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# Immunochromatographic Test with Recombinant Em18 Antigen for the Follow-Up Study of Alveolar Echinococcosis▽

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**The performance of a rapid and simple immunochromatographic test (ICT) with recombinant Em18 (rEm18) antigen for serological follow-up of *Echinococcus multilocularis* infection was evaluated by comparison with that of an enzyme-linked immunosorbent assay (ELISA) with rEm18, using serum samples from patients who underwent surgery and/or received antiparasitic chemotherapy. The degree of Em18-band intensity on the ICT correlated highly with the absorbance value obtained by the ELISA. The kinetics of antibody levels obtained by the ICT paralleled those of the ELISA. These data suggest that the ICT has high potential as an easy-to-handle, fast, and reliable follow-up tool to monitor the status of alveolar echinococcosis in different stages.**

Alveolar echinococcosis (AE), caused by the larval stage (metacestode) of *Echinococcus multilocularis*, is a serious parasitic disease of humans in countries in higher latitudes of the Northern Hemisphere. In the previous decade, a lot of new data have been published on the prevalence of *E. multilocularis* in final and intermediate hosts in areas where it had previously not been recorded (5). The metacestodes propagate asexually, like a tumor, leading to organ dysfunction, mainly in the liver. Because of their slow growth, clinical symptoms usually do not become evident until 10 or more years after initial infection (13). Complete removal of alveolar lesions by surgery has been strongly recommended as a primary treatment (3). In addition, therapy with benzimidazole derivatives is also important in patients with AE. Although such imaging techniques as ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) with [$^{18}$F]fluorodeoxyglucose are used to monitor the efficacies of treatments, atypical imaging results lead to difficulties in terms of interpreting disease status (progression or regression) (2). In contrast, serologic analysis is considered to be useful for monitoring disease activity (4, 8, 10). Studies by an enzyme-linked immunosorbent assay (ELISA) with recombinant *E. multilocularis* 18-kDa antigen (rEm18) (14) and serum samples from patients in different clinical stages of AE according to the World Health Organization (WHO)-PNM (P, parasitic mass in the liver; N, involvement of neighboring organs; M, metastasis) system (11) have revealed that specific immunoglobulin G (IgG) antibody levels in a patient's serum shows a close relationship between the clinical status and the individual treatment (7, 9, 17, 18). However, the ELISA is time-consuming and requires special materials and equipment, which renders this test unsuitable for direct clinical applications. To overcome this problem, we recently developed an immunochromatographic test (ICT) using rEm18 and demonstrated its reliability (15). Another group also has developed an immune filtration assay with multiple native antigens for rapid serodiagnosis of human cystic echinococcosis and AE (6). The ICT has been known as a simple and rapid method for detection of specific antigens of infectious agents or specific antibodies to them semiquantitatively. In this study, we evaluated the ICT with rEm18 as a follow-up tool for monitoring AE patients after treatment in different stages.

## MATERIALS AND METHODS

**Patients.** All patients described in this study were seen at the University Hospital and Medical Center Ulm, Ulm, Germany. A total of 12 patients (72 sera) with a history of hepatic AE and a follow-up period of 2.5 to 6.5 years were included in the study. The patients were assigned to different clinical WHO-PNM stages of the disease (11). All patients had acquired AE in Germany and received benzimidazole therapy. Three patients had curatively resected lesions, 3 had recurrences after surgery, 5 had unresectable lesions but stable disease, and 1 had apparently dead, fully calcified lesions (Table 1). All serum samples were tested at the Department of Parasitology, Asahikawa Medical University, Japan, in a blind test. The classification of curative resection, stable disease, progressive disease, or presence of an apparently dead, fully calcified lesion was established by magnetic resonance imaging based on lesion size and morphology at the respective follow-up intervals. Ethical approval was obtained from the University of Ulm.

**Enzyme-linked immunosorbent assay and immunochromatography test.** For the ELISA, recombinant Em18 antigen (14) was used to coat microtiter plates at a concentration of 10 ng/well. The wells were blocked with 250 μl of blocking buffer (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% casein) at 37°C for 1 to 2 h. After the wells were rinsed twice with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBST), 100-μl serum samples diluted 1:100 in blocking buffer were added, and the mixtures were incubated at 37°C for 1 h. The wells were rinsed five times with PBST, incubated with 100 μl of recombinant protein

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TABLE 1. Characteristics of patients with alveolar echinococcosis included in this study

| Patient no. | Stage | PNM code | Status[a] | Age (yr)[b] | Sex | Follow-up duration (yr) |
|---|---|---|---|---|---|---|
| 1 | I | P1N0M0 | Apparently dead, fully calcified lesion | 58 | M | 4 |
| 2 | I | P1N0M0 | Unresectable, stable disease | 61 | M | 3.5 |
| 3 | II | P2N0M0 | Curative resection | 38 | F | 3 |
| 4 | II | P2N0M0 | Unresectable, stable disease | 59 | F | 6.5 |
| 5 | II | P2N0M0 | Recurrence after resection | 74 | F | 2.5 |
| 6 | IIIa | P3N0M0 | Recurrence after resection | 17 | F | 3.5 |
| 7 | IIIa | P3N0M0 | Recurrence after resection | 19 | F | 3 |
| 8 | IIIb | P4N0M0 | Unresectable, stable disease | 57 | M | 3 |
| 9 | IIIb | P4N0M0 | Unresectable, stable disease | 86 | F | 5.5 |
| 10 | IV | P4N1M0 | Curative resection | 30 | M | 3 |
| 11 | IV | P4N1M0 | Curative resection | 52 | M | 2.5 |
| 12 | IV | P4N1M0 | Unresectable, stable disease | 54 | M | 4 |

[a] Assessed by imaging (apparently dead lesion, progressive disease, and stable disease) or imaging and histologic analysis (curative resection).
[b] Age when first blood sample was obtained.

G conjugated with peroxidase (Zymed) at 37°C for 1 h, and rinsed five times with PBST. After incubation with 100 μl of substrate (0.4 mM 2,2′-azino-di-[3-ethylbenzthiazoline sulfonate] in 0.2 M citric acid buffer, pH 4.7) for 30 min at room temperature, the absorbance at 405 nm with a reference wavelength of 630 nm was measured. For the ICT, the immunochromatographic strip and cassette were prepared as described previously (15). For the assay, 10 μl of the respective serum sample was mixed with 20 μl of a serum dilution buffer containing 0.1 mg/ml alkaline phosphatase-conjugated goat anti-human IgG antibody (Dako, Japan) in a tube, and the mixed serum sample was applied to the sample window of the plastic device. Soon after applying the serum sample (within 30 s), 200 μl of the substrate solution was loaded onto the substrate reservoir pad and the result was judged after 20 min. 5-Bromo-4-chloro-3-indolylphosphate was used for color development. A sample was considered positive if two color lines (control line, anti-goat IgG; test line, rEm18) appeared in the result window, and a sample was considered negative if only a control line appeared in the result window (see Fig. 1). In the case of no appearance of a control line, the assay was invalid even if a colored test line appeared. The color intensity of the stained blue line was confirmed by observation with the naked eye and quantified by an immunochromatography reader, C10066 (Hamamatsu, Japan) according to the manufacturer's instructions. The results are expressed as milli-absorbance (mABS) units per capture zone, and relative intensity was calculated by dividing mABS of the test line by mABS of the control line.

In both methods, serum samples giving values greater than the mean value plus four standard deviations for negative-control samples collected from healthy Japanese people not showing any clinical features of AE were considered seropositive.

**Statistical analyses.** The binomial test was applied to compare the sensitivity of the ICT and the ELISA, and Spearman's rank test was used for the correlation analysis of the ICT and the ELISA.

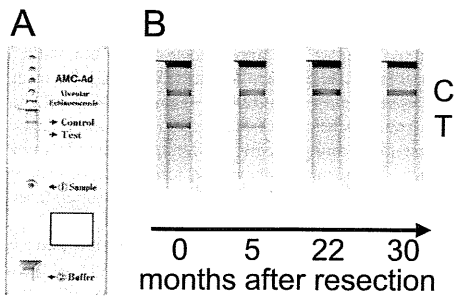


FIG. 1. Examples of ICT tests with negative and positive sera. (A) Result with a negative serum sample. The inscriptions "Sample" and "Buffer" represent the positions for loading of sample and of substrate solution, respectively. (B) Results with positive sera from patient 11. Only result windows are shown. C, control line; T, test line.

TABLE 2. Comparison of ICT with ELISA

| ICT result | No. (%) of samples with ELISA result | | Total (%) |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 64 (88.9) | 5 (6.9) | 69 (95.8) |
| Negative | 0 (0) | 3 (4.2) | 3 (4.2) |
| Total (%) | 64 (88.9) | 8 (11.1) | 72 (100) |

## RESULTS AND DISCUSSION

A total of 72 serum samples from 12 AE patients were examined by the ICT and the ELISA. For the ICT, the intensity of the band which appeared at the test line (Fig. 1) was quantified to obtain objective judgment after observation by the naked eye. No discrepancies between the results based on relative intensity values and visual inspections were observed. Five ELISA-negative samples, one from patient 1 and four from patient 5, with absorbance values close to the cutoff absorbance value, showed very weak ICT-positive results. There was no significant difference in detection of specific antibody to rEm18 between the ICT and the ELISA (Table 2, binomial test; $P = 0.0625$). As shown in Fig. 2, the values obtained by the ICT and the ELISA for individual sera correlated very well (Spearman's rank test; $R_s = 0.916$; $P = 0.0000002$), which indicated that the ICT had ability to assay antigen-specific IgG semiquantitatively and the band intensity was in proportion to antibody levels.

