

Research Article

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## Ultrasound and Serology For Diagnosis of Human Cystic Echinococcosis in An Endemic Zone in China

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### Abstract

Cystic echinococcosis (CE) is a public health concern in China. However, the incidence and prevalence of CE in the Chinese province of Gansu is not well known. CE diagnosis is often limited to ultrasonography (US) and serological tests. We have assessed the use of these two approaches for diagnosis of CE in the Xiahe county (Gannan prefecture, Gansu province), an endemic area in the northwest of China. This was a community-based cross-sectional study involving 972 people selected by multi-stage cluster random sampling. CE was investigated by US and serology, using a commercially available ELISA test. Eighty-four individuals tested positive for serology, out of whom 74 (88.10%) were 21-60 years old. Twelve individuals were diagnosed by US. Overall, seroprevalence was 8.63%, and the rate of individuals diagnosed by US was 1.23%. Seroprevalence was higher among females than males (10.16% vs. 6.5%), while the rate of individuals diagnosed by US was slightly higher among males (1.22% vs. 0.89%). Our

results demonstrate that CE remains prevalent in the Gansu province. Serological monitoring can be a valuable tool for detection of infected individuals.

**Keywords:** Ultrasound; Sera,; Screening; cystic echinococcosis; Gansu province; Endemic

## 1. Introduction

Cystic echinococcosis (CE) is a parasitic zoonosis that affects humans and domestic animals and is mainly caused by infection with cyclophyllid cestodes of *Echinococcus granulosus* [1]. Infection in humans, who are intermediate hosts, occurs by accidental ingestion of the worm's eggs that are released in the stool of animals and end up contaminating foods, water, or soil [1]. The clinical features of human CE range from absence of symptoms to severe disease and eventually death [1]. Echinococcosis affects several rural communities worldwide, mainly due to its high-cost treatment and the usually low income of individuals in these communities [6,8]. CE is currently classified as a neglected tropical disease [7], represents an important public health concern worldwide, and is considered endemic in some regions [1], including Southern Europe, East Africa, Australia, New Zealand, Latin America, and Central Asia. Outbreaks have been reported in the Central Asian region, which includes Mongolia, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, Afghanistan, parts of Iran, and Pakistan [8], leading to substantial health and economic issues [2,3,4,7].

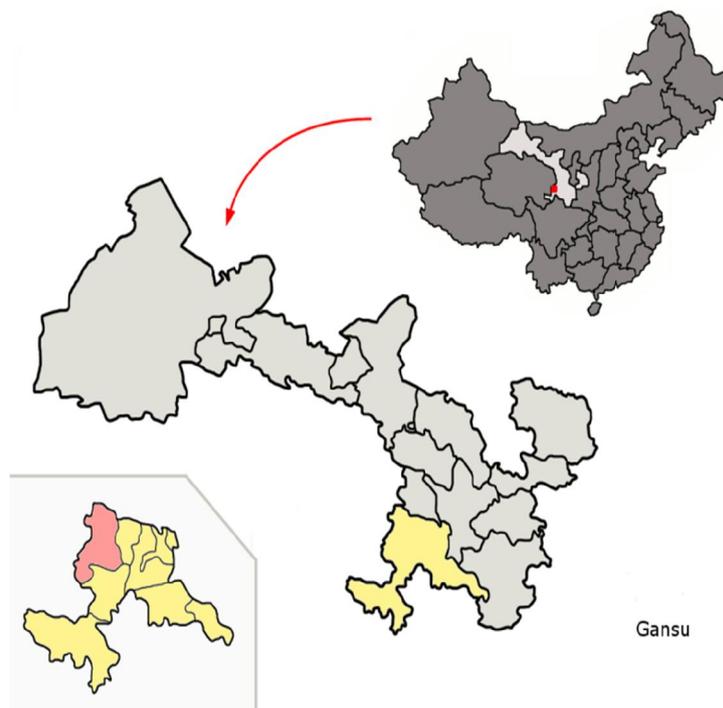
In China, Human CE was reported to be spread in 21 provinces, especially Qinghai, Gansu, Sichuan, Ningxia, Xinjiang, Inner Mongolia, Tibet, and Yunnan (Brunetti et al. 2011), which cover 87% of the total Chinese territory [9-13]. From 2011 to 2015, 16,114 new cases were detected among 9.08 million people. For 263 enteric counties, EC detection rate was 0-11.93%, and the average rate was 0.5%. In 2015, the sera positive rate in case of 4-12 years old patients was 1.22%, of which 1.66% rate was associated with the Tibetan zone [13]. The Gansu province is located near the Tibetan zone, and there are a number of reported cases of human and animal echinococcosis in this region [17]. However, no epidemiological surveys using serological monitoring have been performed in the last 5 years. The present study aimed to investigate the prevalence of echinococcosis in an endemic area of the Gansu province, China, using an enzyme-linked immunosorbent assay (ELISA) based test for detection of specific serum antibodies to *E. granulosus* and ultrasonography (US) for detection of hydatid cysts.

