COMBINED ULTRASOUND AND SEROLOGIC SCREENING FOR HEPATIC ALVEOLAR ECHINOCOCOSIS IN CENTRAL CHINA

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Abstract. Alveolar echinococcosis (AE), caused by Echinococcus multilocularis, is a zoonotic helminthic disease that can mimic malignancy. In the 1970s, foci of the disease were found in central China. The aim of the present study was to estimate the prevalence of AE in humans in 2 districts of south Gansu Province, China, by use of ultrasound and Echinococcus serology. After answering an epidemiological questionnaire, 2,482 volunteers from 28 villages underwent ultrasound. Serology via enzyme-linked immunosorbent assay for antibody activity was performed on whole blood collected on filter paper in all subjects; on serum from subjects with an abnormal ultrasound image; and on randomly chosen subjects that either had no lesions or had atypical lesions. At least one (25.3%) abnormal ultrasound image was observed in 630 of the subjects screened. A typical lesion of progressive AE was found in 84 subjects (3.4%). Serologies were positive in 77 (96%) of 80 of patients who had lesions typical of progressive AE. Ultrasound is useful for screening for AE in endemic regions.

INTRODUCTION

Alveolar echinococcosis (AE) is a relatively rare zoonotic helminthic disease that can mimic malignancy. It is caused by the development of the larval form of the canine tape-worm Echinococcus multilocularis in the liver. Invasion of surrounding organs, vessels, or both and distant metastases can result in obstruction of bile ducts and hepatic vessels. The disease can be severe and can lead to death within 5–10 years unless treated. Early detection and treatment of AE has shown to significantly improve survival. Even though the odds of asymptomatic AE lesions progressing to severe disease are not known, it is reasonable to hypothesize that population-based screening is justified to identify asymptomatic forms of the disease in endemic countries.

Areas endemic for AE include the central part of western Europe, parts of the Near East, Russia, and central Asian Republics, northern Japan, and Alaska. A focus of high prevalence of the disease in central China was suggested in the early 1970s by examination of hospital records from Gansu, Ningxia, Qinghai, and Sichuan Provinces. The high prevalence of AE in these provinces was confirmed in the 1990s by a pilot screening program that used a combination of serologic testing and ultrasound examination. The disease can be severe and can lead to death within 5–10 years unless treated.

The disease can be severe and can lead to death within 5–10 years unless treated. Progress in surgical management of the disease and the use of albendazole and mebendazole at high dosages have improved the prognosis of AE. Early detection and treatment of AE has shown to significantly improve survival. Even though the odds of asymptomatic AE lesions progressing to severe disease are not known, it is reasonable to hypothesize that population-based screening is justified to identify asymptomatic forms of the disease in endemic countries.

The screening program was undertaken in 1994–1997 in cooperation with county Public Health Offices, commune (district) leaders, local doctors, and local teachers. Background information and the reasons for the study were also discussed with the local population. The study was approved by the Lanzhou Medical College Ethics and Thought Committee. Informed consent was obtained from all adult subjects and from the parents of minors after translation into the local Han Chinese dialect by staff members of Lanzhou Medical College or by local authorities.

Twenty-eight villages in Zhang and Puma Xian counties populated by Han Chinese peasants were included (Figure 1). The average population per village was 350 inhabitants (range, 200–1,500 inhabitants), and the total population was ~15,500 inhabitants. This area was ~250 km (18 hr by car) from the provincial capital, Lanzhou (34°33 N, 104°34 E), and had a mean altitude of 2,500 m. Ultrasound and serology were performed on 2,482 volunteers (mean age, 41 years; range, 11–72 years; 1,402 men, 1,077 women, with the sex ratio of 1:1.3). The screened population was 16% of the estimated total population, and per village, it averaged 27.5% of the village population (range, 5–92%).