Comparisons of relative intensity values with absorbance values in individual patients revealed that the kinetics of levels of antibody to rEm18 obtained by the ICT were similar to those obtained by the ELISA (Fig. 3). In cases with curative resections, continuous and dramatic decreases in antibody levels after resection of an alveolar hydatid cyst were observed. These results are consistent with those of previous studies using Em18 and other native *E. multilocularis* metacestode antigens (1, 7, 9, 12, 16, 17, 18). In cases with unresectable and stable diseases, the kinetics of antibody levels varied in individual patients. However, antibody levels had an inclination to

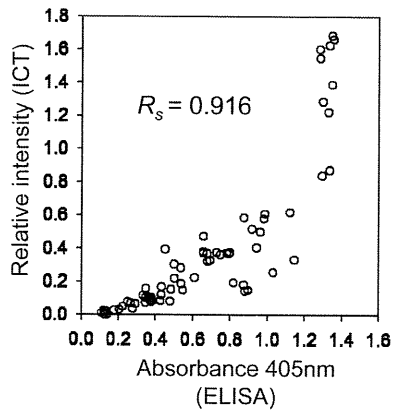


FIG. 2. Scatter graph showing the correlation between relative intensity in the ICT and absorbance values in the ELISA using 72 serum samples from AE patients. Spearman's rank correlation coefficient ($R_s$) was 0.916 ($P = 0.0000002$).

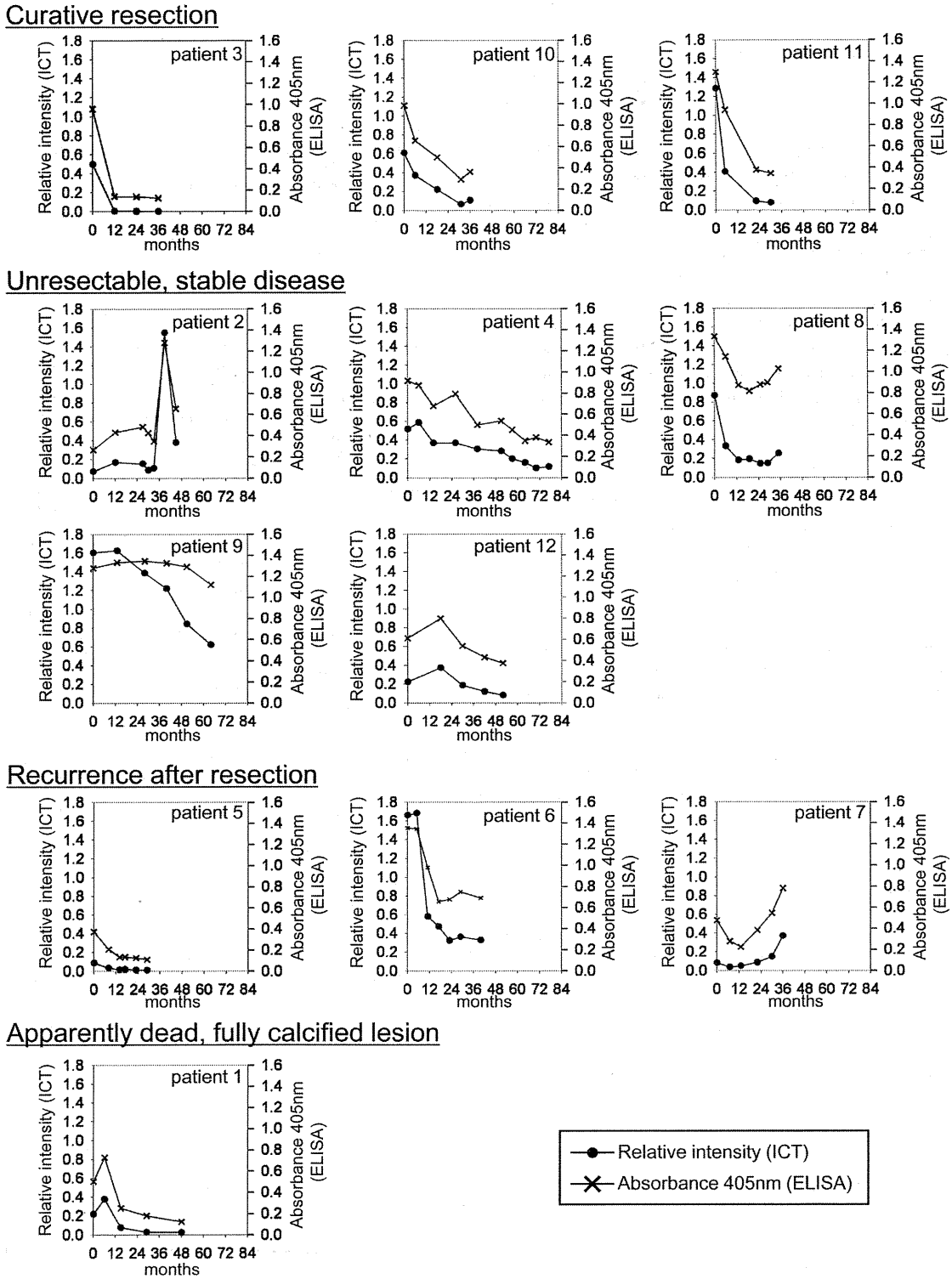


FIG. 3. The kinetics of anti-rEm18 antibody levels obtained by using the ICT and the ELISA in patients after surgical operation and/or chemotherapy. The cutoff values for the ICT and the ELISA were 0.009 and 0.162, respectively. Time of resection and start of chemotherapy are at month 0.

decrease slowly during the observation period. A drastic increase in the antibody level was observed in patient 2 after 39 months. In patient 8, antibody levels began to increase slowly after 29 months. In patient 9, ELISA absorbance values did not change until 51 months, although relative intensities of the ICT began to decrease from 13 months. Because high antibody levels in serum samples until 51 months possibly made ELISA absorbance values reach a plateau level, a change in ELISA

absorbance values might not have been observed. Further experiments with a higher serum dilution for ELISA are needed to make clear the reason for the difference in kinetics of antibody levels observed between two methods. In cases with recurrences, the initial decreases in antibody levels were observed for all three patients. For patient 5, after 14 months, antibody levels dropped below the cutoff as determined by the ELISA and remained undetectable throughout the observation period, in contrast to all samples being positive by the ICT. In two other patients, increases in antibody levels were observed after 29 months (patient 6) and 13 months (patient 7). In a case with a calcified lesion (patient 1), a slow decrease in antibody levels was observed, and the last serum sample became negative by the ELISA but was ICT positive. Because antibody levels in patients are very closely related to the progression of AE (1, 7, 9, 12, 16, 17, 18), the efficacy of chemotherapy using benzimidazole against parasites might influence the variability of antibody responses.

In conclusion, the results of this study strongly indicated that the band intensity observed in ICT-positive tests was reflected in the height of antibody levels against rEm18. In other words, the ICT has high potential as a follow-up tool for monitoring the progression of AE, since antibody levels against rEm18 are closely related to the parasite's activity in the human host. In addition, the ICT shows several advantages: (i) expertise, experience, and special equipments which are required for conventional laboratory-based ELISA are not needed; (ii) time consumption is only 20 min, which is much lower than the 4 h taken by the ELISA. Therefore, as a rapid and easy-to-handle (bedside) test, the ICT is able to monitor levels of antibody to Em18, which is a marker for the presence of active or inactive/abortive lesions and the lesion status after radical resection, which can facilitate and accelerate proper clinical management. However, further analysis of reproducibility among different lots of ICT devices and a large-scale evaluation might be necessary, and in the case of positive ICT results without prior imaging, imaging should still be included as a diagnostic tool.

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PLOS NEGLECTED TROPICAL DISEASES

# Post-Treatment Follow-Up Study of Abdominal Cystic Echinococcosis in Tibetan Communities of Northwest Sichuan Province, China

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## Abstract

***Background:*** Human cystic echinococcosis (CE), caused by the larval stage of *Echinococcus granulosus*, with the liver as the most frequently affected organ, is known to be highly endemic in Tibetan communities of northwest Sichuan Province. Antiparasitic treatment with albendazole remains the primary choice for the great majority of patients in this resource-poor remote area, though surgery is the most common approach for CE therapy that has the potential to remove cysts and lead to complete cure. The current prospective study aimed to assess the effectiveness of community based use of cyclic albendazole treatment in Tibetan CE cases, and concurrently monitor the changes of serum specific antibody levels during treatment.

***Methodology/Principal Findings:*** Ultrasonography was applied for diagnosis and follow-up of CE cases after cyclic albendazole treatment in Tibetan communities of Sichuan Province during 2006 to 2008, and serum specific IgG antibody levels against *Echinococcus granulosus* recombinant antigen B in ELISA was concurrently monitored in these cases. A total of 196 CE cases were identified by ultrasound, of which 37 (18.9%) showed evidence of spontaneous healing/involution of hepatic cyst(s) with CE4 or CE5 presentations. Of 49 enrolled CE cases for treatment follow-up, 32.7% (16) were considered to be cured based on B-ultrasound after 6 months to 30 months regular albendazole treatment, 49.0% (24) were improved, 14.3% (7) remained unchanged, and 4.1% (2) became aggravated. In general, patients with CE2 type cysts (daughter cysts present) needed a longer treatment course for cure (26.4 months), compared to cases with CE1 (univesicular cysts) (20.4 months) or CE3 type (detached cyst membrane or partial degeneration of daughter cysts) (9 months). In addition, the curative duration was longer in patients with large (>10 cm) cysts (22.3 months), compared to cases with medium (5–10 cm) cysts (17.3 months) or patients with small (<5 cm) cysts (6 months). At diagnosis, seven (53.8%) of 13 cases with CE1 type cysts without any previous intervention showed negative specific IgG antibody response to *E. granulosus* recombinant antigen B (rAgB). However, following 3 months to 18 months albendazole therapy, six of these 7 initially seronegative CE1 cases sero-converted to be specific IgG antibody positive, and concurrently ultrasound scan showed that cysts changed to CE3a from CE1 type in all the six CE cases. Two major profiles of serum specific IgG antibody dynamics during albendazole treatment were apparent in CE cases: (i) presenting as initial elevation followed by subsequent decline, or (ii) a persistent decline. Despite a decline, however, specific antibody levels remained positive in most improved or cured CE cases.

***Conclusions:*** This was the first attempt to follow up community-screened cystic echinococcosis patients after albendazole therapy using ultrasonography and serology in an endemic Tibetan region. Cyclic albendazole treatment proved to be effective in the great majority of CE cases in this resource-poor area, but periodic abdominal ultrasound examination was necessary to guide appropriate treatment. Oral albendazole for over 18 months was more likely to result in CE cure. Poor drug compliance resulted in less good outcomes. Serology with recombinant antigen B could provide additional limited information about the effectiveness of albendazole in CE cases. Post-treatment positive specific IgG antibody seroconversion, in initially seronegative, CE1 patients was considered a good indication for positive therapeutic efficacy of albendazole.

