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Prevalence and Mode of Transmission of Echinococcosis in Dogs of Kashmir Valley

Hidayatullah Tak[1], Abas Andrabi[2], Rukhsana Akhter[3], Bilal Ahmad Para[4] and Ishfaq Majeed[5*]

[1]Associate Professor, Department of Zoology, University of Kashmir, Srinagar (J&K), India.
[2]Research Scholar, Parasitology Research Laboratory, Department of Zoology, University of Kashmir, Srinagar (J&K), India.
[3]Department of Pathology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar (J&K), India.
[4]Department of Mathematical Sciences, Islamic University of Science and Technology, Kashmir (J&K), India.
[5]Parasitology Research Laboratory, Department of Zoology, University of Kashmir, Srinagar (J&K), India.

(Corresponding author: Ishfaq Majeed)*
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ABSTRACT: *Echinococcus granulosus* parasite is known to cause echinococcosis in dogs and hydatid disease or hydatidosis in ruminant animals and accidently in humans. In Kashmir valley dogs have a significant role in transmission of zoonotic parasites, as they often come in close contact with local inhabitants, mostly the Gujjars and Bakarwals. Challenges for *Echinococcus granulosus* detection and control exist for Kashmir valley as well as for those nations that may be considering hydatid disease in control now or in the future. A variety of methods are available for its diagnosis in humans but a universal gold standard is lacking. However the use of modern techniques of immunodiagnosis has proved to be the best tool for the diagnosis of intestinal echinococcosis at a larger scale and thus resulted in conducting epidemiological studies on large number of individuals. The prevalence of Echinococcosis infection in canines was determined by examining fecal samples collected from different districts of the Valley. For detection of coproantigens of this helminth in dogs an immunodiagnostic test sandwich ELISA was used. A total number of390 dog fecal samples were tested (from December 2021 to October 2022) and out of them 38 samples were found to be positive in sandwich ELISA., the fecal sample collection was made from different collection sites like streets, playgrounds, open fields, parks, etc.

Keywords: Zoonosis, Sandwich ELISA, Coproantigen, Kashmir.

INTRODUCTION

Echinococcus granulosus is a tapeworm belonging to the family Taeniidae. This zoonotic parasite maintains itself primarily in a life cycle between domestic dogs (definitive host) and domestic ungulates (intermediate host). This tapeworm has been found to cause Echinococcosis in dogs and hydatidosis in ruminants and accidently in humans (Chhabra and Singla 2009). The two states of Andhra Pradesh and Tamil Nadu have been found to have the greatest prevalence of echinococcosis in India when compared to other regions of the nation (Nepalia *et al.,* 2006). There are also various reports from Kashmir, where studies on different aspects of the same parasite were conducted (Fomda *et al*., 2015; Chisti *et al.,* 2000; Fomda *et al.,* 2002). However, adequate data on the incidence and prevalence of canine echinococcosis infection in Kashmir Valley are still lacking. In Kashmir valley, dogs have a significant role in transmission of zoonotic parasites, as they are in close contact with humans, but people are less interested in controlling Echinococcosis. In definitive hosts (living dogs) the small proglottids discharged along with the dog feces are usually overlooked so the diagnosis of intestinal Echinococcosis infection is very difficult. Routine coprological techniques can not differentiate their eggs with other eggs of Taenia species because the eggs of *Echinococcus* show an extreme morphologic similarity with them (Dinkel *et al.,* 1998). Purgation with arecoline compounds and necropsy of the small intestine of canine definitive hosts are two of the most frequently used diagnostic techniques in dogs (Unruh *et al.,* 1973; Craig *et al.,* 1995). Necropsy is regarded as the gold standard and the preferred method; however both of these approaches have a number of drawbacks (Jenkins *et al.,* 2000; Lopera *et al.,* 2003). However the use of modern immunodiagnostic techniques lead to the improvement of detection of *Echinococcus* spp. in definitive hosts (domestic and wild carnivores). A very good approach in this field is the detection of parasitic antigens from fecal samples (coproantigens) by the ELISA immunoenzymatic assay. It has been noted that this method has already demonstrated its value for the diagnosis in both live and dead animals. The enzyme-linked immunosorbent assay (ELISA), which is used for the detection of *Echinococcus* specific coproantigens, has been considered as a novel approach for the diagnosis of intestinal Echinococcosis infection (Deplazes *et al.,* 2003). Babos and Nemeth were the

first to describe the discovery of parasite-specific antigen in host faeces for canine *Echinococcus granulosus* (1962). When looking for coproantigens in feces, ground-collected fecal samples have more epidemiological significance (Cavagion *et al.,* 2005). Adequate information on the incidence and the prevalence of echinococcosis infection of different dog types in our Kashmir valley is not yet available. Therefore, the present study is undertaken to examine the prevalence of Echinococcosis and its mode of transmission from canids to cattle in the valley.