After answering an epidemiological questionnaire, 4 drops of blood were taken from an earlobe of each person and placed onto Whatman number 1 filter paper (Whatman International, Maidstone, Kent, UK). Each patient underwent an ultrasound liver examination during which the clinician used a portable scanner with a 3.5-MHz transducer (Sonoline SX, Siemens, Erlangen, Germany). Examinations were performed in schools, dispensaries, or family houses. Patients

Our aim here is to describe ultrasound images and the AE pathology of asymptomatic patients identified during population screening in south Gansu and to compare the results of ultrasound to those obtained by serology by use of 4 different Echinococcus antigen preparations via enzyme-linked immunosorbent assay (ELISA). Detailed epidemiological transmission and ecological studies are reported elsewhere.
stood for the examination. The liver was scanned in axial and longitudinal sections for the left hepatic lobe and in axial, subcostal, and recurrent subcostal sections for the right hepatic lobe. All examinations were performed or supervised (for teaching local doctors) by a single experienced examiner (B.B.). A 5-mL sample of venous blood (in addition to filter paper blood spots) was taken from patients whose ultrasound image suggested AE; from one out of every 5 people (chosen randomly) with isolated punctate calcifications in the liver; and from one out of every 50 people without evidence of liver lesions.

Serologic tests for AE antibodies were performed on whole blood after dilution of a filter paper blood disk 5 mm in diameter in 250 μL of elution buffer. An ELISA that used a *E. multilocularis* protoscolex fraction as antigen (EmP) was used as previously described. An ELISA that used a crude *E. multilocularis* cyst extract (EmC), the purified *E. multilocularis* antigenic fraction Em2, and *E. granulosus* hydatid fluid (Eg) as antigens in ELISA as previously described. For each antigen-antibody system used in the ELISA, sensitivity, specificity, positive predictive value, and negative predictive value were calculated for the 299 subjects who had venous blood samples drawn. Subjects with typical ultrasound images of AE were considered the gold standard positive cases; subjects with any other lesions or with no lesions at ultrasound examination were considered to be negative cases.

Patients with typical lesions of AE were given albendazole treatment for 6 months; local doctors were in charge of giving albendazole for the next 6 months, and the screening team distributed albendazole for previously screened patients at every survey mission. Albendazole was given to the patients according to the recommendation of the World Health Organization Working Group on Echinococcosis. A list of patients with AE with operable hepatic lesions was scheduled for surgical intervention in Zhang, Puma, or Lanzhou hospitals.

**RESULTS**

At least one abnormal ultrasound image was observed in 630 (25%) of 2,482 subjects examined. The mean time of examination was 1 min per subject and was always < 5 min, even in patients with abdominal ultrasound AE lesions in whom a careful check-up of the lesions was done. An average of 120 subjects (range, 60–180 subjects) were examined per day, typically screened over the course of 8–12
Typical AE lesion with central necrosis: large hypoechoic area surrounded by hyperechoic parasitic tissue (arrows). AE lesion: heterogeneous hyperechoic partially calcified area, 9 cm in diameter (arrows). Infiltrating AE lesion: heterogeneous hyperechoic partially calcified area, 9 cm in diameter (arrows), without central necrosis.

A typical lesion of progressive AE was found in 84 subjects (3.4% of the screened population): 52 women and 32 men, mean age 38.7 years (range, 12–70 years), from 28 villages. Although cases of AE usually presented as a single individual in a household, 4 cases of apparent familial clustering occurred. In 46 patients, the ultrasound image appeared similar to a neoplastic intrahepatic mass with a heterogeneous structure, mainly hyperechoic, containing scattered calcifications, and with irregular, poorly defined edges. A central necrotic cavity with a hypoechoic pseudoliquid structure and irregular borders was observed in 37 patients (Figure 2a). In 54 cases, the infiltrative lesions measured > 10 cm in diameter and invaded or surrounded vascular structures, biliary structures, or both (Figure 2b). In 29 cases, lesions were nodular, 3–6 cm in diameter in the longest dimension, and had calcifications. Twenty-four single AE lesions were located in the right hepatic lobe and 8 in the left hepatic lobe. Involvement of both right and left hepatic lobes by a single lesion was observed in 34 patients. Two or more distinct foci were observed in 18 patients: one focus in right and left hepatic lobes in 13 patients, many foci in the right hepatic lobe in 5 patients (2, 3, and 4 foci in 3, 1, and 1 patients, respectively). In 4 patients, a hyperechoic nodular lesion, 2 cm in diameter, was associated with typical lesions. In one patient, portal hypertension, ascites, and splenomegaly were present. Serology was positive with at least one of the tested antigens in 77 (96.2%) of 80 tested patients with typical lesions at ultrasound examination (Table 1).