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## Author Summary

Cystic echinococcosis is a serious public health problem in Tibetan communities of northwest Sichuan Province, China. Antiparasitic treatment with albendazole remains the only choice in most cases, due to the poor socio-economy and inadequate hospital facilities in this area. A post-treatment follow-up study was carried out in community-detected 49 CE cases by application of abdominal ultrasound and serology with recombinant antigen B (rAgB) in a Tibetan region of Sichuan from 2006 to 2008. Following 6 to 30 months regular albendazole therapy, 32.7% of CE cases were considered cured at ultrasound, 49.0% were classed as improved, 14.3% remained unchanged or static, and 4.1% of cases became aggravated. The treatment course for cure was longer in patients with CE2 type cyst pathology compared to cases with CE1, CE3a or CE3b type cysts. In addition, patients with large cysts (≥10 cm) had a longer curative duration compared to those with medium cysts (5–10 cm) or small cysts (<5c m). The changes of serum specific IgG antibody levels against rAgB were not strongly associated with the viability of cystic echinococcal lesions, however, post-treatment specific IgG antibody positive sero-conversion in initially seronegative CE1 patients, was an indicator for the albendazole efficacy in specific CE patients.


## Introduction

Human cystic echinococcosis (CE), caused by the metacestode stage of *Echinococcus granulosus*, is a complex, chronic disease with a cosmopolitan distribution, and the liver is the most frequently affected organ [1]. Clinical manifestation of this disease ranges from asymptomatic infection to severe, or rarely even fatal disease. Diagnosis of CE remains highly dependent on imaging techniques, due to the fact that immunodiagnosis frequently lacks sensitivity [2], with about 20% of clinically or surgically confirmed CE cases, and up to 50% of community-detected patients presenting negative serology [3–6]. The most common applied imaging techiniques include magnetic resonance imaging (MRI), ultrasonography (US) or radiography, for detection of characteristic space-occupying cysts [7,8]. MRI is able to show highly specific features of CE, but it is prohibitively expensive and not available in rural areas of many endemic countries. In contrast, US is accessible, much less expensive, and can identify hydatid cyst pathological type (CE1-CE5) [9].

Approaches in clinical management for CE include surgery, percutaneous techniques and antiparasitic treatment for active cysts, and the so-called watch and wait approach for inactive cysts [9]. Currently, surgery remains the most common approach for CE treatment that has the potential to remove cysts and lead to complete cure, but it involves risks including those associated with any surgical intervention, anaphylactic reactions, and secondary CE owing to spillage of viable parasite (protoscoleces) material [10–12]. Drug therapy with benzimidazoles (albendazole or mebendazole) has increasingly been used to treat CE, and proved to have efficacy against the parasite in humans, with about 30% of patients cured and 30%–50% of cases improved after 12 months follow-up [10]. However, the response to drug therapy is unpredictable, and the optimum duration has not been definitively determined [11,13]. Moreover, risk of recurrence remains the major problem in surgical or medical treatment [10,12,13]. Therefore, post-treatment or post-surgical follow-up of CE patients for several years is usually indicated.

Imaging techniques such as MRI, X-ray or ultrasonography, are useful tools for follow-up of CE patients. However, these techniques are sometimes difficult to detect the newly growing small cyst and also to discriminate between dead and viable cysts [14]. Therefore, efforts have been directed at applying immunological tests of significantly diagnostic and prognostic values. ELISA and immunoblotting for serum antibody detection using various antigen preparations, including crude hydatid cyst fluid, purified fractions of antigen 5 or B, and *E. granulosus* protoscolex soluble extract, have been applied to follow up CE patients [15–20]. However, all of these tests exhibited problems mainly related to temporally delayed reactions to clinical changes [18,20]. Recombinant antigen B (rAgB) proved to have similar diagnostic value to native antigen B in CE patients [21,22]. However, there has been little or no application of rAgB for post-treatment follow-up of CE patients.

In Tibetan regions of China, human cystic echinococcosis is highly endemic [23]. Albendazole therapy is the primary choice of treatment in the majority of patients owing to remote communities, poor socioeconomics and basic hospital facilities in Tibetan Autonomous Prefectures/communities. The current prospective study was designed to assess the effectiveness of cyclic albendazole treatment in community detected CE patients using ultrasonography as well as ELISA with rAgB as diagnostic/follow-up tools, and also to monitor the changes of serum specific IgG antibody levels against rAgB in these patients during treatment.

## Materials and Methods

### Ethics statement

The study protocol was approved by the Ethical Committee of Sichuan Centers for Disease Control and Prevention (Sichuan CDC). Clearance to carry out the study was obtained from Shiqu County CDC. Information about the purpose of the post-treatment follow-up study was spread to the villagers. Persons with confirmative ultrasound images of CE were voluntarily self-selected to be involved in this study by written informed consent and were assured free medical treatment with cyclic albendazole therapy if necessary. Recommendations were also provided for possible surgical intervention (cyst removal). At confirmed ultrasound diagnosis, each patient was requested to complete a questionnaire which was designed to get information on demographics. Questions were mainly designed to identify clinical manifestations, history of any previous treatment with albendazole (regular or irregular, duration), as well as history of surgery. At each follow-up, another questionnaire was completed to obtained information on administration of albendazole, surgery associated with echinococcosis, improvement of symptoms if any, adverse effects such as gastrointestinal disturbances, alopecia, jaundice, skin itch, hepatic pain/sting, dizziness etc. Chinese-Tibetan translators were employed when necessary.

### Criteria for diagnosis and classification of cystic echinococcosis

Diagnosis and classification of cystic echinococcosis (CE) was made using portable ultrasound according to the criteria proposed by the World Health Organization Informal Working Group on Echinococcosis for CE [12,24]. On the basis of patho-morphological features of cysts, CE lesions were differentiated into six types: CL, CE1, CE2, CE3, CE4 and CE5 (Figure 1). Briefly, the CL type cyst refers to a cystic lesion of parasite origin without a clear rim indicating a very early stage of parasite development, while the presence of CE1 (unilocular cyst with thick endo membrane) or CE2 (daughter cysts present) is suggestive of active


**Figure 1. Ultrasound classification of cystic echinococcosis based on WHO expert consensus.** CL: as a potentially parasitic cyst, indicates a very early stage of parasite development. CE1 or CE2: suggests active parasite. CE3a: is characterized by detachment cyst membrane and/or partial degeneration of cyst content, without daughter cysts, indicates a transitional stage. CE3b: suggests a transitional stage of parasite, partial degeneration of daughter cysts. CE4 or CE5: indicates an inactive parasite.
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stages of the disease. While CE3 is broken into CE3a and CE3b characterized by detached cyst membrane and partial degeneration of daughter cysts, respectively, indicating the parasite is at a transitional stage, and CE4 and CE5 implies cyst involution, necrosis, partially calcified or inactive parasite [1,12,24].

Application of chest X-ray for diagnosis of lung CE was not carried out in this study.

## Origin of cystic echinococcosis (CE) patients

During May 2006 to November 2008, mass ultrasound examination was carried out in eight Tibetan townships of Shiqu County (Ganzi Prefecture, Sichuan Province) for detection of individuals with abdominal cystic echinococcosis infection. Patients with CE1/CE2/CE3a/CE3b type cysts were invited to enroll in the current prospective follow-up study. All CE patients, whether enrolled or not, were offered free albendazole treatment.

## Albendazole therapy

Cyclic treatment with albendazole was provided freely to each patient as 100-mg tablet at a daily dose of 10–15 mg/kg body weight (in two divided doses, together with fat-rich meal). Cyclic treatment of 30 days was followed by a 'wash out' period of 7–10 days without albendazole [1]. Albendazole tablets sufficient for six months application were delivered to patients at each follow-up, to whom possible adverse effects were explained. In addition, albendazole was also available freely in the local county CDC clinic.

Follow-up was carried out at six months intervals. Once a cystic lesion changed to CE4 type, the patient was requested to cease albendazole, but further regular ultrasound examination was necessary to understand if the cyst remained inactive. According to the questionnaire investigation, patients who took albendazole as requested during follow-up period were included in the regular-treated group, whereas others who did not take albendazole as requested due to poor compliance belonged to the irregular-treated group.

## Responses to albendazole therapy

The effectiveness of albendazole in CE patients assessed by ultrasound was described as follows: cured, improved, unchanged/static or aggravated. "Cured" was defined as disappearance of cysts, or degeneration of cyst contents. In other words, 'cured' referred to a cyst changing to a CE4 or CE5 type from a CE1, CE2, CE3a or CE3b type cyst. 'Improved' was determined as detachment of cyst membrane, partial degeneration of cyst contents (or daughter cysts) and/or reduction of cyst size, indicative of the cyst converting to CE3a/CE3b type from a CE1 or CE2 cyst. A 'static' or unchanged cyst showed no morphological and/or size changes. 'Aggravated' CE disease was defined as enlargement of the cyst and/or recurrence of daughter cysts.

## Collection of serum samples and image data

Approximately 3 ml of venous blood was taken voluntarily from patients at diagnosis (during mass ultrasound screening) as well as at each follow-up, and then centrifuged on the same day. Sera were aliquoted and stored at −20°C for later serological analysis. Blood transaminase levels were not monitored in the current study, due to the difficulty of doing liver function tests in the field.

Information about the characteristics of hydatid cysts for new CE cases and follow-up CE patients was documented in detail, including the cyst type (CE1-5), the number of cysts (single or multiple), location (the lobe of the liver, abdominal cavity, pelvic cavity, spleen or kidney), and the size (cm).

## Serology

ELISA with recombinant antigen B (rAgB) based on previous description [22] was performed on each serum sample for determination of *Echinococcus* specific IgG. Samples from the same patient were analyzed concurrently. The cut-off point was determined as the mean optical density plus 3 times standard deviation for a panel of serum samples obtained from healthy donors (n = 30).