## 2. Methods

### 2.1 Study area and population

The Gansu province is located in the northwest of China, at 32°31'N and 42°57'E longitude, lying among the Tibetan plateau, Loess plateau, Inner Mongolia plateau, and Qinghai-Tibet plateau. Its pasture areas comprise 144.7 million ha, accounting for 31.68% of the total land area, being one of the five largest pasture areas in China. The Gannan prefecture, with an average elevation of 3,000 m and a population of 0.75 million inhabitants, is located in the south of the Gansu province. The pasture area of the Gannan prefecture is located in the edge of the Tibetan plateau. The Xiahe county is located in the south of the Gannan prefecture, has a population of 80.5 thousand inhabitants (2010), an area of approximately 8,038 square kilometers, altitude of 2,200-3,600 m above the sea level, and is home to 18

ethnic groups, which comprise mainly the Tibetans but also the Hans, Huis, and Mongolians. The Xiahe County houses the main pasture areas in the Gansu province, and priority was given to nomadic individuals, among whom the prevalence of hydatid disease was higher. In 2012, a World Health Organization (WHO) project in China, which focused on CE epidemiology in selected rural communities of the Gansu province was launched aiming at estimating the prevalence of human abdominal CE using ultrasound (US) and ELISA as diagnostic methods. The present study was carried out in the Gannan prefecture and aimed to assess the current prevalence of human echinococcosis based on results of a serological test, US, and clinical status so as to help health policy makers to identify the main public health issues in the community and to elaborate strategies for prevention and control of transmission and dissemination of the parasite in humans.



**Figure 1:** Location of the Xiahe county (pink) and the Gannan prefecture (yellow) within the Gansu province

## 2.2 Serum samples

The study was reviewed and approved by the Medical Ethics Committee of the Beijing Friendship Hospital, Capital Medical University, Beijing, China. Participants or legal guardians (when the participant was less than 14 years old) provided written informed consent.

This was a community-based cross-sectional study and multi-stage cluster random sampling methods were used to determine the study's sample size. The formula " $N = t^2PQ/d^2$ " was used to calculate the sample size, where  $t$  is the theoretical value according to the  $t$  table at a 95% confidence interval,  $P$  is the expected population value of the proportion,  $Q$  corresponds to  $1 - P$ , and  $d$  is the margin of error.

### **2.3 Sampling techniques**

Multi-stage cluster random sampling methods were used. First, three municipalities were randomly selected using SPSS version 10.0 (SPSS, Chicago, IL, United States). Then, 15 villages were randomly selected from all villages of the three selected municipalities, and 972 individuals from 300 households were enrolled in the study.

### **2.4 Diagnostic criteria**

All cases were diagnosed based on the national standardized diagnostic criteria for CE, published by the China Ministry of Health (WS257-2006). These criteria consider the epidemiological history, clinical features, and cysts detected by abdominal US. Epidemiological history concerns any history of exposure to livestock, such as dogs, cattle, sheep, and other domestic animals from endemic areas. Clinical manifestations of hepatic CE include signs associated with hepatic space-occupying lesions or cyst rupture or leakage.

