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Infectious Diseases Diagnosis & Treatment

Research Article

Foglia F. and Folliero V., et al. Infect Dis Diag Treat 5: 172.
DOI: 10.29011/2577-1515.100172

The Prevalence of Human Cystic Echinococcosis in Campania Region: Result of a Regional Screening Project

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Citation: Foglia F, Folliero V, Della Rocca MT, Corvino G, Rinaldi L, Franci G, Sarnelli P, Finamore E, Galdiero Massimiliano (2021) The Prevalence of Human Cystic Echinococcosis in Campania Region: Result of a Regional Screening Project. Infect Dis Diag Treat 5: 172. DOI: 10.29011/2577-1515.100172

Received Date: 21 January 2021; **Accepted Date:** 29 January 2021; **Published Date:** 05 February 2021

Summary

Cystic echinococcosis is a world-wide parasitic infection mainly caused by *Echinococcus granulosus (E. granulosus)*. The purpose of our study was to assess the current status of human Cystic Echinococcosis (CE) epidemiology in Campania region. In view of the severity of this human disease, the early diagnosis of the presence of the cyst/s avoid surgery practice and allow the admission of drug therapy. In this regard the project has offered farmers, their families, and farm workers the possibility of CE serological and clinical diagnosis. The study was conducted between 2014 and 2018 in 650 farms, located in four provinces of Campania region (Salerno, Avellino, Caserta and Benevento). Seroprevalence was evaluated through Enzyme-Linked Immunosorbent Assay (ELISA), Indirect Hemagglutination Test (IHA) and Western Blot (WB). A seroprevalence of 0.4% was documented, in particular 0,3 % in Salerno and 0,1 % in Avellino. Out of a total of 4 positive subjects, 3 were male, all were recorded in over 40 years' age group, located in sheep farms (0,6%). Liver cyst, as CE3a stage, was identified in one subject from Salerno province. This is the first study on human hydatidosis in Campania region so could have a huge impact to define the strategies and monitoring activities for spread reduction of human CE.

Keywords: Campania; Cystic echinococcosis; *Echinococcus granulosus*; Epidemiology; Italy; Ultrasound

Introduction

CE or hydatid cyst is a zoonotic parasitic infection, caused by larval stage of cestode, member of the genus *Echinococcus* [1]. Currently, six species of *Echinococcus* have been identified, but the member of greatest clinical relevance and geographic distribution is *Echinococcus granulosus* (*E. granulosus*) [2]. The life cycle of this parasite includes carnivores (dogs and wolves) which represent definitive hosts and farm animals (sheep and cattle) which are intermediate hosts. The humans constitute the aberrant intermediate hosts [3]. Larval stage of parasite is located in the small intestine of mammals. Every day the adult cestode produces thousands of eggs, which are released through the faeces of carnivores into the surrounding environment. Eggs can be infectious for months or up to a year depending on the environment conditions. When the intermediate host ingests the eggs, they hatch, forming the oncosphere. It crosses the intestinal mucosa and reaches the internal organs such as the liver and lungs through the bloodstream. If the liver filter is passed, the parasite can reach the lungs, which represent the second site of definitive localization. If the lung barrier is also overcome, the parasite can reach and develop in any district of the body. The definitive host is infected through the ingestion of organs of the infected intermediate host. After ingestion, the parasite evaginate and attach the mucosa of its small intestine. Here the parasite develops until it reaches the adult stage after 30 - 60 days. The most common form of transmission to humans is the consumption of water or food contaminated with the faeces of infected carnivores [4-6]. The main risk factors are

