E-ISSN 2822-3152
E-mail: echinococcosis.editor@gmail.com
Journal homepage: www.echinococcoses.org

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International Journal of Echinococcoses 2022;1(2):26-32
DOI: 10.5455/IJE.2022.03.05

International Journal of Echinococcoses

Journal homepage: www.echinococcoses.org

Original Article

Epidemiological Indicators of the State of Cystic Echinococcosis in Republic of Bulgaria for the Period 2011-2020

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ARTICLE INFO

Received: Mar 01, 2022
Accepted: May 04, 2022
Available online: May 19, 2022

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CITATION

Rainova IG, Harizanov RN, Tsvetkova ND, Kaftandjiev IT, Enikova RB, Ivanova M, Videnova MV, Kaneva EM, Mikov OD . Epidemiological Indicators of the State of Cystic Echinococcosis in Republic of Bulgaria for the Period 2011-2020 International Journal of Echinococcoses 2022;1(2):26-32. DOI: 10.5455/IJE.2022.03.05

The journal is the official publication of The Turkish Association of Hydatidology

ABSTRACT

Objective: Cystic echinococcosis (CE) is one of the most severe parasitoses, which leads to prolonged disability, frequent recurrences, and even to death. In Bulgaria it is one of the most frequently registered and severe helminthic infection.
The aim of this study is to determine the epidemiological indicators as morbidity, mortality and lethality from CE in Bulgaria for the period 2011-2020, as well as a comparative analysis of morbidity in the country with that of other EU member states.
Materials and Methods: The study is a retrospective analysis of confirmed cases of CE, as well as of the deceased from this parasitic disease. Data from several sources were used - the annual analyzes of parasitic morbidity in the country, prepared by the NCIPD, as well as information from the National Center for Public Health and Analysis and the National Statistical Institute. Statistical methods were used to calculate morbidity and mortality rates.
Results: From 2011 to 2020, a total of 2626 cases of CE are registered in Bulgaria (95% Confidence Interval: 263 ± 3.16), with an average incidence for the period of 3.7‰ (95% CI: 3.65 ± 0.357). The registered primary cases are 2336 (95% CI: 233.6 ± 3.03) in total and 290 (95% CI: 29 ± 1.04) are recurrences. Cystic echinococcosis is comparatively more prevalent in women than in men, respectively 1397 (95% CI: 139.7 ± 2.39) and 1229 (95% CI: 122.9 ± 2.14) registered cases for the period and for morbidity values are 3,7‰ (95% CI: 3.71 ± 0.36) to 3,5‰ (95% CI: 3.53 ± 0.343). The most affected is the age group 20–59 years, i.e. people of active working age. The number of affected children aged 1 to 19 (509 cases) is relatively high, which indicates an active transmission of the parasite. During the studied period deceased patients due to echinococcosis are 65 of whom 38 men and 27 women. The average mortality and the lethality are respectively 0.09‰ (95% CI: 0.09 ± 0.103) and 2.49% (95% CI: 2.49 ± 0.405).
Conclusion: The results show a reduction in examined indicators regarding cystic echinococcosis compared to data for the 1990s and the studied from us period, but these values are still higher than the established EU averages.

Keywords: Cystic echinococcosis, morbidity, mortality, lethality

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DOI: 10.5455/IJE.2022.03.05

INTRODUCTION

Zoonoses are diseases transmitted between animals and humans. They have a cosmopolitan distribution (1). About 150 infectious and parasitic diseases common to animals and humans are known. Their relevance and health significance have increased significantly in recent years worldwide (2). Among helminthic zoonoses the most significant are echinococcosis, trichinosis, toxocariasis, filariasis, fasciolosis (3). Cystic echinococcosis (CE) is a foodborne parasitic disease which may take a severe clinical course that often leads to prolonged disability. The disease tends to relapse frequently after treatment and sometimes can cause permanent disabilities and even death (4). In Bulgaria, studies on the prevalence of CE have been conducted since the beginning of the 20th century. Over the years, a significant number of large-scale studies have been conducted on the dynamics of morbidity, mortality and lethality in this parasitosis (5, 6). In addition CE in Bulgaria is subject to mandatory notification and registration, including both primary cases and recurrences. An investigation record form approved by the Ministry of Health is filled in for each registered case.

Surveillance of CE is important for health care due to the possibility of long-term disease, frequent recurrences, permanent disability and death. After the political and economic changes in 1989, the incidence of CE in Bulgaria increased significantly, and in 1998 and 2002 two peaks were registered - 8.47 per 100,000 and 8.32% per 100,000 (7).

Therefore between 2004 and 2008 a National program for the control of echinococcosis in humans and animals was conducted. After the end of the program and so far a tendency for gradual reduction of the incidence from CE was established in Bulgaria and in 2020 reached their lowest values - 95 primary cases and relapses, as well as an incidence of 1.37‰.

Despite successes in reducing reported cases and morbidity, cystic echinococcosis deaths have been reported each year, most commonly in cases of multiple echinococcosis, lung involvement, or cyst rupture with anaphylactic shock. As one of the most important helminthiasis in Bulgaria in this study, we tried to update the data on the main epidemiological indicators concerning CE. Objectives of this report are to determine the epidemiological indicators as morbidity, mortality (mortality is the number of deaths per hundred thousand citizens of a given community over a given period of time) and lethality (lethality, usually expressed as a percentage, is the ratio of the number of deaths to the total number of patients with certain disease over a period of time) from cystic echinococcosis (CE) in Bulgaria for the period 2011-2020, as well as a comparative analysis of morbidity in the country with that of other EU member states.

MATERIALS AND METHODS

Study design

The study is a retrospective analysis of confirmed cases of CE, as well as of the morbidity and lethality from this parasitic disease in Bulgaria for ten year period. Only aggregate data were used in the study and it was not necessary to obtain permission from the Institutional Ethics Committee.

Data collection

Currently, surveillance and control of echinococcosis is carried out in accordance with Regulation №5 of Ministry of Health for the diagnosis, prevention, and control of local parasitic diseases. All newly registered cases are subject to rapid notification and epidemiological investigation by the Regional Health Inspectorates, which send these data to the National Centre of Infectious and Parasitic Diseases for further processing and analysis. In addition to this data, we also used data from other sources such as the National Center for Public Health and Analysis and the National Statistical Institute regarding mortality rates and the number of Bulgarian population by year.

Patients

The current study includes all registered cases of CE for a ten-year period 2011-2020, with patients grouped by gender and age (children and adolescents up to 19 year, and adults).

Descriptive statistics were used to determine some statistical indicators such as mean, standard deviation and 95% confidence interval of the mean..

