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Seroprevalence and risk factors of human cystic echinococcosis in Central and North Kashmir, India

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Abstract

Cystic echinococcosis (CE) is a dreadful zoonotic neglected disease that affects public health globally. The infection is highly endemic in northern India (Kashmir valley). The present survey based study was done to know the seroprevalence of cystic echinococcosis infection among residents of north and central Kashmir valley and to identify risk variables linked to this infection. In addition to collecting 462 serum samples (244 males and 218 females), a questionnaire was also filled out for each participant utilizing systematic random sampling from June to November 2019 in 16 villages and 6 districts. Using a commercial enzyme-linked immunosorbent assay (ELISA) kit, the samples were tested for immunoglobulin G. Of the 462 serum samples obtained, 33 (7.14 percent) were found to be positive. Results showed that age group, gender, contact with dogs, occupation, and literacy rate were significantly related with the rate of seroprevalence of cystic echinococcosis infection in Central and North Kashmir. Statistics showed that the observations were significant (P< 0.05). Children, men, and those who were uneducated had considerably greater rates of seropositivity for this infection.

Keywords: *Zoonotic, seroprevalence, ELISA, immunoglobulin G.*

The zoonotic infection, cystic echinococcosis, caused by the metacestode stage of the helminth *Echinococcus granulosus*, a cestode worm, is a noteworthy public health issue that affects people all over the world and is the cause of both economic hardship and public health related issues in numerous nations (Rahimi *et al.*, 2011). There are very few countries that are considered to be completely free of *E. granulosus* (Sréter *et al*., 2003). A crucial fact to keep in mind is that grazing areas where dogs are able to consume infected animal offal often coincide with those regions where the incidence of *E. granulosus* infection is high (Thompson, 1995). Dogs and

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carnivores serve as the pathogen's final hosts, although a variety of cattle and people serve as its intermediate hosts (Eckert and Deplaze, 2004). By accidentally consuming the larvae containing embryonated eggs of *E. granulosus* through contaminated food, water, and soil, or by coming into direct proximity with dogs, humans can contract cystic echinococcosis, a disease that naturally spreads from predatory animals to domestic ruminants (Pednekar *et al*., 2009; Moro and Schantz 2009; Salem *et al*., 2011; Wang *et al*., 2014). Cystic echinococcosis infection is a complicated, dreadful and neglected infection that mainly affects the important body organs like liver and/or lungs. They constitute a public health issue, affecting more than one million population (World Health Organization, 2011). Cystic echinococcosis infection is a complex and significant infection in a number of the countries around the whole world where the migration of canines and livestock is more dominant (Zhang and McManus 2006). In human beings, the cystic echinococcosis infection can cause a deadly disorder associated with clinical symptoms like liver malfunction, pulmonary swelling (Brehm *et al*., 1999), and breakage of the cysts of hydatidosis, which may bring about the anaphylactic shock (Regassa *et al*., 2010; Mandal and Mandal, 2012). Moreover, the infection may badly affect the welfare, socioeconomic pursuit of the humans or at-risk person (Battelli, 2009). Diagnosis is presently based on imaging methods, involving ultrasound, computerized tomography and magnetic resonance imaging (MRI) technique (Carmena *et al*., 2007). However, although being more advanced, these methods of diagnosis do not always provide the best opportunities for early detection, and the information they produce can be challenging to interpret mostly because it is frequently confused with information from tumours and abscesses. Furthermore, imaging techniques are not always available in poor countries with inadequate medical infrastructure (Eckert *et al.,* 2001; McManus and Smyth,1986). Furthermore, small hydatid cysts that occur in the early stages are very difficult to detect using the radiological observation method (Harandi *et al*., 2011). These cysts act like tumour growths that can disrupt the proper function of the body organs where they are found. Anti-echinococcus Ig-G and Ig-M immunoglobulins to *E. granulosus* can be determined by immunochemical techniques like ELISA, which are readily available (Moro *et al*., 1997; Sbihi *et al*., 1996). Recent studies have also reported that the method of ELISA is more sensitive and specific than the other tests that are available for diagonosis of cystic echinococcosis, particularly lung infection (Wattal, 1986). The primary goal of the present investigation was to identify seroprevalence of human cystic echinococcosis and related risk elements in Central and North Kashmir.

