

Frequency of Echinococcosis Granulosis in High-Risk Group, Experience in District Mardan, Khyber Pakhtunkhwa

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ABSTRACT

Objective: To know the prevalence of Cystic Echinococcosis in high risk population in district Mardan.

Study Design: Prospective study

Place and Duration of Study: This study was conducted at the Department of Medicine, Mardan Medical Complex Mardan and Department of Biotechnology Abdul Wali Khan University, Mardan in Khyber Pakhtunkhwa from January 2021 to March 2021.

Materials and Methods: Mardan is the second biggest district of KP. After informed consent, blood samples were collected from high risk group and were transferred to health Biotechnology laboratory Abdul Wali Khan University. Serological identification was performed by Elisa method. Results were calculated according to the formula; Sample O.D / cut off serum O.D x 10.

Results: Total of 80 blood samples were enrolled, out of which 07(8.75%) were found positive for Cystic Echinococcosis. Maximum samples (20%) were reported from Takht Bhai region. Most effected (42.85%) age group was 31-45 years.

Conclusion: Strategies to create awareness and effective preventive and treatment programmes against this disease are needed.

Key Words: Cystic Echinococcosis, E. granulosis, ELISA, Mardan, Khyber Pakhtunkhwa

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INTRODUCTION

Cystic Echinococcosis (CE) or hydatid cyst disease is a parasitic disease. CE is also known by name of Hydatid cyst and also by Hydatidosis. It is caused by the metacestode of Echinococcus granulosis. It is a zoonotic infection and the cause of human infection in eggs of E. granulosis when humans unintentionally eat the eggs of tapeworm¹.

CE is a zoonotic infection that affects and damages animals and humans' vital organs, especially the liver and lungs. The main cause of the infectious disease is E. granulosis².

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It silently destroys the health of humans and animals and produces major public and economic loss around the globe especially in those areas and regions where dogs and animals are raised in number and share human life activities³⁻⁶. Two distinct mammalian hosts are needed for the life cycle of the echinococcosis.

Definitive hosts such as foxes, dogs, and wolves and others are intermediate hosts including herbivores like as hoarse, sheep cattle and hardly humans.⁷ The regions where the hydatid cyst disease is endemic include, Mediterranean, Northern China, North Africa, Australia, Turkey, Indian subcontinent, South America and New Zealand,⁸

The disease observed in human and mammalian tissues, particularly in the liver and lungs, causes echinococcosis.⁹ Other mammalian organs effected include pancreas, heart, spleen, brain¹⁰⁻¹². The cycle of E. granulosis is naturally a dog or sheep cycle in which an infected dog implies contamination of a sheep through its feces. In the parasitic natural life cycle, humans are not a part, in the life cycle of parasite; humans unintentionally take the place of the sheep through the close interaction of the infected dog.¹³ Human beings are usually dead ends for the parasite¹⁴. Various risk factors are involved in causing the infection. These include poor open slaughter house practices, abundance of stray dogs, contaminated vegetables and other foodstuffs available in the market and poor healthcare facilities.¹⁵ Defensive approaches would, therefore, include health education,

reduced interactions with dogs and sheeps and effective removal of their wastes.¹⁶ The inaccessibility of clean drinkable water and the close communication of human beings with sheep and dogs make Pakistan an endemic region to this disease.¹⁷

To recognize distinct parasite genotypes, molecular characterization of Echinococcosis granulosis is necessary. Genotyping enables to manage their life cycle and transmissions¹⁸. Ten distinct strains of this parasite are reported worldwide. All strains are disease-causing agents, excluding G4 strain. Most prevalent and main causative agent of the infection in both humans and livestock is *E. granulosis sensu stricto* (G1-3) across the globe. In Pakistan, the G3 and G6 strains of the parasite have been reported in Punjab and Khyber Pakhtunkhwa provinces while in Indian occupied Kashmir the G1 and G3 strains have been reported.¹⁹⁻²¹

Discussing characteristics are antigenicity, sensitivity to chemotherapeutic agents, transmission dynamics, and patterns of pathology life-cycle, development rate and host specificity.²² For the diagnostic reagents, vaccine development and impacting of drugs on the control and epidemiology of echinococcosis may have important implications for these characteristics.²³ Certainly, *Echinococcus* species individually keeps a specific genetic identity with host-adapted that infrequently overlaps in some geographical areas.²²

Different serological methods are being used for the diagnosis and analysis of hydatid cyst disease such as latex agglutination, hemagglutination, ELISA, skin test and immune electrophoresis.²⁴ Previously no such studies were done in KPK. Aim of our study is to find out the prevalence of hydatid cyst disease in high risk population of KPK using ELISA technique.