human contact with infected dogs and farm animals, rural lifestyle and lack of public health education [7]. The primary stage of infection in humans is manifested by an asymptomatic course, with well encapsulated cysts, which expand slowly and often go unnoticed for years [8]. The symptoms are related to several factors, such as: i) the number and size of the cyst; ii) location of the cyst within the affected organ; iii) the pressure exerted by the cysts on the adjacent tissues and structures [9]. In addition, the sudden onset of symptoms may be related to cyst rupture, leading to anaphylactic shock [10]. CE represents a socio-economic problem for humans and farm animals in different countries of the world [11]. Currently, CE have shown a prevalence of 2-3 million cases and an incidence of 200000 new infections per year worldwide [12]. The highest incidence of CE in humans and farm animals has been registered in Central Asia, northern and eastern Africa, Australia and Mediterranean regions [13]. This disease is endemic in Italy, especially in the southern regions. The reported prevalence of CE in southern Italy varies from 2.33% to 9.77% in the different regions [14]. Furthermore, it has been estimated that the impact of the disease, in terms of Disability Adjusted Life Years (DALYs), it is around 871000 with an annual cost of 3 billion US$ accounting for human treatment and livestock production losses [15]. Despite this strong socio-economic impact, CE is considered one of the 17 Neglected Tropical Diseases (NTD) by the World Health Organization (WHO) [16]. European Centre for Disease control (ECDC) has highlighted that Italy lacks a surveillance system for CE and, until few years ago, no official data are transmitted to European authorities (EFSA 2013) [17]. Since the beginning of the 2000s, the Campania Region has addressed the problem of parasites in organic and planned way, in particular with zoonotic aspect. With the aim of outlining the regional epidemiological framework and the role of some animal species in parasitosis chain, our region has been activated a series of regional projects. The land survey covered the Campania livestock population consisting of cattle, buffalo, sheep and goats. Surveys were carried out to assess the prevalence of Echinococcosis/Hydatidosis in sheep and goats' farms. The actual spread of the CE in humans is not easy to define for the latency (often long) of the infection, before cyst detection, and for discontinuous information flows. The aim of the present study was to assess the current status of human CE epidemiology in Campania region, focused on the spatial distribution and epidemiological dynamics of this endemic zoonosis.

Materials and Methods

Study Design

Our work represents medical component results of regional project named: "Control and reduction of Echinococcosis/ Hydatidosis in animal populations and prevention of related human pathology - EchinoCamp1000". The operating units of this project have been the Regional Center for the Monitoring of Parasitosis / Department of Veterinary Medicine and Animal Productions, University of Naples "Federico II " (CREMOPAR), Zooprophylactic Institute of Mezzogiorno (IZSM) and the medical component made of Clinical Microbiology, University of Campania "Luigi Vanvitelli". The current study was conducted from 2014 to 2018 in 650 farms, located in four provinces (Salerno, Avellino, Caserta and Benevento) of the Campania region in southern Italy. Geographic Information System (GSI) was used for analysis of study area. The aim was delaine the epidemiological aspect of Echinococcosis/Hydatidosis in Campania region to prevent the related human pathology. In view of the human severity disease, the early diagnosis of the cyst/s presence avoid surgery practice and allow the admission of drug therapy. In this regard the project has offered farmers, their families, and farm workers the possibility of CE serological and clinical diagnosis. Laboratory and clinical diagnosis were carried out. The laboratory diagnosis was performed through the use of two serological methods: Enzyme-Linked Immunosorbent Assay (ELISA) and Indirect Hemagglutination Test (IHA). These techniques have a good specificity and sensitivity; however, it is possible to find false positives in the case of subjects with neoplasms or other parasitosis [18]. As a result, they make it necessary to use a confirmation investigation, represented by the Western Blot (WB) [19]. While the clinical diagnosis provides ultrasound examinations for further confirmation to the patient's clinical/surgical management. In the first project phase, the positive serological cases, in farms with suspected cases of zoonosis infection, were directed to instrumental diagnostic-clinical path at our University Hospital. In light of this finding and for the importance of related human diagnosis, the second-phase, in positive sheep farms, has included diagnostic research with ultrasound portable machine (MyLab™ Omega Esaote, MyLab). In this way was ensured complete human diagnosis avoiding follow-up losses in positive or potentially positive subjects.

