UNIQUE FAMILY CLUSTERING OF HUMAN ECHINOCOCCOSIS CASES IN A CHINESE COMMUNITY

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Abstract. We have identified a significant focus and unusual clustering of human cases of cystic echinococcosis (CE) and alveolar echinococcosis (AE) in the village of Nanwan, Xiji County, Ningxia Hui Autonomous Region, in one of the most highly endemic areas for both diseases in China. The village, a Chinese Hui Islamic community, is composed of 167 members of four extended families. A total of 28 people died (12 of echinococcosis) since the village was first settled in the 1950s. Despite similar life patterns, the number of AE and CE cases occurring in each family was different. Overall, the prevalences of AE and CE were 9% (20 cases) and 5.9% (13 cases), with a combined prevalence of 14.9%. In contrast to CE, a comparison of the prevalence of AE indicated significant differences between the four family clusters. Although suggestive that host genotype might play a role in susceptibility to AE, this hypothesis requires further investigation.

INTRODUCTION

Echinococcosis is a zoonosis caused by adult or larval stages of cestodes belonging to the genus *Echinococcus* (family Taeniidae). Larval infection is characterized by long term growth of metacestode (hydatid) cysts in the intermediate host. The two major species of public health importance are *Echinococcus granulosus* and *E. multilocularis*, which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. They are serious life-threatening diseases, especially AE, and they have high fatality rates and poor prognosis if careful clinical management is not carried out.

Echinococcosis is recognized as an increasing health problem in China with 60 million persons at risk of infection and 1.3 million reported cases, one third of whom are children. Most of the at-risk population is resident in seven provinces/Autonomous regions of central and western China that include Ningxia Hui Autonomous Region (NHAR). Unlike other parts of the world, *E. granulosus* and *E. multilocularis* occur sympatrically in most Chinese disease-endemic areas, including southern NHAR; however, few clinical or community studies have been reported from this area.

We describe here a community investigation of AE and CE undertaken in 2001 and 2002 as part of an ongoing investigation of the epidemiology of echinococcosis in NHAR. The focus was Nanwan, a small village situated in the northeastern part of Xiji county and inhabited only by Chinese Hui, a minority Islamic group (Figure 1). The village and surrounding farmland, which occupy an area of approximately 10 km², are close to the border of Guyuan, another county. Its unique population resulting from a 50-years settlement by only four families gave us the opportunity to assess potential family clustering of echinococcosis in an area highly endemic for both AE and CE. The results of the survey showed a very serious focus of human echinococcosis (both CE and AE), a rare case of AE and CE in a single patient, and an unusual family clustering of AE cases. As a result of the usually low prevalence of AE in disease-endemic areas (mostly western European countries) studied, family clustering of cases has been considered to be extremely rare.

METHODS

**Study area and community.** The village of Nanwan (Figure 1) is located on the slopes of Yueliang Mountain in northeastern Xiji, part of the Liupan Mountain range. Residents are farmers growing crops (potatoes, carrots, soy beans, wheat). In recent years, with dramatic population growth, low economic development and poor communication with the outside, large areas of local forest were cut down for economic purposes and the grassland was transformed into areas for cultivating crops, resulting in serious erosion around Nanwan.

Domestic dogs and cats were previously very numerous (almost every household had one dog and/or cat) although the numbers had decreased dramatically by 1997 when only three dogs remained in the entire village. This was due to the introduction in the early 1990s of poisoned baits to control the large rodent population present in the village. The poisoned baits or rodents were inadvertently eaten by the dogs, resulting in a sharp decrease in their numbers. Sheep farming, home slaughter, and throwing viscera to dogs is a common practice, particularly during religious (Islamic) festival seasons. Other animals such as donkeys, rabbits, cats and horses, although not abundant, were also commonly kept. The whole village used valley rivulets and two shallow ground springs for its water supply that could be accessed by both animals and humans until 1997. Because of food shortages after frequent annual natural disasters, collecting herbs and wild vegetables and hunting wild animals, especially large mammals, were usual daily occurrences. However, some recent changes have
occurred. After 1997, a reticulated water system was set up to provide protection of the water source from contamination by animals. Current extension of plowing fields has resulted in a continuous increase in the local rodent population, and there has been a reduction in the wild fauna because of deforestation. Numerous wild animals including foxes, wolves, rodents, and rabbits previously inhabited the area surrounding Nanwan, where most of the slopes were previously covered by shrubs, forest and grassland.