MATERIALS AND METHODS

This prospective was conducted at department of Medicine, Mardan medical complex and department of Pathology, Abdul Wali Khan University, Mardan in Khyber Pakhtunkhwa from 1st January 2021 to 31st March 2021. A total of 80 blood samples were collected from high-risk population. After collection, samples were transferred to the health biotechnology laboratory of Abdul Wali Khan University Mardan KPK for further processing using stranded protocols.

Patients of all age and of either gender were included in the study. Data was collected using a structured data collection proforma. Data was entered and analyzed using statistical package for social sciences (SPSS) Version 22. Our study was approved by ethical committee of Mardan Medical Complex.

Serological identification for the detection of *Echinococcus granulosis* in the high-risk groups was done through ELISA method. All the samples for hydatid Cyst infection were processed according to the manufacturer's instructions (Viracell® Hydatidosis Elisa

IgG 96, Germany). Samples were run along with positive and negative controls.

We adopted the following procedure:

- Incubation was done at 37°C
- The temperature of ingredients was lower down to room temperature.
- Besides, labeling was based on a sample set, including positive negative cut off serum for the estimation of plate validity.
- Initially 100 µl of serum diluent of the kit was added followed by the addition of 5 µl serum sample and shaking in plate shaker.
- Plates were sealed using the sealing sheet supplied in kit followed by incubation at 37°C up to 45min.
- After completion of incubation the liquid content was aspirated completely and the wells were washed five times by pouring 300µl washing solution in each well.
- After performing washing 100µl IgG solution of the kit was added and the plate was further incubated for 30min at 37°C
- Furthermore, after incubation liquid was aspirated and 100µl substrate was added on the spot, the plate was again incubated for 20min at room temperature the plate was protected from light.
- The incubation period was followed by the addition of 50µl stopping solution to each well provided in the kit and was read under ELISA reader at 450nm

RESULTS

Results were calculated according to the formula, (Sample O.D/ cut off serum O.D) × 10, from the reading taken by ELISA reader from the ELISA plate. Figure 1 shows the positive and negative samples general outcomes.

Negative Control		
Ctrl0001	Blank 1	Std0001
3.159	0.111	1.366
Un0001	Un0002	Un0003
2.832	3.058	0.173
Un0004	Un0005	Un0006
0.759	0.207	0.224
Un0007	Un0008	Un0009
0.278	0.160	0.169
Un0010	Un0011	Un0012
2.727	0.295	2.696
Un0013	Un0014	Un0015
0.352	0.939	0.221
Un0016	Un0017	Un0018
0.206	0.271	0.154
Un0019	Un0020	Un0021
0.445	0.188	0.214

Figure No.1: Figure displaying the samples read by ELISA

Ctrl 0001 designated the positive control, Blank 1 represents Negative control and cut-off control designated for Std 0001. The positive tests are the samples Un 0001, Un 0002, Un 0010, and Un 0012.

Out of 80 samples 07(8.75%) were found positive for Cystic Echinococcosis as shown in figure 2.

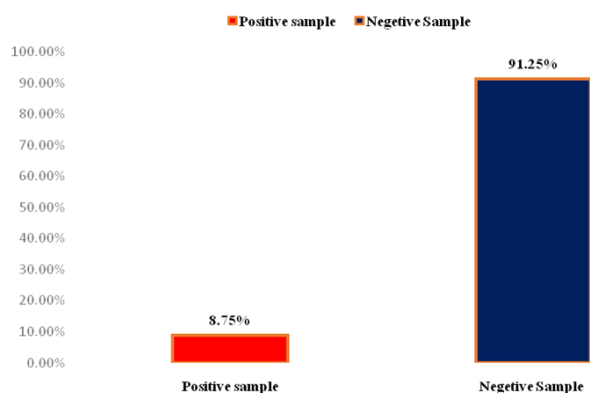


Figure 2: Showing positive and negative samples

Maximum number of samples were from Takht Bahi region of district Mardan, On the other hand less number of samples were collected from Shankar area, as shown in figure 3.