Samples Collection and Analysis

In our study, we have collected 1497 peripheral blood samples for laboratory diagnosis. The samples were centrifuged, using a benchtop centrifuge (ThermoFisher, Massachusetts, USA), and the serum was separated and stored at -20 ˚C until analysis. All serum samples were screened by a commercial ELISA (Cypress Diagnostic, Langdorp, Belgium) and IHA (Dade Behring, Newark, NJ, USA), according to the manufacturer's instructions. Samples that were positive or borderline to previous tests had been confirmed through WB (Euroimmun Labordiagnostika, Luebeck, Germany), according to the manufacturer's recommendations. Seropositivity was determined when the WB result was positive.

Ultrasound

Positive participants were subjected to ultrasound examinations for hydatid cysts at University Hospital "Luigi Vanvitelli" of Naples. The ultrasounds were performed in the first phase at our University Hospital. In the second phase an internist doctor, with significant experience in the diagnosis of cystic echinococcosis, has been appointed with ultrasound portable machine. Ultrasound images were resolved according to WHO Informal Working Group On the Classification of Echinococcosis (WHO-IWGE). This classification ranks liver cysts into Cystic Lesions (CLs) and cystic echinococcosis in stages 1-5 (CE1-CE5). CE1 and CE2 represent active disease stages. CE3a is considered

transitional disease stages. At the last CE4 and CE5 corresponds to inactive disease stages.

Data Collection and Analysis

The process of informed consent occurs with all the study participants that gave their authorization and agreement. Data and results were statistically analyzed by SPSS software (version 22.0, Chicago, IL, USA) [20].

Results

In the current study, 650 farms were visited, in particular 168 in Avellino, 123 in Benevento, 102 in Caserta and 257 in Salerno (Table 1). On the basis of the regional livestock population, cattle, sheep and buffalo of breeding farms were selected in several municipalities across the study area [21]. Analysis, performed by Geographic Information System (GSI), showed the distribution of farms in the region with small cluster in the south-eastern and north part of the study area (Figure 1) [22]. For each province, the distribution of the farms is shown in figure 2. A total of 1497 farmers were part of our study: 23,7% from Avellino, 18,0% from Benevento, 19,0% from Caserta and 39,3% from Salerno. This study population was composed most of male (61,5%) than female and regarding age distribution, the most representative was over 40 years (64,3 %) (Table 2). Peripheral blood sample were collected for each farmer. The serological ELISA and IHA screening detected 38 potential positive samples (2,5 %) out of 1497 blood specimens. Immunoblotting analysis confirmed 4 positive samples (0.4%) of which 3 in Salerno and 1 in Avellino (Table 3). All positives were found in over 40 years' age group and 3 of them were male. CE was diagnosed in individuals, located in sheep farms (0,6%). The positivity to the echinococcosis through the serological screening has allowed to identified subjects without symptoms or confirmed the positivity of those already undergoing previous pharmacological or surgical treatment. So that, perform an analysis of the risk factors, like ultrasound examination, could be indicative of the human transmission of this parasitic infection. The serological positive participants were subject to ultrasound examinations for hydatid cysts. We reported a liver cyst detected by ultrasound portable machine diagnosis in one participant from Salerno province. The cyst was classified as CE3a stage (Figure 2).

VARIABLE COLLECTED	AVELLINO	BENEVENTO	CASERTA	SALERNO
n(%) farmers	355 (23,7)	269 (18,0)	284 (19,0)	589 (39,3)
n farms	168	123	102	257

Table 1: Collected farms and farmers' distribution rates in analyzed Campania provinces.

