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Original Article

Microbiology Section

A Serological Study on Hydatid Disease from a Tertiary Care Hospital in Southern India: A Retrospective Observational Study

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ABSTRACT

Introduction: Echinococcus species are zoonotic parasites that cause hydatid disease, affecting humans and animals. The seroprevalence of hydatid disease reflects the level of exposure to this parasite and can be used as an indicator of the burden of the disease in a population.

Aim: To determine the seroprevalence of hydatid disease.

Materials and Methods: The study was a retrospective observational study conducted at JIPMER hospital, a tertiary care centre located in Puducherry, Southern India. In this study, hydatid serological data from January 2019 to December 2022 were collected in March 2023 and retrospectively analysed in April 2023. All the serum samples that tested positive for Enzyme Linked Immuno Sorbent Assay (ELISA) for echinococcosis during the period from January 2019 to December 2022 were included. The demographic details such as age, gender, place of residence, etc., of the patients whose samples were included were collected from their case records. These samples were then analysed using Microsoft Excel for proportions and percentages.

Results: The study showed that the seroprevalence of hydatid disease was 70 (34%) out of 206 samples tested. The seroprevalence was higher in the older age group (>45 years), with 41 (58.6%) cases. It was observed that males and females were almost equally infected, with minor differences in the prevalence of 37 (52.9%) males and 33 (47.1%) females.

Conclusion: As the prevalence of hydatid disease in Southern India was unknown, it was observed to be lower when compared to other states of India. This study has provided valuable information on the level of exposure of hydatid disease in the population and will help formulate disease control and prevention strategies.

Keywords: Echinococcosis, Human hydatidosis, Seroepidemiology, Seroprevalence

INTRODUCTION

Hydatid disease is a zoonotic disease caused by the *Echinococcus* species. *Echinococcus granulosus (E. granulosus)* and *Echinococcus multilocularis (E. multilocularis)* are the most pathogenic human species. Echinococcosis, commonly known as Cystic Echinococcosis (CE), is caused by *E. granulosus*, and alveolar hydatid disease is caused by *E. multilocularis*. Polycystic neotropical echinococcosis is caused by *E. vogeli* infection, whereas uni-CE is caused by *E. oligarthrus* infection [1]. The definitive host of *E. granulosus* is dogs, and *E. multilocularis'* definitive hosts are wild foxes, coyotes, and dogs. Sheep and cattle serve as intermediate hosts by ingesting eggs discharged in the feces of the definitive host. Humans act as accidental hosts by eating diseased livestock as well as polluted food and water [1].

According to the World Health Organisation (WHO), more than 50 individuals per 100,000 population worldwide are affected by hydatid disease at any given time. Endemic areas for CE include Argentina, Peru, East Africa, Central Asia, and China, with prevalence rates of 5-10% [1]. Several temperate countries have human and animal hosts, including many areas of the Mediterranean, southern and central Russia, central Asia, China, Australia, portions of the America (especially South America), and northern and eastern Africa [2,3]. India is one of the endemic countries for echinococcosis in Southeast Asia, and various seroprevalence studies from northern India have shown a prevalence of 28.6% [4]. Hydatid disease is prevalent in a majority of Indian states, with Andhra Pradesh and Tamil Nadu being prominent [5].

The need for the study was to analyse the true seroprevalence of hydatid disease in Southern India, as the existing literature predominantly consists of case reports and no original research is available. The novelty of this study was that the seroprevalence rate for echinococcosis has not been previously reported from Southern India. The aim of the study was to assess the seroprevalence of hydatid disease in Puducherry, India.

MATERIALS AND METHODS

This retrospective observational study was conducted at JIPMER Hospital, a tertiary care centre located in Puducherry, Southern India. Hydatid serological data from January 2019 to December 2022 were collected in March 2023 and retrospectively analysed in April 2023. Ethical approval was obtained from the Institute Ethics Committee (JIP/IEC/2021/247), and informed written consent was obtained from all the patients to include their data in this study.

Inclusion criteria: All the patients who were found positive for echinococcosis from January 2019 to December 2022 were included in the study.

Exclusion criteria: Patients who did not give consent were excluded from the study.

Sample size: The authors did not perform any sample size calculation as this was a retrospective study. Out of 206 samples subjected to ELISA testing for echinococcosis from January 2019 to December 2022, 70 patients were found to be positive which formed the sample size.

