

Seroprevalence of human cystic echinococcosis in South Kashmir, India

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ABSTRACT

Cystic echinococcosis is a zoonotic disease that causes economic losses and public health problems throughout the globe. Present study was undertaken to estimate the seroprevalence of cystic echinococcosis in humans of South Kashmir and to determine the risk factors associated with this disease. The present study was carried out from April 2017 to March 2018, during which 458 blood samples (from 222 males and 236 females) were collected from selected subjects from 12 villages of four districts along with demographic characteristics. Samples were analyzed by an Enzyme-linked Immunosorbent Assay (ELISA) (commercially prepared kit) for detection of immunoglobulin IgG against cystic echinococcosis. Out of 458 samples, 20 (4.36%) samples were found positive. Results implied that the seroprevalence of cystic echinococcosis in South Kashmir had significant relationship with age, gender and occupation ($P < .05$). Seroprevalence for cystic echinococcosis was significantly higher among males, children, and illiterate persons.

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1. Introduction

Cystic echinococcosis caused by the larvae of cestode parasite *Echinococcus granulosus*, is a significant public health problem and zoonotic disease that occurs throughout the world and causes economic losses and public health problems in many countries. Domestic intermediate hosts (sheep, goats and cattle) are major reservoirs for the disease in humans (Thompson, 1995). This zoonotic parasite maintains its life cycle primarily between domestic dogs (definitive host) and domestic ungulates (intermediate host). Humans become infected by the accidental ingestion of *E. granulosus* eggs shed in the faeces of infected canids resulting in cystic echinococcosis (Moro, 2013; Eckert and Deplazes, 2004). Cystic echinococcosis is one of the important and serious diseases in many countries in the world because the migration of infected dogs and livestock. Moreover, it is more prevalent in those areas where intermediate hosts and definitive hosts such as dogs are in close relationship (Zhang and Mc Manus, 2006). The clinical features depend mainly upon the organ involved and mimic many other diseases, thus the diagnosis is difficult and is currently based on identification of the parasite's structures by imaging techniques, including ultrasound, computerized tomography and magnetic resonance imaging (Benito-Carmena and Eraso, 2007). However, these techniques are relatively com-

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plex and do not always provide good prospect for early diagnosis and sometimes produce data that are difficult to interpret, being often confused with abscesses and neoplasm. In addition, imaging technology is not always available in developing countries with inadequately equipped medical facilities (Eckert et al., 2001; McManus and Smyth, 1986). Furthermore, small cysts in the very early stages are not easily detectable by radiological examination (Harandi et al., 2011). Clinical symptoms are caused by these cysts, which are slow growing fluid-filled structures that contain the larvae and are most often located in the liver or lungs called hydatid cysts, they act like tumours that can disrupt the function of the organ where they are found. Therefore, the clinical diagnosis is only based on assumption and needs specific tests. ELISA based tests are available to detect IgG and IgM antibodies to *E. granulosus* (Moro et al., 1997; Sbihi et al., 1996). Serological techniques are also very useful for the follow-up of patients after surgical or pharmacological treatment (Fujimoto et al., 2005; Ma et al., 1997). In many countries including India cystic echinococcosis is important both to human and animal health, because of the morbidity and occasionally mortality cases by the infection of humans with this disease. The aim of this study is to find out the seroprevalence of human cystic echinococcosis and associated risk factors in south Kashmir.

2. Material and methods

2.1. Ethical clearance

The study was approved by the ethical clearance committees of Sher-i-Kashmir Institute of Medical Sciences Srinagar, Kashmir No. SIMS 1131/IEC-SKIMS/2018/334.

2.2. Data collection procedures

The study was conducted from April 2017 to March 2018 by using a simple random sampling from 12 areas in south Kashmir from 4 districts viz., Anantnag (33.73°N 75.15°E, altitude 1601 masl, population 10.8 lakhs), Kulgam (33.64°N 75.01°E, altitude 1739 masl, population 4.24 lakhs), Pulwama (33.87°N 74.89°E, altitude 1630 masl, population 7.77 lakhs) and Shopian (33.72°N 74.83°E, altitude 2057 masl, population 2.67 lacs) Fig. 1. The main occupation in all the four districts is agriculture with the literacy rate 60% average. An informed consent was obtained from subjects before collecting sample from them and for minor consent was obtained from their parents. A questionnaire was designed for each participant, to obtain information regarding demographics data including age, sex, occupation and educational level.