**Questionnaire and data collection.** A single-page questionnaire was used to register individuals in the survey and record information on each person in the village. The questions were designed to record personal data including name, age, sex, previous and present address, marital status and family lineages, behavioral information, including dog ownership, fox hunting history, and contact with fox skins, eating of wild fruit or vegetables, and a medical history of either AE or CE for subjects and their relatives. All inhabitants ranging from in age from 7 to 70 years were invited to participate in the survey in 2001 and 2002, usually in family groups. The completion of the questionnaire was carried out at an interview mainly with the husbands from each family (total = 23 home units in 2001), with the help of local county leaders. Additional questions included the history of the settlement, land use around the village in the past 30 years (forests, shrub or scrub land, plowed fields and crops), water source(s) for the community, and presence of animal hosts for both *E. granulosus* and *E. multilocularis* in the immediate village environment. All procedures related to the study and the purposes of collecting blood were thoroughly explained to the subjects. They were told that this was totally voluntary and the subjects were informed that, if they refused to partake, it would not affect the
standard care given to patients in the study site. All human adult participants or the parents or legal guardians of minors were then asked to sign an informed consent. The study was reviewed and approved by the Ethical Review Board of Ningxia Medical College and the Regional Clinical Ethical Committee, Besancon, France, according to National Institutes of Health (Bethesda, MD) procedures.

**Screening for human AE and CE.** After completion of the questionnaire, a liver scan was performed on all volunteers by a sonographer using an ultrasound scanner (model 3.5 MG67N-35F2.4; Aloka, Tokyo, Japan). Sonograms were recorded using a thermal printer (Sony, Tokyo, Japan). There is generally no difficulty in differentiating established AE from CE in the liver by ultrasonography, according to a validated procedure. The CE cases were recorded using the World Health Organization Informal Working Group classification of ultrasonographic images. A small sample of blood was taken from the ear lobe onto strips of Whatman (Brentford, United Kingdom) no. 1 filter paper. The strips were air-dried, stored at 4°C, and then transported to the laboratory for specific antibody testing. A 5-mL venous blood sample for serum was taken from any person with an abnormal liver image. The serum was kept at 4°C during transport and then stored at −20°C before testing. An individual was confirmed as having AE or CE because of a pathognomonic image by ultrasound (with supporting serology) or after positive serologic confirmation of a query image. All identified AE cases were offered a minimum treatment of a three-month course of albendazole, and CE cases were advised to seek treatment at local hospitals. Further follow-up of the patients was organized by the investigators.

**Serologic tests.** A rapid dot immunogold filtration assay (dot-immunogold filtration assay) combined test was used to detect antibodies to AE/CE, according to the instructions provided by the manufacturer (Approval no. [2000] 400004; Xinjiang Bio. Ltd., Urumqi, Xinjiang, China). The antigens used in this combined test were crude hydatid cystic fluid, antigen B from hydatid fluid, and a protoscolex extract derived from E. granulosus metacestodes. The Em2 antigen was an affinity-purified cyst homogenate antigen from E. multilocularis. An enzyme-linked immunosorbent assay (ELISA) for serum and filter paper blood samples using E. granulosus antigen B or E. multilocularis protoscolex extract was conducted using the procedures of Craig and others and Bartholomot and others. The native Em18 and recombinant Em18 antigen preparations from E. multilocularis and their use in an ELISA and immunoblotting assay followed the methods of Ito and others and Xaio and others.

**Data management and analysis.** Chi-square values and 95% confidence intervals were calculated using Epi-Info (Centers for Disease Control and Prevention, Atlanta, GA) and SPSS version 11.5 (SPSS, Inc., Chicago, IL) for analyses of prevalence. Multiple variate logistic regression was used to calculate adjusted odds ratios to assess the relationship of dog ownership to Echinococcus spp. infections while controlling for factors as age, sex, and families. The Statistical Analysis for Genetic Epidemiology Release 5.0 program (S.A.G.E. version 5.0, Cleveland, OH) was used for family correlation estimation and homogeneity tests were used in the correlation estimations.

