

Human cystic echinococcosis in nomads of south-west Islamic Republic of Iran

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مسح وبائي مصلي لداء المشوكات المثاني في بدو جنوب غرب جمهورية إيران الإسلامية
عبد الله رفيعي، أحمد حمادي، شريف مراغي، بيجن كيكخائي، فيليب سيمون كريج

الخلاصة: إن مرض داء المشوكات المثاني، ذلك المرض الديداني الحيواني المصدر، هو مرض واسع الانتشار ولاسيما في المناطق الريفية. وقد تَقَصَّى الباحثون مدى انتشار داء المشوكات المثاني البشري بين المجتمعات البدوية في أربعة أماكن من مقاطعة خوزستان، وهي: بهبهان، وشوش، ومسجد سليمان، وإيزه. وقد جمعت عينات دم عشوائية من 3446 فرداً من 700 عائلة بدوية، وتم فحصها لتحري أضداد المشوكة الحبيبية. كما تم لقاء أفراد العائلات لتقييم عوامل الاختطار المحتملة لحدوث العدوى، كالمجموعة العمرية، والجنس، واقتناء الكلاب. وكانت نسبة الإصابة بداء المشوكات المثاني في بهبهان 13.8٪، وفي شوش 17.3٪، وفي مسجد سليمان 17.3٪، وفي إيزه 18.2٪، وهي نسب يُعْتَدُّ بالفوارق في ما بينها من الناحية الإحصائية. ولم يكن هنالك أي تَرَابُطٌ يُعْتَدُّ به إحصائياً بين الإيجابية المصلية لداء المشوكات المثاني وبين المجموعة العمرية أو الجنس أو اقتناء الكلاب.

ABSTRACT Cystic echinococcosis (CE) is a widespread helminth zoonosis, especially in rural areas. We investigated the prevalence of human CE among nomadic communities in 4 areas of Khuzestan province: Behbahan, Shoush, Masjed Soleiman and Izeh. Blood samples from 3446 individuals from 700 randomly selected families were examined for detection of antibody against *Echinococcus granulosus*. Family members were interviewed to assess possible risk factors for infection such as age, sex, dog ownership. The prevalence of CE was 13.8%: 1.9% in Behbahan, 12.4% in Shoush, 17.3% in Masjed Soleiman and 18.2% in Izeh. These differences were statistically significant. There was no significant association between CE seropositivity and age, sex and dog ownership.

L'échinococose kystique humaine chez les nomades du sud-ouest de la République islamique d'Iran

RÉSUMÉ L'échinococose kystique est une zoonose helminthique répandue, en particulier en zone rurale. Nous avons évalué la prévalence de l'échinococose kystique humaine dans des communautés nomades de 4 régions de la province du Khuzestan, à savoir Behbahan, Shoush, Masjed Soleiman et Izeh. Les échantillons sanguins prélevés sur 3446 sujets appartenant à 700 familles sélectionnées au hasard ont été examinés afin de détecter l'anticorps dirigé contre *Echinococcus granulosus*. Les membres de chaque famille ont été interrogés afin d'évaluer les facteurs de risque d'infection possibles comme l'âge, le sexe, la possession d'un chien. La prévalence de l'échinococose kystique était de 13,8 % : 1,9 % à Behbahan, 12,4 % à Shoush, 17,3 % à Masjed Soleiman et 18,2 % à Izeh. Ces différences se sont avérées statistiquement significatives. Il n'est apparu aucune association significative entre la séropositivité à l'échinococose kystique et l'âge, le sexe ou la possession d'un chien.

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Introduction

Human cystic echinococcosis (CE) is the larval stage of *Echinococcus granulosus*. People acquire infection by accidental ingestion of *Echinococcus* eggs voided in the faeces of infected dogs. It is one of the most geographically widespread helminth zoonoses [1]. The disease has a global distribution in most regions where sheep-rearing is a major industry. Human CE is known as an occupational public health problem for farmers and shepherds in endemic areas [2].

