**Epidemiological Report**

Cystic and Alveolar Echinococcosis: an Epidemiological Survey in a Tibetan Population in Southeast Qinghai, China

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**SUMMARY:** An epidemiological investigation on echinococcosis was made in Jiuzhi County of Qinghai Province, western China. Ultrasoundography and an indirect hemagglutination test revealed a morbidity of 8.0% (124/1,549) and a seroprevalence of 25.8% (287/1,113), respectively, in the Tibetan population. The morbidity in herdsmen (16.6%) and Buddhist priests (15%) was significantly higher than that in other occupation groups (3.2%), and it was higher in females (9.8%) than in males (6.2%). The ultrasound images showed a coexistence of cystic echinococcosis (CE) and alveolar echinococcosis (AE), occupying 69 and 31% of the cases, respectively. An *Echinococcus* Western blot assay was performed as a serological backup test for differentiating CE and AE. The assay revealed that serum samples from most cases with a positive AE image showed a specific antibody against antigen bands at 16/18 kDa. Autopsy proved that 9 out of 12 stray dogs were infected with *Echinococcus granulosus* (*n* = 8) and *E. multilocularis* (*n* = 1). Inspection at the abattoirs demonstrated a hydatid rate of 78.5% in yaks and 82.6% in sheep. The data indicate that Jiuzhi County is an important endemic area for both CE and AE, in both human and animal populations.

**INTRODUCTION**

Both cystic echinococcosis (CE) and alveolar echinococcosis (AE) are endemic in China, and they are one of the most challenging problems for public health and animal husbandry in the western region. A national investigation of the major parasitic diseases, carried out in 2002-2004, revealed that by enzyme-linked immunosorbent assay (ELISA) and ultrasonography (US) for echinococcosis in randomly sampled populations of 39,826 and 34,486 people in 12 provinces and autonomous regions, respectively, the serological prevalence and morbidity were 12.0 and 1.1%, respectively (1). Qinghai, with a population of 5.3 million, is one of the provinces in the northeastern region where, due to the poor socioeconomic development, the prevalence of the disease has been increasing and even expanding from agro-pastoral areas to urban areas.

In 1997-1998, a seroepidemiological investigation with PVC-Dot-ELISA revealed a seropositive rate of 14.0% in the residents of south Qinghai, with rates of 16.8% in females and 11.5% in males, and the seroprevalence increased with age (2). As part of the national investigation 2002-2004, a survey on CE and AE was conducted in Qinghai in 2003. An ELISA test given to 3,989 randomly sampled people revealed a positive rate of 14.6%, with rates of 12.0 and 16.4% in males and females, respectively, while US examination in 5,274 people demonstrated an overall incidence of 1.8%, with rates of 1.4 and 2.1% in males and females, respectively; 5 out of 95 cases with clinical signs were patients of AE (3). Earlier data indicated that in some areas of the province, the infection rate of echinococcosis in dogs was 36%, and the rate of hydatid disease in yaks and sheep was 46 and 63%, respectively (4).

To understand the epidemiological conditions of echinococcosis in a county where the predominant population is ethnic Tibetan, we conducted an investigation using parasitological, immunological and clinical examinations in humans and animals. In this report we describe the results of our investigation.

**MATERIALS AND METHODS**

**Areas and subjects for the study:** Jiuzhi County of the Guoluo Tibetan Autonomous Prefecture is situated in southeastern Qinghai, bordering Sichuan Province (longitude 101°00'-101°50' and latitude 32°50'-33°80' (Fig. 1). This area is located on the eastern part of the Tibetan Plateau, with approximately 8,700 km², between 3,600 and 4,500 m above sea level. The human population of Jiuzhi County is about 20,000, and 98% are ethnic Tibetans. Villages and towns in the sparsely populated area with animal husbandry as the major economy were identified for the survey. All the local residents aged 5 and above were included as subjects, if they were present during the survey.