**RESULTS**

**Community survey.** Nanwan village is comprised of four families with 168 inhabitants resident in late December 2001 when the first survey was undertaken. Of these, one 37-year-old man died of AE before the 2002 follow-up survey. Originally, three families (H, W, and M) settled in this village in the 1950s. Family H was composed of three brothers who arrived with their parents, and produced three family lineages (H1, H2, and H3). A fourth, family L, settled in the village in the 1960s. A daughter of family M married a son of family L in the late 1970s. All individuals who were registered as living village residents for the 2001 survey are listed in Table 1. This population included 159 individuals (82 females and 77 males) with ages ranging from 3 months to 70 years for females and 1–60 years for males. There were 68 villagers less than 12 years of age, 4 in family H1, 28 in family H2, 6 in family H3, 16 in family W, 12 in family M, and 2 in family L.

When the 2001 and 2002 community surveys were combined, 113 villagers (72%) ≥7 years of age were examined by liver ultrasound scanning. Of these, 100 provided blood samples for serologic testing. The ultrasound results showed that there were 8 individuals with AE, 11 with CE, and 1 with both AE and CE (Table 1). Information obtained from the questionnaire indicated that one additional AE case and one additional CE case occurred in two family members that were resident outside the village at the time of the survey (Table 1).

<table>
<thead>
<tr>
<th>Family</th>
<th>Registered villagers</th>
<th>Ultrasound</th>
<th>Questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Living resident</td>
<td>Absent</td>
<td>Deceased</td>
</tr>
<tr>
<td>H1</td>
<td>10</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>H2†</td>
<td>61</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>H3</td>
<td>10</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>W</td>
<td>36</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>M</td>
<td>35</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>L</td>
<td>7</td>
<td>0‡</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>159</td>
<td>35‡</td>
<td>27</td>
</tr>
</tbody>
</table>

* AE = alveolar echinococcosis; CE = cystic echinococcosis; AE/CE = mixed/simultaneous infection.
† One male member of family H2 died of AE after the 2001 survey but before the 2002 follow-up survey. Therefore, family H2 was composed of 72 persons in 2002 and the total number of deceased persons in 2002 was 28.
‡ Nine persons were temporarily absent from the village at the time of the 2001 survey. The remaining 26 persons had left permanently.
In addition, the questionnaire recorded 10 AE cases and 1 CE case in deceased Nanwan villagers, with most confirmed by local authority records. Therefore, there were 20 AE cases (9%) and 14 CE cases (6%), including one mixed AE/CE case, in the 221 villagers.

The results of serologic analysis correlated well with those obtained by ultrasound for diagnosis of AE but less so for CE (Table 2). Three villagers were seropositive for antibodies only to *E. multilocularis*, 9 were seropositive for antibodies to both *E. multilocularis* and *E. granulosus*, and 20 were seropositive for antibodies only to *E. granulosus*.

Of the 8 ultrasound-positive AE cases detected by the survey, the one serologically negative individual had fully calcified images (1 × 1.28 cm in the right liver lobes and 0.8 × 1 cm in the left liver lobes). Five of the cases were co-positive by serologic analysis for antibodies to *E. multilocularis* and *E. granulosus*. Of 11 ultrasound-positive CE cases detected by screening, six were seronegative, indicating a false-negative rate of 54%. Three were from family H2, with ultrasound images from two of the cases showing very early cyst development, and the third showing a type 5 CE ultrasound image\(^2\) with multiple, small calcified cysts. The remaining three were males from family M, one elderly person with a type 4 CE ultrasound image (solid mass) and two younger men, one with type 3 and type 4 CE images, and the other with an irregular residual cavity remaining after a previous surgery for CE. Overall, the false-negative serologic rate for AE and CE was 35%. The rate of disagreement between ultrasound and serologic diagnosis was 26%, and this included the seven false seronegative cases described earlier in this report. In addition, there were 19 seropositive individuals who were not confirmed by ultrasound although cysts in other organs other than the liver or abdomen would have been missed.

**Current situation and family history.** The distribution of AE and CE in individuals from the four families representing the entire population of Nanwan is shown in Table 3. Up to 2002, the overall prevalences for AE and CE were 4.7% and 6.2%, respectively, with one simultaneous AE/CE case. The combined prevalence of AE and CE was 11.4% for living family members of Nanwan village (Table 3).