In these assays, 100-µl volume was applied throughout unless otherwise stated. 96-well microtiter plates (MaxiSorp; Nalge Nunc International, Roskilde, Denmark) were coated with diluted rAgB at 0.5 µg/ml in PBS overnight at 4°C. Plates were rinsed 3 times with PBST and blocked with 300 µl of 1% casein buffer at 37°C for 1 hr. Sera were diluted 1:100 in 1% casein buffer. Plates with diluted sera were incubated in duplicate wells at 37°C for 1 hr and then washed five times with PBST. Rabbit anti-human horseradish peroxidase-conjugated protein G (Zymed Laboratories, Inc., South San Francisco, Calif.) was diluted at 1:4000 in 1% casein buffer and incubated at 37°C for 1 hour. Plates were washed five times with PBST. For colour development, substrate solution (0.4 mM 2,2′-azino-bis[3-ethybenzthiazoline-6-sulfonic acid] in 0.1 M citric acid buffer and 0.2 M $Na_2HPO_4$) was added into each well and incubated at room temperature for 30 min. Colour reaction was then stopped by application of 1% SDS in each well. The optical density at 405 nm was evaluated with an ELISA reader.

## Statistical analysis

Chi-square test was used to compare the occurrence rate of spontaneous involution between males and females, and the cure rate between the patient groups with albendazole course ≤6 months and those >6 months, ≤12 months and >12 months, ≤18 months and >18 months, and ≤24 months and >24 months. Significance was set at $P \leq 0.05$.

# Results

A total of 196 persons with CE infection were registered in this study (male = 83, female = 113), with a mean age of 37.5 years at diagnosis (range 4–80 years). Of these 196 cases, 55 (28.1%) had

received prior regular albendazole therapy, while an additional 15 (8.2%) had prior surgery. However, only 4 of 15 operated cases received regular albendazole treatment following surgery.

Totally 98 of 108 CE patients without any previous albendazole treatment at diagnosis were investigated about clinical symptoms and signs, 55.1% (54) reported various degrees of discomfort, while 44.9% (44) were asymptomatic. The most common discomfort was hepatic or epigastric pain in 49.0% of patients, other complaints included abdominal distention, palpable abdominal mass, etc. All the 32 patients with cysts of CL, CE4 or CE5 type were asymptomatic, while 74.2% (49/66) of patients with cysts of CE1, CE2 or CE3 presented various degrees of symptoms.

Of these 196 cases, 37 (18.9%) were observed at first examination, to have evidence of spontaneous involution of cystic lesions without any interventional procedures, presenting inactive cysts (CE4 or CE5) in the liver. Persons with CE4 cysts had a mean age of 39.2 years (n = 27), while individuals with CE5 cysts had an average age of 61.3 years (n = 10). Of the 37 patients with evidence of spontaneous involution, 23 were male and 14 were female. In other words, spontaneous cure of cystic echinococcosis occurred more frequently in male (27.7%) than in female (12.4%), and the difference was significant ($\chi^2 = 7.3$, $P<0.01$).

A total of 49 CE patients received regular albendazole treatment for 6 to 30 months, including 19 males and 30 females. The youngest CE case was 4 years old and the oldest was 80 years, with a mean age of 37.7 years. Cystic lesions were confined in the liver in 43 cases, and the remaining 6 cases had lesions not only to the liver, but also in the abdominal cavity. Of these 49 patients, 16 had CE1 type cysts, 17 had CE2 cysts, 10 had CE3a type cysts, and the remaining 6 had CE3b cysts (Table 1). The cyst measured ≥10 cm in 25 patients, the cyst varied in size 5 cm–10 cm in 20 cases, whereas the remaining 4 CE cases had cysts less than 5 cm (Table 1).

Following 6 to 30 months regular therapy, 16 (32.7%) of 49 patients were observed to have cysts that changed to CE4 type (ie. considered cured), 24 (49.0%) were observed to have cysts converted to CE3a or CE3b from CE1 or CE2 type (ie. improved) (mean duration = 14 months), cysts remained unchanged in the other 7 (14.3%) patients (mean = 10.3 months), and enlargement of hydatid cysts was observed in the remaining 2 (4.1%) patients (Figure 2.1; Table 2). The cure rate was 15.4% (2/13) and 38.9% (14/36) in the patient groups with albendazole course ≤6 months and those >6 months, 21.4% (6/28) and 47.6% (10/21)) for the group with treatment duration ≤12 months and those >12 months, 22.9% (8/35) and 51.7% (8/14) for cases with treatment course ≤18 months and those >18 months, and 26.8% (11/41) and 62.5% (5/8) for patients with albendazole course ≤24 months and those >24 months. Further statistical analysis revealed that the cure rate was significantly different only between the patient group with treatment course ≤18 months and those >18 months ($\chi^2 = 5.24$, $P<0.05$). The 16 'cured' patients were composed of 5 CE1 cases, 5 CE2, 4 CE3a and 2 cases with CE3b cyst. The treatment course for cure varied in patients with cysts at different stages, that is, the mean curative course was 26.4 months in CE2 patients, 20.4 months in CE1 cases and 9 months in CE3a/CE3b patients. Moreover, the curative duration also differed in cases with cysts at different size. Patients with large hydatid cysts (≥10 cm) needed 22.3 months treatment (n = 7), whereas cure was achieved following 17.3 months therapy in cases with medium cysts (5 cm to 10 cm) (n = 8) and 6 months in patients with small cysts (≤5 cm) (n = 1). In addition, 5 of these 16 cured CE patients were further followed up with ultrasound for 6 to 24 months, in whom the cysts remained inactive (CE4 type), indicative of no recurrence.

**Table 1.** The cyst stage and size in 49 CE cases.

| Size | No. cases | | | | Total |
|---|---|---|---|---|---|
| | CE1 | CE2 | CE3a | CE3b | |
| Small | 2 | 1 | 1 | 0 | 4 |
| Medium | 8 | 4 | 6 | 2 | 20 |
| Large | 6 | 12 | 3 | 4 | 25 |
| Total | 16 | 17 | 10 | 6 | 49 |

Small: the cyst measured <5 cm at maximum diameter;
Medium: the cyst measured 5–10 cm at maximum diameter;
Large: the cyst measured ≥10 cm at maximum diameter.
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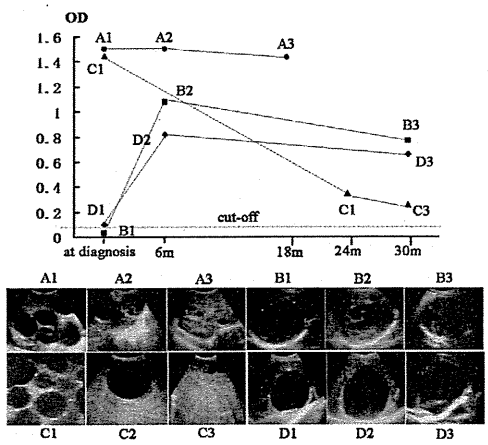
In contrast, 12 CE patients who poorly complied with albendazole treatment were observed to have much poorer prognosis during 6 to 30 months follow-up observation (mean = 17.0 months). Of these 12 patients, cysts remained unchanged in 8 cases, while enlargement of the cyst or recurrence of daughter cysts was observed in the remaining 4 patients (Figure 2.2).

Of 13 CE1 patients without any previous albendazole treatment, 7 (53.8%) were sero- negative for a specific IgG response to rAgB (Figure 2.3). However, following 3 to 18 months albendzole therapy, positive IgG seroconversion was observed in 6 (no. p1-p6) of these 7 initially seronegative cases (Figure 2.3 and figure 2.4). Concurrently, ultrasound scan detected detachment of cyst membrane and/or partial degeneration of cyst content (i.e. CE1 type changed to CE3a type) in all these six patients (no. p1-p6) (Figure 2.4). In another patient (no. p7) in whom serum specific IgG antibody remained negative during 6 months follow-up period (Figure 2.4), ultrasound scan did not detect any changes of the cyst (the image was not shown). A questionnaire investigation revealed that this patient had reported a poor compliance with albendazole therapy.

Sequential serum samples (n = 36) were obtained from 8 CE patients (CE1 = 4, CE2 = 3 and CE3b = 1), who did not receive any previous chemotherapy at diagnosis and were considered to be cured according to ultrasound images following albendazole therapy in the current study. At least 3 serum samples were monitored in each patient. The follow-up period ranged from 24 months to 78 months (mean 39.8 months), and an average 4.5 serum samples were taken from each patient. Longitudinal assessment of specific IgG antibody against rAgB in ELISA revealed that serum antibody levels of IgG were initially elevated, and subsequently decreased in five (4 CE1 and 1 CE2) of these 8 patients (Figure 2.5). In another two patients (1 CE2 and 1 CE3b), specific IgG antibody levels decreased but with a minor fluctuation during albendazole administration. In the remaining patient with a CE2 type cyst, a cured CE end-point was achieved following treatment, but there was no significant change of specific IgG antibody levels during the period of treatment (Figure 2.6A). Serum specific IgG antibody in fact remained positive in all the 8 patients where CE was considered 'cured' even 24 months after cure in one CE patient (ie. P2) (Figure 2.5A).