Material and methods

Data collection

The study was done from June to November 2019 by applying random sampling from 16 areas in North and Central Kashmir from 6 districts *viz*., Budgam (33.93°N 74.64°E, altitude 1610 masl, population 7.54 lakh), Ganderbal (34.21°N 74.77°E, altitude 1619masl, population 2.97 lakhs), Kupwara (34.53°N 74.25°E, altitude 1615 masl, population 70 thousand), Bandipora (34.50°N 74.69°E altitude 1581-1578 masl, population 3.92 lakh), Baramullah (34.15°N 74.35°E, altitude 1593masl, population 10.08 lakhs) and Srinagar (34.08°N 74.79°E, altitude 1585 masl, population 11.8 lakh) as shown in Fig 1. The Sher-i-Kashmir Institute of Medical Sciences' ethical clearance committees in Srinagar, Kashmir, gave its approval for the study plan (IEC-SKIMS/2018/334, No. SIMS 1)

Before collecting samples from participants, we received their informed consent; in the case of minors, we also got their parents' consent. A questionnaire was prearranged for each participant, to learn about demographic information, such as age, sex, dog contact, occupation and educational level.

Sample size determination

G*Power version 3.1.9.2 (Faul *et al*., 2007) statistical tool was employed to determine the size of the sample for the study. The formula for sample size determination is given as

$$n \geq \frac{Z^{2}{}_{1-\frac{\alpha}{2}}\, p(1-p)}{d^{2}}$$

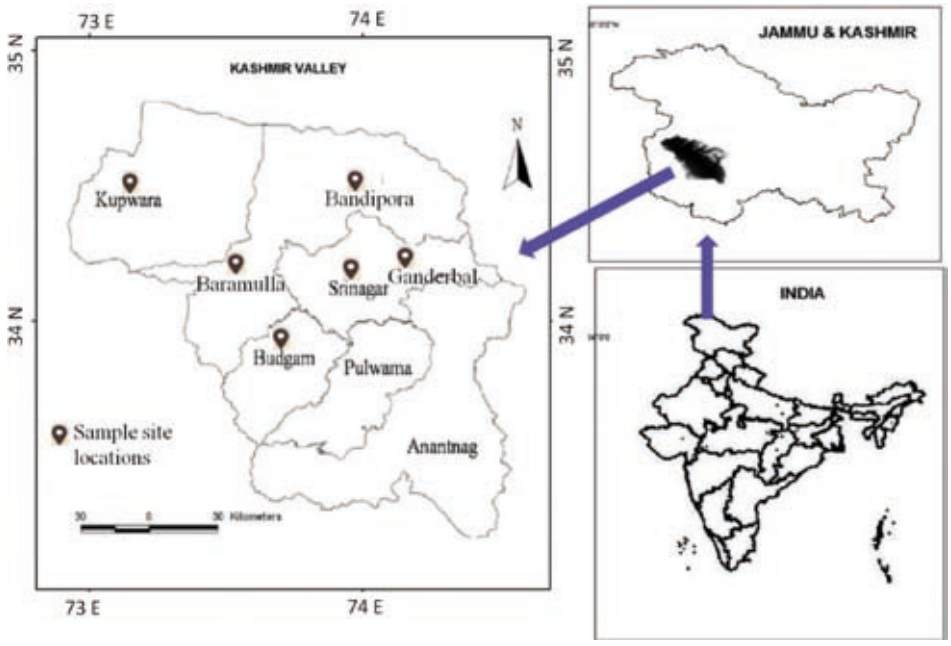

Fig.1. Map showing sample collection sites.

Least sample size required to carry out the study, with extent of significance (α) 5%, permissible error (d) 0.02 and prevalence (p) 5% (Fomda *et al*., 2015) was found to be 462. Random sampling was applied to choose the 462 study participants from various North and Central Kashmir areas.