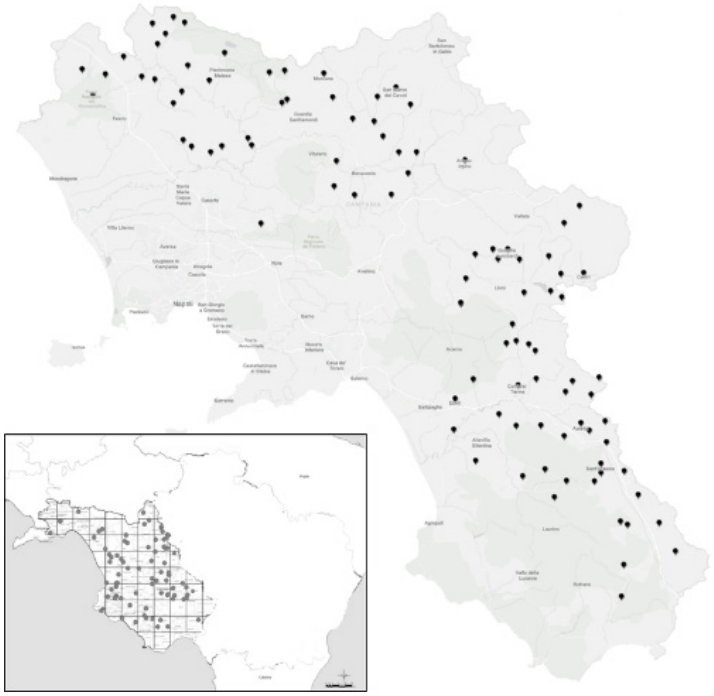

Figure 1: Farm animal's echinococcosis study areas. In small square the restrictive distribution area of positive CE sheep farms in Salerno.

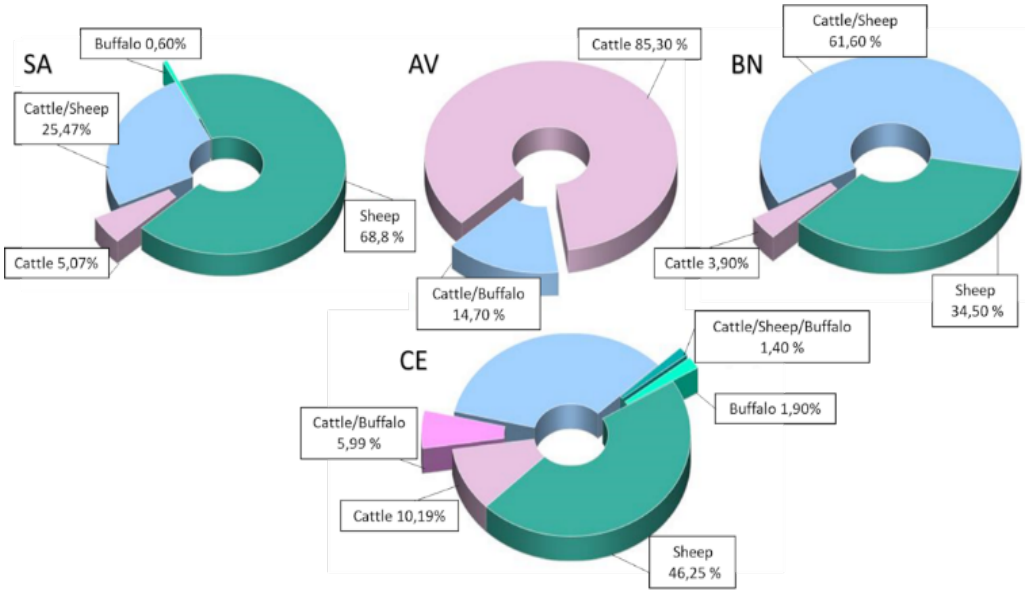

Figure 2: Percentage distribution of type of farms in different Campania provinces.

(CE: Caserta; SA: Salerno; AV: Avellino; BN: Benevento).

Gender	**n (%)**
Male	921 (61,5)
Female	576 (38,5)
Age groups	**n (%)**
Under 10 years	35 (2,3)
10-20 years	133 (8,9)
20-40 years	367 (24,5)
Over 40 years	962 (64,3)

Table 2: Sex and age distribution of collected population in Campania.

Number of patients examined	**Number of potential positive IHA/ELISA infected**	**Infection rate (%)**	**Positive provinces n (%)**	
			Salerno	**Avellino**
1497	38	0,4	3 (0,3)	1 (0,1)

Table 3: Seroprevalence of antibodies against *E.granulosus* in selected patients resident in different provinces of Campania.