Consecutively collected 25 sera from 6 patients (CE1 = 2 and CE2 = 4) with improved CE following albendazole treatment were also assessed for specific IgG antibody against rAgB. Of these 6 cases, four (1 CE1 and 3 CE2) did not receive any previous albendaozle therapy at diagnosis, and initial elevation and subsequent decline of specific IgG antibody levels occurred in all these 4 patients (Figure 2.6B). For the other 2 cases with previous albendazole treatment at diagnosis, all antibody levels were

1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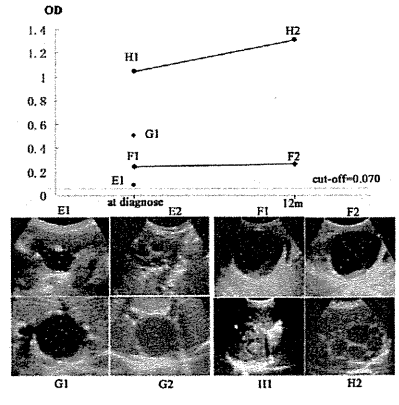
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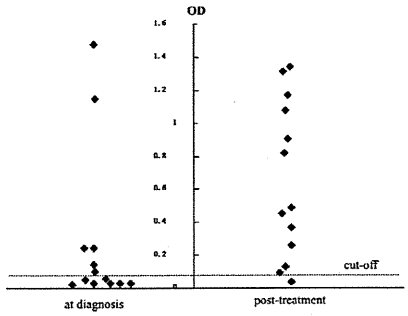
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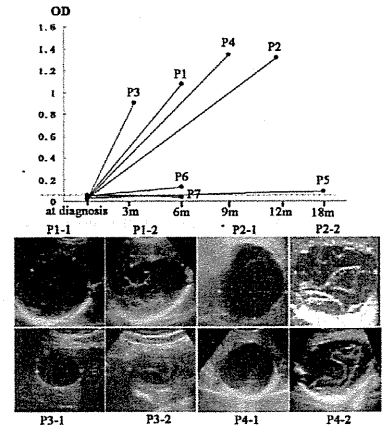
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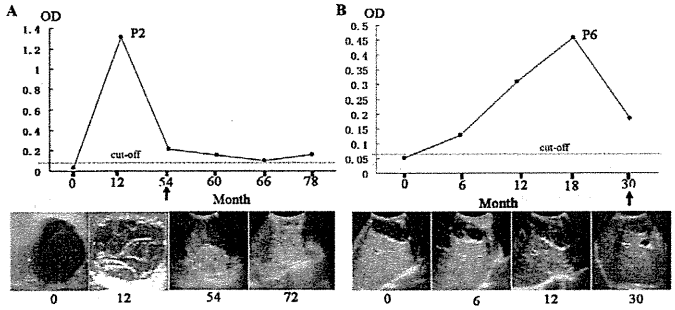
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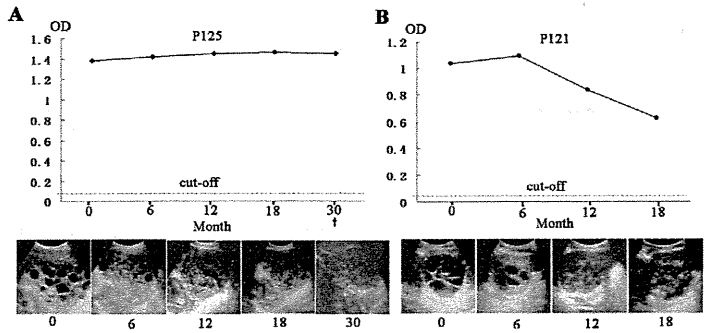
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**Figure 2. Changes of images and serum antibody levels against rAgB in CE cases during albendazole therapy. 1.** Patients with regular treatment. (A): Hepatic CE2 was cured after 18 months treatment (A-1: at diagnosis; A-2: 12 months; A-3: 18 months). (B): Hepatic CE1 was cured following 30 months treatment (B-1: at diagnosis; B-2: 6 months; B-3: 30 months). (C): CE2 was improved in abdominal cavity following 42 months treatment (C-1: at diagnosis; C-2: 36 months; C-3: 42 months). (D): Hepatic CE1 was aggravated following 30 months treatment (D-1: at diagnosis; D-2: 6 months; D-3: 30 months). **2.** Patients with poor compliance of treatment. (E): Hepatic CE (E-1: CL type cyst at diagnosis; E-2: CE3 type cyst with daughter cyst at 30 months). (F): Hepatic CE (F-1: CE1 type cyst at diagnosis; F-2: unchanged cyst at 12 months). (G): Hepatic CE (G-1: CE1 type cyst at diagnosis; G-2: the unchanged cyst at 6 months). (H): Hepatic CE (H-1: CE3 type cyst at diagnosis; H-2: CE2 type cyst at 12 month). Patient E and G refused to donate blood samples when followed up. **3.** ELISA OD values in 13 CE1 cases before and after treatment (3 months to 18 months). **4.** In seven CE1 cases (P1-P7) with seronegative response at diagnosis, six (P1-P6) converted to be seropositive following 3 months to 18 months treatment. Concurrently the cyst changed to CE3a from CE1 type in all these six cases. **5.** In two albendazole-cured CE1 cases, specific antibody levels were initially elevated and subsequently declined during treatment, but remained positive when CE was cure. **6.** In two albendazole-cured or improved CE2 patients, (A): stability of specific antibody levels in a cured CE2 case; (B): initial elevation followed by subsequent decline of specific antibody levels in an improved CE2 patient. The arrow referred to the time when CE was found to be cured.
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observed to progressively decrease following therapy. In contrast, specific IgG antibody levels were observed to sharply increase in one patient with evidently aggravated CE following irregular albendazole administration, presenting enlargement of the cyst and recurrence of daughter cysts (Figure 2.2 H).

Of 83 CE patients who were investigated about adverse effects related to albendazole administration, 37 (44.6%) reported no subjective adverse reactions, while 45 (54.2%) reported gastrointestinal disturbance, exhibiting stomach ache, acid regurgitation, nausea or diarrhea. Additionally, headache, dizziness and hepatic pain (or 'sting') each occurred in at least one case besides gastrointestinal disturbance. Evident alopecia was not reported and observed in the current study. Therapy with albendazole was ceased in 2 cases during follow-up, due to occurrence of serious dizziness and stomach ache. Transaminase levels were not monitored in the current study, due to the difficulty of doing liver function tests in the field.

## Discussion

Albendazole became available for treatment of human hydatid disease in 1983 [25,26], and its effectiveness in cystic echinococcosis (CE) has been evaluated in a series of studies [27–30], from which approximately 30% of patients were cured, a further 30–50% were improved, and 20–40% remained unchanged following 3 months to 6 months treatment. However, 25% of albendazole (ABZ) treated patients relapsed in long-term follow-up studies [30]. This indicated the need for a prolonged treatment greater than the standard 3 to 6 months in some cases. In the present study, the effectiveness of albendazole was assessed in 49 Tibetan CE patients, detected after mass screening, using ultrasound during a 6 to 30 months regular treatment and based on the WHO consensus classification [12]. Therapy was continued until hydatid cyst morphology changed to a CE4 type (ie. a solid mass with or without partial calcifications), but further regular ultrasound follow-up was required. As a result, 32.7% of CE patients with regular albendazole therapy showed involution of cysts to a CE4 type (cured), 49.0% of CE cases exhibited the cyst degenerating into CE3 (improved), whereas 14.3% remained unchanged and 4.1% were observed to have enlarged cysts or have recurrence of daughter cysts. Our current study also indicated that the cure rate increased with a prolonged treatment course, but the patient group with >18 months albendazole course was more likely to exhibit a cyst that changed to a CE4 type (57.1%) compared to those (22.9%) ≤18 months. This observation suggested that longer-term albendazole treatment caused a greater positive therapeutic impact as has been observed in other CE case series [28,31]. Though no recurrence of CE was detected in the cured patients during 6 to 24 months ultrasound follow up in the current study, further ultrasound examination is necessary for years in the future. In contrast spontaneous involution of CE without treatment was observed in 18.9% of CE patients in the current study, which was consistent with previous reports of natural degeneration of hydatid cysts, for example in 13.6% and 21% of cysts in CE patients from Kenya and Argentina, respectively [32,33].

Many factors can influence the effectiveness of anti-hydatid treatment, for example previous studies demonstrated that benzimidazole treatment was more efficacious against smaller and younger cysts, and the type of cyst may also have an effect [1,27]. Results from the current study indicated that patients with CE2 type cysts (ie. with daughter cysts) needed the longest treatment course (mean 26.4 months) before cure, whereas the curative duration was shortest (mean 9 months) in cases with CE3a or CE3b type cysts (ie. with evidence of detached membrane and/or degraded daughter cysts). In addition, our study also suggested

**Table 2.** Outcomes of cyclic albendazole treatment in 49 CE cases.

| Course (month) | No. cases treated | Cured | | Improved | | Static | | Aggravated | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. cases | % | No. cases | % | No. cases | % | No. cases | % |
| 6 | 13 | 2 | 15.4 | 7 | 53.8 | 3 | 23.1 | 1 | 7.7 |
| 12 | 15 | 4 | 26.7 | 8 | 53.3 | 3 | 20.0 | 0 | 0 |
| 18 | 7 | 2 | 2/7 | 4 | 4/7 | 1 | 1/7 | 0 | 0 |
| 24 | 6 | 3 | 3/6 | 3 | 3/6 | 0 | 0 | 0 | 0 |
| 30 | 8 | 5 | 5/8 | 2 | 2/8 | 0 | 0 | 1 | 1/8 |
| Total | 49 | 16 | 32.7 | 24 | 49.0 | 7 | 14.3 | 2 | 4.1 |

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that CE patients with large cysts (≥10 cm) needed a longer curative treatment period (mean 22.3 months), compared to patients with medium size (5–10 cm) cysts (mean 17.3 months) or those presenting with small (<5 cm) cysts (mean 6 months). Therefore, the putative duration of albendazole curative treatment may differ greatly in individual CE patients. Thus, it is strongly recommended that regular imaging monitoring, even in remote communities, be continued during the period of albendazole treatment to guide the appropriate medical treatment.

Antigen B (AgB), as a major component of *E. granulosus* hydatid cyst fluid, has been proved to have a high diagnostic value in the serological diagnosis of cystic echinococcosis in humans [34–37]. Our most recent serological study indicated that recombinant antigen B (rAgB) positive serum samples in ELISA were significantly lower in patients with CE1 type cysts, compared to other patients with CE2 or CE3 type cysts [38]. Similarly, in the current follow-up study only 46.2% (6/13) of patients with CE1 cyst showed positive antibody responses against rAgB before ABZ treatment. Interestingly, serum specific IgG antibody levels were observed to convert in six of the seven initially sero-negative CE1 patients following albendazole therapy. Concurrently, ultrasound scan revealed that hydatid cysts progressed from a CE1 type to a CE3a type in all these 6 CE patients with positive IgG antibody seroconversion. In the remaining one case (P7), specific serum antibody still remained negative by the end of follow-up, and ultrasound scan could not detect any improvement of the cyst. Subsequent questionnaire investigation showed that this patient failed to take albendazole as requested. This finding suggests that serum specific IgG antibody (against rAgB) was initially absent in a great proportion (53.8%) of CE1 patients, probably due to the typical intact cyst wall in this type of cyst which limits the release of antigenic cyst fluid into the circulation. Therefore, occurrence of serum specific IgG antibody in initially sero-negative CE1 hydatid patients after albendazole administration, not only conversely verified the clinical diagnosis, but importantly also acted as a positive indicator for the potential therapeutic effectiveness of antiparasitic drugs.