RESULTS

From 2011 to 2020, a total of 2626 cases of CE are registered in Bulgaria, with an average incidence for the period of 3.7‰(Table. 1).

The highest values of the incidence are reported in 2012 and 2014 - 4.75‰ and 4.6‰, but the general trend for the whole period is gradual decrease of the registered cases and morbidity, which in 2020 reached 1.37‰ and is the lowest of 40 years (5).

The registered primary cases of CE clearly shows the trend of gradual decline, while recurrences lack special dynamics and they vary from 20 to 40 per year. In total for the period the cases of recurrence represent 11.08% of all registered cases of CE. In 2020, there are only nine relapses, most likely due to missed diagnostics related to the pandemic of SARS-CoV-2.

The follow-up of the dynamics of the incidence of CE by

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Table 1: Cases of CE divided by type and sex

Year	Registered cases (n)	Primary cases (n)	Relapses (n)	Male sex	Female sex	Annual incidence per 100 000 population
2011	347	312	35	159	188	4.7
2012	346	312	34	162	184	4.75
2013	307	266	41	148	159	4.2
2014	332	296	36	156	176	4.6
2015	313	283	30	141	172	4.37
2016	269	238	32	120	149	3.78
2017	218	196	22	118	100	3.1
2018	207	178	28	90	117	2.93
2019	192	168	24	90	102	2.74
2020	95	86	9	45	50	1.37
Total	2626	2335	291	1229	1397	36.54
Mean	262.60	233.5	29.1	122.9	139.7	3.65
Standard deviation	26.16	74.75	9.06	38.22	45.55	1.10
95% CI of mean	263 ± 3.16	233.5 ± 3.03	29.1 ± 1.04	122.9 ± 2.14	139.7 ± 2.39	3.65 ± 0.357

gender for the studied period shows that both the cases and the incidence in women prevail over those in men - 1397 to 1229 registered cases and 3.8‰ to 3.5‰ established average incidence (Figure 1).

Cystic echinococcosis (CE) affects all age groups. According to age and gender, the highest number of patients in males is in the groups 30-39 years (212) and 20-29 years (209), and in females - in the groups 30-39 years (212) and 60-69 years (210) (Figure 2, Figure 3). Also in the groups between 40 and 60, women (616) are significantly more affected than men (421). Our data show that affected by this helminth infection are mainly people of working age, 58% of registered patients are in the age group of 20 to 59. The incidence in the age groups of 20-29 (4.85‰), 30-39 (4.2‰), 40-49 (3.48‰) and 50-59 (3.39‰) shows decreasing values and only in the group of 60-69 (3.6‰) this indicator is higher, probably due to the higher number of registered women with cystic echinococcosis (Figure4).

In children, the registered cases of cystic echinococcosis in boys aged 0 to 19 are 281, and in girls - 228. In the age groups from 0 to 9, and 10 to 19 the number of affected boys is more than girls - 112 to 70, and 169 to 158. Although reported cases of cystic echinococcosis are higher in the age groups between 20 and 40 years in both men and women, the highest incidence is found in the age group between 10 and 19 years. (5.06‰) (Figure 4).

During the studied period, both the number of cases and the incidence rate vary in different regions of the country (Fig. 5). Most patients with CE are from the districts of Plovdiv (276), Sliven (249), Burgas (199), Varna (192) and Kardzhali (161), although the morbidity is highest in the districts of Sliven (13.09‰), Kardzhali (10.5‰), Shumen (7.75‰) and Dobrich (7.36‰).

During the studied period by reason of echinococcosis there were 65 deceased patients, the largest number - 23, is in the age group 70-79 year followed by the groups 60-69 year with 13 patients and 50-59 year with 9. Among the group of children and adolescents, two cases with fatal outcome were registered in two boys aged 4 and 15.

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DOI: 10.5455/IJE.2022.03.05

Depending on gender, 38 of the deceased are men and 27 are women. Forty-two of the deceased lived in rural areas, and they are more than those from the urban areas, where they are 23. Both from the villages and from the cities the number of deaths among men were higher - 24 males vs 18 females among the rural and 14 vs 9 among the urban residents.

The average rate of mortality and lethality from CE for the period from 2011 to 2020 were 0.09‰ and 2.49% (Figure 6, Figure 7). The annual values of this indicators were the highest in 2012, respectively 0.19‰ and 4%, and in 2016 - 0.15‰ and 4%.

Data on the organ location of echinococcal cysts show that 70% of them are in the liver, 20% in the lungs and in 6.5% of patients cysts are with extra hepatopulmonary localization.