**Human echinococcosis screening:** Image examination using a portable ultrasonograph (SSD-500; Aloka, Tokyo, Japan) was applied for the clinical diagnosis of all residents in each village/town who voluntarily participated in the abdominal examination. The classification of CE images followed the criteria proposed by the World Health Organization (WHO) (5), and that of AE images followed those recommended by Xu and associates (6). All individuals were registered by name, age, sex, occupation and any previous history of the disease recorded (usually surgical). Venous blood samples were taken from the same participants. A total of 1,549 residents living...
in three villages (Waeryi, Baiyu and Suohurima) and a county town were screened by abdominal US, and 1,113 participants agreed to give blood samples for serology in October 2005.

**Serologic tests:** A conventional indirect hemagglutination (IHA) test was made for serum antibody detection for all samples. For the IHA test we used crude antigen, which was sterile *Echinococcus granulosus* hydatid cyst fluid from the livers of infected sheep. Serum samples of those cases that showed positive US images with CE or AE were used for a confirmatory test by the *Echinococcus* Western Blot IgG (LDBIO Diagnostics, Lyon, France). Serum samples of those cases that showed negative US images with CE and AE but that were positive in the IHA test were also evaluated by an immunoblot assay. This immunoblot assay, using a whole larval extract from *Echinococcus multilocularis* as the antigen, was carried out according to the manufacturer’s instructions (7).

**Survey of animals:** As the definitive host for both *E. granulosus* and *E. multilocularis*, the local dog population was screened for infection. Because of religious beliefs, the Tibetan people did not allow the investigators to conduct postmortem diagnosis on their house dogs, so an autopsy survey was performed on a limited number of stray dogs captured around the villages.

Canine fecal samples were collected from around the houses/villages and subjected to coprological diagnosis. *Echinococcus* coproantigen was screened using an ELISA kit from the Sichuan Provincial Institute of Parasitic Diseases (8). Microscopic egg detection was also conducted with a modified Wisconsin procedure using sucrose solution with a specific gravity of 1.27. Since the eggs of *Echinococcus* are morphologically indistinguishable from the other members of Taeniidae (9), such eggs were subjected to additional molecular analysis. Extraction of DNA from egg materials was performed using a protocol described by Bretagne et al. (10) and subjected to polymerase chain reaction (PCR) as described by Nickisch-Rosenegk et al. (11). Each PCR product of the 12S ribosomal RNA gene was then digested with *Ase*I and *Ssp*I, and individually identified using the species-specific restriction fragment length polymorphism (RFLP) patterns.

Yaks and sheep are the major livestock and are also thought to be important intermediate hosts for *E. granulosus* in the area. The animals slaughtered for human consumption in October 2005, and organs, mainly livers and lungs, were examined macroscopically at the local abattoirs. Occurrence of cystic lesions of *E. granulosus* was recorded.

**Ethical consideration:** Ethical clearance for the investigation was received in July 2005 from the Qinghai Provincial Center for Disease Prevention and Control. Explanation was made for why and how the survey was to be conducted to the leaders of the county and the related townships to help their understanding and gain their collaboration. Considering the high illiteracy rate of the local residents, only oral individual consent was required before the examination. Those examinees with positive US were advised to take treatment with albendazol, which was arranged after the examination at the local health centers.

**Data analysis:** A Pearson’s chi-square test with Yates’ continuity correction (2-tailed) was performed to examine differences between groups. When the overall value of $\chi^2$ was significant, a post-hoc multiple comparison was made using the confidence intervals (CIs) to find the difference in proportions between any two groups. Statistical analysis and calculation of CIs were carried out using SPSS 13.0 (SPSS Inc., Chicago, Ill., USA) and Microsoft Excel 2000 (Microsoft Corp., Redmond, Wash., USA), respectively.