The present study on community detected Tibetan CE cases disclosed two broad profiles of specific IgG antibody dynamics in a majority of improved or cured CE patients during albendazole therapy. One profile was typified by specific antibody levels that were initially elevated then subsequently decreased, whereas the second antibody profile was characterized by progressive decline in serum IgG antibody levels. However, all cured CE patients still remained seropositive at the end of medical treatment. Therefore, assessment of serum rAgB-specific IgG antibody levels in this group of Tibetan CE patients could only provide limited information about the effectiveness of albendazole. Nevertheless, occurrence of post-chemotherapeutic IgG antibody seroconversion in CE1 patients appeared to be an important indicator for effectiveness of albendazole in such cases.

The most frequent adverse reactions associated with long-term albendazole treatment have been reported as gastrointestinal disturbances, reversible alopecia, elevation of liver transaminases, and pancytopenia [29,39,40]. In general, significant reversible abnormalities of liver function occur in about 20% of cases, reversible alopecia appears in about 5% of cases, while fatalities due to pancytopenia have so far been reported in only 3 cases of echinococcosis [29,41]. In the current study, gastrointestinal upset was commonly reported by 54.2% of Tibetan CE patients taking oral ABZ therapy as out-patients, but was generally well tolerated by the majority. However, regular monitoring of parameters indicative of liver function were not carried out in the current community based study, as nomadic lifestyle and absence of medical facilities in this exceptional area limited the ability to use liver function testing. Importantly albendazole has proved to be relatively safe with normally only mild side-effects when they occurred [29,42,43].

In conclusion, this was the first attempt to assess the effectiveness of cyclic albendazole treatment for Tibetan CE patients treated as out-patients in their own communities. Though the sample size was small (n = 49), results from this study indicated that albendazole treatment showed beneficial efficacy in over 80% of CE patients, and the treatment course for a curative indication was strongly associated with hydatid cyst pathological type as well as the cyst size. In addition, the changes of specific serum IgG antibody levels against rAgB in CE patients provided a degree of limited but useful information about the effectiveness of albendazole during treatment. It should also be noted that nearly 19% of community diagnosed hepatic CE cases showed evidence of spontaneous cure. Further community based post-treatment follow-up studies of human CE needs to be continued in resource poor Tibetan areas of western China.

### Accession Number

The accession number in GenBank for recombinant antigen B applied in the current study is Z26336.

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## Author Contributions

Conceived and designed the experiments: TL AI PSC. Performed the experiments: TL RP YS XC DQ NX. Analyzed the data: TL. Contributed reagents/materials/analysis tools: YS AI. Wrote the paper: TL PSC.

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BMJ Case Reports

Novel diagnostic procedure

# Capsule endoscopy is a feasible procedure for identifying a *Diphyllobothrium nihonkaiense* infection and determining the indications for vermifuge treatment

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## Summary

*Diphyllobothrium* is a member of *Cestoda* family, which is the largest parasite of humans. The diagnosis of diphyllobothriasis is based on the detection of eggs in the stool. Because the remainder of the scolex causes a relapse in diphyllobothriasis, the scolex must be completely discharged to cure the parasite infection. However, the scolex or forefront of the *Diphyllobothrium* is difficult to detect with gastroduodenoscopy and colonoscopy, because most *Diphyllobothrium* attach to the jejunal wall. In the present case, capsule endoscopy detected proglottids as well as forefront of the parasite at jejunum. Based on the results of capsule endoscopy, the patient underwent additional vermifuge (anthelminthic) treatment to cure the diphyllobothriasis and discharged a worm measuring 3 m in length with a scolex. Capsule endoscopy is a practical option to determine whether additional vermifuge treatment is required through the detection of the proglottids as well as a scolex or forefront of the parasite.

## BACKGROUND

*Diphyllobothrium* is a member of the *Cestoda* (tapeworm) family and it is the largest parasite of humans. Among *Diphyllobothrium* tapeworms, more than 10 species are known to cause human infection, which is called diphyllobothriasis,[1] and *Diphyllobothrium nihonkaiense* is the main causative species of diphyllobothriasis in Japan.[2] The second intermediate hosts of *D nihonkaiense* are anadromous Pacific salmons such as masu salmon *Oncorhynchus masou masou*, pink salmon *Oncorhynchus gorbuscha* and chum salmon *Oncorhynchus keta*, and humans normally acquire the parasite by eating raw or undercooked fish.[1 3]

The diagnosis of diphyllobothriasis is based largely on the detection of eggs in the stool of the patients. Because the remainder of the scolex causes a relapse in diphyllobothriasis, the scolex must be completely discharged to cure the parasite infection. However, the scolex or forefront of the *Diphyllobothrium* is difficult to detect by gastroduodenoscopy and colonoscopy, because most *Diphyllobothrium* attach to the jejunal wall. The current case report describes how capsule endoscopy can directly detect live *Diphyllobothrium*, including the forefront of the parasite; thus, suggesting the usefulness of this examination for determining the necessity of performing vermifuge (anthelminthic) treatment.

## CASE PRESENTATION

A 23-year-old man visited our hospital due to numerous white string-like excretions. No particular abnormality was noted with the physical or blood examinations. The patient had eaten no raw salmon within the period of several months. Eggs of ovoid shape with an operculum on a narrowed pole were detected in the patient's stool. Therefore, the patient was diagnosed to have a *Diphyllobothrium* infection. To confirm whether the scolex of the parasite remained, capsule endoscopy was performed. The examination detected the forefront of the parasite at the upper jejunum (figure 1) and numerous proglottids from the upper to lower small intestine (figure 2). Mature proglottids were also found in the small intestine. No other abnormality was observed during the examination. Subsequently, an additional vermifuge treatment using 200 ml of gastrografin (Nihon Shering, Osaka, Japan) was performed and the patient discharged a worm measuring 3 m in length with a scolex and many proglottides after approximately 1 h. To identify the tapeworm at species level, cytochrome *c* oxidase subunit I (*cox1*) and cytochrome *b* (*cob*) genes of mitochondrial DNA (mtDNA) were analysed according to Yanagida *et al*.[4] Both *cob* and *cox1* sequences showed more than 99.7% identities with those of *D nihonkaiense* (AB522615 and AB508838, respectively). These results demonstrated the tapeworm obtained in the present case to be *D nihonkaiense*.

## OUTCOME AND FOLLOW-UP

The patient was completely cured by an additional vermifuge treatment.

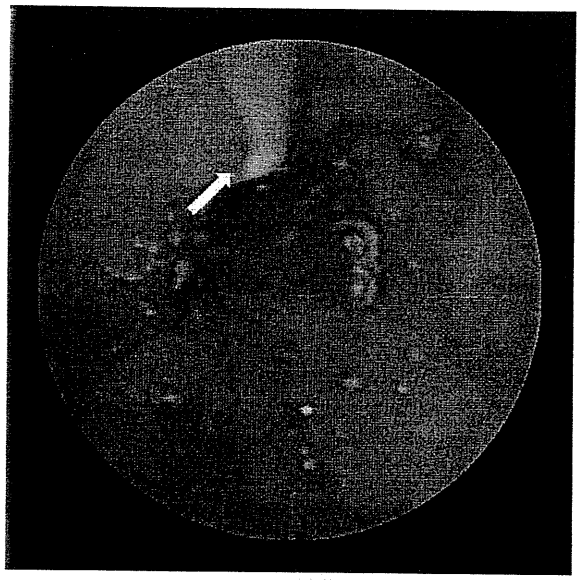

**Figure 1** The forefront of *Diphyllobothrium nihonkaiense* was observed in the patient's jejunum.

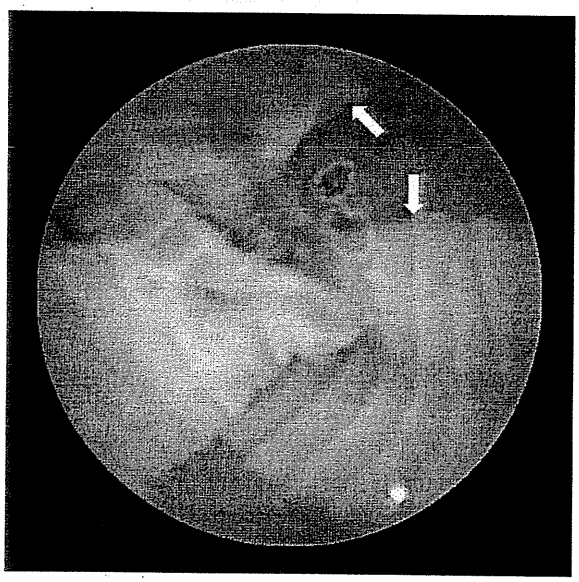

**Figure 2** Numerous proglottids were detected in the jejunum and ileum.

## DISCUSSION

In the present case, capsule endoscopy contributed to the diagnosis of diphyllobothriasis infection through the direct observation of the live parasite in the small intestine. It is noteworthy that the capsule endoscopy identified numerous proglottids as well as forefront of the parasite; thus, suggesting that this examination is a practical and effective procedure for determining whether additional treatment is required to treat a *Diphyllobothrium* infection. Seven reported cases and the present patient with tapeworm infection detected by capsule endoscopy[5–10] are summarised in table 1. Three subjects were infected by *Diphyllobothrium spp*, while the other five were infected by *Taenia*. Four subjects had histories of eating raw fish or meat. Capsule endoscopy detected a scolex or forefront of the parasite in six cases and proglottids in all eight cases. In four cases, the cause of anaemia was attributed to tapeworm infection. In the present case, capsule endoscopy was useful to confirm the remaining scolex or forefront of the parasite, which might cause a relapse of the diphyllobothrium infection and it is also a safer and more comfortable method for the patient. Based on the results of capsule endoscopy, the patient underwent additional vermifuge treatment to cure the diphyllobothriasis.

In summary, the present study demonstrates that capsule endoscopy is a feasible option to identify tapeworm infection and also to determine whether additional vermifuge treatment is required through the detection of the proglottids as well as a scolex or forefront of the parasite. Therefore, capsule endoscopy, which is a safe and less invasive procedure for observing the small intestine, may be applied to accurately diagnose parasite infections in the intestinal tract.