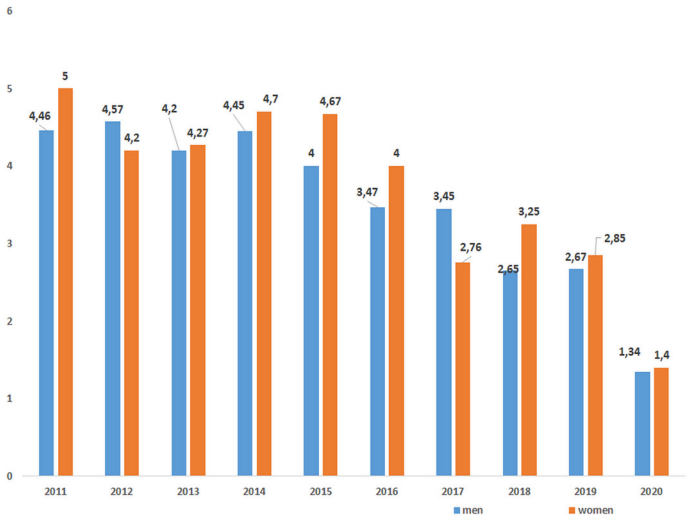

Figure 1. Incidence by years distributed by gender per 100 000 population

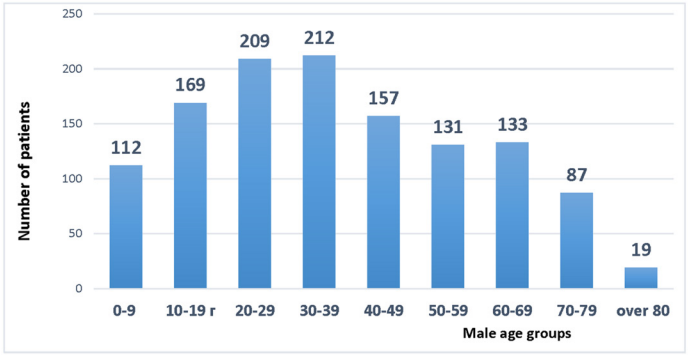

Figure 2. Registered cases of cystic echinococcosis by age groups in males

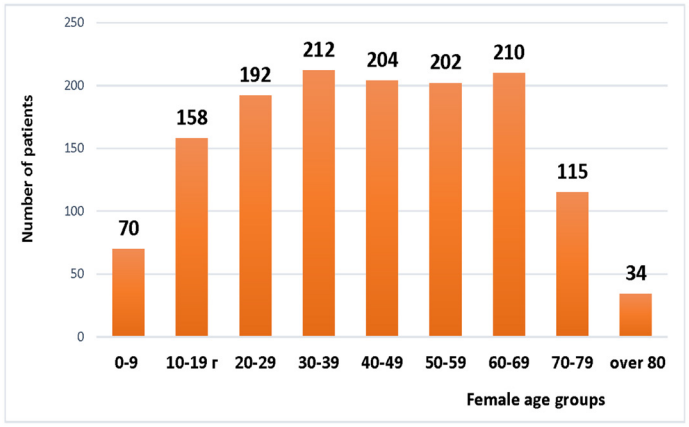

Figure 3. Registered cases of cystic echinococcosis by age groups in females

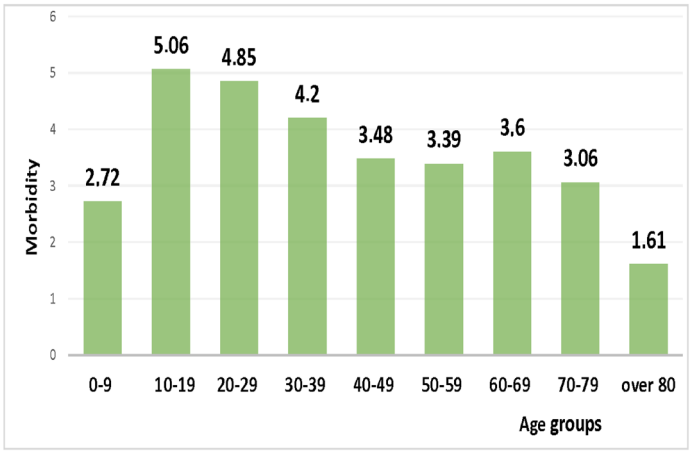

Figure 4. Morbidity by age groups for the studied period 2011-2020 per 100 000 population

Figure 5. Registered cases of cystic echinococcosis by regions of the country

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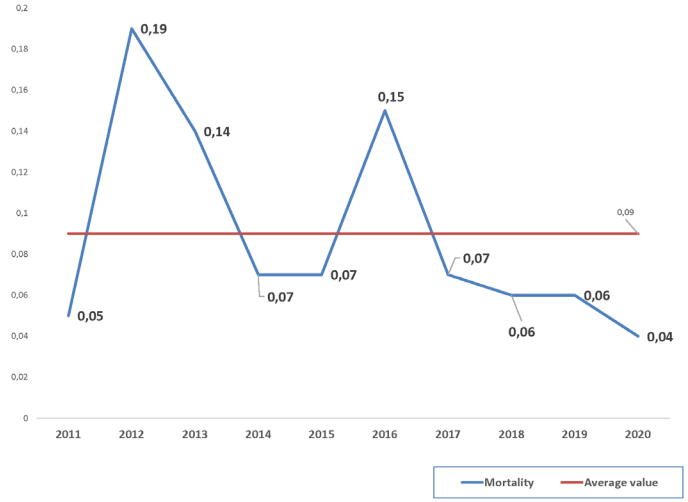

Figure 6. Mortality of cystic echinococcosis for the period 2011-2020 per 100 000 population

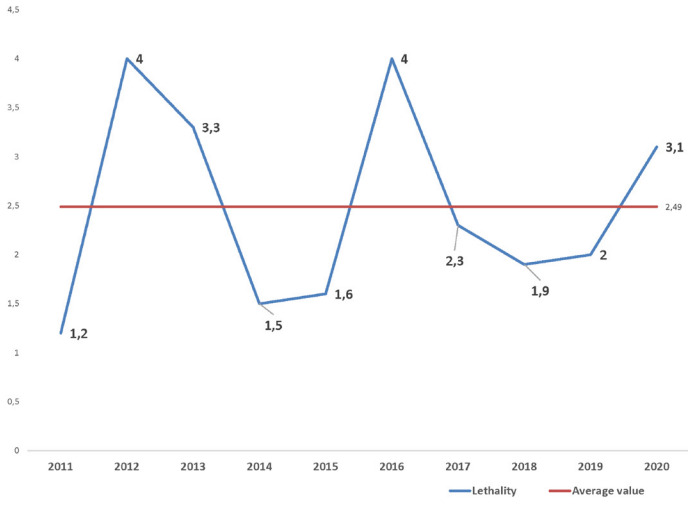

Figure 7. Lethality of cystic echinococcosis for the period 2011-2020 in %

DISCUSSION

CE is one of the parasitic diseases of greatest medical importance in Bulgaria (7). For the studied ten-year period the average incidence is 3.7 per 100,000. A comparison of the data obtained with those of a previous study conducted in the period 2001-2010 with an established average incidence of 6.77 per 100,000 shows that an almost twofold reduction in the incidence of CE has been achieved (8). Probably the decrease in the annual incidence rates of CE is largely due to

implementation of the National Program for the Control of Echinococcosis among Humans and Animals conducted between 2004 and 2008. Within the framework of this program, a mass serological screening of at-risk contingents of 9 390 individuals was carried out throughout the country, as a result of which 66 new undiagnosed cases of CE were detected. A diagnostic-therapeutic algorithm was prepared to be applied by medical specialists, as well as measures for health information for the population on measures to prevent infection. The veterinary part was mainly related to the implementation of preventive measures for deworming, mainly of stray dogs. However, both registered cases and morbidity in Bulgaria remain higher than in other EU countries. During the 2004 - 2008, a total of 4 306 cases of CE were reported in the EU countries, and these data show that 61% of those registered with this parasitic disease are from Bulgaria (9). The recent cross-sectional ultrasound-based survey that recruited volunteers from 50 villages in four districts of Bulgaria was with data for 31 persons with abdominal CE of 8602 people screened in Bulgaria (10).

In total for the period the cases of recurrence represent 11.08% of all registered cases of CE. Previous study by Harizanov et al. covering the period 2006 - 2017 presents data for 9.9% recurrences for the period (11). This shows that the relative part of recurrences remains at relatively constant levels in the country, despite the fact that according to Bulgarian legislation, all cases of CE treated surgically or with PAIR technique are subject to subsequent anti-relapse treatment with albendazole, which is reimbursed 100% by the National Health Insurance Fund. This gives us reason to believe that not all cases subject to anti-relapse treatment are covered by the health system in the country. Our data for the cases of recurrence are similar with these of other authors. According to a study by Velasco-Tirado et al. cases of recurrence are 11.5% in the contingent of CE patients studied by them (12).