CE in the Islamic Republic of Iran is an important but neglected public health and veterinary problem, especially in rural and nomadic communities [3,4]. Human CE has been reported from different parts of the Islamic Republic of Iran [3–5]. In 1998, Saberi et al. reported 13.7% seropositivity in a semi-nomadic community in the country [6]. We have reported an overall 1.7% seropositivity in Khuzestan and 7.7% seropositivity in rural areas of Ahvaz (capital city of Khuzestan province) [7]. As a rule, sheep act as the intermediate and dogs as the main host of *E. granulosus* in the Islamic Republic of Iran. The prevalence of CE in intermediate hosts from different parts of the country indicates an average rate of about 2%–20% [8,9]. The prevalence of *E. granulosus* infection in dogs has also been reported to range from 3.3% to 63.3% in sheepdogs [10]. The same study indicated that 20% of *E. granulosus* infection in dogs was from Khuzestan province, which is in the south-west of the country. Half of the more than 60 million Iranian inhabitants live and work in rural areas as farmers, ranchers and shepherds. Thus CE is a human health hazard and results in economic loss.

Nonetheless, there have been few sero-epidemiological and mass screening studies of CE in the Islamic Republic of Iran. Due

to the importance of this helminth zoonosis and the lack of information about its prevalence in nomadic communities in the country, we conducted a seroepidemiological study from 2001 to 2003 to assess the prevalence of human CE among Khuzestan nomads. We recruited a large sample to determine CE seropositivity and to provide baseline information about the disease prior to the possible implementation of a regional hydatid control programme.

Methods

Study area

Khuzestan is located in south-west Islamic Republic of Iran. This province covers an area of 64 746 km² and has a population of approximately 3 746 772; 48% female and 52% male. It consists of mountainous regions in the north and east, and plateau regions in the south and west. The climate is dry and semi-dry. Four different cities located in the north-east (Izeh and Masjed Soleiman) south-east (Behbahan) and west (Shoush) of Khuzestan province were selected. These cities are known to have 90% of the nomadic population settled around them. Nomads settle in the rural areas around these cities during autumn and summer for 6 months, and thus these communities fall under the governmental administration of the cities for the purposes, for example, of primary health care.

Sample selection

A representative sample of primary health care centres was randomly selected from a sampling frame of all public health care centres in the 4 cities. From the selected health centres, 7000 nomad households were registered from which 700 were randomly selected; thus finally 3446 people were selected for inclusion in the study.

Questionnaire

A questionnaire was designed to provide the demographic details of the respondents and information about the keeping and handling of domestic livestock and dogs. The questionnaire also enquired about the number of previous surgical operations for CE the respondents had had in the past.

Data and blood sample collection

Selected households were visited and the questionnaire completed. Each family member was interviewed face-to-face to determine what factor(s) related to their living environment might predispose them to transmission of *E. granulosus*.

Venous blood samples (5 mL) were taken from all randomly selected family members. All blood samples were transferred to the laboratory on ice on the same day of collection, and sera were collected and stored at -70°C until tested.

Serological test

Different methods have been used for community screening of CE, but good results have been reported using enzyme-linked immunosorbent assay (ELISA) methods with an overall sensitivity of 94% and specificity 90% [11]. Thus all serum samples were tested for IgG antibodies to *E. granulosus* by microplate ELISA as described previously [11].

Briefly, Immulon I plates (Dynatech, United Kingdom) were coated (100 μL /well) overnight at 4°C with antigen B (5 μL /mL) in bicarbonate coating buffer, pH 9.6. Plates were washed 3 times with phosphate-buffered saline (PBS) + 0.1% Tween 20. The plates were then blocked with PBS + 5% skimmed milk for 1 h at room temperature. Sera were diluted 1:100 in PBS, added to the plates, and incubated for 1.5 h at room

temperature. Plates were washed as above, then alkaline phosphate-conjugated anti-human IgG (whole molecule, Sigma, United Kingdom) was used at 1:2000 dilution for 1 h at room temperature. After washing the plate, para-nitrophenyl phosphate (Sigma) in 1 M diethanol amine buffer and 0.5 mM magnesium chloride, pH 9.6, was used as the substrate. Absorbance was measured at 450 nm after 20 minutes using a Dynatech automatic microplate reader.