MATERIAL AND METHODS

Study design. A cross-sectional study was carried out in order to acquire latest information on prevalence of echinococcosis in dogs. The samples were collected from nine (09) districts of the Kashmir valley using simple random sampling *viz.*, Anantnag (33 '73° N 75' 15°E, with altitude of 1601masl and population of 10.8 lakh), Pulwama (33'.87°N 74'.89°E, with altitude of 1630 masl and population of 7.77 lakh), Shopian (33.72°N 74.83°E, with altitude of 2057 masl and population 2.67 lakh), Srinagar (34'.08°N 74'.79°E with altitude of 1585 masl and population of 11.8 lakh), Ganderbal (34.21°N 74.77°E, with altitude of 1619 masl and population 2.97 lakhs), Budgam (33.93°N 74.64°E, with altitude of 1610 masl and population 7.54 lakh), Baramullah (34.15°N 74.35°E, with altitude of 1593 masl and population 10.08 lakhs), Kupwara (34'.53°N 74.25°E with altitude of 1615 masl and population of 70 Thousand) and Bandipora (34.50°N 74.69°E, with altitude of 1581-1578 masl and population 3.92 lakh). The main occupation of locals in all these nine districts is based primarily on agriculture.

Sample size determinations and sampling method. Sample size was determined by considering 50% expected prevalence rate and 95% confidence interval with a 5% desired absolute precision (Thrusfield, 2005). Thus,

$$N=\frac{1.962\ [Pexp*(1-Pexp)]}{d2}$$

Where: N= is the required sample size
Pexp = the expected prevalence (50%)
d = is the desired absolute accuracy (0.05)
z= value at 95% (1.96)

An expected prevalence of 50% was used to increase the degree of precision and considering a 5% absolute precision and 95% confidence level was given 384 sample sizes. To minimize errors 390 samples were collected and examined in the study. These sample sizes were selected by simple random sampling method during the collection.

These 390 samples included in the study were collected from different districts in the Kashmir valley using simple random sampling.

Study duration and Study Methodology. The studies have been conducted during December 2021 – October 2022 in Kashmir valley for detecting eggs and coproantigens of *Echinococcus granulosus*. During the said time period, 390 fecal samples of dogs were examined. 127 samples were collected from south Kashmir, 142 from central Kashmir and 121 from north Kashmir. Fecal samples were collected from different dog types like stray dogs, dogs wandering near butcher shops & slaughter houses, dogs of tribal people especially Gujjars and Bakerwals who visit Kashmir valley in summer season and from pet dogs in different seasons like winter, spring, summer and autumn of different districts of Kashmir valley. Both fresh as well as dried fecal samples were collected during the study. The samples collected were preserved in 10% formalin to inhibit hatching of eggs. After that the fecal samples were stored for at least 14 days at -20°C before being further processed. In first step floatation method (Charles and Josephine 1970; Soulsby, 1982) was employed for isolating parasitic eggs. This method is a qualitative test for the detection of parasite eggs and is based on the separation of eggs from fecal material and then concentrating them by means of a floatation fluid having appropriate specific gravity, so that the parasitic eggs rise to the surface and can be skimmed out of surface film. The commonly used suspending media (floatation fluid), are saturated solution of sodium chloride (NaCl 400g; H_2O 1000ml.) and sugar solution. However during our study it was very difficult to differentiate the *Echinococcus granulosus* eggs from other taenid eggs as the eggs of Taenia species are indistinguishable from each other as well as from other members of Taeniidae family. So to find out the prevalence rate of *Echinococcus granulosus* in the fecal sample of dogs immunodiagnostic technique sandwich ELISA was used for the detection of specific coproantigens of *Echinococcus* spp. from the samples of dog faeces and for this purpose commercially available kit Qayee-Bio Canine *Echinococcus* kit was purchased. The Qayee-Bio Canine echinococcus kit is specially designed for the detection of *Echinococcus granulosus* species. The test was performed by using dog fecal material diluted in the kit's sample dilution buffer as per instructions. After centrifugation for 20 minutes, the supernatants obtained were used for ELISA test. Coproantigen estimation levels were carried out. The interpretation of results was made following the attached protocol from the above producer.