Analysis of the survey data for AE and CE (Table 3) indicated a higher prevalence of AE infection in family H than in the others (\(\chi^2 = 8.36, \text{df} = 3, P < 0.05\)). There was no significant difference for CE prevalence or combined AE and CE prevalence between the four families. There was no significant difference in the prevalence for AE and CE within each family, although family M had a higher prevalence of CE prevalence than of AE (\(\chi^2 = 3.8, P = 0.051\)).

A comparison of accumulative prevalence between and within the four families based on the ultrasound and questionnaire data is shown in Table 3. The prevalence of AE was significantly higher in family H than in the others (\(\chi^2 = 10.29, \text{df} = 3, P < 0.05\)), especially when compared with family W (\(\chi^2 = 5.61, P < 0.05\)). There were no statistical differences in CE prevalence and combined AE and CE prevalence between the various families, except between the families H and W (\(\chi^2 = 4.31, P < 0.05\)). The prevalence of AE was significantly higher than that of CE within family H (\(\chi^2 = 4.95, P < 0.05\)), but not in the other families. Multiple comparisons for AE/CE between and within families indicated that the current (2001–2002) and accumulative prevalences were generally similar, although some differences were apparent (Table 3). Within family H, the accumulative prevalence of AE was significantly higher than that of CE (\(\chi^2 = 4.9, P = 0.026\)). Within family M, the current (\(\chi^2 = 3.80, P = 0.051\)) and cumulative (\(\chi^2 = 0.98, P = 0.32\)) CE prevalences were higher, although not significantly higher, than those for AE.

**Family correlation with echinococcosis in Nanwan villagers.** The questionnaire data were combined with the male/female resident local government registered documents for compilation of family member information. Ultrasound scanning and serologic analysis showed that the confirmed AE and CE cases were mainly distributed in the second and third generations of each family. Because the L and M families were related through marriage, we combined them into one family (L-M); thus, family correlations were determined for three families (Table 4). The results indicated that for AE, sibling relationships (brother-brother, sister-brother, and sister-sister) showed significant correlations with infection, as did the relationship between mother and offspring (son and daughter). In contrast, there was no significant correlation between infection with CE and any of the various family relationships. Using homogeneity tests, we observed that comparisons for parents and offspring showed significant differences (\(P < 0.001\)) in AE infection between mother and son, mother and daughter, father and son, and father and daughter, but not between siblings; no differences were evident between any of the family relationships for CE.

**Risk factors.** The questionnaire data showed that dog ownership and dog ownership history had changed in the recent past. Thus, if the questionnaire for teenagers (young children) showed no dog, while an answer from their parents showed that they had a dog several years ago, the risk factor will be

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**Table 2**

Results of ultrasound scanning compared with serology from the Nanwan, China village surveys (2001 and 2002)*

<table>
<thead>
<tr>
<th>Ultrasound scanning</th>
<th>AE (n=2)</th>
<th>AE/CE (n=5)</th>
<th>CE (n=1)</th>
<th>Negative (n=1)</th>
<th>Total seropositive (n=8)</th>
<th>Ultrasound positive (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>1</td>
<td>17</td>
<td>61</td>
<td>68</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>9</td>
<td>20</td>
<td></td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

* For definitions of abbreviations, see Table 1.
† This indicates positive serologic results for both AE and CE.
‡ Both AE and CE lesions (simultaneous infection) were evident in ultrasound images.
used as a dog owner for all family members. Because of water shortages and limited area for habitation, the entire Nanwan village obtained water from the same valley rivulets and shallow ground springs; thus, all villagers shared the same water supply until 1997. Although general discussions in the village indicated a history of hunting by many older villagers, this practice was completed denied in individual questionnaires. This biased answer probably occurred because hunting of large wild animals is forbidden by the Chinese authorities. Other behavioral traits were comparable because all villagers shared the same religion, poor economy, and education levels. Therefore, logistic regression analysis with dog ownership/ownership history as exposure variables and other potential factors such as age, sex, and families/households was used to calculate adjusted odds ratios to assess relationships of the multiple variants to Echinococcus infection (Table 5). There was no significant difference between the three families for AE or CE infection or echinococcosis when dog ownership/ownership history was compared. The differences that occurred in those less than 30 years of age did not indicate a significant risk factor for CE and echinococcosis. When the family group as a risk factor was compared, the H family, in particular, and the L-M family showed significant associations with AE infection. In contrast, there was no significant association for CE within any of the families.