Basic demographic details of the patients, including their age and the medical services where they presented, were obtained from the Hospital Information Systems (HIS). A blood sample was collected from each patient in a clot activator tube and transported to the Parasitology laboratory, Department of

Microbiology, JIPMER Puducherry, for hydatid serology as part of routine investigations.

The qualitative immune-enzymatic determination of IgG antibodies was based on the serology format of *Echinococcus* antigen-Linked Immuno Sorbent Assay (ELISA) from Nova Tec Immunodiagnostic GmbH, Germany [6]. The ELISA method was used for detecting the seroprevalence of CE [7-9]. Before the assay, all samples were diluted 1:100 with IgG sample diluents (10 µL and 1 mL serum). Standards/controls and diluted samples were dispensed into their respective wells on the microtiter plates coated with specific antigens (*Echinococcus* antigen) as per the manufacturer's instructions. The wells were covered with aluminum foil and incubated for one hour at 37°C. Following incubation, the well contents were aspirated, and each well was washed three times with 300 µL of washing buffer using a Robonik wash well ELISA washer from India. After washing, 100 µL of Horseradish Peroxidase (HRP) labeled conjugate was added, which binds to the captured antibodies. A second washing step removed unbound conjugate. The immune complex formed by the bound conjugates was visualised by adding Tetramethylbenzidine (TMB) substrate, producing a blue reaction product. The intensity of this product is proportional to the number of specific antibodies in the sample. Sulfuric acid was added to stop the reaction, resulting in a yellow endpoint colour. Absorbance at 450/620 nm was read using an ELISA microwell plate reader (Merilyser EIAQuant, Delhi, India), and the values were noted and analysed.

STATISTICAL ANALYSIS

The data was analysed using Microsoft Excel. All the continuous variables were expressed as proportions. The data was analysed using descriptive statistics.

RESULTS

The results of the 206 samples tested showed 34% (70/206) IgG seropositivity for echinococcosis. Among the 206 samples analysed, there were 25 people under the age of 18. The age group of (46-60 years) accounted for the maximum number of 27 (38.6%) cases. In the elder age group of over 45 years, the seroprevalence was greater, with 41 (58.6%) cases [Table/Fig-1].

Age group (years)	n (%)
<15	2 (2.9)
15-30	6 (8.6)
31-45	21 (30)
46-60	27 (38.6)
>60	14 (20)

[Table/Fig-1]: Distribution of the number of positives among various age groups.

Among the 206 samples collected, 86 (41.7%) and 120 (58.3%) were from females and males, respectively. It was also observed that males and females were almost equally infected, with minor differences in the prevalence [Table/Fig-2].

Gender	Number of positives N (%) n=70
Male	37 (52.9)
Female	33 (47.1)

[Table/Fig-2]: Gender-wise distribution of the number of positives.

Among the 70 positive cases, state-wise distribution is shown in [Table/Fig-3]. Among the 70 patients, 20 patients (29%) were associated with an agricultural occupation [Table/Fig-4].

The district-wise distribution of positive cases is depicted in [Table/Fig-5].

State which the patient belongs to	n (%)
Tamil Nadu	52 (74.3)
Puducherry	6 (8.6)
West Bengal	5 (7.11)
Other states	7 (10)

[Table/Fig-3]: Demographic distribution of positive patients.

Occupation which the patient belongs to	n (%)
Agriculture	20 (29)
Others	50 (71)

[Table/Fig-4]: Occupational distribution among IgG seropositive patients.

District Name	n (%)
Vilupuram	20 (28.6)
Cuddalore	14 (20)
Thiruvannamalai	7 (10)
Puducherry	6 (8.6)
Trichy	3 (4.3)
Chengalpattu	2 (2.8)
Kallakurichi	2 (2.8)
Other TN districts	4 (5.7)
West Bengal	5 (7.1)
Other states in India	7 (10)

[Table/Fig-5]: IgG seropositivity based on district-wise spread and few other states of India.

The surgery department had the highest prevalence with 34 (48.6%) cases, followed by the medicine department and the emergency department [Table/Fig-6].

Department	n (%)
Medicine	31 (44.3)
Surgery	34 (48.6)
Emergency medicine	5 (7.1)

[Table/Fig-6]: Department-wise distribution of positive cases.

During the study period from 2019 to 2022, it was observed that the prevalence varied from 26.5% to 39.6% [Table/Fig-7].