2.3. Sample collection

In the present study 458 blood samples were collected from different districts of south Kashmir using simple random sampling and were asymptomatic. Ten positive participants for ascariasis by stool examination and 10 samples seropositive for amoebiasis, toxoplasmosis were included to assess the cross reactivity. For positive controls 5 confirmed cystic echinococcosis patients were included and 10 healthy participants excluded for hydatid infection by specific antibody detection by ELISA and intestinal parasitic infections by stool examination served as negative controls. 5 ml of venous blood from each individual was collected for serologic testing and then was centrifuged at 2000g for 10mins to obtain serum. All serum samples were stored at -70°C until antibody determination. Specific ELISA was used for detecting specific anti *Echinococcus granulosus* antibodies in the sera of participants as described earlier (Torgerson and Budke, 2003; Chiraga et al., 2014. Specific ELISA used was a commercially prepared kit (Echinococcus ELISA IgG- NovaTec, Germany -D-63128).

2.4. Statistical analysis

Statistical analysis was done using Statistical Package R version 3.5.3. Chi square test with 5% level of significance was used to assess the overall association between seroprevalence of cystic echinococcosis and each of risk factors. Univariate logistic regression is used assess the significance of each factor level with respect to seropositivity. Multivariate analysis was done to eliminate the confounding factors with respect to seropositivity. Adjusted and unadjusted odds ratios for risk factors along with 95% confidence intervals are reported. The results were considered statistically significant when the p -value was <0.05 .

3. Results

Four hundred fifty eight subjects were included in the present study with the grouping in the age range of 1–17 years (150), 18–55 years (156) and > 55 years (152). Out of 458 subjects 222 were males and 236 were females. 20 subjects were found positive out of 458 for cystic echinococcosis IgG antibodies by ELISA with a seroprevalence of 4.37% (females 2.54% and males 6.31%). Present study was conducted in four districts of Kashmir with seroprevalence of cystic echinococcosis in Anantnag (8.00%) followed by Kulgam (6.00%), Pulwama (4.00%) and Shopian (2.00%) (Table 1).

Multivariate statistical analysis was conducted in relation to age, gender, residence. Age group of 1–17 years had significantly higher number of seropositive subjects (RR = 3.237, 95%CI = 0.998–0.51, $P < .05$). Association between gender and cystic echinococcosis were statistically significant ($p < .05$), males were significantly more positive than females (RR = 3.610, 95%CI =