The registration of CE to a large extent in the active, working age can be explained by the chronic course of the disease, progressing over time and often diagnosed years after infection. And the higher incidence among females compared to males is probably due to more frequent contact of women with pets and the work done to grow low-stem vegetables and fruits on the private farm.

The data on the established highest morbidity in the age group 10-19 years (5.17 per 100,000) is quite alarming and shows that there is an active transmission of the disease (13). The number of affected children and adolescents up to 19 years of age in our study is relatively high (509 cases) but is more than twice lower than the number of affected persons in the same age group for the period 2000 - 2010 (1193 cases, incidence 6.4 per 100.000) (13). The established high values of the incidence in the age group 10-19 years are possible due to insufficient hygiene habits in children and their more frequent contact with dogs for play. Also since 1990, the country's population of stray dogs has increased significantly that are not regularly dewormed, particularly in areas with developed livestock

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DOI: 10.5455/IJE.2022.03.05

breeding and especially sheep breeding (13). Sheep are the most important intermediate host of *E. granulosus*. Their role in the spread of the cystic echinococcosis is determined by their constant contact with dogs and the high percentage of fertile echinococcal cysts. This explains the widespread prevalence of echinococcosis in countries with developed sheep breeding (14). The highest average incidence rate of CE was recorded mainly in four regions:

Sliven (13.09 per 100,000), Kardzhali (10.5 per 100,000), Shumen (7.75 per 100,000) and Dobrich (7.36 per 100,000). Sheep farming is well developed in all four areas. Sliven District is located in the Southeastern region of the country where 21.5% of the sheep are raised, Kardzhali is in the South Central region, and 23.2% of the sheep are raised there, and Shumen and Dobrich are located in the Northeast region of the country, where more than 15% of sheep are bred (15).

In terms of mortality and lethality, the data obtained can be explained by the relatively large number of cases of cystic echinococcosis registered in the country and unfortunately delayed diagnosis due to lack of clinical symptoms, creating preconditions for spontaneous or traumatic rupture of echinococcal cysts and/or cysts, which almost always leads to anaphylactic shock with lethal outcome. Of course, this can include complications arising after surgery for the disease, especially in multiple and multi-organ involvement, as well as rare localizations affecting vital organs and systems (heart, CNS). However, it should be noted that compared to a previous study covering the period from 1991 to 2002, the mortality rate from cystic echinococcosis in Bulgaria decreased 2 times and the lethality 1.6 times (6).

The data on the organ localization of echinococcal cysts are not surprising, because according Eckert et al., the relative percentages of liver and lung localizations together account for at least 90% of the hydatid cysts in humans (16). Data from previous study in Bulgaria presents data for location the first place in the liver (65.47%) and in the second in the lungs (23.48%) (6). In the Timis County, Romania, of the 182 individuals studied between 2004 and 2010 with CE, localization in the liver was found in 81.9% of them (17). However, the relative part of cases with extrahepatopulmonary localization of 6.5% shows that they are also quite common and mainly affected are the spleen, abdominal cavity, kidneys and muscles/ subcutaneous tissue. Similar data were presented from Harizanov et al, according which for the period 2010–2019, the cases with extra-hepatopulmonary localization were 5.17% (18).

Analyzing the data, the logical question arises: Why are there so many cases of echinococcosis in Bulgaria? In our opinion, the answer is multifaceted and the reasons for this are different. In the first place are the measures against the main reservoir source of the disease in synanthropic foci - the dog. The control over the number of the stray dog population is weak or absent, and the measures for their regular deworming are fragmentary and only in some settlements. This also applies to domestic and shepherd dogs, especially in small settlements, where animal husbandry and basic sheep breeding are practiced, because sheep are the most important intermediate host of *E. granulosus* (14). We believe that in the field of human medicine, with the exception of prevention, there are no particular problems, both in terms of diagnosis and in terms of treatment, surveillance and control of the disease. However, serious veterinary control is required with regard to the final host (dog) and the main intermediate (sheep).

CONCLUSION

In conclusion, it can be said that despite the successes achieved in the surveillance and control of CE in Bulgaria, the disease is still of great health and public impact. Measures to reduce the incidence, mortality and lethality include enforcement of the preventive actions by the public health authorities with media collaboration and involvement.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: The work is supported by the Executive Agency Science and Education for Smart Growth Operational Program (Project BG05M2OP001-1.002-0001 "Fundamental, Translational and Clinical Investigations on Infections and Immunity") to the Ministry of Education and Science of the Republic of Bulgaria.

Ethical approval: Only aggregate data were used in the study and it was not necessary to obtain permission from the Institutional Ethics Committee.

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International Journal of Echinococcoses 2022;1(2):33-7
DOI: 10.5455/IJE.2022.02.04

International Journal of Echinococcoses

Journal homepage: www.echinococcoses.org

Original Article

Investigation of Direct Microscopy and Indirect Hemagglutination Test Results in the Diagnosis of *Echinococcus granulosus* in Selçuk University Hospital

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ARTICLE INFO

Received: Feb 24, 2022
Accepted: Apr 12, 2022
Available online: May 19, 2022

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CITATION

Macin S, Samadzade R. Investigation of Direct Microscopy and Indirect Hemagglutination Test Results in the Diagnosis of *Echinococcus granulosus* in Selçuk University Hospital. International Journal of Echinococcoses 2022;1(2):33-7. DOI: 10.5455/IJE.2022.02.04

The journal is the official publication of The Turkish Association of Hydatidology

ABSTRACT

Objective: Cystic echinococcosis (CE) is a zoonotic disease in humans and herbivorous animals caused by cestods of the genus Echinococcus. Humans are incidental hosts in the infection caused by *Echinococcus granulosus*. The aim of this study is to retrospectively investigate the direct microscopy and seropositivity of patients diagnosed with CE.
Materials and Methods: Between January 2017 and January 2021, cyst fluid and serum samples from 55 patients were sent to the Parasitology Laboratory from various units of the hospital. Cyst fluid samples were examined microscopically after condensation. Specimens showing hooks or protoscolex on microscopy have been reported as positive. Serum samples of the patients were investigated for cystic echinococcosis by indirect hemagglutination test (IHA). Those with a titer of ≥160 were considered positive.
Results: Twenty two (40%) cyst specimens with hooks or protoscolex on direct microscopy were reported as positive. Of the patients included in the study, 38 (69.1%) were male and 17 (30.9%) were female. Both direct microscopy and IHA test were studied from 30 patients. In terms of CE, the serum of 17 (56.6%) patients was found to be seropositive. In addition, both direct microscopy and IHA results of 8 (36.3%) patients were found to be positive.
Conclusion: Although CE is quite common in Turkey, it is highly neglected because it usually does not cause symptoms for years and is not reported frequently even though it is mandatory. For this reason, since the clinical findings of CE, which is still an important public health problem in our region, can be confused with other system pathologies, it would be useful to evaluate clinical, radiological, serological and biochemical findings together in the diagnosis.