### **2.5 Serological evaluation**

A commercially available ELISA kit for diagnosis of human echinococcosis (Zhuhai Haitai Combined Biotech Co. Ltd, Guangdong, China), with sensitivity of 88.5% and specificity of 91.7%, was used for the serological analyses. The test consists of an indirect ELISA technique, and the antigen is composed of partially purified recombinant protein subunits derived from human hydatid cyst fluid. A total of 2.0 mL of peripheral venous blood was collected from all enrolled participants, from which serum was separated and stored at -80 °C until analysis. Each sample was diluted 1/100 in phosphate buffer saline supplemented with tween (PBST), and 100 µL of each diluted sample were added to the wells of a flat-bottom 96-well microplate (Nunc, Denmark) previously coated with the antigen and incubated at room temperature for 1.5 hours. After washing, horseradish peroxidase (HRPO)-conjugated antibodies (diluted 1/1000 in PBST) were added, and the plate was incubated at room temperature for 1 hour. After subsequent washing, 100 µL of a chromogenic substrate (o- phenylenediamine dihydrochloride, 0.4 mg/mL + 0.025% hydrogen peroxide in citrate buffer; 0.1 M, pH 5.0) were added to each well and the absorbance values were read at 405 nm on a microplate reader. The cut-off for determining a positive result corresponded to the sum of the negative control serum's mean optical density (OD) and thrice the standard deviation.

### **2.6 Ultrasound (US) examination**

Abdominal US scans were performed by two specialized radiologists using a Terason t2000 + Color Doppler ultrasound (3.5 Hz) system (Teratech Corp., Burlington, MA). In patients with space-occupying lesions due to CE, power Doppler sonography was performed to observe the blood flow distribution in and around the lesions. CE and AE were classified according to the WHO criteria and the criteria established by the Health Industry Standards of the People's Republic of China. Based on imaging characteristics of the lesion, CE was categorized into the following five types: CE1 (hypodense unilocular cysts), CE2, CE3 (a and b), CE4, and CE5. AE, characterized by irregular lesions, was categorized into three types: infiltrative (heterogeneous, hyperechoic, partially calcified area), calcified (nodular, hyperechoic lesion), and central necrotic fluid (pseudo cystic aspect due to a large area of central necrosis surrounded by an irregular hyperechogenic ring). A total of 972 people underwent abdominal US during the study.

### 2.7 Statistical analysis

Statistical analyses were performed using SPSS version 10.0 (SPSS, Chicago, IL, United States). Associations between the variables of interest and US-confirmed hepatic CE were investigated using univariable and multivariable analyses. Age, gender, living environment, and a number of occupational risk factors were considered as variables of interest, and the chi-square test was used to identify potential associations. Unconditional multivariable logistic regression analysis was used to calculate the odds ratios (ORs) and corresponding 95% confidence intervals (CIs).

For all tests, a P-value lower than 0.05 was considered statistically significant.

Data were analyzed using SPSS version 10.0 (SPSS, Chicago, IL, United States) and EpiInfo (version 7.2.2.6) software (Developed by Centers for Disease Control and Prevention, U.S. Department of Health and Human Services). ORs, calculated by multivariate logistic regression analysis, were used to determine risk factors. A p-value < 0.05 was considered statistically significant and a p-value < 0.10 was considered borderline significant. All the tests were two sided.

### 3. Results

Age distribution: 972 people polled, all ages have distribution, from 5 to 91 years, with 11-50 people questioned most, accounting for 95.06% of the total group (924/972), give priority to with 21-60 patients with positive serum, accounting for 88.10% of the positive serum (74/84), ultrasound in patients with positive was given priority to with 31 to 60 years old, 83% of the patients with ultrasound positive (10/12) (See Table 1).

Regarding gender, 411/972 (42.28%) individuals were male, among whom 27 (6.57%) tested positive for serology and five (1.22%) were diagnosed by US; 561 (57.72%) were female, among whom 57 (10.16%) tested positive for serology and five (0.89%) were diagnosed by US.