Sample collection

The four hundred and sixty-two serum samples used in this investigation were all asymptomatic and were drawn from various districts in the North and Central parts of the Kashmir valley. Thirteen positive subjects for ascariasis by faecal observation and 8 samples each seropositive for toxoplasmosis, amoebiasis were collected to study the cross reactivity. Three observed cystic echinococcosis case subjects were included as positive controls and 8 healthy cases that were excluded for CE infection by specific immunoglobulin detection by ELISA method as well as intestinal parasitic diseases by faecal observation served as negative controls. The serum samples were stored at -70 °C until antibody determination. ELISA method was applied to identify specific anti-*Echinococcus granulosus* immunoglobulins in the serum of the individuals (Chirag *et al*., 2015). A commercially available ELISA kit (*Echinococcus* ELISA Ig-G-NovaTec, Germany -D-63128) was applied for the specific ELISA test.

Statistical analysis

Statistical examination was done by applying Statistical Package R version 3.5.3. In order to know the importance of the relation between the seroprevalence rate of cystic echinococcosis and other risk variables in North and Central Kashmir, univariate logistic regression examination was used. Confounding variables that were strongly correlated with seropositivity were eliminated using multivariate binary logistic regression analysis. There are reported adjusted and unadjusted odds ratios for the risk factors, along with 95 percent confidence intervals. The p-value less than 0.05 were applied to know the statistical importance of the results.

Results and discussion

Present survey done in 6 districts of North and Central Kashmir recorded seroprevalence rates of CE in Budgam (11.0%), Srinagar (7.9%), Ganderbal (7.1%), Bandipora (6.6%), Kupwara (6.1%) and Baramullah (4.2%). Age groups of 462 subjects included

in this surveyed study were in the range of <17 years (146), 17–55 years (164) and > 55 years (152). There were 218 women and 244 men among them. By using ELISA, 33 samples out of 462 were shown to have IgG antibodies, with a seroprevalence of 7.14 per cent (females 6.0 % and males 8.2 %).

Table 1 shows the correlation between seroprevalence of cystic echinococcosis and demographic characteristics of the people who were under investigation, including place of habitation, gender, occupation, dog contact, and age group. Gender and cystic echinococcosis were statistically associated (p <0.05). No significant relationship was reported between echinococcosis seropositivity and region (P > 0.05). Age, sex, dog contact and occupation are the positive risk elements of the cystic echinococcosis with significant association (P-value < 0.05) (Table 1).

The results of Univariate and multivariate regression analysis of CE seropositivity by sex, occupation, age, proximity with dogs and district are presented in Table 2 (location). Adjusted and unadjusted odds ratios for risk factors along with 95% confidence intervals are reported. The results were found to be statistically significant when the p-value was <0.05.

Cystic echinococcosis is characterized by hydatid cyst– occupying space in different body organs like lungs, liver and other tissues of the human body (Fakhar and Sajjadi, 2007). In India the yearly occurrence of this disease varies from 1–200/100,000 population. High incidence rate is found from Kashmir valley and the states of Tamil Nadu, Andhra Pradesh, and Central India (Parikh, 2012).

In the present study the immunochemical test ELISA was done for or determining antibody against CE. ELISA is a better technique for the initial screening of the expected cases of cystic echinococcosis due to its higher sensitivity, specificity and simplicity in practice (Rajaji, 2005). Kaur *et al*. (1999) reported a sensitivity of 100% and a specificity

Table 1. Seropositivity of cystic echinococcosis in different groups of Central and North Kashmir