Keywords: Cystic echinococcosis, *Echinococcus granulosus*, Indirect Hemagglutination Test, protoscolex, direct microscopy

INTRODUCTION

Cystic echinococcosis (CE), also known as Hydatid Cyst Disease, is a zoonotic infection caused by *Echinococcus granulosus (E.granulosus)*, a cestode species. The adult parasite lives in the breed of dogs (dog, fox, wolf). It infects animals such as sheep, goats and cattle, which are natural intermediate hosts, through its larval form in the metacestode period (1). Humans are incidental hosts in the infection caused by *E.granulosus*. CE disease develops as a result of the ingestion of eggs excreted with the feces of carnivores, which are definitive hosts, with contaminated

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DOI: 10.5455/IJE.2022.02.04

hands and drinking water, or by consumption of raw vegetables and fruits (2).

E.granulosus can be involved in any organ at random. CE, which is not encountered in most of the developed countries around the world, is frequently seen in developing countries (3). CE, is a common disease in both animals and humans in Turkey, it also causes significant economic loss. The economic loss caused by hydatid cyst in our country are mainly due to the expenses of diagnosis and treatment of the disease in humans. In farm animals, depending on the spread in the organs during the development of the cyst; factors such as low wool quality, increase in sterility rate, decrease in meat and milk yield are the most important detrimental effects (4).

Since the clinical findings are nonspecific, radiological and serological diagnostic methods are used in the diagnosis of the disease. Radiologic diagnostic methods such as USG, CT and MRI are frequently used in the follow-up of patients with cyst rupture and in the postoperative period.In such cases, serological methods are very useful both in confirming the diagnosis and in the follow-up of postoperative recurrences (5). Most of these methods are based on the detection of antibodies specific to E. granulosus in patient's sera. ELISA and indirect hemagglutination (IHA) methods are frequently preferred for diagnosis because of the ease in application, low cost, and high sensitivity and specificity (6).

In the microscopic examination of the cyst fluid, seeing the protoscolex of the parasite or even one hook belonging to the protoscolex is sufficient for a definitive diagnosis. Some of the ruptured cysts can be seen in the bronchi, intestine, urinary and biliary tract. Therefore, protoscoleces, hooks and germinal membrane particles of the parasite can be seen in samples taken from these regions (7). However, the absence of typical structures in microscopic examination does not rule out the disease. Because some cysts may be sterile (they may not contain any scolex and daughter cysts) (8).

The aim of this study was to retrospectively investigate the correlation of direct microscopy and seropositivity of patients diagnosed with cystic echinococcosis. In addition, it was aimed to investigate the distribution of antibodies positivity of *E.granulosus* according to hospital units and gender.

MATERIALS AND METHODS

The results of direct microscopic examination of cyst fluid samples sent to the Medical Microbiology Laboratory from various clinics of Selçuk University Medical Faculty Hospital between January 01, 2017 and January 01, 2021 were examined. In case of protoscolex in cyst fluids, it was centrifuged at 4000 rpm for 15 minutes in order to precipitate. Macroscopically, after centrifugation, a sedimentation or turbidity was detected at the bottom of the liquids, which are protoscoleces. After centrifugation, 1-3 cc droplets were dropped on the slide with a pipette from the sediment, and covered with a coverslip. The preparation was examined under a microscope with 40 objective. Specimens showing hooks and/or protoscolex on microscopy have been reported as positive.

Serum samples of the patients had been investigated for cystic echinococcosis by IHA. Blood had been withdrawn from the patients in 10 cc vacuum tubes with yellow caps. The sera separated by centrifugation for 10 minutes at 4000 rpm, (xg) were used for the study. The IHA reagent is diluted with 2.5 µl of deionized water and vortexed. On 175 µl buffer was placed in the well and 25 µl patient serum was added in the well. Then 50 µl buffer was placed in 2nd,3rd,4th,5th,6th,7th wells and diluted by 50 µl dilutions. Finally, 25 µl antigen is placed on it and shaken for 20 seconds. It was incubated for 2 hours. Those with a titer of ≥160 were considered positive. The results were analyzed retrospectively through hospital automation.

Ethical Approval

Blood samples sent for routine microbiological examinations from patients with pre-diagnosis of hydadic cyst were included in this study. Voluntary consent form was read and signed by the people whose sample was used. The study protocol followed ethical guidelines of the Declaration of Helsinki.

RESULTS

Fifty-five cyst fluid samples were examined in our laboratory in a three-year period. Hooks and/ or protoscoleces was observed in direct microscopic examination of 22 (40%) of them. Of the cyst fluids examined microscopically, 52 (94.5%) were defined as liver and 3 (5.5%) as lung samples. In addition, 38 (69.1%) of the patients studied for CE were male and 17 (30.9%) were female (Figure 1).

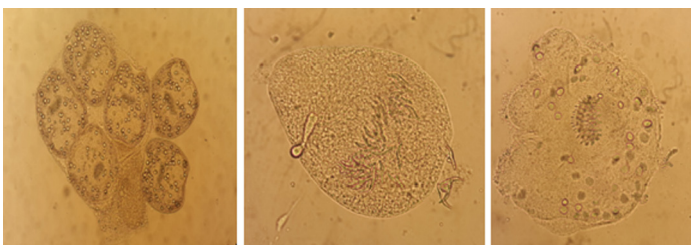

Figure 1. Protoscoleces detected on direct microscopy of cyst fluid

IHA test was studied in the sera of 30 patients from whom direct microscopy had been requested. It was determined that the serum of 17 (56.6%) patients was seropositive

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DOI: 10.5455/IJE.2022.02.04

for CE. The IHA test was also studied in 12 (54.5%) of the patients with positive direct microscopy. According to the results, although the number of patients was small, the sensitivity of both direct microscopy and IHA was found to be high in 8 (66.6%) patients (Figure 2).

In addition, cyst samples were sent mostly from General Surgery (47.2%) and Radiology (35.4%) units.