Data analysis. Statistical analysis was done using Statistical Package R version 3.5.3. Descriptive statistics is used to get the prevalence of Echinococcosis infection in dogs in different districts of Kashmir valley. Chi-square test is employed to test the association of Echinococcosis infection in dogs and different locations (districts) and in different dog types in Kashmir valley. The results were considered statistically significant when the p-value was <0.05.

RESULTS

Of the 390 dog fecal samples collected from different areas, 38 fecal samples were coproantigen positive for *Echinococcus* sp., representing overall coproantigen prevalence for *Echinococcus* sp. in Kashmir valley as 9.74% (Table 1). Out of 38 positive fecal samples, district Kupwara was found to have the highest prevalence rate of infection (7/37: 18.91%), followed by district Anantnag (6/39, 15.38%), district Budgam

(5/46, 10.86%), district Bandipora (4/40, 10%), district Pulwama (4/43, 9.30%) district Ganderbal (4/46, 8.69%), district Baramullah (3/44, 6.81%) and district Shopian (3/45, 6.66%), however the lowest rate of infection was found in district Srinagar (2/50, 4%).

Besides, the prevalence rate in the dogs of Gujjars and Bakerwals was found to be higher (17/85:20.00 positive %) than dogs wandering near butcher shops (11/130: 09.20 positive %) and stray dogs (10/175: 5.71 positive %) (Table 2).

Table 1: Prevalence of Echinococcosis infection in dogs in different districts of Kashmir valley.

Sr. No.	District	Total	Positive	Negative	%age
1.	Pulwama	43	4	39	9.30
	Shopian	45	3	42	6.66
2.	Bandipora	40	4	36	10
3.	Budgam	46	5	41	10.86
4.	Anantnag	39	6	33	15.38
5.	Kupwara	37	7	30	18.91
	Baramullah	44	3	41	6.81
6.	Srinagar	50	2	48	4
	Ganderbal	46	4	42	8.69
7.	Total	390	38	390	9.74

Chi-Sq = 7.878, DF = 8, P-Value = 0.446

Table 2: Prevalence of Echinococcosis infection in different dog types.

Sr. No.	Dog types	Total	Positive	Negative	Percentage
1.	Stray dogs	175	10	165	5.71
2.	Dogs near butcher shops	130	11	119	9.20
3.	Tribal watch dogs	85	17	68	20.00
4.	**Total**	**390**	**38**	**352**	**9.7**

Chi-Sq = 13.641, DF = 2, P-Value = 0.001

DISCUSSION

Around the world, there are wide variations in the prevalence of *E. granulosus* in dogs and its metacestode in herbivores (Matossian *et al.,* 1977). Domestic canines serve as the definitive host for this zoonotic parasite, and domestic ungulates serve as its primary reservoir (intermediate host). The five highly important zoonotic species in the genus *Echinococcus* include *Echinococcus granulosus*, *Echinococcus multilocularis*, *Echinococcus oligarthus, Echinococcus vogeli*, and *Echinococcus shiquicus* (Thompson and Lymbery 1995); (Xiao *et al.,* 2006). In both their adult and larval stages, each of them has a different morphology. It is one of the six priority neglected zoonotic illnesses and has been listed as one of the neglected tropical diseases (W.H.O., 2014). In addition to being a major health and economic issue in Kashmir, Hydatidosisis a significant cause of death in many regions of the world. Rural areas make up the majority of the Kashmir Valley region. For the people who inhabit the majority of these rural areas, agriculture and livestock grazing (sheep and cattle) are the main sources of income. Further most of the tribal people especially Gujjars and Bakerwals having large number of watch dogs prefer to visit Kashmir valley during summer season for grazing their animals. Additionally, there is a sizable population of stray dogs; nevertheless, the Kashmir Valley has a ratio of 1:12 compared to the national average of 1:36 for the country as determined by a WHO-sponsored national multi-centric survey (Sudarshan *et al.,* 2006). Mode of transmission for the spread of this disease to domestic livestock (occasionally humans) is through dogs as the dogs act as reservoir for this parasite. There are various agents that are responsible for the spread of this disease to humans and animals. Domestic animals acquire this parasite though grazing or browsing and the humans can be exposed to the eggs of this parasite by "hand-to-mouth" transfer or contamination by ingesting food or soil and drinking water contaminated with stools from infected canids. Large populations of stray dogs on main roads and in rural areas close to butcher shops may be a source of the echinococcosis infection. Especially on the occasion of Eid-ul-Adha, home butchering of ungulates without sufficient veterinary oversight and the common habit of feeding dogs the viscera of deceased animals have become pervasive rural practises in Kashmir valley. All of these conditions make echinococcosis transmission quite likely.