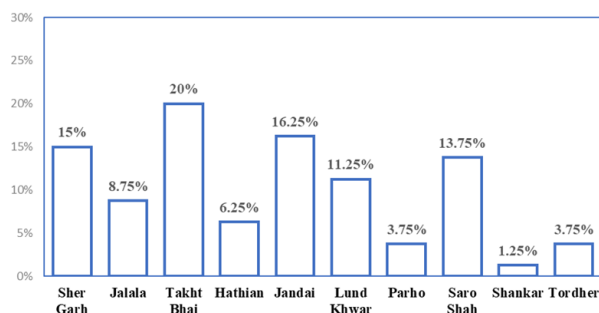


Figure No.3: Showing Area-wise collection of samples

As far as age is concerned, age of the study participants ranged from 01 to 60 years. 21.25% of blood samples were collected from patients with age of 1-15 years, 38.75% from age of 16-30 years. While 28.75% and 11.25% of the samples were collected from patients with age in range of 31-45 years and 46-60 years respectively.

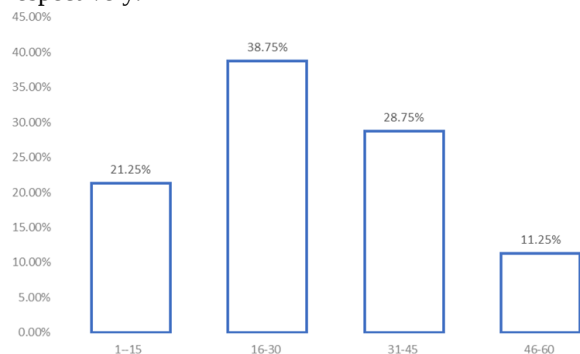


Figure 4: Showing Age-wise distribution of samples

Maximum number of samples (42.85%) were found positive between the age of 31 to 45 years. One sample (14.28%) was tested positive in each age group of 01-15 years and 46-60 years. Patients in age group of 16-

30 years had 02(28.56%) samples tested positive for Cystic Echinococcosis.

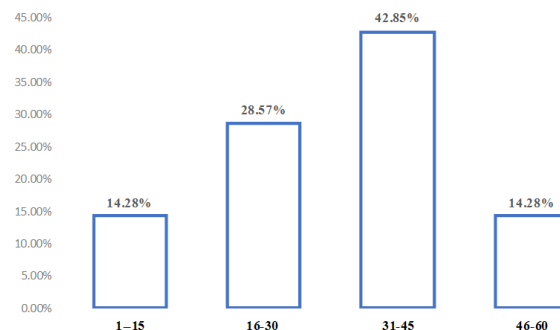


Figure No.5: Showing the percent of positive samples age-wise.

DISCUSSION

Echinococcosis soundlessly destroys the livestock industry and human health. Echinococcosis is a problem for livestock and public health, affecting distinct countries' economies. The previous retrospective study and genotyping in Pakistan and India showed that this disease in these nations is widespread and prevalent.²⁵ It is mentioned in tropical countries as a neglected tropical disease²⁶. *E. granulosus* is identified as widespread throughout the country, according to the literature, "camel-dog" and "sheep-dog" are strains that infect humans^{12,27}. There are Several reports on molecular detection of *E. granulosus* genotypes are available in domestic animals.^{24,28,30} Three genotypes of *E. granulosus* were reported in different organisms.

In Khyber Pakhtunkhwa, there is no previously published research study on the retrospective and genotypical characterization of hydatid cyst. To avoid its life cycle, it was important to approximate the disease burden. This is the first research of its kind that was conducted in KP.

A total of 80 blood samples were collected from the high risk group. We found 07 samples as positive. Our study demonstrated that most morbidity of CE was in the age of 31 to 45 years.