Finding calcified lesions in the liver was common in this survey. In addition to calcifications associated with typical aspects of AE lesions, different types of calcifications were observed in 451 examined subjects. They could be divided into 2 subgroups according to size: calcified lesions, 1–3 cm in diameter (Figure 3a), and punctate calcifications, < 1 cm in diameter (Figure 3b). The first type (medium-sized calcified lesions) was observed in 19 women and 25 men (mean age, 30 years, range, 11–60 years) from 22 villages. The calcified focus was unique in 43 cases; 2 discrete foci were observed in one subject. In 40 subjects, the calcified lesion was located in the right liver and in 4 cases in the left liver. The size of the calcified focus was 1–2 cm in 20 cases and 2–3 cm in 25 subjects. A hyperechoic lesion was associated with the calcified focus in 3 subjects.

Serology on whole blood performed by use of the EmP antigen in ELISA was positive in 13 of 44 subjects with medium-sized calcifications. A more specific serology that used EmC, Em2, and Eg antigens in ELISA was performed in 43 subjects on venous blood samples and was positive in

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**Table 1**

Serological results in screened subjects with and without a suspected lesion of alveolar echinococcosis at ultrasound examination*

<table>
<thead>
<tr>
<th>Ultrasound image</th>
<th>No. cases</th>
<th>No. tested sera</th>
<th>Serology on serum samples</th>
<th>Serology on whole blood†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical image</td>
<td>84</td>
<td>80</td>
<td>74 67 74 3 77</td>
<td></td>
</tr>
<tr>
<td>Calcified lesion (1 cm &lt; size &lt; 3 cm)</td>
<td>44</td>
<td>43</td>
<td>13 11 13 6 13</td>
<td></td>
</tr>
<tr>
<td>Punctiform calcifications</td>
<td>363</td>
<td>64</td>
<td>2 2 1 14 6</td>
<td></td>
</tr>
<tr>
<td>Without specific arrangement</td>
<td>31</td>
<td>10</td>
<td>2 2 3 2 1</td>
<td></td>
</tr>
<tr>
<td>With linear arrangement</td>
<td>13</td>
<td>10</td>
<td>1 1 2 4 1</td>
<td></td>
</tr>
<tr>
<td>With circular arrangement</td>
<td>40</td>
<td>40</td>
<td>6 5 8 17 7</td>
<td></td>
</tr>
<tr>
<td>Nodular hyperechoic lesion</td>
<td>40</td>
<td>40</td>
<td>6 5 8 17 7</td>
<td></td>
</tr>
<tr>
<td>Other lesions (unrelated to AE)</td>
<td>68</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>No abnormal image</td>
<td>1,852</td>
<td>52</td>
<td>1 0 1 1 1</td>
<td></td>
</tr>
<tr>
<td>Total (subjects)</td>
<td>2,482†‡</td>
<td>299</td>
<td>99 88 102 47 106</td>
<td></td>
</tr>
</tbody>
</table>

* αass. = associated with a positive serology using EmC, Em2, or both; Eg = Echinococcus granulosus crude antigen (hydatid fluid); EmP = Echinococcus multilocularis protoscolex purified antigen, according to Craig and others; Em2 = Echinococcus multilocularis purified specific antigen, according to Gottstein and others; EmC = Echinococcus multilocularis crude antigen.