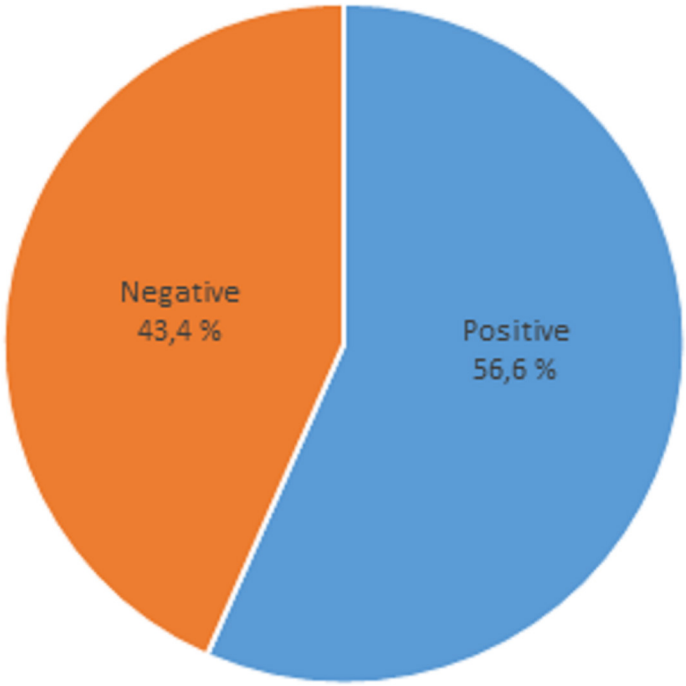

Figure 2. Distribution of IHA results in serum samples

Table 1: Distribution of cyst samples according to clinics	
Hospital Units	**%**
General Surgery	47.2
Radiology	35.4
Pediatric surgery	7.8
Thoracic surgery	5. 5
Others* Gastroenterology, Neurosurgery, Pediatric Gastroenterology and Hepatology	

DISCUSSION

CE is an important zoonotic parasitic infection that threatens public health all over the world. CE spreads widely in Turkey due to reasons such as zoogeographic differences, climatic conditions, lack of education about the disease and transmission routes in the society. It is stated that the prevalence of CE in Turkey is 50 per 100,000 and its incidence is around 2 per 100,000 (9, 10). Serum of suspected CE patients sent to the National Parasitology Reference Laboratories of the Public Health Institution of Turkey as a result of the investigation of anti-*E.granulosus* antibodies in various serological methods in the samples, it was determined that KE still continues to be an important public health problem, although it is decreasing gradually in Ankara and its surroundings (11).

Possanti et al. evaluated 1367 articles on potential risk factors in a systematic review and meta-analysis study investigating the relationship between CE and risk factors in humans, and selected 37 studies including case-control and cross-sectional studies as meta-analysis. As a result of the study, they stated that the biggest risks statistically, were stray dogs living in endemic areas, reaching offal, owning a dog and keeping a dog in their homes. However, they also emphasized in their research that the risk factors will differ between geographically different regions and communities (12).

In order to determine positivity of CE in Konya region, many studies have been carried out using the IHA method. Başer et al. found that 332 (21.6%) of 1543 patient samples were seropositive for CE using the IHA method (13). Tasbent et al. seropositivity was detected in 143 (15.2%) of 938 patients included in the study by IHA test (14). In our study, positivity was found to be higher because only specific patients who underwent direct microscopy were included (56,6 %). For this reason, similar studies conducted in Konya show that CE still remains as one of the important health problems in our region.

The absence of specific diagnostic clinical findings in human CE has led to the use of laboratory findings rather than clinical findings in the diagnosis of the disease. On the other hand, the preference of surgical treatment due to the inadequacy of medical treatment in the treatment of the disease and the possibility that CE surgery may bring some complications increase the importance of diagnosis in CE (15). Radiological diagnostic methods have an important place in the diagnosis of CE. However, radiological diagnosis should be supported by serological diagnostic methods in order to make the differential diagnosis of cyst with other space-occupying cases such as tumor, abscess, and simple cyst and to evaluate recurrences after the operation in a healthier way (16).

IHA was first used in the diagnosis of CE in 1957 by Garabedian et al. and it was found positive in 13 (81%) of 16 patients (17). Although the sensitivity of the test generally varies between 80-94%, some researchers found sensitivity values as low as 54-65%. The specificity of the test varies between 92-100%. It is known that the sensitivity of the antibody response and serological tests

International Journal of Echinococcoses 2022;1(2):33-7
DOI: 10.5455/IJE.2022.02.04

varies according to the localization of the cyst. Some researchers found the IHA test positivity in 73% of lung cysts and 89% of liver cysts. Other researchers, on the other hand, found IHA positivity in 59% of lung cysts and 76% of liver cysts (18).

Toraman et al. investigated anti-*E.granulosus* IgG antibodies by IHA method in the serum samples with the suspicion of CE. According to the results, seropositivity was found in 12.9% of 162 patients at dilutions of 1/160 and above (19).

Presence of immunocomplexes may also cause low seropositivity with IHA. Many researchers focusing on false positivities in the test attribute them to the type and preparation of the antigen used. In other studies, it was associated with cross-reactions between the aforementioned disease antigens and serum antibodies against *E.granulosus* in low serum dilutions of people with diseases such as taeniosis, fascioliosis, schistomiosis, cysticercosis, liver cirrhosis, and malignancy (20).

Studies have shown that the lack of a test that can definitively detect the presence of hydatid cyst, which has a complex antigenic structure, causes different seropositivity and seronegativity in terms of diagnosis in different patient groups (21). Kaya et al. examined blood samples of 314 people in the settlements within a 5 km area around the slaughterhouses, for *E.granulosus* antibodies by ELISA and IHA methods (22). The study was planned based on the high number of dogs around the slaughterhouses. As a result of ELISA test, 11 people (3.50%) and as a result of the IHA test, 12 people (3.82%) were seropositive. Both ELISA and IHA tests were positive in 9 patients (2.8%).Artun et al. analyzed serum samples of 100 patients with pre-diagnosis of hydatid cyst using IgG-ELISA, IHA and Western blot methods. As a result, specific antibodies were detected in 20 patients with IgG-ELISA, 16 patients with IHA and 31 patients with Western Blot (18).

There are publications reporting that the incidence of CE is higher in women than in men. In addition, there are also studies showing CE at equal rates in men and women (23). It is thought that this difference between studies may be due to women working more in rural areas and dealing with animals more in some regions (24). In our study, because you only examined the results of patients with pre-diagnosis, it supports that more men than women admitted to the hospital, and that the disease is more common in men than in women.