Khan D et al and Pal R along with his colleagues in their research work showed the incidence of CE in cattle as 5.5 to 9.6%, 12.3% in buffalo, 8.3% in sheep and 7.5% in goats.^{31,32}

Several investigators reported the occurrence of hydatidosis in Pakistan. Iqbal Z and his co researchers studied the existence of cyst in different animals slaughtered at Faisalabad abattoir and reported 14.8% sheep, goats 5.9%, buffaloes 4.9% and cattle 3.3% infected with the disease.³³ Pal R in his research work reported the existence of hydatidosis in sheep 5.3%, goats 1.79% and cattle 31.5% Rawalpindi abattoir individually.³²

The molecular characterization of echinococcus species in all areas of Pakistan requires large-scale surveillance.

This type of large-scale study will surely help to estimate the disease burden in Pakistan. It is the need of the hour to create awareness about this disease in the rural population of Pakistan. Necessary prevention, control measures, diagnosis, and treatment are required to improve public health and reduce economic loss.

CONCLUSION

It is concluded from our study that *E. granulosus* is an important source of economic loss in our community. All over the country molecular characterization of *Echinococcus* species needs extensive surveillance. Moreover, large-scale studies will help to evaluate the disease burden in Pakistan.

Author's Contribution:

Concept & Design of Study: Ziauddin
 Drafting: Shah zeb, Seema Khan
 Data Analysis: Arif Khan, Sumbal Hussain
 Revisiting Critically: Ziauddin, Sajjad Ali
 Final Approval of version: Ziauddin

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Ibrahim I, Tuerdi M, Zou X, Wu Y, Yasen A, Abihan Y, et al. Laparoscopic versus open surgery for hepatic cystic echinococcosis: a systematic review and meta-analysis. *Int J Clin Exp Med* 2017;10(12):16788-97.
2. Rahimi MT, Ahmadpour E, Esboei BR, Spotin A, Koshki MHK, Alizadeh A, et al. Scolicidal activity of biosynthesized silver nanoparticles against *Echinococcus granulosus* protoscolices. *Int J Surg* 2015;19:128-33.
3. Sarıözkan S, Yalçın C. Estimating the production losses due to cystic echinococcosis in ruminants in Turkey. *Veterinary Parasitol* 2009;163(4):330-4.
4. Šnábel V, Altintas N, D'amelio S, Nakao M, Romig T, Yolasigmaz A, et al. Cystic echinococcosis in Turkey: genetic variability and first record of the pig strain (G7) in the country. *Parasitol Res* 2009;105(1):145.
5. González-Sapienza G, Cachau RE. Identification of critical residues of an immunodominant region of *Echinococcus granulosus* antigen B. *J Biological Chemistry* 2003;278(22):20179-84.
6. Driscoll CA, Macdonald DW, O'Brien SJ. From wild animals to domestic pets, an evolutionary view of domestication. *Proceedings National Acad Sciences* 2009;106(Supplement 1):9971-8.
7. Çelik C, Şaşmaz M, Uçan H. Spinal hydatid cyst. *Türkiye Klinikleri tıp Bilimleri Dergisi* 2010;30(3):1073-7.
8. Buttenschoen K, Buttenschoen DC. *Echinococcus granulosus* infection: the challenge of surgical treatment. *Langenbeck's Archives Surg* 2003;388(4):218-30.
9. Harandi MF, Hobbs R, Adams P, Mobedi I, Morgan-Ryan U, Thompson R. Molecular and morphological characterization of *Echinococcus granulosus* of human and animal origin in Iran. *Parasitol* 2002;125(4):367-73.
10. Yalçın E, Dogru D, Özçelik U, Kiper N, Göçmen A, Akhan O. Cardiac hydatid cyst and pulmonary hydatidosis in a child. *Pediatr Infectious Disease J* 2002;21(12):1178-80.
11. Köylüoğlu G, Öztoprak I. Unusual presentation of pancreatic hydatid cyst in a child. *Pancreas* 2002;24(4):410-1.
12. Cebollero M, Cordoba E, Escartin J, Cantin S, Artigas J, Esarte J. Hydatid cyst of spleen. *J Clin Gastroenterol* 2001;33(1):89-90.
13. Pezeshki A, Kia E, Gholizadeh A, Koohzare A. An analysis of hydatid cyst surgeries in Tehran Milad Hospital, Iran, during 2001-2004. *Pak J Med Sci* 2007;23(1):138.
14. Macpherson CN. An active intermediate host role for man in the life cycle of *Echinococcus granulosus* in Turkana, Kenya. *Am J Tropical Med Hygiene* 1983;32(2):397-404.
15. El Berbri I, Ducrotoy MJ, Petavy A-F, Fassifihri O, Shaw AP, Bouslikhane M, et al. Knowledge, attitudes and practices with regard to the presence, transmission, impact, and control of cystic echinococcosis in Sidi Kacem Province, Morocco. *Infectious Diseases Poverty* 2015;4(1):48.
16. Palmer S, Biffin A, Craig P, Walters T. Control of hydatid disease in Wales. *BMJ* 1996;312(7032):674-5.
17. Hussain A, Maqbool A, Hussain S, Athar M. Studies on prevalence and organ specificity of hydatidosis in ruminants slaughtered at Karachi & Faisalabad abattoir, Pakistan. *Ind J Dairy Sci* 1992;45:454-.
18. Pour AA, Hosseini SH, Shayan P. Comparative genotyping of *Echinococcus granulosus* infecting buffalo in Iran using *cox1* gene. *Parasitol Res* 2011;108(5):1229-34.
19. Latif AA, Tanveer A, Maqbool A, Siddiqi N, Kyaw-Tanner M, Traub RJ. Morphological and molecular characterisation of *Echinococcus granulosus* in livestock and humans in Punjab, Pakistan. *Veterinary Parasitol* 2010;170(1-2):44-9.
20. Dogging F. Epidural Steroid Warning Controversy Still. *Pain Physician* 2014;17:E451-E74.
21. Sharma M, Sehgal R, Fomda BA, Malhotra A, Malla N. Molecular characterization of *Echinococcus granulosus* cysts in north Indian patients: identification of G1, G3, G5 and G6

