obihiro	12.6	28
Mori	6.9	105	Teshio	36.7	325	Sarabetsu	15.8	172
Minamikayabe	6.4	46	Haboro	10.6	391	Memuro	13.2	74
Shikabe	9.5	77	Engaru	13.1	149	Makubetsu	9.9	97
Bibai	9.0	420	Wakkanai	21.4	290	Otofuke	12.3	52
Esashi	6.1	434	Sarufutsu	19.5	465	Shihoro	19.5	21
Kaminokuni	2.7	118	Utanobori	16.2	612	Kamishihoro	8.2	246
Assabu	6.4	250	Oumu	18.5	217	Teshikaga	12.3	141
Kumaishi	3.9	279	Kunneppu	8.1	139	Taiki	16.4	253
Otobe	5.4	179	Bihoro	18.8	186	Shintoku	18.6	61
Imagane	9.0	349	Memanbetsu	10.4	54	Shimizu	22.1	66
Kitahiyama	4.0	268	Abashiri	13.0	54	Shikaoi	13.9	59
Kuttyan	11.1	603	Shari	15.6	241	Ikeda	8.9	4
Kimobetsu	8.3	501	Kiyosato	14.4	271	Toyokoro	11.6	40
Kuromatsunai	16.8	344	Koshimizu	16.2	271	Kushiro (City)	20.9	0
Yoichi	10.4	435	Soubetsu	16.0	251	Hamanaka	18.3	0
Yubari	6.8	186	Otaki	7.7	526	Shiranuka	8.7	5
Kuriyama	12.7	272	Noboribetsu	23.9	152	Onbetsu	12.2	64
Iwamizawa	15.1	483	Atsuma	3.2	66	Akkeshi	14.7	41
Mikasa	12.7	374	Hayakita	9.1	134	Nemuro	39.8	27
Takanosu	13.5	482	Mukawa	14.3	0	Nakashibetsu	24.3	162
Aibetsu	18.3	553	Shiraoi	7.2	176	Bekkai	39.2	52
Nayoro	17.4	487	Oiwake	4.5	134	Sapporo	9.5	294
Bifuka	23.2	585	Shizunai	13.2	116	Hakodate	14.0	54
Shibetsu	11.2	538	Biratori	6.8	131			

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Production and characterization of monoclonal antibodies against excretory/secretory products of adult *Echinococcus granulosus*, and their application to coproantigen detection

Cecilia Casaravilla[a], Ramiro Malgor[a], Andrea Rossi[a], Hirofumi Sakai[b], Nariaki Nonaka[b], Masao Kamiya[b], Carlos Carmona[a,*]

[a]*Unidad de Biología Parasitaria, Facultad de Ciencias, Instituto de Higiene, Universidad de la República, Av. A. Navarro 3051, Montevideo CP11600, Uruguay*

[b]*Laboratory of Parasitology Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan*

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Abstract

Two IgM murine monoclonal antibodies (MAbs), EgC1 and EgC3, were produced against the excretory/secretory (E/S) products of *Echinococcus granulosus* adult worms. Immunoblotting revealed that both predominantly recognized a 50 kDa antigen in the somatic extract and an 85 kDa component in the E/S products. Immunolocalization showed that both MAbs reacted with the tegument of the parasite, and additionally EgC3 reacted with parenchyma and the tegument lining the external surface of the reproductive organs. A coproantigen capture ELISA was developed using a rabbit polyclonal antibody against E/S products from adult tapeworms as catching antibodies, and each one of MAbs as detecting antibody. The assays detected seven out of eight (EgC1), and eight out of eight (EgC3) experimentally infected dogs (worm burdens ranging from 61 to 57,500), using heat-treated samples obtained at prepatent period, and none (n=8) of helminth-free samples. Time course analysis showed that, after a 12–25 days lag, coproantigen levels rose above cut off O.D. values and typically peaked around 30 days post-infection (DPI) at the end of the experiment. One dog experimentally infected with *Taenia hydatigena* metacestodes was slightly detected as positive at different time points after 30 DPI. Both MAbs showed a similar pattern of recognition, but *T. hydatigena* antigens were undetectable for a longer period, and reached lower O.D. values with EgC1. Interestingly, fecal samples from two experimentally infected dogs with *Echinococcus multilocularis* were not recognized by the EgC1 assay, suggesting a potential value as species-specific diagnostic tool.

Keywords: Coproantigen; *Echinococcus granulosus*; Monoclonal antibodies

1. Introduction

Echinococcus granulosus, the dog/sheep tapeworm, is the causative agent of cystic echinococcosis, an important zoonosis widely distributed throughout the rural areas of the world. Many affected countries have established control programmes predominantly based on regular dosing of dogs, and in some cases a marked reduction in the transmission of the disease has been achieved [1–3]. Accordingly, accurate assessment of *E. granulosus* in dog populations is a critical requirement for evaluating the programme efficacy, and for estimating the potential infection risk for both human and ruminants. The purgation technique with arecoline hydrobromide has been widely used as the standard method for screening dog populations, but the examination of removed material is time-consuming, requires trained personnel, and it is not sensitive enough, as a single dose could detect less than 50% of *E. granulosus* infections [4].

* Corresponding author. Fax: +598 2 4801597.
E-mail address: ccarmona@higiene.edu.uy (C. Carmona).

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