† The data only refer to those subjects with venous blood samples.

‡ The number of subjects is lower than the number of cases because several screened subjects were found to have various types of lesions at ultrasound examination.
19 cases; in 6 subjects, the serology was positive only when Eg was used as antigen (Table 1). In 407 subjects (15% of screened subjects), one or more punctate calcifications were observed (< 1 cm in diameter). They were solitary in 303 subjects and multiple in 104 subjects, and they were located in the right hepatic lobe in 85% of cases. In 44 subjects, these calcified lesions were linear (31 subjects) or circular (13 subjects) (Figure 3c). By use of EmC, Em2, or Eg ELISA, 17 of 64 subjects were seropositive without specific features of the calcified lesions, 4 of 10 cases with linear arrangement, and 5 of 10 cases with circular arrangement.

Most often, the positive serology was observed only when Eg was used as antigen.

Well-delimited, nodular, hyperechoic, angiomma-like lesions, always < 2 cm in diameter, were found in 40 subjects (20 women, 20 men; mean age, 34.6 years, range, 13–65 years), from 25 villages (Figure 4). In 37 subjects, the lesion was unique; the lesion was in the right hepatic lobe in 33 subjects; and there were 2 discrete lesions in 2 subjects, and 3 lesions in one subject. In 5 subjects, the hyperechoic lesion was associated with punctate calcifications. Serology was positive in 23 subjects; in 17 of them, antibodies were only detected by means of E. granulosus cyst fluid antigen (Table 1).

Other asymptomatic liver abnormalities found in the screened subjects are summarized in Table 2. They included biliary cysts in 23 subjects, gallstones in 15 subjects, kidney cysts in 12 subjects, and pleural effusion in 10 subjects. All these subjects had a negative serology.

Among the 1,852 subjects without any ultrasound abnormality in the liver, 2 were determined to have positive serology, as assessed by the EmP ELISA on filter paper blood spot eluates. Examination of one of these people a year after the first screening showed that he was seronegative, and a

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biliary cysts</td>
<td>23</td>
</tr>
<tr>
<td>Gallstones</td>
<td>15</td>
</tr>
<tr>
<td>Common bile duct stones</td>
<td>2</td>
</tr>
<tr>
<td>Polycystic liver/kidney disease</td>
<td>1</td>
</tr>
<tr>
<td>Hepatic anatomical variants</td>
<td>1</td>
</tr>
<tr>
<td>Postcholecystectomy aerobilia</td>
<td>1</td>
</tr>
<tr>
<td>Right kidney cyst</td>
<td>12</td>
</tr>
<tr>
<td>Right nephrolithiasis</td>
<td>3</td>
</tr>
<tr>
<td>Right kidney angiomyolipoma</td>
<td>2</td>
</tr>
<tr>
<td>Schwarts dysplasia</td>
<td>1</td>
</tr>
<tr>
<td>Ascites</td>
<td>1</td>
</tr>
<tr>
<td>Right pleural effusion</td>
<td>10</td>
</tr>
<tr>
<td>Retroperitoneal tumor*</td>
<td>2</td>
</tr>
<tr>
<td>Ovarian cyst</td>
<td>2</td>
</tr>
</tbody>
</table>

* One of these subjects was symptomatic, with evidence of peritoneal carcinomatosis and chronic, progressive illness.
the first screening showed that he was seronegative, and a
careful ultrasound examination could not find any suspect
lesion in the liver or other abdominal locations. Among the
52 subjects without any ultrasound lesion whose serum sam-
ple were tested, 1, 0, 1, and 1 were seropositive when EmC,
Em2, Eg, and EmP were used as antigens, respectively. Cal-
culated from the data obtained in subjects whose both serum
and whole blood were tested in ELISA, sensitivity was 0.92,
0.83, 0.96, and 0.96; specificity was 0.88, 0.94, 0.66, and 0.
87; positive predictive value was 0.75, 0.75, 0.50, and 0.73;
and negative predictive value was 0.97, 0.94, 0.98, and 0.98
when EmC, Em2, Eg, and EmP were used as antigens, re-
spectively.