Age (years)	N	Positive serology (N)	Diagnosed by US (N)
0-10	9	0	0
11-20	112	3	0
21-30	196	19	1
31-40	298	27	3
41-50	217	14	3
51-60	101	14	4
61-70	33	6	1
>=71	6	1	0
Total	972	84	12

**Table 1:** Age distribution of the 972 individuals enrolled in the study.

Variables	N (%)	Positive serology (N)	Diagnosed by US (N)
Gender			
Male	457(47.0)	27	4
Female	515(53.0)	57	8
Ethnicity			
Tibetan	910(93.6)	83	12
Han	55 (5.7)	1	0
Huizu	7 (0.7)	0	0
Occupations			
Herder	811 (83.4)	73	7
Part-time herder	140 (14.4)	10	5
Peasant	12 (1.2)	1	0
Education			
Student	1 (0.1)	-	-
Preschooler	136 (14.0)	-	-
Illiterate Primary	372 (38.3)	-	-
school	288 (29.6)	-	-
Middle school	143 (14.7)	-	-
High school or above	14 (2.3)	-	-
Religion			
Buddhism	964(99.2)	83	12
Islam	6(0.6)	1	0
Other	2 (0.2)	0	0
Resident			
Permanent residence	423(43.5)	49	2
Nomadism	402 (41.4)	20	7
Temporary residence	132 (13.6)	15	3

**Table 2:** Demographic information of the 972 individuals enrolled in the study.

Regarding ethnicity, 910 individuals were Tibetan, among whom 83 (9.12%) had positive serology and 12 (1.32%) were diagnosed by US; 55 individuals were Han, among whom one (1.82%) had positive serology and none were diagnosed by US; and seven individuals were Hui. Different years' comparison of positive serum and ultrasound data in Gannan prefecture: According to Wang et al. (1995), of 233 people, the IHA positive rate was 51.5%, in 1998, a survey of 933 people, the infection rate of 5.57%, the IHA positive 52 (5.57%), and 2008 Zhao Yumin et al. to 1040 people in Gannan, hydatid prevalence was 2.2%, the survey of 972 people, 84 (8.64%), the number of positive serum B to exceed the number 12 (1.23%) were positive (Figure 2) [30,31].

Xiahe county in Gansu province: In comparing human and animal infection rate. 2008, Zhao Yumin et al studied hydatid infection rate in different animals in the Xiahe region, and found that the infection rate in mouse was 1.15%, the phenol 3.03%, sheep 10.61%, and yaks, Canidae, were 9.16%, 23.38% more than human being of 8.64% (Figure 3) [36,37].

#### 4. Discussion

CE is globally distributed and found in Europe, Asia, Oceania, and South America. China is one of the countries where CE is considered endemic; however, the importance of CE to public health remains overlooked in some areas of China [30,31]. As the early phase of infection and most cases of long-term infection are asymptomatic, many cases are incidentally identified in imaging findings when other conditions are investigated [32,33]. Thus, most cases remain undiagnosed and underreported, and proper diagnosis of human echinococcosis is an essential step to accurately evaluate the true burden of the disease.

Although the prevalence of hydatid disease in the Gansu province has decreased, compared to previous decades, hydatid disease is still considered endemic in this region. However, until the present study, no surveys on hydatid disease had been carried out in the last 5 years in the Gansu province of Xiahe. The present study reveals that human echinococcosis remains a serious problem in the Gansu province and is closely related with animal husbandry, which can be observed by the higher prevalence in pasture than in non-pasture areas, due to the large amount of livestock in pasture areas, like shepherd dogs, which can act as intermediate hosts, infecting yaks and sheep. The frequent and close contact between shepherd dogs and humans was probably the main reason for the high prevalence of human echinococcosis in the Xiahe County.