**RESULTS**

**Prevalence of echinococcosis in the human population:** Image examination revealed that among the total population examined by US (1,549), 124 persons showed positive results with 85 cases of CE (5.5%) and 39 cases of AE (2.5%) occupying 69 and 31% of the total, respectively, and revealing an overall morbidity of 8.0%. The incidence in males and females was 6.2 and 9.8%, respectively ($\chi^2 = 6.32, d.f. = 1, P < 0.05$).

Serological tests demonstrated an overall IHA positive rate of 25.8% (287/1,113), with rates of 20.3% in males and 31.1% in females ($\chi^2 = 16.40, d.f. = 1, P < 0.01$). Age distribution of the prevalence is shown in Table 1. The prevalence of CE and AE through US examination by age groups and the relationship between the prevalence of echinococcosis and sex are shown in Figures 2 and 3, respectively.

A significant difference in prevalence by occupation was found in the US examination ($\chi^2 = 90.35, d.f. = 8, P < 0.01$). The highest incidence of the disease was found in herdsmen (16.6%, 81/487), followed by Buddhist priests (15%, 12/78). A comparison of CIs showed a significant difference between herdsmen and others, and between Buddhist priests and others ($P < 0.01$). However, there was no significant difference at within herdsmen and between Buddhist priests ($P > 0.05$) (Table 2). The IHA result showed a positive rate of 36.7, 31 and 16.5% in the three groups, respectively ($\chi^2 = 59.14, 9.03$ and 1.01, $P < 0.01$, $< 0.01$ and $> 0.05$, respectively).

By areas, the US incidence in inhabitants from the three villages and from a county town was 11.9 and 2.7%, respectively, and there was a significant difference between the villages and the town ($\chi^2 = 41.54, d.f. = 1, P < 0.01$). Similarly, the IHA positive rate was 35.1 and 13.8%, respectively.
and this difference was also significant ($\chi^2 = 64.35$, d.f. = 1, $P < 0.01$).

Result of the immunoblot assay: The immunoblot assay was used for analyzing the specific antibody of the serum samples of 93 out of 112 cases showing positive images of CE or AE. Table 3 summarizes the number of serum samples showing different immunoblot patterns described by the manufacturer. The presence of bands at 7, 16, 18 and 26 to 28 kDa is indicative of the presence of *Echinococcus*-specific IgG in serum. The antigen recognition patterns for each of the serum samples and their interpretation are shown in Table 3. The immunoblot assay showed 88% (82/93) sensitivity for the detection of such positive patterns from P1 to P5. P1 and P2 were considered specific for CE, and 33 out of 63 serum samples (52%) from cases with a positive CE image were typed as P1 or P2. P3 was considered specific for AE. Of serum samples from 30 cases with positive AE images, 27 (90%) were typed as P3. Of serum samples from 63 cases with CE images, 5 (8%) were also typed as P3. P4 and P5 were considered to be indistinguishable between the two species. Interestingly, all 17 serum samples typed as P4 or P5 were simply from cases with CE images.

The immunoblot assay was also used for analyzing the specific antibody of the serum samples of 138 out of 175 cases who showed negative by US but positive in IHA. Among these serum samples, the positive rate of the immunoblot assay was 18.1% (25/138): 6 were typed as P1 or P2, 5 were typed as P3, and 14 were typed as P4 or P5.

Survey on animals: Dogs: Of 12 stray dogs captured around the villages, 9 were found to harbor adult worms at autopsy, with 8 cases of *E. granulosus* and one case of *E. multilocularis* morphologically identified. The latter one was further confirmed by experimental infection of mice and dogs (to be reported separately).

A total of 149 canine fecal samples were collected and examined by coprological diagnosis. Of the samples, 14 and 58 dogs were found to be taenid egg (9.3%) and coproantigen (38.9%) positive, respectively. Species identification of each taenid egg by PCR-RFLP analysis revealed that 7 dogs were infected with *E. granulosus*, 2 had *Taenia hydatigena* and 5 showed concurrent infections of the 2 species. Among 10 samples with eggs of *E. granulosus*, 8 were coproantigen positive.