Factors	Category	ELISA				Chi-statistic (p-value)
		Negative	Positive	Total	Positive %	
Gender	Male	224	20	244	8.2	0.352
	Female	205	13	218	6.0	
	Total	429	33	462	7.14	
District	Budgam	65	8	73	11.0	0.738
	Ganderbal	78	6	84	7.1	
	Kupwara	77	5	82	6.1	
	Bandipora	71	5	76	6.6	
	Baramullah	68	3	71	4.2	
	Srinagar	70	6	76	7.9	
	Total	429	33	462	7.14	
Age	<17	121	25	146	17.1	0.001
	17-55	158	6	164	3.7	
	>55	150	2	152	1.3	
	Total	429	33	462	7.14	
Occupation	House wife	163	3	166	1.8	0.001
	Student	81	21	102	20.6	
	Farmer	162	8	170	4.7	
	Others	23	1	24	4.2	
	Total	429	33	462	7.14	
Contact with dog	Contact	271	29	300	9.7	0.004
	No contact	158	4	162	2.5	
	Total	429	33	462	7.14	

Table 2: Univariate and Multiple logistic regression analysis of CE seropositivity by sex, occupation, age and contact with dogs in North and Central Kashmir

Risk Factor	Frequency	(Seropositive/ total) (%)	Univariate Analysis			Multivariate Analysis		
			Odds Ratio	95% CI	p-value	Odds Ratio	95% CI	p-value
Sex								
Male	244	(20/244) 8.2%	1.40	(0.68, 2.91)	0.354	4.28	(1.48, 12.34)	0.007
Female	218	(13/218) 6.0%						
Occupation								
Housewife	166	(3/166) 1.8%	0.42	(0.04, 4.24)	0.632	1.06	(0.07, 14.39)	0.967
Student	102	(21/102) 20.6%	5.96	(0.76, 46.73)	0.089	12.04	(1.25, 116.2)	0.031
Farmer	170	(8/170) 4.7%	1.13	(0.13, 9.51)	0.906	5.16	(0.46, 57.76)	0.183
Others	24	(1/24) 4.2%						
Age(years)								
<17,	146	(25/146) 17.1%	15.49	(3.59, 66.72)	<0.001	9.43	(1.57, 56.57)	0.014
17–55,	164	(6/164) 3.7%	2.84	(0.56, 14.33)	0.204	3.24	(0.61, 17.16)	0.167
> 55	152	(2/152) 1.3%						
Contact with Dog								
Contact	300	(29/300) 9.7%	4.23	(1.45, 12.24)	0.008	5.19	(1.69, 15.91)	0.004
No contact	162	(4/162) 2.5%						
Distric (Location)								
Bandipora	76	(5/76) 6.6%						
Baramullah	71	(3/71) 4.2%	0.62	(0.14, 2.72)	0.533	0.42	(0.08, 2.17)	0.303
Budgam	73	(8/73) 11.0%	1.75	(0.54, 5.61)	0.348	1.72	(0.46, 6.41)	0.419
Ganderbal	84	(6/84) 7.1%	1.09	(0.32, 3.74)	0.888	0.89	(0.23, 3.42)	0.867
Kupwara	82	(5/82) 6.1%	0.92	(0.26, 3.32)	0.901	1.21	(0.29, 4.89)	0.795
Srinagar	76	(6/76) 7.9%	1.22	(0.36, 4.17)	0.755	1.17	(0.29, 4.74)	0.822

of 90.27% for the standard ELISA test as compared to rapid ELISA method (82.3%) and IHA (70.58%).

In this study, the overall prevalence of CE was found to be 7.14 percent. These sero-prevalence rates, however, was lesser compared to that in other regions, such as Uruguay, where a seroprevalence of 20% has been noted in humans (Cohen *et al*., 1998). Highest prevalence was recorded in age group below 17 years old as children have the highest possibility of exposure with sources of disease such as canines, soil, vegetables, etc. Canines act as the main source of transmission of *Echinococcus* species as they defecate in non restrictive fields like playgrounds and parks which are places where children prefer to play thus, they are exposed to the parasitic eggs. These embryonated eggs can remain alive and contagious for months in favorable environments with high humidity and low temperature. Canine existence was thought to be critical for cystic echinococcosis transmission (Wang *et al*., 2014), and many other studies also indicated that dog-related risk factors were primarily significant (Craig *et al*., 2000). Seropositivity in children may or may not be associated to the progression of hydatid cyst types, as an initial exposure can result in progressive abscesses, abortive lesions, or spontaneous cure. Before the cyst appears, echinococcus infection is asymptomatic for a very long time. In Bulgaria, the yearly incidence of cystic echinococcosis in children has raised from 0.7 per 105 between 1971 and 1982 to 5.4 in 1995 (Todorov and Boeva, 1999) in this survey males had notably more seropositivity than females (P = 0.007). A possible justification for this observation could be the fact that because males are more involved in farming and herding livestock. Furthermore, males are more likely to come into touch with canines or contaminated vegetables during food processing and preparation, and therefore are more susceptible to *Echinococcus* infection. Our findings are in line with those of Heidari *et al*., (2011) who observed that males had a higher incidence of CE than females. However, Bchir