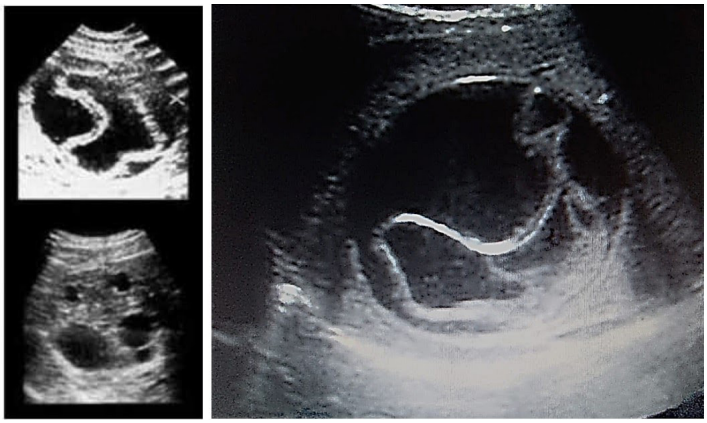

Figure 3: Compared ultrasonography images of cystic echinococcosis CE3a type between WHO-IWGE classification and positive patient liver from Salerno province.

Discussion

CE represents a neglected zoonotic parasitic disease that affects humans and livestock [23]. This disease is frequently caused by *E. granulosus*, species of greatest clinical relevance and geographic distribution [8]. CE determines serious socio-economic and public health damage worldwide [24]. Over the past two decades, several studies focused on the global CE prevalence, in order to better define the endemic areas [13]. In contrast, few studies on CE epidemiology were conducted in Italy [14]. The purpose of this study was to evaluate the current state of human CE epidemiology in Campania region with the aim to build a surveillance network for this illness. In this regard has been implemented the disclosure activity to doctors and members of civil society with a focus on medical aspects. Surveillance of humans CE is difficult, mainly due to the asymptomatic infection in dogs and livestock and the long latency of parasite before the detected human cysts [25]. Study area involved 650 farms, spread over four provinces of Campania: 168 in Avellino, 123 in Benevento, 102 in Caserta and 257 in Salerno. We visited cattle, sheep and buffalo farms in the several municipalities of the study provinces. A total of 1497 peripheral blood samples were collected by each farmer and subjected to laboratory analysis. In this study, the diagnosis was performed through ELISA and IHA screening and WB confirmation. The serological screening found 2,5% of potential positive samples. WB analysis confirmed the 0.4% of positive cases of which 0,3 % in Salerno and 0,1 % in Avellino. Our results confirmed that the CE monitoring by ELISA and IHA overestimated the human exposure to *E. granulosus* in environments where contact with other helminths can occurs [26]. As proposed by WHO, the implementation of a confirmation technique had allowed to eliminate the false positives findings due to the cross reactivity with other parasites [27]. In our region, information and awareness campaigns to control echinococcosis through health education programs and information on disease severity and transmission modalities have contributed to the reduced prevalence of CE. Our findings showed a lower prevalence than observed in Iran (5%), Kenya (3.8%) and China (3.2%) [26-28]. Differences in CE prevalence in several areas could be caused by: i) different climate that affects the viability of the parasite's eggs; ii) the type of livestock farms in each country; iii) the role of the study population; iv) CE surveillance and monitoring systems [29]. Most of positive patients were males and belonged to over 40 years' age group. These results were probably due to currently contact with farm animals, when cleaning or feeding them or during milking [30]. Positive cases possessed sheep farms (0,6%). Sheep are an intermediate host with high reception. In their livers and lungs, hydatid cysts mature easily and become potentially infectious to dogs and consequently to humans [31]. All serological positive participants were subject to ultrasound examinations for hydatid cysts. Ultrasound represent an effective, safe and inexpensive method that allows the recognition of this disease even in the asymptomatic phase [32]. According to WHO-IWGE, the ultrasound images of the patients were interpreted. This study, showed liver cyst in one participant (Salerno), classified as CE3a type. This cyst, detected by ultrasound portable machine, was in transitory stage, where the integrity of the cyst was compromised, producing the typical "water lily" sign [33]. Our study is the first that underline the epidemiological scenario in Campania region for the endemic human CE. Prevalence of human CE remains difficult, costly and time-consuming. For this reason, early diagnosis of cyst/s with ultrasound portable machine may prevent the routine surgery for CE and can offer to farmers and relatives a simple clinical-diagnostic path. It is now widely demonstrated that CE have a serious impact on welfare animal health, quality and quantity of production and also on professional's health. In this scenario, the active collaboration between all the clinical figures involved, can help define a control common program for micro, small and medium-sized enterprises workers with the goal of testing, transferring and disseminating innovative techniques to detected this parasitosis.