The seroprevalence of human echinococcosis in the Xiahe county had a slight decrease from 9%, in 1995 [30,31], to 8.63%, in 2013, and the rate of individuals diagnosed by US changed from 0.1%, in 1998, to 1.2345% in 2013, indicating the significant burden of echinococcosis in the last 20 years. Similar with 1.55%, the prevalence rate of five provinces (Xinjiang, Gansu, Tibetan, Qinghai, and Sichuan) surveyed herding families in 2012, higher than 0.4% of Gannan prefecture in 2007, it indicated that studies conducted in Gansu remained a severe public health problem. Here, this is the first time that the prevalence of CE was evaluated using a serological test, for which 8.63% (2013) of individuals tested positive, a higher rate when compared to those found in the Qom Province (1.6%, in 2015), Central Iran [27], Thessaly (Greece) (1.1%, in 2012)[14], Kashmir (5.03%, in 2015), and North

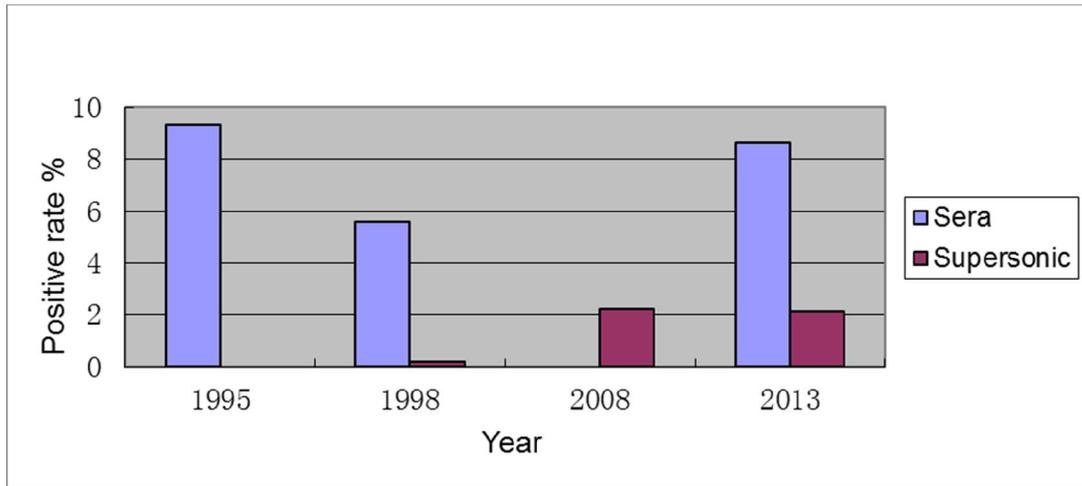
India [15], but lower when compared to that found in the Maqing County (23.8%, in 2015), located in the Qinghai province, 500 km west of the Gannan prefecture.

The highest seroprevalence of hydatid disease in this study was found among individuals aged 21-60 years old (88.95%), as well as the highest rate of individuals diagnosed by US (91.7%). This may be linked to a number of factors, such as culture, lack of basic habits of hygiene, lifestyle, and geographical region. In addition, individuals aged 21-60 years old make up the bulk of the workforce; thus, they are likely to be more exposed to the disease. Khazaei et al. have also reported a higher rate of hydatid disease among individuals within this age range. In their study, 64.5% of individuals aged 20-60 years old tested positive for serology [24].