DISCUSSION

Ultrasonography has been proven to be useful in mass
screening for some infectious diseases involving the viscera,
and because of its low cost, it is particularly useful in de-
veloping countries to diagnose parasitic diseases.3,10 Ultra-
sound has been validated as being a key tool for diagnosing
AE in clinical settings.1–3,11–13 and preliminary reports have
suggested that its use is also appropriate in the field.6 This
study confirms that ultrasound is feasible and efficient in
screening for AE lesions and results in a rapid diagnosis of
the disease at low cost for a large population in an endemic
area. Ultrasound is more accurate than serology, which has
been the most widely used diagnostic tool in AE screenings
for the past 20 years.4,16 The number of such cases, how-
ever, was far lower than that observed in this ultrasound-
screening in hospitals.4,16 Taking typical lesions at ultrasound as the gold standard
for the diagnosis of AE, sensitivity and specificity of the
EmP ELISA performed on whole blood taken on filter paper
were the same as or better than the EmC and Eg ELISA
used in this and other screening programs.4 Em2 ELISA ser-
ology was negative in 13 patients with typical lesions. Em2
ELISA had the lowest sensitivity and positive predictive val-
ue. In addition to typical AE lesions, EmC—and especially
Eg—ELISA were positive in a number of patients with cal-
cifications, characteristic of AE, that masked part of the lesion.
This suggests that the use of crude antigens in ELISA may find “abortive” AE lesions and early cases be-
fore the development of typical images. Abortive cases have
been reported in screening programs that are based on ser-
ology, followed by ultrasound and computed tomography
confirmation in hospitals.4,16 The number of such cases, how-
ever, was far lower than that observed in this ultrasound-
screening. The origin of the numerous punctate calcifications
is more open to debate: tuberculosis is rare in this commu-
nity and should have been visualized as multiple calcifica-
tions scattered in the liver; ascariis infection seems to be the

SCREENING FOR ECHINOCOCCOSIS IN CENTRAL CHINA

27

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nity and should have been visualized as multiple calcifica-
tions scattered in the liver; ascariis infection seems to be the
most probable differential diagnosis because serological cross-reactivity, although rare, cannot be totally excluded.17

Etiology of the nodular hypechoic lesions could only be suggested further by magnetic resonance imaging18 or confirmed by surgical biopsy. Both are difficult because these villages are far from radiological or surgical facilities. Prevalence of hemangiomas of the liver averages 2% in patients with an ultrasound examination in Western countries19; their prevalence in Chinese populations is unknown. In our survey, more than half of the subjects with such a lesion were found to have positive serology (most of them were assessed by means of Eg ELISA), and in 4 patients, a hypechoic nodule was associated with a typical form. Hypechoic nodular appearance of AE lesions has been reported to correspond to early stages of the disease on the basis of observations of recurrence of AE after a so-called radical resection20 or after transplantation from perihepatic remnants.21,22 It must be noted that in our experience, recurrences of AE after transplantation were associated with a positive Eg ELISA serology before the appearance of serum antibodies detected by EmC, and especially Em2, antigens.21,22 At least a part of the nodular hypechoic lesions observed in this screening could be related to AE. Because these lesions develop slowly, ultrasound and serological follow-up of these subjects will be performed every 2 years.

Mass screening for AE by means of ultrasound as a first-intention examination in populations far from any hospital facilities is not only feasible and well accepted by the patient population, but it is also more efficient than serology-based screenings. Serology that uses the EmP antigen in ELISA on whole blood smears may be a useful complementary test. To confirm the interpretation of isolated calcifications as suggestive of abortive forms and of hypechoic nodules as representative of early cases, prolonged follow-up of the screened subjects will be necessary. Such studies will provide a more precise assessment of the prevalence of AE in this Chinese community, which appears to have the highest E. multilocularis infection rate in the world.

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