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### **Abstract**

Echinococcus granulosus is known to cause echinococcosis in dogs and hydatid disease or cystic echinococcosis in ruminant animals and accidentally in humans. Dogs have a crucial role in the transmission of zoonotic parasites in the Kashmir valley, as they frequently come into touch with humans. Cysts developed as a result of this condition are diagnosed using a variety of procedures, including computed tomography, ultrasonography, and magnetic resonance imaging (MRI). The adoption of contemporary immunodiagnostic techniques, on the other hand, has improved the diagnosis of intestinal echinococcosis on a larger scale, allowing epidemiological studies to be conducted on a larger number of people. In the present study, the prevalence of echinococcosis infection in dogs was determined by examining faecal samples collected from different districts of the Kashmir Valley. An immunodiagnostic test, sandwich ELISA, was used for coproantigen detection of Echinococcus granulosus infection in dogs. A total of 476 faecal samples were tested, out of which, 48 samples were found to be positive in sandwich ELISA, which were mostly collected from different districts of the Kashmir valley and the collection sites included streets, playgrounds, open fields, parks, etc. of the Kashmir valley.

Keywords: Zoonosis; prevalence; sandwich ELISA; coproantigen

Echinococcosis is a widespread dreadful parasitic disease with high zoonotic potential that has major medical and socio-economic costs for human beings and is also a great threat to livestock productivity (Battelli, 2009; Budke *et al.*,2006). The highest prevalence of echinococcosis in India has been reported in two states *viz*. Andhra Pradesh and Tamil Nadu when compared to other parts of the country, (Nepalia *et al.*,2006).

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Hospital-based studies and numerous other reports on this infection are also available from Srinagar, Kashmir (Chisti et al., 2000; Fomda et al.,2015; Fomda et al.,2002). However, in the Kashmir valley, adequate information on the incidence level and the prevalence of echinococcosis infection of dogs is not yet available. Accurate diagnosis of echinococcosis in definitive hosts (dogs) for controlling livestock and human losses due to this infection has always been an important factor for establishing parameters for the epidemiological study of echinococcosis (Sakai, 1996). Routine coprological techniques cannot differentiate the Echinococcus eggs from those of Taenia species due to morphologic similarity (Dinkel et al., 1998). The most extensively used diagnostic methods in dogs are purgation with arecoline compounds and examination of the small intestine after necropsy (Unruh et al., 1973; Craig et al., 1995). Though necropsy is considered as the gold standard, this method has its limitations (Jenkins et al., 2000; Lopera et al., 2003). Alternatively, immunodiagnostic techniques for detecting specific antigens or antibodies have recently been used. During the last few decades, several serological and immunological tests have been introduced the diagnosis of Echinococcus spp. in definitive hosts (dogs). The enzymelinked immunosorbent assay (ELISA) used for the detection of Echinococcus specific coproantigens has been considered as a novel approach for diagnosis of intestinal echinococcosis infection and this method has already proved its value for the diagnosis of infection both in live and dead animals (Deplazes et al., 2003). Parasite-specific antigen detection in host faeces was first reported for canine Echinococcus granulosus by Babos and Nemeth (1962). Coproantigen tests or arecoline tests with rectal faecal samples of dog provided the information on individual prevalence, while the use of ground collected faecal samples is of greater epidemiological value for the detection of coproantigens in the faeces (Cavagion et al.,2005).

The objective of the present work was to establish the prevalence of *Echinococcus granulosus* in dogs of Kashmir valley by using a Copro-ELISA technique which is considered as a more reliable diagnostic method.

Α cross-sectional study conducted to obtain up-to-date information on the prevalence of echinococcosis in dogs. By using a simple random sampling technique, the samples were collected from six districts of the Kashmir Valley viz. Anantnag (33.73°N 75.15°E, altitude 1601masl, population 10.8 lakh), Pulwama (33.87°N 74.89°E, altitude 1630 masl, population 7.77 lakh), Srinagar (34.08°N 74.79°E, altitude 1585 masl, population 11.8 lakh), 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) and Budgam (33.93°N 74.64°E, altitude 1610 masl, population 7.54 lakh) (Fig. 1). Agriculture is the most common occupation in all six districts, with an aggregate literacy rate of 60 per cent.

The study was conducted from February, 2018 to December, 2019 at the Department of Zoology, Kashmir University for detecting coproantigens of Echinococcus granulosus. A total of 476 faecal samples were collected and examined as part of this study. All faecal samples were collected from stray dogs of different districts of the Kashmir valley; both fresh, as well as dried faecal samples, were collected. After collection of the faecal samples, they were stored for at least 14 days at -20°C before being further processed. Immunodiagnostic test ELISA was used for the detection of specific coproantigens of Echinococcus spp. from the samples of dog faeces. Commercially available kit (Qayee-Bio Canine Echinococcus kit) was used for the detection of Echinococcus granulosus species according to the instructions of the manufacturer. The test was performed by using dog faecal material diluted in the kit's sample dilution buffer as per the instructions. After centrifugation of the sample suspension for 20 minutes at room temperature, the supernatant obtained were used for the ELISA test. Coproantigen levels were estimated and interpretation of the results was made following the attached protocol.