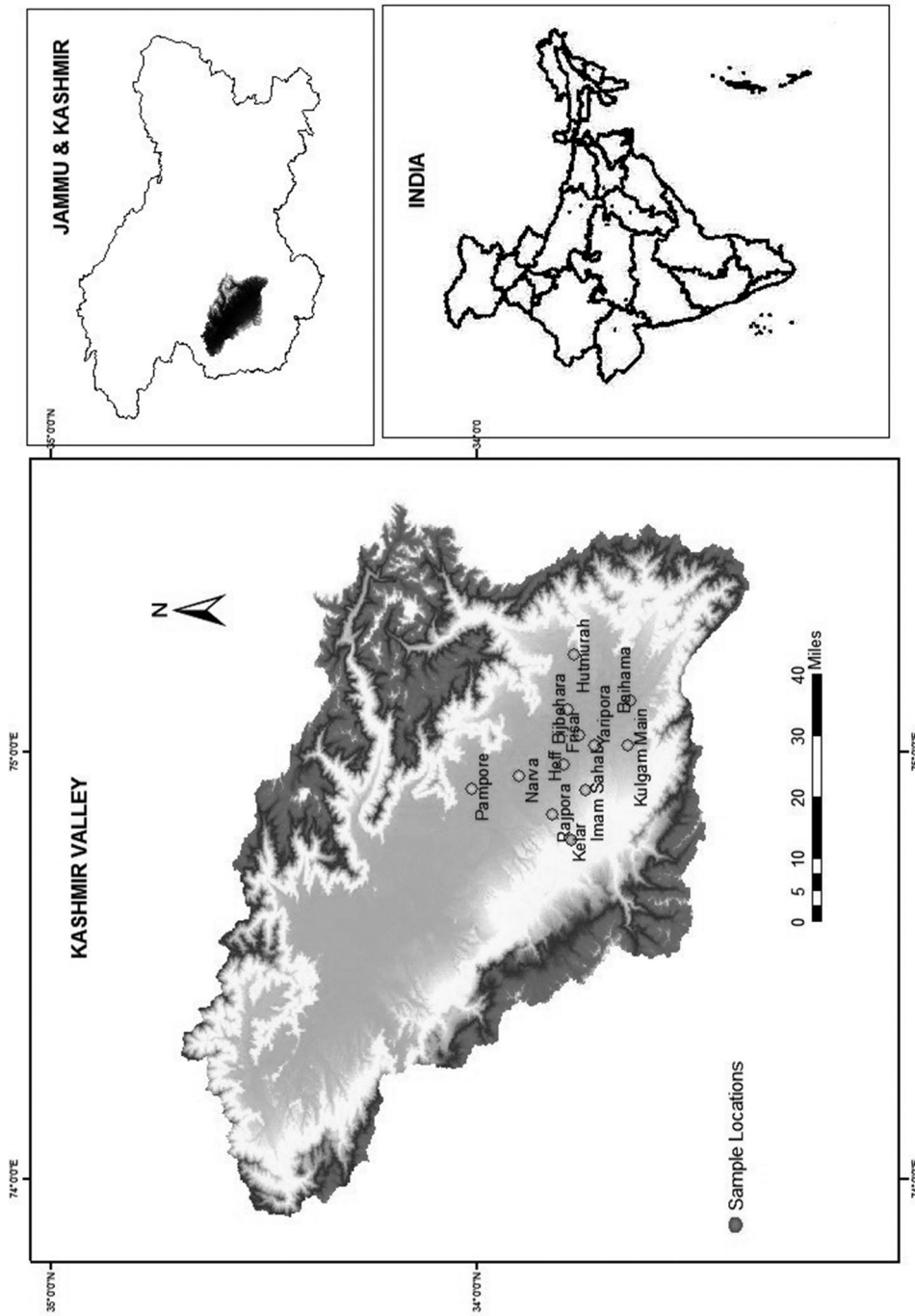


Fig. 1. Map of Kashmir showing sample collection sites.

Table 1

Univariate and Multiple logistic regression analysis for association between the different risk factors and seroprevalence to cystic echinococcosis.

Risk factor	Frequency	(Seropositive/total) %	Univariate analysis			Multivariate analysis		
			Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Sex								
Male	222	(14/222) 6.31%	2.58	(0.974, 6.837)	0.057	3.610	(1.048, 12.43)	0.042
Female	236	(06/236) 2.54%	Reference					
Occupation								
Housewife	100	(2/100) 2.0%	3.02	(0.27, 33.76)	0.003	3.42	(0.381, 30.73)	0.018
Student	96	(12/96) 12.5%	21.14	(2.70, 165.47)		9.48	(2.01, 44.71)	
Farmer	114	(6/114) 5.3%	8.22	(0.98, 69.29)		3.16	(0.608, 16.40)	
Others	148	(0/148) 0.0%	Reference					
Age (years)								
1–17	150	(12/150) 8.0%	6.52	(1.43, 29.66)	0.015	3.237	(0.998, 10.51)	0.052
18–55	156	(06/156) 3.8%	3.01	(0.596, 15.10)		1.951	(0.524, 7.274)	
> 55	152	(02/152) 1.3%	Reference					
District (Location)								
Anantnag	118	(8/118) 6.8%	4.00	(0.831, 19.26)	0.328	3.27	(0.65, 16.48)	0.150
Kulgam	116	(6/116) 5.2%	2.04	(0.365, 11.35)		2.05	(0.358, 11.74)	
Pulwama	112	(4/112) 3.6%	3.01	(0.593, 15.19)		3.16	(0.593, 16.83)	
Shopian	112	(2/112) 1.8%	Reference					

1.048–12.43, $P = .04$). Place of residence and age are high risk factors of cystic echinococcosis with significant association (P -value $<.05$) (Table 1).

4. Discussion

Cystic echinococcosis, caused by *Echinococcus granulosus* is one of the most important zoonotic diseases and having worldwide distribution with the highest prevalence in Russia, China, north and east Africa, Australia, South America and Europe. In India the annual incidence varies from 1 to 200/100,000 population. High prevalence was reported from Kashmir, Andhra Pradesh, Tamil Nadu and Central India (Parikh, 2012).