Yaks and sheep: During the study period, 106 of 135 yaks (78.5%) and 95 of 115 sheep (82.6%) from local abattoirs were found to have cystic lesions of *E. granulosus*.

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**Table 1. Results of ultrasound examination and IHA test by age groups**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Ultrasound</th>
<th>IHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of examined</td>
<td>No. of positives (%)</td>
</tr>
<tr>
<td>~9</td>
<td>83</td>
<td>0 (0)</td>
</tr>
<tr>
<td>~19</td>
<td>721</td>
<td>25 (3.5)</td>
</tr>
<tr>
<td>~29</td>
<td>186</td>
<td>18 (9.7)</td>
</tr>
<tr>
<td>~39</td>
<td>283</td>
<td>37 (13.1)</td>
</tr>
<tr>
<td>~49</td>
<td>127</td>
<td>18 (14.2)</td>
</tr>
<tr>
<td>~59</td>
<td>79</td>
<td>10 (13)</td>
</tr>
<tr>
<td>~60</td>
<td>70</td>
<td>16 (23)</td>
</tr>
<tr>
<td>Total</td>
<td>1,549</td>
<td>124 (8.0)</td>
</tr>
</tbody>
</table>

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**Table 2. Results of ultrasound examination and IHA test by occupation groups**

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Ultrasound</th>
<th>IHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of examined</td>
<td>No. of positives (%)</td>
</tr>
<tr>
<td>Buddhist priests</td>
<td>78</td>
<td>12 (15)</td>
</tr>
<tr>
<td>Cadres</td>
<td>62</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Doctors</td>
<td>21</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Herdsmen</td>
<td>487</td>
<td>81 (16.6)</td>
</tr>
<tr>
<td>Houseworkers</td>
<td>37</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Merchants</td>
<td>26</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Students</td>
<td>755</td>
<td>19 (2.5)</td>
</tr>
<tr>
<td>Teachers</td>
<td>56</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Others</td>
<td>27</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Total</td>
<td>1,549</td>
<td>124 (8.0)</td>
</tr>
</tbody>
</table>

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Fig. 2. Prevalence of cystic and alveolar echinococcosis (CE and AE) by ultrasound in age groups.

Fig. 3. Prevalence of echinococcosis by sex and age.
DISCUSSION

The majority of the population in Jiuzhi County are Tibetans with animal husbandry as their primary economy. During the seasons from spring to autumn, the Tibetan families move about in search of pasture with their herds of yaks or sheep. This typical nomadic life, almost always with dogs, and the unhygienic conditions on the grasslands and in their tents form highly favorable circumstances for the transmission of echinococcosis.

The examination by abdominal US revealed an echinococcosis morbidity of 8.0% (124/1,549) in the inhabitants, which is much higher than the rates previously reported in other areas of the province (12), which indicates that Jiuzhi is one of the important endemic areas for human echinococcosis. Among the total population examined by US, 124 people showed positive results, with 85 cases (5.5%) of CE and 39 cases (2.5%) of AE occupying 69 and 31% of the total, respectively. In a co-endemic area of CE and AE like this region, differentiation of the two species of Echinococcus is important. The diagnosis of human echinococcosis remains highly dependent on imaging techniques to detect the larval infection of E. granulosus or E. multilocularis. US is the only technique that can be used in the field and can virtually differentiate hepatic AE from CE (13). However, with an extraparenchymal infection, US examination is not helpful in most cases. In addition, the efficacy of an US-based screening relies on the skill of the ultrasonographer. Therefore, a serological backup test is expected to play a complementary role for diagnosis (14). A commercial test kit, the Echinococcus Western Blot IgG, is available for differentiating CE and AE. Importantly, 27 out of 30 serum samples (90%) from cases with AE images showed the positive pattern of P3, including narrow bands at 16/18 kDa, which was considered to be specific to AE (7,15,16). On the other hand, 5 serum samples (8%) from 63 cases with CE images also showed the same pattern. Apparently, the specificity of the positive pattern P3 needs to be further studied in sera of CE cases in a co-endemic area like Jiuzhi.