**Learning points**

- Capsule endoscopy, which is a safe and less invasive procedure for observing the small intestine, is a practical procedure to detect the proglottids as well as a scolex or forefront of the parasite.
- Capsule endoscopy is useful to determine the indication of an additional vermifuge treatment.
- Therefore, capsule endoscopy may be applied to accurately diagnose parasite infections in the intestinal tract.



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**Table 1** Reported cases with tapeworm infection detected by capsule endoscopy

| Age/sex | Species | History of eating raw fish or meat | Detectability of the scolex or forefront of the parasite | Detectability of proglottids | Efficiency of capsule endoscopy | Reference |
|---|---|---|---|---|---|---|
| 23/Female | *Diphyllobothrium latum* or *D nihonkaiense* | Raw salmon | Detected | Detected | Capsule endoscopy confirmed the Diphyllobothrium infection | 5 |
| 75/Male | *Taenia saginata* | Raw meat | Detected | Detected | Capsule endoscopy indicated that the cause of iron deficiency anaemia was a tapeworm infection | 6 |
| 14/Male | *T saginata* | Not described | Detected | Detected | Capsule endoscopy indicated that the cause of iron deficiency anaemia was a tapeworm infection | 7 |
| 50/Female | *T saginata* | Raw beef | Not described | Detected | Capsule endoscopy identified the possible cause of anaemia to be a tapeworm infection | 8 |
| 54/Female | *D latum* | Not described | Not described | Detected | Capsule endoscopy identified the cause of anaemia was a Diphyllobothrium infection | 9 |
| 44/male | *Taenia* | Not described | Detected | Detected | Capsule endoscopy detected the remaining proglottids and scolex of the parasite; thus, additional treatment was conducted | 10 |
| 47/Female | *Taenia* | Not described | Detected | Detected | Capsule endoscopy detected the remaining proglottids and scolex of the parasite; thus, additional treatment was conducted | 10 |
| 23/Male | *Di nihonkaiense* | No history of eating raw salmon | Detected | Detected | Capsule endoscopy detected the remaining proglottids and forefront of the parasite thus additional treatment was conducted | Present case |

**Competing interests** None.

**Patient consent** Obtained.

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BMJ Case Reports

Novel diagnostic procedure

# Neurocysticercosis case with tuberculoma-like epithelioid granuloma strongly suspected by serology and confirmed by mitochondrial DNA

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## Summary

An Indian woman with multiple and nodular cerebral lesions presenting epithelioid granulomas was diagnosed as suspected malignant tumours and the biopsy was carried at local hospital in Japan. The specimen was pathologically diagnosed as tuberculoma. However, due to her nationality, the authors suspected it to be neurocysticercosis (NCC) and carried out serology and applied mitochondrial DNA analysis of *Taenia solium*. These diagnostic tools revealed it as NCC radiologically and pathologically mimicking central nervous tuberculomas. Molecular confirmation of NCC cases is strongly recommended when we can get the biopsy specimens.

## BACKGROUND

Neurocysticercosis (NCC), caused by the larval stage of *Taenia solium* (*T solium*), is the most common and serious parasitic disease of the central nervous system (CNS) in the majority of developing countries where people eat pork.[1 2] Clinical manifestations of NCC are highly variable depending on the number, type, size, localisation and developmental stage of cysticerci with the host immune response.[3] NCC, echinococcosis, anthrax and rabies are now recognised as re-emerging, but neglected, infectious diseases worldwide.[2 4] If NCC is suspected through neuroimaging, either typical or atypical, and questionnaire on visiting and/or living history in endemic areas of *T solium*, it is strongly recommended to carry out serology using highly specific antigens.[2 5–7]

NCC cases with multiple cysts are rather easily detectable by such a serology.[2] However, even highly specific serology is not always 100% sensitive especially in cases with solitary cyst, with calcified cysts only or with background of immunodeficiency.[2] Without typical neuroimaging or serological confirmation of NCC, patients are often reluctantly treated surgically with suspected diagnosis of malignant tumours, tuberculosis or any other diseases causing neurological disorders especially in non-endemic countries.[2] Therefore, pathological approaches on NCC cases could inevitably become feasible for crucial diagnosis to identify such NCC cases. It is not always easy that the infectious diseases including atypical NCC are confirmed with the surgical specimens of the brain in non-endemic countries. However, molecular diagnosis using PCR has been introduced and established for molecular diagnosis of many pathogens and provided satisfactory results in NCC as well.[8–11]

## CASE PRESENTATION

An Indian woman was referred to our hospital for the evaluation of multiple and nodular cerebral lesions. Two months before admission to our hospital, she noted occasional headache and paraesthesia of right forearm and MRI, examined in a local hospital, revealed Gd-well enhanced multiple nodular lesions with peripheral oedema and scattered calcifications (figure 1). Because no neoplastic lesions could be excluded on the basis of these radiological findings and because NCC is not indigenous in Japan[1 2] and most of clinicians have no or poor knowledge on NCC without their own experiences, NCC unfortunately was not suspected as a causative disease, and a brain biopsy was performed in a local hospital. However, figure 2 strongly suggested somewhat typical image of NCC with oedema, and if the clinicians tried to get second opinions from parasitologists, chemotherapy without neurosurgery could strongly be recommended with sound serological evidence.[1 2 6 12] The H&E-stained microsections derived from one of the cerebral nodules showed presence of epithelioid granulomas surrounded by reactive gliosis (figure 3A). These granulomas were also divided into three layers including plasma cell-rich mononuclear cell outer layer (mo), epithelioid-cell middle layer (epi) and central necrosis (nec) (figure 3B). Although these pathological findings were mimicking tuberculomas, Ziehl-Neelsen stains of the microsections as well as PCR amplification for *Mycobacterium tuberculosis* DNA were negative (data not shown) and also no microorganisms were detected by cultures.

On admission to our hospital for the evaluation as a second opinion, laboratory data showed a total white cell count of 6290/ml (with a differential of 58.0%

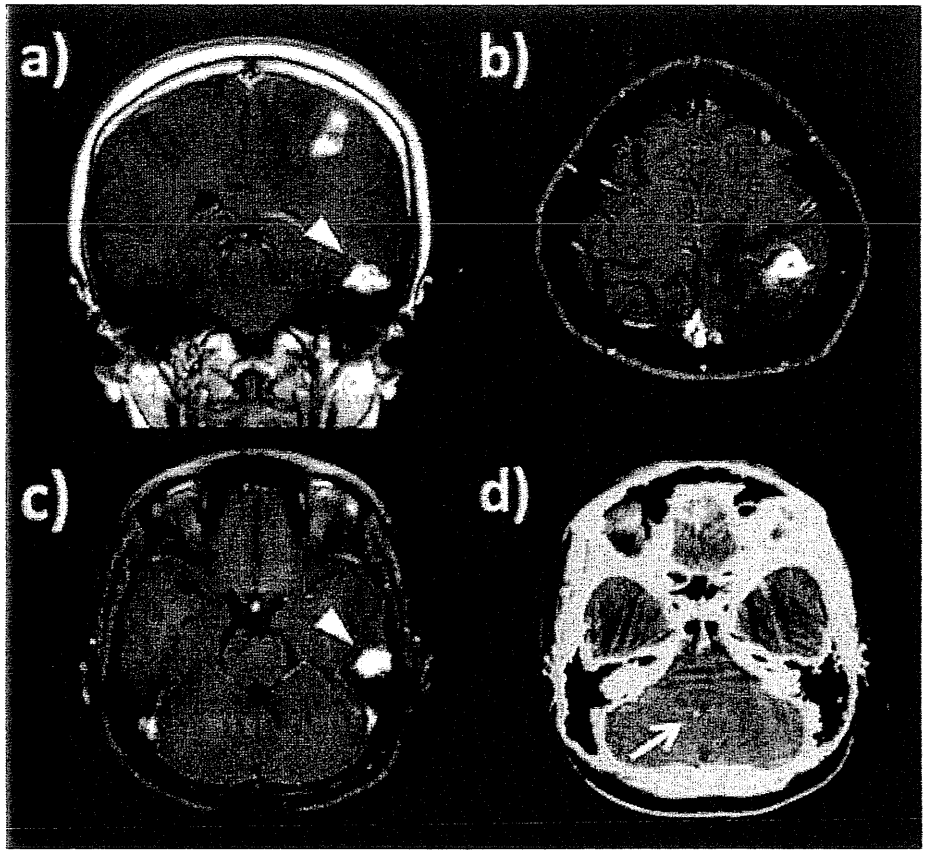


**Figure 1** Neuroimaging figures. (A–C) T1-weighted MRI after contrast enhancement presented high signal intense lesions with gadolinium enhancement. Arrowheads show the biopsied lesion at left temporal lobe. (d) This brain CT image shows calcification scattered in the parenchyma (arrow).

neutrophils, 26.5% lymphocytes, 12.5% eosinophils), C reactive protein level of 0.04 mg/dl, and IgE level of 16.0 U/ml. NCC was highly suspected because of her nationality, multiple nodular lesions with peripheral oedema, eosinophilia and brain nodules with scattered calcifications. On the basis of those findings, serological test was performed for detection of antibodies specific to *T solium* cysticercus.[2 6 12] The specific antibodies against *T solium* cysticercus were detected by Western blots using both native antigens purified by isoelectrofocusing from *T solium* cyst fluids[6] and recombinant chimeric protein,[12] and by ELISA using recombinant chimeric protein (see figure 2).[12]

In order to confirm whether the epithelioid granulomas were really derived from *T solium* cysticerci or not, mitochondrial DNA (mtDNA) analysis was performed. DNA samples from formalin-fixed paraffin-embedded brain specimens were prepared according to the previously described methods.[9] DNA sequence analysis of these PCR products was performed and revealed the causative agent to be a *T solium* Asian genotype (data not shown).[15] Before antiparasitic treatment with albendazole,[14] MRI revealed significant decrease in oedema and transformation into cystic nodules with thin wall, and neurological symptoms were completely disappeared (figure 2). The antibody titres and neuroimagings were monitored after chemotherapy.

## OUTCOME AND FOLLOW-UP

After 2 years and 10 months from an attack of disease, reactivation was occurred with symptoms resolved spontaneously within several days. Inflammatory finding showed in MRI was also spontaneously improved and the antibody titres were gradually decreased from 0.121 to 0.066 (cut-off value: 0.038, figure 2).