The positive control consisted of serum samples from surgically and pathologically confirmed CE patients from Khuzestan province ($n = 132$). The negative control consisted of serum samples from blood donors or randomly selected blood samples from endemic normal individuals, who clinically had no cystic lesions ($n = 300$). They were all visited by a general practitioner. The positive-negative cut-off value was calculated as the mean +2 SD of the endemic normal individuals; therefore the ELISA result was on highest achievable test sensitivity so as to maximize the chances of case-finding [12].

Ethical approval

Ethical approval for the study was given by Ethics Committee of Jundishapur University of Medical Sciences. Informed consent was obtained from the individuals or heads of households prior to screening family members.

Statistical analysis

Epi-Info, version 6 was used for data entry from the questionnaires. A *t*-test was used for determination of the significant differences in the antibody responses. Differences between the groups were compared by the chi-squared test.

Results

A total of 3446 individuals provided serum samples which were tested for antibody detection against hydatid cyst by ELISA; 13.8% of those tested were seropositive (Table 1). The ELISA absorbance value for seropositive individuals was +2SD above the mean of the negative control (optical density 0.211 nm). The prevalence of CE in the 4 cities surveyed was 1.9% in Behbahan, 12.4% in Shoush, 17.3% in Masjed Soleiman and 18.2% in Izeh (Table 1) and the differences were statistically significant.

There were no statistically significant differences between CE seropositivity and age group or sex, with the exception of significantly higher infection in females than males in the age group of 31–50 years ($P = 0.04$) (Table 2). However, the rate of seropositivity in females compared with males was not significantly different in the whole community. As regards previous surgery for CE, 10 out of 3446 (0.29%) persons screened had undergone such surgery in the past.

A total of 2863 dogs were kept by the surveyed sample, i.e. approximately 0.8 dogs per person. There was no significant difference between dog ownership and number of kept dogs and CE seropositivity (Table 3). The largest number of dogs per

family was 24 dogs. Dogs were used for guarding the home and livestock and were usually unleashed and even had access to inside the tents and houses. There was a statistically significant association between CE seropositivity and non-home slaughtering ($P = 0.001$) and occupation; shepherds and farmers were more infected ($P = 0.016$) (Table 3).

Discussion

The overall prevalence of human CE among Khuzestan nomads of the Islamic Republic of Iran was 13.8%, which confirms that CE is highly endemic in this population. No previous large-scale CE seroepidemiological study has been conducted among such communities in this country. These communities are similar to other Iranian nomadic populations which suggest all such communities are at high risk of infection because of their specific lifestyle and occupation (mostly shepherds).

A study conducted by Saberi et al. reported 13.7% seropositivity in 1000 individuals randomly selected from among Fars nomadic tribes in southern Islamic Republic of Iran [6]. The ELISA method using antigen B that was used in our investigation is a more sensitive serological

Table 1 Cystic echinococcosis seropositivity among Khuzestan nomads

City	Total no. screened	Seropositive		Seronegative		OR (95% CI)
		No.	%	No.	%	
Behbahan	566	11	1.9	555	98.1	1
Shoush	894	111	12.4	783	87.6	7.15 (3.7–14.2)
Masjed						
Soleiman	1010	175	17.3	835	82.7	10.57 (5.5–20.7)
Izeh	976	178	18.2	798	81.8	11.25 (5.9–22.1)
Total	3446	475	13.8	2971	86.2	

$P < 0.0001$.

OR = odds ratio; CI = confidence interval.