Acknowledgment

We would like to show our gratitude to Campania Region that supported the project: "Control and reduction of Echinococcosis/Hydatidosis in animal populations and prevention of related human pathology - EchinoCamp1000" and to all the partners of the project who provided insight and expertise that greatly assisted the research**.**

References

1. Agudelo Higuita NI, Brunetti E, Mc Closkeyc C (2016) Cystic Echinococcosis. J Clin Microbiol 54: 518 -523.
2. Ma X, Zhang L, Wang J, Luo1 Y (2019) Knowledge domain and emerging trends on Echinococcosis research: a scientometric analysis. Int J Environ Res Public Health 16: 842.
3. Cerda JR, Buttke DE, Ballweber LR (2018) Echinococcus spp. tapeworms in North America. Emerg Infect Dis 24: 230-235.
4. Romig T, Deplazes P, Jenkins D, Giraudoux P, Massolo A, et al. (2017) Ecology and life cycle patterns of Echinococcus species. Adv Parasitol 95: 213-314.

5. Cucher M, Prada L, Mourglia-Ettlin G, Dematteis S, Camicia F, et al. (2011) Identification of Echinococcus Granulosus microRNAs and Their Expression in Different Life Cycle Stages and Parasite Genotypes. Int J Parasitol 41: 439-448.

6. Tappe D, Stich A, Frosch M (2008) Emergence of polycystic neotropical Echinococcosis. Emerg Infect Dis 14: 292-297.

7. Wang Q, Huang Y, Huang L, Yu W, He W, et al. (2014) Review of risk factors for human echinococcosis prevalence on the Qinghai-Tibet Plateau, China: a prospective for control options. Infect Dis Poverty 3: 3.

8. Eckert J, Deplazes P (2004) Biological, epidemiological and clinical aspects of Echinococcosis, a zoonosis of increasing concern. Clinical Microbiol Rev 17: 107-135.

9. Pakala T, Molina M, Wu GY (2016) Hepatic Echinococcal cysts: a review. J Clin Transl Hepatol 4: 39-46.

10. Akbulut S, Ozdemir F (2019) Intraperitoneal rupture of the hydatid cyst: four case reports and literature review. World J Hepatol 11: 318-329.

11. Oguz B, Ozdal N, Kilinc O, Deger MS (2018) Preliminary studies on the prevalence and genotyping of *Echinococcus granulosus* infection in stray dogs in Van Province, Turkey. J Vet Res 62: 497-502.

12. Rossi P, Tamarozzi F, Galati F, Pozio E, Akhan O, et al. (2016) The first meeting of the European Register of Cystic Echinococcosis (ERCE). Parasit Vector 9: 243.

13. Grosso G, Gruttadauria S, Biondi A, Marventano S, Mistretta A (2012) Worldwide epidemiology of liver hydatidosis including the Mediterranean area. World J Gastroenterol 18: 1425-1437.

14. Manfredi MT, Di cerbo AR, Zanzani S, Moriggia D (2011) Prevalence of echinococcosis in humans, livestock and dogs in northern Italy. Helminthologia 2: 59-66.

15. Budke CM, Jiamin Q, Qian W, Torgerson PR (2005) Economic effects of Echinococcosis in a disease-endemic region of the Tibetan Plateau. Am J Trop Med Hyg 73: 2-10.

16. Piseddu T, Brundu D, Stegel G, Loi F, Rolesu S, et al. (2017) The disease burden of human cystic echinococcosis based on HDRs from 2001 to 2014 in Italy. PLOS Negl Trop Dis 11: e0005771.