**DISCUSSION**

This community survey identified a major echinococcosis focus and an unusual clustering of human cases of CE and AE in Nanwan village in NHAR, People’s Republic of China, with a combined prevalence of 11%. Alveolar echinococcosis was a significant cause of death in this village, which was founded in the 1950s. This study confirmed the particular severity of this disease when patients have no access to any type of treatment. The village was composed of four family clusters and the inhabitants lived in the same ecologic and social environment and shared a similar life style and religion. However, the prevalence of AE and CE cases in the four families differed significantly. The most striking feature of this analysis was the clustering of AE cases in family H and the family composition, which showed a correlation between mother-offspring and sibling-sibling. All AE/CE patients were distributed in the second and third generations, which probably reflects higher levels of Echinococcus transmission in the past, compared with the current situation, and provides potential insight into the sibling correlation obtained. There were no significant differences in environment between the families/households detected in this regression analysis, indicating that the difference in prevalence between the families may be due to genetic factors, which could also, at least in part, account for the strong mother-offspring correlation. Other studies have shown only limited correlations of family members being affected by echinococcosis, although village clustering has been reported frequently.5,17,18

The coexistence of both AE and CE in the same community indicates that the environment was conducive to the transmission of both forms of the disease. Diagnosis of a combined case of CE and AE in the same patient, albeit not unique,12 is an example of the infection pressure by both Echinococcus species in this community.

Among the well recognized behavioral factors for both CE
and AE, poor life style of villagers, severe water shortages, contacts with infected dogs, lack of knowledge regarding prevention of echinococcosis, and eating of Echinococcus eggs could play an important role in the existence of both AE and CE. Islamic culture could also play a role in increasing AE prevalence because numerous rodents that may act as intermediate hosts for E. multilocularis. Similar factors were noted in surveys of echinococcosis in southern Gansu Province. The influence of permanent pasture increasing AE transmission was also reported in Europe and on the Tibetan plateau. With regard to human transmission in the future, further landscape and biocological studies will be required to clarify the transmission factors involved and the risks for AE and CE infection at the village level in Ningxia. Persons who are currently less than 12 years of age (40% of the population of Nanwan village) should be screened again because Echinococcus infection may occur early in life and there is a long-lasting latent phase in both diseases.

The unique history of this village, which has a limited number of families, a common life style and culture, a limited mobility outside the county, and an unusually high prevalence of both types of echinococcosis ascertained by systematic combined ultrasound and serologic screening on the living population and medical records for the deceased, allowed us to undertake correlation analysis among family members. To our knowledge, such a family analysis has never been re-
ported for echinococcosis. Genetic variations might account for the prevalence differences evident in this rural group. Genetic correlations with resistance or susceptibility to other, including parasitic, diseases have been recognized and extensively investigated, but they have been largely ignored for echinococcosis. No genetic studies have been reported for CE, although two family-aggregated CE cases have been reported and only population studies are available for AE. Cases of AE observed in central China were shown to be associated with HLA-DR4, but no immunologic correlations were reported. 

Although immunogenetic correlations with AE have been made, until now the absence of a well-studied community with a high prevalence of the disease that shared common risk factors for a prolonged period of time has been an obstacle to any further analysis. In this community analysis, there was a highly heterogenous distribution of prevalence in four families and evidence of segregation of echinococcosis infection in two of the family clusters. This suggests that this familial pattern is most likely due to genetic factors. Future linkage analysis designed to localize the specific loci involved would be informative. Furthermore, whole genome scanning using highly polymorphic genetic markers to define specific loci involved in susceptibility/resistance to AE and CE, may permit identification of the individual genes influencing the progression of these serious diseases. The combination of ultrasound examination, which may assess the actual presence of the disease, and highly specific serologic analysis, which may reflect the contamination pressure and individual resistance/susceptibility to disease development after Echinococcus infection, is particularly appropriate for investigating the variety of factors influencing the etiology of echinococcosis in a community such as Nanwan. The high frequency of positive serologic results without lesions or associations with calcified lesions in healthy subjects living in areas endemic for AE has been stressed in several studies and was found in ours. These subjects and their particular response to various antigenic components of the parasite should also be carefully taken into account in further family immunogenetic studies because they may represent examples of genetically resistant hosts.

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