Year	Number of samples received	The number of samples tested positives.
2019	58	23 (39.6)
2020	18	7 (38.8)
2021	64	17 (26.5)
2022	66	23 (34.8)

[Table/Fig-7]: Yearly distribution of positives among all tested samples.

It has also been observed that the fewest number of patient samples were tested during 2020, which indicates underdiagnosis and fewer patient presentations to hospitals during the Coronavirus Disease-2019 (COVID-19) pandemic.

DISCUSSION

The authors examined the seropositivity data of individuals suspected of having hydatid disease over a four-year period (2019-2022) in this study. The seroprevalence was 34% in southern India, which was comparable to the rate of 38.5% estimated by Sinha S et al., in their study on north Indian population [10]. Moreover, the seroprevalence rates were also found to be on the rise in northern India [4,11]. Zaman K et al., and Khurana S et al., demonstrated seroprevalence rates of 28.6% (2004-2015) and 23.1% (1999-2003), respectively [4,11]. This indicates an increasing trend in the seroprevalence rate in northern India. The high prevalence observed in this study might be underestimated since only clinically suspected individuals were included, and hospital-based studies are not always accurate predictors of population prevalence. Although seroprevalence may not be a precise measure of disease burden, the WHO suggests that hospital cases provide a highly valuable and useful indicator of the incidence and efficacy of control efforts, even if they underestimate the disease's true prevalence [4]. A seroprevalence of 2.9% was observed for CE in children, which was much lower than the seroprevalence of CE observed by Sarkari B et al., (6.7%) [12]. This was primarily due to the lower proportion of children included in this study. However, in western studies, the seroprevalence ranged from 1-4%, which was comparable with this study [13,14].

In this retrospective data, the most common age group involved was 46-60 years. Men and women both actively participate in farming, household chores, and animal husbandry in rural India, making both genders vulnerable to the disease. In contrast, Khabisi SA et al., from southeast Iran discovered that women were more afflicted by this illness than males, and the age group with the highest seroprevalence rate for hydatid cysts was 31 to 50, which differs from this study [15]. Kayal A and Hussain A, conducted a clinical study of Hydatid disease and found that there were significantly more women than men, with an M:F ratio of 1:2, who were infected with this disease, and the average age of disease onset was 40 years [5]. Similarly, in a study done by Sanaei Dashti A et al., males accounted for 42.1% and females for 56.1%, and in the study by Aslanabadi S, et al., males were 54.2% and females were 45.8% [16,17]. Another study from Kashmir showed a sharp contrast to this study in that the most common age group affected was children under 15 years old. The Kashmir study stated that children's exposure to stray dogs in the parks was a significant risk factor for the transmission of this disease [18].

This conflicting report of seropositivity in various age groups is based on hospital studies, which primarily comprise symptomatic people. The higher incidence in adults in this study compared to children shows a reduction in disease burden due to the control measures used. In this study, authors were unable to demarcate any significant difference between urban and rural populations. In rural locations, farmers and housewives are especially vulnerable since they are active in household tasks connected to animal breeding and agriculture [19]. The seroprevalence of hydatid in the rural population was about 6%, and in the urban population, it was around 3.5% in the population of Sena Madureira [20].

Due to its varying clinical presentation, no significant differences were found in consulting departments. This study shows that seroprevalence has decreased from 39.6% to 34.8%. The decreased seropositivity for hydatid disease in this study underscores the importance of implementing control measures. With this seroprevalence rate, targeted preventive measures can be implemented. These preventive measures can be targeted to the age group (>45 years), which showed high seroprevalence, and to the specific districts that showed higher seroprevalence.

Limitation(s)

As this was a retrospective study, patient treatment and follow-up were not assessed. Another drawback of this study was that it was a hospital-based study, which will not reflect the exact prevalence of hydatid disease in the community.

CONCLUSION(S)

The present study has provided background knowledge about hydatid disease, and it was observed that its prevalence in Southern India was lower when compared to other states of India. The disease can be prevented by improving animal husbandry techniques, such as regulating the home slaughter of sheep and other livestock, controlling the stray dog population, limiting the locations where dogs are permitted, and keeping them from ingesting cyst-infected meat. Educating the public on simple personal hygiene measures, such as washing hands with soap and warm water after handling dogs and before handling food, thereby avoiding direct intake of food contaminated with fecal matter from dogs, could be extremely effective in breaking the chain of transmission of this important medical entity. Meanwhile, active surveillance to determine infection incidence in humans and cattle across the country will be critical in understanding the disease's epidemiology and aiding in its control.

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