Table 2 Cystic echinococcosis seropositivity by age and sex among Khuzestan nomads

Age group (years)	Total		Seropositive				Relative risk for seropositivity (by age and sex) (95% CI)	P-value	
	Male	Female	Male No.	Male %	Female No.	Female %			
0-4	50	44	12	24.0	7	15.9	19/94	1.66 (0.55-3.3)	0.49
5-14	424	526	68	16.0	74	14.1	142/950	1.10 (0.81-1.5)	0.54
15-30	360	861	46	12.8	105	12.2	151/1221	1.08 (0.77-1.5)	0.66
31-50	232	544	21	9.1	87	16.0	108/776	0.63 (0.4-1.0)	0.04
> 50	215	192	29	13.5	26	13.5	55/407	1.04 (0.62-1.7)	0.88
Total	1281	2167	176	13.7	299	13.8	475 (13.8%)	1.02 (0.85-1.2)	0.85

CI = confidence interval.

test in comparison with other tests [13-16]. However, it should be noted that serological tests for CE may have overall reduced sensitivity when used directly in endemic communities compared with advanced symptomatic cases [11], which suggests that the prevalence of CE may in fact be higher

than our results indicate. The results of our community-based study together with those of other studies mostly of hospital records and the high prevalence of *E. granulosus* in dogs [9,10,17,18] confirm that hydatidosis should be considered a major parasitic

Table 3 Cystic echinococcosis seropositivity by dog ownership, occupation and home slaughtering in Khuzestan nomads

Variable	No tested	Seronegative		Seropositive		OR (95% CI)	P-value
		No.	%	No.	%		
Dog ownership							
0	583	501	85.9	82	14.1	-	0.47
1-3	2617	2258	86.3	359	13.7	1	
≥ 4	246	212	86.2	34	13.8	0.99 (0.67-1.48)	
Total	3446	2971	86.2	475	13.8		
Occupation							
Low risk ^a	1261	1121	88.9	140	11.1	1	0.016
Student	451	391	86.7	60	13.3	1.23 (0.89-1.70)	
Farmer	27	22	81.5	5	18.5	1.82 (0.69-4.88)	
Shepherd	1707	1437	84.2	270	15.8	1.50 (1.21-1.87)	
Total	3446	2971	86.2	475	13.8		
Home slaughtering							
Yes	2953	2553	86.5	400	13.5	1	0.001
No	247	200	81.0	47	19.0	1.095 (1.052-1.139)	
Total ^b	3200	2753	86.0	447	14.0		

OR = odds ratio; CI = confidence interval.

^aChildren under 6 years, employees other than farmers and shepherds.

^bSome data were missing for this variable.

helminth zoonosis in the Islamic Republic of Iran.

Regarding the prevalence of CE in the 4 cities screened, the highest and lowest prevalences were detected in Izeh and Behbahan respectively. We do not have any clear explanation for why the rates between the communities differed. It does not appear to be related to dog ownership, but it may be related to their different lifestyles (for example differences in education, hygiene practices, income, which were evident during sample collection); this needs to be investigated in more detail.

The significantly higher rate of CE seropositivity in females in the 15–30 years age group is in agreement with some other epidemiological studies [15]. However, overall, there was no significant association between CE positivity and sex. This contrasts with some previous studies in which the rate of infection was greater in females than males [9,15,19–22]. The reasons for our result could be due to the free movement of dogs among the community and also the lifestyle and culture of the nomads who live in close contact as a big family from their early childhood and so all members are equally at risk of exposure to infection. Our results indicate that there was no significant difference between CE seropositivity and dog ownership, and also no significant association with the number of dogs owned. This could be explained by the fact that the dogs were unleashed and had free access, even to inside the tents which would put non-dog-owner families at the same level of risk of exposure, which would suggest that shepherd dogs play a role in the transmission of CE in such communities; this is in agreement with previous studies [22,23].

It has been reported that home slaughtering of sheep is also an important risk factor

for CE transmission in nomadic communities [24]; the rate of seropositivity among people who practised home slaughtering was significantly higher than people who did not. This is in contrast to our results as we found that CE seropositivity was a significantly higher among people who did not slaughter at home. This observation suggests that the practice of home slaughtering may promote immunological protection among those exposed to the practice, but this suggestion needs further evaluation.

In conclusion, our study confirms the high endemicity of human CE in Khuzestan nomads. Due to the similar living conditions and culture among Iranian nomadic communities and rural areas high CE prevalence is expected to prevail in all such communities also. It is clear therefore that CE is a major public health problem among Iranian nomadic communities and all family members are at the same level of risk of exposure to *E. granulosus* infection.

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