## DISCUSSION

After dissemination into CNS, cysticerci are viable as vesicular or avesicular types for variable period and could elicit little inflammatory responses in the surrounding parenchyma until some cysticerci are damaged. Recent retrospective work by Yanagida *et al*[15] strongly suggested that the parasite could survive at least more than 10 years. Phased degeneration into granuloma, fibrous scar and calcification without their specific structures like suckers and hooklets are followed as in our case.[16] Because the foreign body granuloma of degenerating NCC could show tuberculoma-like lesions as demonstrated here or various non-characteristic morphologies, serology before treatment and monitoring of progression and mtDNA analysis using histopathological specimens are crucial tools for definitive diagnosis after surgery.[2 9–11] Nonetheless, serology before treatment is the essential for diagnosis of NCC as it is well recognised in developed countries outside of Japan. In this case, if we applied serology before surgery (figure 2), we



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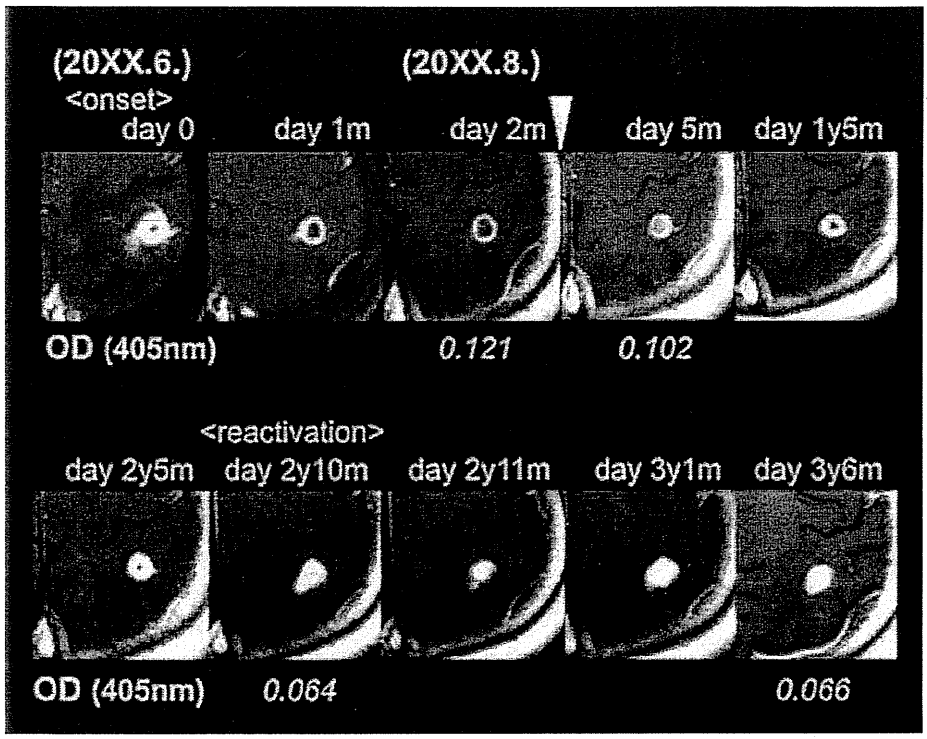


**Figure 2** Time course of both T1-weighted MRI after contrast enhancement and antibody responses. ELISA data were shown as the OD values using recombinant chimeric antigen (cut-off OD value: 0.038).[12] Treatment with ABZ was introduced at arrowheads.

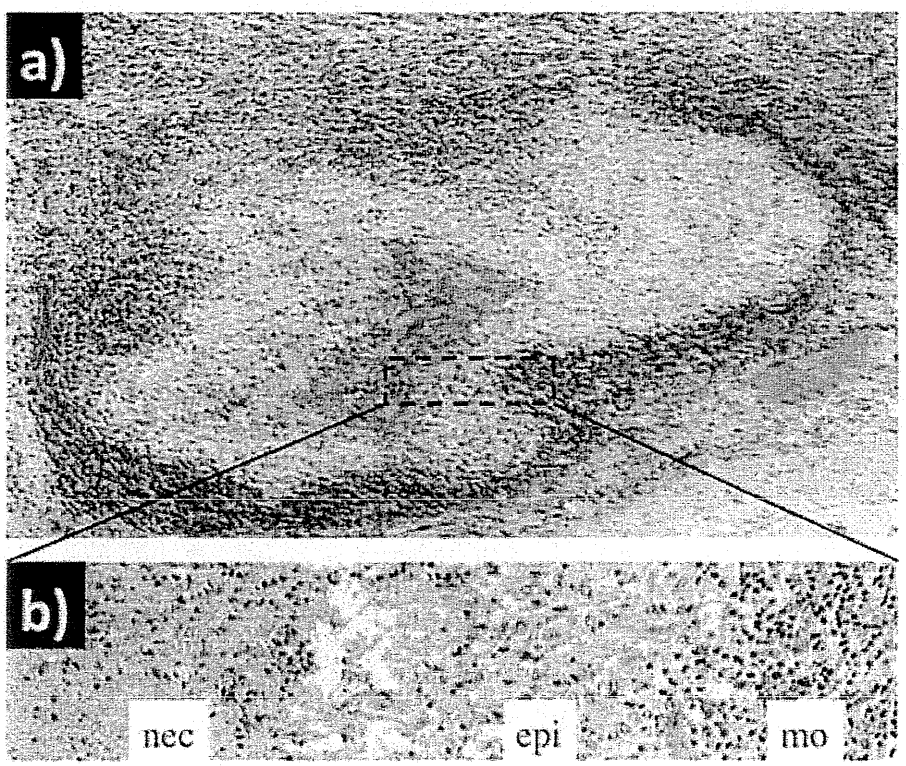


**Figure 3** Histopathologic figures. (A) The low-power view of the section with H&E staining derived from the nodules at left temporal lobe presented epithelioid granulomas. (b) High magnification of a dotted square in figure 3A with three types of distinct layers: plasma cell-rich mononuclear cell outer layer (mo), epithelioid-cell middle layer (epi) and central necrosis (nec).

could avoid the invasive and risky procedure of a brain biopsy.[1 2]

Recent work on mtDNA of *T solium* in Bali, Indonesia has shown minor difference in the nucleotide sequences.[17] Most recent similar work on another NCC case with surgery revealed that a Japanese man with travel and living history in Indonesia, Nigeria, Nepal and Malaysia, where NCC was endemic, was concluded to have been exposed to eggs of *T solium* in Nepal by haplotype network analysis.[15] Another most recent NCC case of one US woman 35 years after her immigration from Lao PD also confirmed that she got infection during her repeated visits to Lao PD and enjoyed local foods through her visiting friends and relatives.[18] Therefore, it may be possible for us to assess where the infection was acquired from when we can get histopathologic specimens and more polymorphic differences in mtDNA sequences in the two genotypes.[10 13]

**Learning points**

- Serologic diagnosis is useful for differential diagnosis of NCC before treatment and mtDNA analysis using histopathologic specimens are crucial tools for definitive diagnosis after surgery to assess where the infection was acquired from.

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**Competing interests** None.

**Patient consent** Not obtained.

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MEETING REPORT Open Access

# The first workshop towards the control of cestode zoonoses in Asia and Africa

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**Abstract**

The first workshop towards the control of cestode zoonoses in Asia and Africa was held in Asahikawa Medical University, Japan on 15 and 16 Feb 2011. This meeting was fully supported by the Asian Science and Technology Strategic Cooperation Promotion Programs sponsored by the Special Coordination Funds for Promoting Science and Technology, the Ministry of Education Japan (MEXT) for 3 years from 2010 to Akira Ito. A total of 24 researchers from 9 countries joined together and discussed the present situation and problems towards the control of cestode zoonoses. As the meeting was simultaneously for the establishment of joint international, either bilateral or multilateral collaboration projects, the main purposes were directed to 1) how to detect taeniasis/cysticercosis infected patients, 2) how to differentiate *Taenia solium* from two other human *Taenia* species, *T. saginata* and *T. asiatica*, 3) how to evaluate *T. asiatica* based on the evidence of hybrid and hybrid-derived adult tapeworms from Thailand and China, 4) how to evaluate *T. solium* and *T. hyaenae* and other *Taenia* species from the wild animals in Ethiopia, and 5) how to detect echinococcosis patients and 6) how to differentiate *Echinococcus* species worldwide. Such important topics are summarized in this meeting report.

## History

The coordinator, Akira Ito at Asahikawa Medical University (AMU) organized a symposium on cysticercosis at the 3rd Seminar on Food-borne Parasitic Zoonoses in Bangkok, 2000 [1]. From 2003, Akira Ito was recommended to establish leadership in science and technology in Asia through his research on cestode zoonoses by a special Japanese Governmental fund for three year project (2003-2005). Akira Ito set up an international symposium every year [2] and seminars for the transfer of technology through joint research projects on cestode zoonoses mainly in Asia and some in Africa. The first seminar was held in AMU from 17 Jan until 2 Feb 2004. We invited 10 guests from Inodonesia (4), Thailand (3), China (3) with international consultants from USA (Peter M Schantz, CDC), Australia (David D Jenkins, the President of Australian Society for Parasitologists) and UK (Philip S Craig, University of Salford, the coordinator for the WHO informal Working Group on Echinococcosis). The second one was held from 14 Sept until 1 Oct 2004. We invited 7 guests from Cameroon (2), Nepal (2), Vietnam (1), Philippines (1) and Australia (1). The third one was held from 17 Jan until 2 Feb 2006. Nine guests were from China (4), Indonesia (2), Philippines (1), Thailand (1) and France (1). The biggest program was to organize the international symposium entitled "Taeniasis/cysticercosis and echinococcosis with focus on Asia and the Pacific" held in the Asahikawa Grand Hotel on 5-8 July 2005. The proceedings of this meeting were published as a supplement of Parasitology International in 2006 [3]. All participants were invited to join together in Asahikawa with full support for travel expenses including air tickets, accommodation and living expenses. Approximately 100 researchers from 30 countries, including Japan, participated. Approximately 80 participants were foreign guests including experts from WHO, FAO and CDC. After this 3 year project, we received another 3 year project for the establishment of the reference center for cestode zoonoses in Asia and Africa from the Japan

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