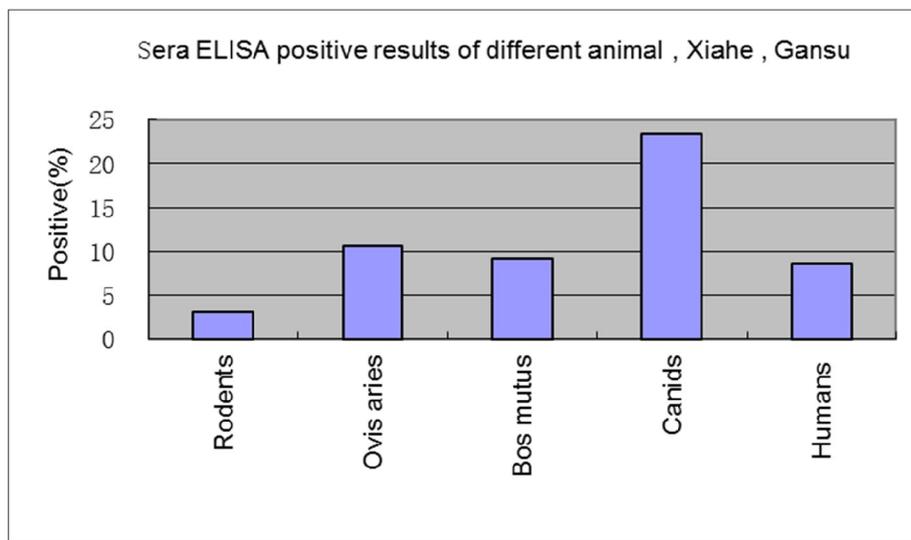
The seroprevalence of echinococcosis was higher among females than males (10.16% vs. 6.5%), although the rate of male individuals diagnosed by US was slightly higher (1.22 vs. 0.89%), which may suggest that women have greater contact with animals than men in the Xiahe county. In an Iranian study, Rokni et al. found that housewives were more likely to come in contact with infection sources, such as vegetables containing parasitic eggs. Additional studies in different countries also found a higher prevalence of echinococcosis in females [19,21,26,35], while other reports showed a higher prevalence in males [19,21,27-29].

Regarding ethnicity, Tibetans accounted for the highest rate of infected individuals in the present study, which was expected as Tibetans make up the majority of the Gansu population, but this does not mean that Tibetans are more prone to develop hydatid disease. The population of the Chinese Han was relatively small, and this phenomenon could not explain why the Tibetan is prone to hydatid infection. Other study reported a higher prevalence of echinococcosis among Tibetan individuals[13]. Such higher prevalence may also be explained by the high number of herders and part-time herders among Tibetan individuals, which implies a greater contact with shepherd dogs and other infected animals.

And of the comparison of infection of human and other animal in this area, we found the *Canids* much higher than human being and other animals, human being similar to the *Ovis aries* and peophagnt mutrs that reminds us the CE remained transmtion more vigorously as the life cycle of *E. granulosus* was actively maintained around canis to human being, *O. aries* and peophagnt mutrs, *Rodents* and *Cricetidae* over the country, maybe the same condition was that of Uzbekistan [22]. It also revealed that in the Xiahe region, echinococcosis remains a serious zoonosis, both for humans and other animals.



**Figure 2:** Prevalence of cystic echinococcosis (CE) in the Xiahe County (Gannan prefecture, Gansu province, China) according to serological tests for *E. granulosus* and diagnostic imaging, from 1995 to 2013.



**Figure 3:** Prevalence of echinococcosis among humans and different kinds of animals in the Xiahe county (Gannan prefecture, Gansu province, China), according to serological (ELISA) tests for *E. granulosus*

Through the comparison of hydatid infection rate in recent 10 years, we found that although the prevention and control of hydatid in Gansu province has started more than 10 years before, hydatid control working remained a long way to go, as long incubation period, children are likely to be infected, adult onset, so the primary and secondary school students knowledge of prevention and control of echinococcosis propaganda was very important.

The sample addressed in the present study was not large, which may have limited our analyses. Overall, we provide a survey on the prevalence of CE in the Xiahe county (Gannan prefecture, Gansu province, west of China) using an ELISA test and US findings, in which we found a relatively high rate of infected individuals. CE eradication is still far from being achieved, and the present findings emphasize the importance of measures for prevention and control of echinococcosis in the Chinese province of Gansu.

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### **Compliance with Ethical Standards**

#### **Disclosure of potential conflicts of interest:**

Data curation: Dan Li.

Formal analysis: Qi Gao

Investigation: Yu Feng, Wenhua Ning, Yanqing Dong, Jingyi Li.

Methodology: Qi Gao, Wenjun Xu.

Project administration: Deli Xin.

Writing – original draft: Jian Liu.

**Research involving Human Participants and/or Animals:** The study was reviewed and approved by the Medical Ethics Committee of the Beijing Friendship Hospital, Capital Medical University, Beijing, China.

**Informed consent:** Participants or legal guardians (when the participant was less than 14 years old) provided written informed consent.

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