CONCLUSION

CE is still a neglected zoonotic disease that affects humans socio-economically, has high morbidity and mortality rates, and causes serious economic losses. CE is one of the important helminth diseases in our country, and the data were mostly obtained from hospitals, and there are a limited number of population-based epidemiological studies. The disease continue to be an important public health problem in our region, especially since the Central Anatolia region and the vicinity of Konya are the places where agriculture and animal husbandry is intense. For this reason, advanced techniques such as serological methods, computed tomography and magnetic resonance imaging are useful in confirming the diagnosis and in preparation for surgical intervention. In addition, it was concluded that the IHA or ELISA method should be preferred in the routine laboratory diagnosis of CE and the results should be confirmed with WB if possible.

Presented as an oral presentation at the 10th National and 3rd International Hydatidology Congress

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: Blood samples sent for routine microbiological examinations from patients with pre-diagnosis of hydadic cyst were included in this study. Voluntary consent form was read and signed by the people whose sample was used. The study protocol followed ethical guidelines of the Declaration of Helsinki.

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International Journal of Echinococcoses 2022;1(2):38-45
DOI: 10.5455/IJE.2022.01.01

International Journal of Echinococcoses

Journal homepage: www.echinococcoses.org

Original Article

Predictive Ability of Prognostic Markers in Cystic Echinococcosis with Bile Leakage

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ARTICLE INFO

Received: February 02, 2022
Accepted: May 15, 2022
Available online: May 21, 2022

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CITATION

Kara S, Korkut E, Aksungur N, Altundas N, Ozturk G, Yildiz M, Yeni M. Predictive Ability of Prognostic Markers in Cystic Echinococcosis with Bile Leakage. International Journal of Echinococcoses. 2022;1(2):38-45. DOI: 10.5455/IJE.2022.01.01

The journal is the official publication of The Turkish Association of Hydatidology

ABSTRACT

Objective: The most important post-surgical complication of cystic echinococcosis (CE) is cystobiliary communication and bile leakage in terms of mortality and morbidity. Therefore, we aimed to predict bile leakage using prognostic markers before the operation.
Materials and Methods: All patients hospitalized with the diagnosis of CE in the Hepatobiliary Surgery Service of Atatürk University Research Hospital between 2011 and 2021 were retrospectively analyzed. Patients who were operated for CE and developed postoperative leakage were included in the study. Prognostic markers were calculated using preoperative laboratory tests. And these values were analyzed according to the recovery time of the patient.
Results: The mean recovery time of postoperative biliary fistula was 13.97 ± 7.33 days. No mortality was observed in the patients. The mean prognostic nutritional index (PNI), the neutrophil–lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR) values were 48.68 ± 8.46, 3.09 ± 2.61, and 149.34 ± 86.17, respectively. PLR significantly affected the recovery times. An increase in PLR meant a prolonged recovery period. PNI and NLR had no significant effect on the recovery times of biliary fistula.
Conclusion: In patients with a high PLR value, caution should be exercised in terms of postoperative biliary fistula. PLR can be used as a marker if the cyst is to be intervened in the preoperative period.

Keywords: Liver hydatid cyst, Bile fistula, prognostic markers

INTRODUCTION

Hydatid cysts are caused by the adult or larval stages of tapeworms of the genus Echinococcus. CE is a zoonotic disease caused by *Echinococcus granulosus*. Its incidence varies in different geographies. CE is an endemic disease in the Middle Eastern and Mediterranean countries (1-3). As recommended by the World Health Organization (WHO), medical and surgical treatment strategies are determined based on the cyst size. Various complications, such as symptoms due to compression on neighboring organs, intra-abdominal rupture, and biliary

International Journal of Echinococcoses 2022;1(2):38-45
DOI: 10.5455/IJE.2022.01.01

tract rupture, can be observed in untreated cases (4,5). Cystobiliary communication occurs at a rate of 13%–37%, whereas jaundice associated with CE is observed at a rate of 8.9%–17% (5-7). Postoperative bile leakage is the main complication in CE after conservative surgery, which increases the morbidity and mortality (8,9). Cyst diameter is an important predictive factor of biliary fistula (10). In addition, the rate of bile leakage is lower in patients who undergo radical surgery such as liver resection or cystectomy (11).

The prognostic nutritional index (PNI) was originally introduced as an index of nutritional status in nonemergency general surgery patients and has been shown to be associated with the risk of postoperative complications (12). This index was then formulated using peripheral blood lymphocyte count and serum albumin concentration and simplified to assess the immune–nutritional status of patients (13). Recently, PNI has been shown to predict survival in gastrointestinal tract carcinomas (14). the Immune–nutritional status is also evaluated using (15) the neutrophil–lymphocyte ratio (NLR) (16) and platelet–lymphocyte ratio (PLR) in addition to PNI.

This study aimed to examine the effects of PNI, NLR, and PLR in the preoperative period on recovery times and morbidity in CE with biliary fistula.

Patient Selection and Methods

All patients who were hospitalized with the diagnosis of CE in the Hepatobiliary Surgery Service of Atatürk University Research Hospital between 2011 and 2021 were retrospectively analyzed (n = 1.058). Patient data were retrieved electronically from the hospital information system by scanning the patient files. Patients who underwent radical or conservative surgical treatment and developed biliary fistula postoperatively and those with preoperative or perioperative cystobiliary fistula were included in the study. Patients who were treated percutaneously by interventional radiology and those who underwent radical or conservative surgery and did not develop postoperative biliary fistula were excluded (Figure 1).

CE localization, CE classification according to WHO (17), and radical surgical technique (cystectomy and resection) or conservative surgical technique (cystotomy and omentoplasty, tube drainage, partial cystectomy, and bile leak repair) were recorded for all patients included in the study. To calculate PNI, PLR, and NLR values, the latest albumin level as well as platelet, neutrophil, and lymphocyte counts in the preoperative period were recorded. PNI was calculated using serum albumin and peripheral blood lymphocyte count according to the following formula: PNI = [10 × serum albumin level (g/dL)] + [0.005 × total peripheral lymphocyte count (per mm^3)]. NLR was calculated by dividing the neutrophil count in the peripheral blood by the lymphocyte count, and PLR was calculated by dividing the platelet count in the peripheral blood by the lymphocyte count.

The recovery times of the patients who developed biliary fistula in the postoperative period and the degree of biliary fistula according to the Clavien–Dindo classification were recorded. All interventions were also recorded.