In the present study specific ELISA was used which is highly sensitive and specific technique for detection of Cystic echinococcosis specific antibody. Kaur et al., 1999 reported a 100% sensitivity and 90.27% specificity for the standard ELISA as compared to rapid ELISA (82.3%) and IHA (70.58%). Chiraga et al., 2014 reported a sensitivity of 95.12% and specificity of 87.5% using crude antigen. Although ultrasonography is essential to reveal cases which should be benefited by treatment, serology may provide useful data for assessing the actual infection.

The overall seroprevalence of human cystic echinococcosis in this study was determined as 4.40%. However, these seroprevalence rates are not as high as those reported from other countries in the Mediterranean basin like Greece where a seroprevalence rate of 29.00% has been reported in humans (Sotiraki et al., 2003). Furthermore our results shows the range of <17 years old as the age group of the highest incidence because of having highest chance of contact with sources of infection such as dog, soil, vegetable etc. Dogs defecate in open areas like parks and playground, these are preferred sites where children play thus are exposed to the eggs of parasite. Parasitic eggs can survive and remain infective for months under favourable conditions such as high humidity and low temperature (Siddharth et al., 2012). Infection due to *Echinococcus* in the initial stage remains asymptomatic for many years until cyst development. The disease symptoms are caused by the cysts, which are slow growing fluid-filled structures that contain the larvae and are most often located in the liver or lungs called hydatid cysts, they act like tumours that can disrupt the function of the organ where they are found, the cysts occasionally rupture and cause severe allergic reactions in humans (Jernej et al., 2008). In many developing countries like Bulgaria the annual incidence of cystic echinococcosis in children increased from 0.7 per 10^5 in 1971–1982 to 5.4 in 1995 (Todorov and Boeva, 1999).

In this study, the seropositivity among asymptomatic males was significantly higher ($P = .049$) than in asymptomatic females. An acceptable justification for this phenomenon might be probably because men are more involved in farming and herding livestock, as well as more likely to have contact with dogs or contaminated vegetables during food processing and preparation and increased susceptibility of male to *Echinococcus* infection. However, our observations does agree with the findings of Torgerson and Heath, (2003) and Heidari et al. (2011) who reported high rates of infection in males than females. Our finding is in sharp contrast with (Yang et al., 2008; Craig et al., 2007; Maysara et al., 2013) who reported echinococcosis predominantly in females because of different geographical locations and different lifestyle. Our results indicate that there was no statistically significant association between cystic echinococcosis and place of residence which is in accordance with Yang et al. (2008) who showed no significant association between cystic echinococcosis and region that may be due to different geographic conditions and dog human ratio of the region. In present study higher number of seropositive asymptomatic subjects clearly shows that unhygienic practices and habits being adopted.

Seroprevalence of cystic echinococcosis had significant relationship with the level of education. Among 20 seropositive persons in south Kashmir 15 (75.00%) were illiterate, this finding supports the relation between health education, hygiene and chance of

getting infection. Our results are in conformity with the findings of Zibaei et al., 2013. Cystic echinococcosis is preventable, it can be achieved through proper education and by imposing strict rules regarding disposal of remains of slaughtered Animals.

In this study 1–17 age group were more likely to be seropositive among different occupations in all four districts of south Kashmir that may have more opportunity to be exposed to echinococcus- infected dogs and the contaminated environment. Students screened were of the age group <17. Thus, those students may have more opportunity to be exposed to cystic echinococcus infected dogs and the contaminated environment Fomda et al., 2015 also has reported <15 years of age as highest infected age group in Kashmir valley. The data indicate that most human infections with *E. granulosus* occur during childhood and adolescence.

Limitation in our study was that only those subjects who were present and who volunteered to participate in the study were examined, so the cystic echinococcus seroprevalence rate may have been underestimated.

5. Conclusion

Based on the results of present study, it can be concluded that seroprevalence of cystic echinococcosis in South Kashmir had significant relationship with age, gender and occupation. The present study also provides information about the cystic echinococcosis in the area, which is valuable in terms of epidemiology. Moreover, there is little previously published study from this region regarding seroprevalence of cystic echinococcosis infection in an asymptomatic population; hence the present study will serve as baseline data for monitoring future changing trends of this infection and thus may help in the formulation of control strategies.

Declaration of Competing Interest

All authors declare that we do not have any conflict of interest.

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