- genotypes. PLoS Neglected Tropical Diseases 2013;7(6):e2262.
22. Thompson RA, Lymbery AJ. Echinococcus and hydatid disease: Cab International; 1995.
 23. Thompson R, Lymbery A. Echinococcus: biology and strain variation. Int J Parasitol 1990;20(4):457-70.
 24. Ramos M, Ph. D, Guillermo, Orduña M, Antonio, García-Yuste M, Mariano. Hydatid cyst of the lung: diagnosis and treatment. World J Surg 2001;25(1):46-57.
 25. Khan A, Naz K, Ahmed H, Simsek S, Afzal MS, Haider W, et al. Knowledge, attitudes and practices related to cystic echinococcosis endemicity in Pakistan. Infectious Diseases Poverty 2018;7(1):4.
 26. Control CfD, Prevention. Echinococcosis. Recuperado de: <https://www.cdc.gov/dpdx/echinococcosis/index.html> 2018.
 27. Abbassioun K, Amirjamshidi A. Diagnosis and management of hydatid cyst of the central nervous system: Part 2: Hydatid cysts of the skull, orbit, and spine. Neurosurg Quarterly 2001;11(1):10-6.
 28. Horchani A, Nouira Y, Kbaier I, Attayaoui F, Zribi AS. Hydatid cyst of the kidney. Eur Urol 2000;38(4):461-7.
 29. Young E. Brucella species in: Mandell, Douglas, Bennett: Principles and practice of infection diseases. Elsevier, Churchill livingstone; 2005.
 30. Ammann RW, Eckert J. Cestodes: echinococcus. Gastroenterol Clinics 1996;25(3):655-89.
 31. Khan D, Haseeb M. Hydatidosis of livestock in Pakistan. Folia parasitologica 1984.
 32. Pal R, Jamil K. Incidence of hydatidosis in goats, sheep and cattle. Pak Veterinary J (Pakistan) 1986.
 33. Iqbal Z, Hayat C, Hayat B, Khan MN. Prevalence, organ distribution and economics of hydatidosis in meat animals at Faisalabad [Pakistan] abattoir. Pak Veterinary J (Pakistan) 1989.