et al. (1988) Shambesh, *et al*. (1992) and Bai *et al*. (2002) reported CE to be more in females than in males. Our findings suggested that there was no statistically significant relation between seropositivity to CE and place of residence. Cystic echinococcosis seroprevalence rates in Budgam, Srinagar, Ganderbal, Bandipora, Kupwara and Baramullah were 11.0 percent, 7.9 percent, 7.1 percent and 6.6 percent, respectively. Our observations are in line with those of Yang *et al*. (2008), who reported no conclusive correlation between seropositivity of CE and location, probably as a result of the area's unique geographical features and canine-to-human ratio. The greater prevalence of seropositive asymptomatic patients points to the unhygienic practices and routines that are being adopted. The level of literacy rate and the seroprevalence of cystic echinococcosis were significantly correlated. Among 33 seropositive individuals in Central and North Kashmir, 23 (69.6%) were uneducated which confirmed the correlation between health education, hygiene, and risk of infection. Cystic echinococcosis can be effectively controlled by implementing stringent regulations for the disposal of the offal of butchered ruminants and by providing appropriate education (Mohammed, 2013; Zibaei *et al*., (2012).

According to this study, people aged 17 years and older, engaged in various professions were mostly seropositive in all six districts of Central and North Kashmir. This could possibly be due to a higher risk of exposure to canines that are infected with the *Echinococcu*s sp. and a contaminated environment. The findings of this survey are congruent with those of Saida and Nouradin (2011) who identified the highest prevalence in the age range of <20 years. Besides Fomda *et al*. (2015), had also reported that infection was more in individuals under the age of 15 years in the valley of Kashmir. The data has shown that most human diseases with *E. granulosus* occur during childhood stage and adolescence period. Parasitic infections including CE are typically related with under developed and often marginalized societies where cattle are still butchered traditionally without any municipal veterinary supervision and carcass wastes (offal) are easily available to scavenging canines and other animals. Awareness of the human population in these areas regarding proper control measures is important, since disease occurs through accidental intake of *Echinococcus* eggs. Feeding of carcass wastes to canines should be prevented as this is the major source of transmission of infection. As the findings of this study indicated a high prevalence in young age group, a childhood education programme for cystic echinococcosis control needs to be implemented. Presence of baseline data about *E. granulosus* sero-reactivity and actual infection rates will help in framing the future control plans by utilizing data that will assist in the computation of fairly precise incidence rates. Because only those people who consented and showed up for the study were observed, the seroprevalence rate of cystic echinococcosis may have been inflated in our observation.

Conclusion

The findings of this observation imply that some practices that contribute to *Echinococcus granulosus* transmission still exist in Kashmir's endemic regions. One of the prophylactic measures that should be implemented is to restrict dogs' access to food material and drink that are meant for human consumption. In this survey, children, men, and illiterates had considerably higher seropositivity of hydatid disease. Our findings imply that preventive interventions such as regular veterinary treatment for animals, educational and supportive initiatives aimed at humans, particularly children and those working in farming and animal husbandry should be implemented as a means to reduce human seroprevalence of cystic echinococcosis in Central and North Kashmir. The findings of this type of study will also serve as a basis of management and decision making for Department of Health, and Department Animal Husbandry of J&K and can facilitate the formulation of more comprehensive plans for controlling this disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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