The overall prevalence of canine echinococcosis infection determined by sandwich ELISA from our study in nine districts in the Kashmir Valley was 9.74%, which is consistent with the findings from earlier studies carried out in other regions of the world. Liu *et al*. (2018) found 18.1% *Echinococcus granulosus* Prevalence in Dogs of southwest Xiji. Chaâbane-Banaoues *et al*. (2015) found 8.3% to 41.3% prevalence of echinococcosis in dogs of Tunisia. Adediran *et al.* (2014) found 12.45% *Echinococcus granulosus* prevalence in Dogs of Southwest Nigeria. Svobodova and Lenska (2002) discovered an 8.1% prevalence of echinococcosis in dogs in the Czech Republic using a coproantigen ELISA test. By using a sandwich ELISA test, Cavagion *et al.* (2005) observed that dogs in Argentina had an echinococcosis prevalence of 7.3%. However, in numerous other studies on dogs, higher prevalence rates of echinococcosis infection have also been reported: Craig *et al*. (1995) used a sandwich ELISA test to determine

the prevalence of echinococcosis in Uruguay, which was 22.7%. According to Moro *et al.* (1999) and Lopera *et al*. (2003), the prevalence of echinococcosis was 46% and 82%, respectively. Additionally, using a sandwich ELISA test, Moro *et al.* (2005) found that the infection rate for *Echinococcus granulosus* was 51% in Peru. In Libya, *Echinococcus granulosus* prevalence in stray dogs was reported by Buishi *et al.* (2005) to be 21.6%. Christofi *et al.* (2002) did find a 0.2% prevalence of *Echinococcus granulosus* infection in dogs in Cyprus (2002).

Echinococcosis infection prevalence rate in dogs was almost found to be similar with minor deviations in different districts of Kashmir valley. Fecal samples collected from tribal areas in summer season have shown higher prevalence rate and the possible reason for this can be that the tribal people carry with them watch dogs (Fig. 2) and perform slaughtering of sheep and cattle without any proper and safe disposal of carcase wastes or offal providing more chances for the dogs to get access to the offal, thus increasing the chances of reinfection in dogs and hence overall prevalence of Echinococcosis infection in dogs. Out of these nine districts, District Srinagar was the only one where its prevalence was reported to be under 4% (Fig. 1). The cause of this may be because dogs in Srinagar City are less vulnerable to the infection due to the proper and safe disposal of waste animal carcasses or offal by the municipal department of Srinagar from butcher shops and from animal carcase carrying dustbins on a regular basis. The second reason behind it is less number of dog populations in this district, as in 2004 Veterinary unit of municipal department has launched a programme of sterilisation of the male dogs, which in turn decrease the population of dogs in Srinagar. Due to Srinagar's sparse dog population and proper offal disposal practises, there are less opportunities for dogs to acquire offal, reducing their risk of reinfection and, consequently, the prevalence of Echinococcosis infection in dogs as a whole.

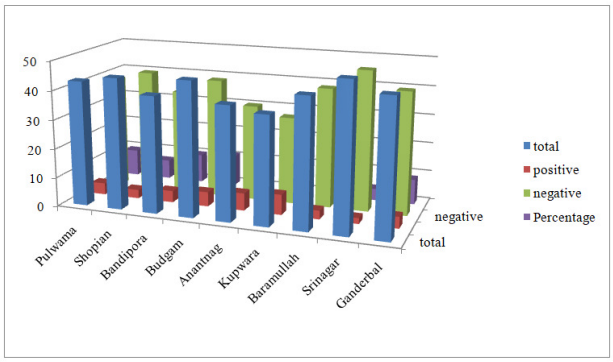

Fig. 1. Prevalence of Echinococcosis infection in dogs of different districts of Kashmir valley.

Fig. 2. Tribal communities with watchdogs.

CONCLUSION

Further research is required to determine the reinfection rate in dogs and the related risk factors of the infection in humans because of the higher prevalence rate and high intensity of infection among dogs in this study. However, this knowledge is crucial for comprehending the *E. granulosus* transmission dynamics in Kashmir valley, which will help in the successful creation of a successful control programme for this significant zoonosis in this region.

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Conflict of Interest. None.

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