17. Herrador Z, Siles-Lucas M, Aparicio P, Lopez-Velez R, Gherasim A, et al. (2016) Cystic Echinococcosis epidemiology in Spain based on Hospitalization records, 1997-2012. PLOS Negl Trop Dis 10, e0004942.

18. Aydin Y, Altuntas B, Kaya A, Ulas AB, Uyanık MH, et al. (2018) The availability of Echinococcus IgG ELISA for diagnosing pulmonary hydatid cysts. Eurasian J Med 50: 144-147.

19. Bass JJ, Wilkinson DJ, Rankin D, Phillips BE, Szewczyk NJ, et al. (2017) An overview of technical considerations for Western blotting applications to physiological research. Scand J Med Sci Sports 27: 4-25.

20. D. Pignataro, F. Foglia, M. T. Della Rocca, C. Melardo, B. Santella, V. Folliero, et al. (2020) Methicillin-resistant Staphylococcus aureus: epidemiology and antimicrobial susceptibility experiences from the University Hospital 'Luigi Vanvitelli' of Naples, Pathogens and Global Health.

21. Yin LK, Lieng TC, Cheong WK (2006) How to analyse your research data? Illustrations with hands-on exercises using Spss. Malays Fam Physician 1: 77-81.

22. Wieczorek WF, Delmerico AM (2010) Geographic Information Systems. Computational Statistics 1: 167-186.

23. Nunnari G, Pinzone MR, Gruttadauria S, Celesia BM, Madeddu G, et al. (2012) Hepatic echinococcosis: Clinical and therapeutic aspects. World J Gastroenterol 18: 1448-1458.

24. Benner C, Carabin H, Sánchez-Serrano LP, Budke CM, Carmena D (2010) Analysis of the economic impact of cystic echinococcosis in Spain. Bull World Health Organ 88: 49-57.

25. Thys S, Sahibi H, Gabriël S, Rahali T, Lefèvre P, et al. (2019) Community perception and knowledge of cystic echinococcosis in the High Atlas Mountains, Morocco. BMC Public Health 19: 118.

26. Li T, Chen X, Zhen R, Qiu J, Qiu D, et al. (2010) Widespread co-endemicity of human cystic and alveolar Echinococcosis on the Eastern Tibetan Plateau, Northwest Sichuan/southeast Qinghai, China. Acta tropica 113: 248-256.

27. S, Mamishi S, Banar M, Pourakbari B, Keshavarz H (2019) Epidemiology of echinococcosis in Iran: a systematic review and meta-analysis. BMC Infectious Diseases 19: 929.

28. Solomon N, Zeyhle E, Carter J, Wachira J, Mengiste A, et al. (2017) Cystic Echinococcosis in Turkana, Kenya: the role of cross-sectional screening surveys in assessing the prevalence of human infection. Am J Trop Med Hyg 97: 587-595.

29. Haleem S, Niaz S, Qureshi NA, Ullah R, Alsaid MS, et al. (2018) Incidence, risk factors, and epidemiology of cystic Echinococcosis: a complex socioecological emerging infectious disease in Khyber Pakhtunkhwa, province of Pakistan. BioMed Res Int 2018: 5042430.

30. Chalechale A, Hashemnia M, Rezaei F, Sayadpour M (2016) Echinococcus granulosus in humans associated with disease incidence in domestic animals in Kermanshah, west of Iran. J Parasit Dis 40: 1322-1329.

31. Bhutania N, Kajalb P (2018) Hepatic echinococcosis: A review. Annals of Medicine and Surgery 36: 99-105.

32. Mihmanli M, Idiz UO, Kaya C, Demir U, Bostanci O, et al. (2011) Current status of diagnosis and treatment of hepatic echinococcosis. World J Hepatol 8: 1169-1181.

33. WHO Informal Working Group (2003) International classification of ultrasound images in cystic Echinococcosis for application in clinical and field epidemiological settings. Acta Tropica 85: 253-261.