Statistical analysis was done using Statistical Package R version 3.5.3. Descriptive statistics was used to get the prevalence of Echinococcosis infection in stray dogs in different districts of Kashmir valley. The Chisquare test was employed to test the association



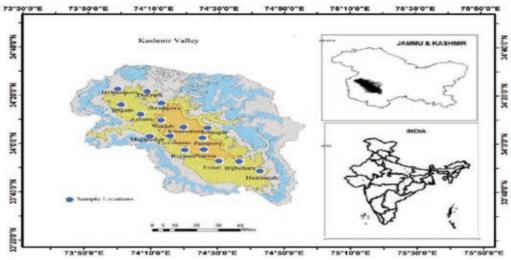


Fig. 1 Map of Kashmir showing sites of sample collection

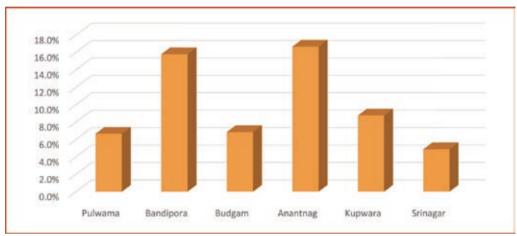


Fig. 2. Prevalence of Echinococcosis infection in stray dogs of different districts of Kashmir valley.

Table 1. Prevalence of echinococcosis infection in stray dogs of different districts of the Kashmir valley

S. No.	District	Total	Positive	Negative	Per cent
1	Pulwama	75	5	70	6.7
2	Bandipora	76	12	64	15.8
3	Budgam	73	5	68	6.8
4	Anantnag	90	15	75	16.7
5	Kupwara	80	7	73	8.8
6	Srinagar	82	4	78	4.9
7	Total	476	48	428	10.1

Chi-Sq = 11.446, DF = 5, P-Value = 0.043

of Echinococcosis infection in stray dogs of different districts in the Kashmir valley. The results were considered statistically significant when the p-value was <0.05.

Ofthe476dogfaecalsamplescollected from different areas, 48 faecal samples were coproantigen positive for Echinococcus spp. representing overall coproantigen prevalence

for Echinococcus spp. in Kashmir valley as 10.1 per cent. Faecal samples were collected from different areas like streets, roadsides, orchards, parks, playgrounds and open fields of Kashmir valley. Out of 48 positive faecal samples, the highest prevalence rate of infection (15/90, 16.7%) was reported from Anantnag district followed by Bandipora (12/76, 15.8%), Kupwara (7/80 8.8%), Budgam (5/73, 6.8%), and Pulwama (5/75, 6.7%) districts. The lowest rate of infection was reported from Srinagar district (4/82, 4.9%). The overall infection rate among dogs of the Kashmir valley was estimated as 10.1 per cent (Table1 and Fig. 2). Chi-Square value calculated was 11.446 and p-value was 0.043.

The prevalence of E. granulosus in dogs and its metacestode in herbivores varies considerably throughout the world (Matossian et al., 1977). This zoonotic parasite maintains itself primarily in a life cycle between domestic dogs (definitive host) and domestic ungulates host). Echinococcosis (intermediate hydatidosis is of significant importance causing economic losses globally. It has been included in the list of neglected tropical diseases and is considered to be one of the six priority neglected zoonotic diseases (WHO, 2014). Most of the regions in the Kashmir valley are covered by rural areas and agricultural lands. Sheep and cattle are important sources of income for people living in the majority of these rural areas. Moreover, a huge population of stray dogs is found in these areas. While WHO-sponsored national multi-centric survey has reported the average human-dog ratio in the country as 1:36 (Sudarshan et al., 2006), this ratio for the Kashmir Valley is 1:12. The high population of stray dogs near butcher shops in agricultural lands and those wandering on main roads may be a source of dissemination of the echinococcosis infection. Livestock slaughter without proper veterinary control and the common practice of feeding dogs with the viscera of home butchered ungulates slaughtered on homes especially on the occasion of Eid-Ul-Adha has become a widespread rural practice in the Kashmir valley. All these factors are highly favourable for the transmission of echinococcosis.

## **Summary**

The results of the present study conducted in six districts of the Kashmir valley indicated that the overall prevalence of echinococcosis infection in dogs by sandwich ELISA was 10.1 per cent which agrees with other studies that were conducted in different parts of the world. In the Czech Republic, Svobodova and Lenska (2002) used coproantigen ELISA test in dogs to determine the prevalence of echinococcosis and their results revealed the prevalence of about 8.1 per cent in the definitive host. In Argentina, Cavagion et al. (2005) found a 7.3 per cent prevalence of echinococcosis in dogs by sandwich ELISA test. However, in other studies on dogs, higher prevalence rates of echinococcosis infection have been recorded, such as Craig et al. (1995) who reported a 22.7 per cent incidence of echinococcosis in Uruguay using a sandwich ELISA test. With the use of sandwich ELISA tests, Moro et al. (1999) and Lopera et al. (2003) revealed 46 per cent and 82 per cent prevalence of echinococcosis, respectively.

However, in different districts of the Kashmir valley, the prevalence of echinococcosis in dogs was nearly similar. The sole exception to this was seen in Srinagar district where a frequency of only 4.9 per cent was observed.

The high prevalence of echinococcosis infection in dogs in the valley of Kashmir shows the poor concern of authorities as well as the general population towards control measures. In view of the increased prevalence rate and high intensity of infection among dogs in this study, further studies are suggested to explore the reinfection rate in dogs. Moreover, it is possible that the associated risk factors of the infection to humans is also very likely. This information is however very important for understanding the transmission dynamics of Echinococcus granulosus in the Kashmir valley, which, in turn, will assist in the fruitful development of an effective control program for this important zoonosis in this area.

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## **Conflict of Interest**

The authors declare that they have no conflict of interest.

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