Our investigation, like previous surveys, showed that more females were infected than males. This may be because Tibetan women were more involved in taking care of livestock, spent more time with the family dogs and were more closely involved with the environment in and around their houses.

It is interesting to note that the incidence in monks and nuns was among the highest, close to that of herdersmen, which is similar to the results of a previous survey (12). This is most likely related to the monks’ and nuns’ religious beliefs. The killing of animals, especially dogs, is forbidden by the Buddhist practice, and this practice encourages benevolence in the form of feeding dogs and results in a huge number of stray dogs in and around the lamaseries (temples) and therefore a higher possibility of infection passing from the dogs to the monks and nuns.

The IHA test using a crude antigen prepared from E. granulosus hydatid cyst fluid is reasonably sensitive; in our study, 91% (112/122) of the examinees with confirmed clinical diagnosis by US showed positive IHA test results. However, it is well known that specificity of the test is not always satisfactory (17). For those with positive IHA but negative findings by US, an infection or historical infection could not be excluded as the examinees were all native residents. In fact, specific antibodies were detected by the immunoblot assay from 18.1% (25/138) of these serum samples.

The data on age distribution shown in Table 1 shows that the prevalence of Echinococcus infection approached a plateau at the age of 20-29 years by serology and 30-39 years by US. US examination disclosed a much lower prevalence (3.1%) among the examinees under 19 than did the serological test (18.1%), which probably reflects the fact that it takes longer for the development of a hydatid cyst in a host to appear as a distinguishable image than it takes for the appearance of a detectable antibody level, and that attention needs to be paid to adolescents to detect infection early.

A high prevalence in dogs and in livestock also revealed a high endemicity of the diseases. Because of the difficulties in dog deworming, observation was made in only a few dogs, and there may have been a bias toward the high prevalence (75%), as only stray dogs were examined. Coproantigen detection showed a rate of 38.9% positive. Further investigation is needed to interpret the result of the fecal antigen test on the basis of parasitological examination on dogs.

As the previous studies revealed, cystic and alveolar echinococcosis are of major public health importance in north-west China (18,19) and the infections are highly pertinent socioeconomical, ecological and environmental factors (20, 21). The rate and impact of infection in the south of Qinghai Province are similar to those of west Sichuan Province, which also has ethnic Tibetans as the predominant population and coexistence of the two types of echinococcosis (22). Further understanding of the epidemiological factors of echinococcosis will therefore be essential for providing a strategy to control the disease in this region, which probably also covers northern Tibet.

ACKNOWLEDGMENTS

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Technical assistance from Han Xi-Min, Zhang Jing-Xiao, Ma Jun-Ying, Liu Hai-Qing and Kyoko Arakawa is highly appreciated.

REFERENCES


Table 3. The immunoblot results of sera from cases with an image of alveolar (AE) or cystic (CE) echinococcosis at ultrasound examination

<table>
<thead>
<tr>
<th>Cases with AE/CE image</th>
<th>No. of serum samples with the following immunoblot pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested sera</td>
</tr>
<tr>
<td>AE</td>
<td>30</td>
</tr>
<tr>
<td>CE</td>
<td>63</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
</tr>
</tbody>
</table>

"Echinococcus Western Blot IgG pattern" Interpretation

- Negative: No bands in the range of 7 through 28 kDa. : not echinococcosis
- P1: Bands 7 kDa only. : CE
- P2: Bands 7 kDa + fuzzy 16-18 kDa. : CE
- P3: Bands 26-28 kDa + both narrow bands. : AE
- P4: Bands 26-28 kDa only. : CE or AE
- P5: Bands 7 kDa + 26-28 kDa. : CE or AE

93 (in Chinese with English Summary).