CE management

Medical, percutaneous, and surgical treatment is applied depending on the type, localization, and complexity of the cyst in CE. According to WHO classification, medical treatment was applied for CE1 cysts with a diameter of <5 cm, whereas surgical treatment was applied for CE1 with a diameter of > 5 cm, CE2, CE3, and CE4 cysts with with complex fluid content and calcified wall. No intervention was applied to CE5 cysts. Patients who had recurrent cysts or cyst collections in CE1, CE3A, CE3B and some CE2 cysts; those who did not accept surgery; or those whose medical conditions were not suitable for surgical treatment were treated percutaneously via interventional radiology. While emergency surgical treatment was applied for ruptured hydatid cysts in the intraperitoneal area, elective surgical treatment was applied for CE1 cysts that could not be treated percutaneously, CE2, CE3A and CE3B cysts with many daughter vesicles, CE4 cysts with fluid collection, and cysts with cystobiliary communication. Albendazole treatment was initiated 2 weeks before the operation or intervention in all patients and continued for 2 months after the intervention. Andazol was given at 15 mg/kg per day divided into two, with an interval of 12 hours (maximum daily dose was 800mg).

CE surgical procedure

As part of the conservative surgical method, the cyst content was evacuated initially by securing the surrounding tissues with compresses and sponges to prevent intraperitoneal recurrence. Then, the cavity was sterilized by injecting a scolicidal agent into the cavity. Chlorhexidine or 3%–30% NaCl solution were used as the scolicidal agent. The agents were left for 5–10 min in the cavity, and the cavity was then aspirated. Contact with the biliary tract was avoided as much as possible to minimize the risk of cholangitis. After the cavity was sterilized, biliary fistula control was performed. If a cystobiliary duct was visible and it was not the main bile duct, the duct was sutured carefully. If there was no visible duct and there was bile in the cyst, leakage was checked by injecting 0.9% NaCl from the cystic duct via cholecystectomy. Partial cystectomy, tube drainage, and omentoplasty were used for cavity management. Hepatectomy and cystectomy were performed as radical surgical techniques.

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Table 1: Patients Characteristics

		Frequency	p value
Age		40.78 ± 15.48 (20-83)	
Gender	Female	82 (% 56.6)	0.513
	Male	63 (% 43.4)	
PNI		48.68 ± 8.46 (27.5-72.7)	
NLR		3.09 ± 2.61 (0.7-19.8)	
PLR		149.34 ± 86.17 (45-552)	
Localization	Right Lobe	95 (% 65.5)	0.662
	Left Lobe	17 (%11.7)	
	Bilobar	10 (% 6.9)	
	Centre	21 (% 14.5)	
	Caudat	2 (% 1.4)	
WHO Classification	CE 1	27 (%18.6)	0.667
	CE 2	56 (%38.6)	
	CE 3A	30 (% 20.7)	
	CE 3B	22 (%15.2)	
	CE 4	10 (% 6.9)	
Surgical Tecnique	Cystotomy	68 (%46.9)	0.559
	Partial cystectomy+Cholecystectomy+Cysticostomy	2 (%1.4)	
	Partial cystectomy+Cholecystectomy+Omentoplasty	2 (% 1.4)	
	Partial cystectomy	18 (% 12.4)	
	Cystotomi + Omentoplasty	20 (%13.8)	
	Cystotomy + Cholecystectomy	16 (% 11)	
	Cystotomy+ cholecystectomy+cysticostomy	5 (% 3.4)	
	Cystotomy+cholecystectomy+omentoplasty	6 (% 4.1)	
	Cystotomy+cholecystectomy+omentoplasty+	4 (% 2.8)	
	cysticostomy	1 (% 0.7)	
	Partial cystectomy+omentoplasty	3 (%2.1)	
Bile leak repair in operation	Partial cystectomy+Cholecystectomy	123 (%84.8)	
	Yes	22 (%15.2)	
Radiologic prevention	None	13 (% 9)	
	Percutan intervention	132 (%91)	
surgical prevention	None	136 (%93.8)	
	None	6 (%4.1)	
	Abscess Drainage	2 (%1.4)	
	Wound Debridement	1 (0.7)	
Endoscopic prevention	Hepaticojejunostomy	94 (% 64.8)	
	none	4 (% 2.8)	
	preoperative sphincterotomy	10 (%6.9)	
	preoperative sphincterotomy + stent	26 (% 17.9)	
	sphincterotomy	11 (%7.6)	
Bile Fistula Type	sphincterotomy + stent	131 (%90.3)	
	Occult	14 (%9.7)	
Recovery Time	Frank	13.97 ± 7.33 (3-41)	

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DOI: 10.5455/IJE.2022.01.01

Table 2: Multivariate Linear Regression Analyse

		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	8.430	5.505		1.531	.128					
	PNI	.040	.092	.046	.430	.668	-.135	.036	.035	.583	1.716
	NLR	.122	.293	.044	.417	.677	.175	.035	.034	.609	1.643
	PLR	.022	.010	.254	2.173	.031	.251	.180	.177	.487	2.054

Table 3: Binary Logistic Regression

Variables in the Equation

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	highplr(1)	-1.891	.767	6.077	1	.014	.151	.034	.679
	Constant	2.197	.745	8.690	1	.003	9.000		

a. Variable(s) entered on step 1: highplr.

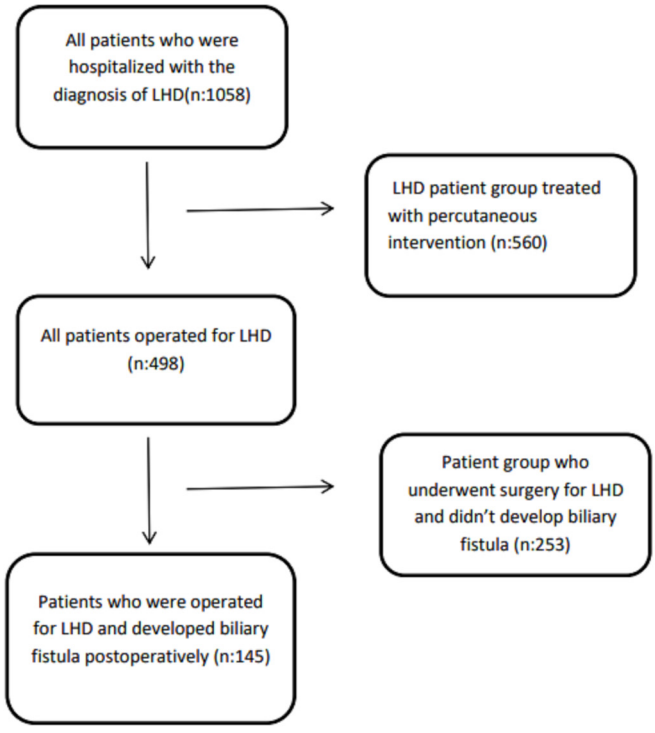

Figure 1. Patient selection

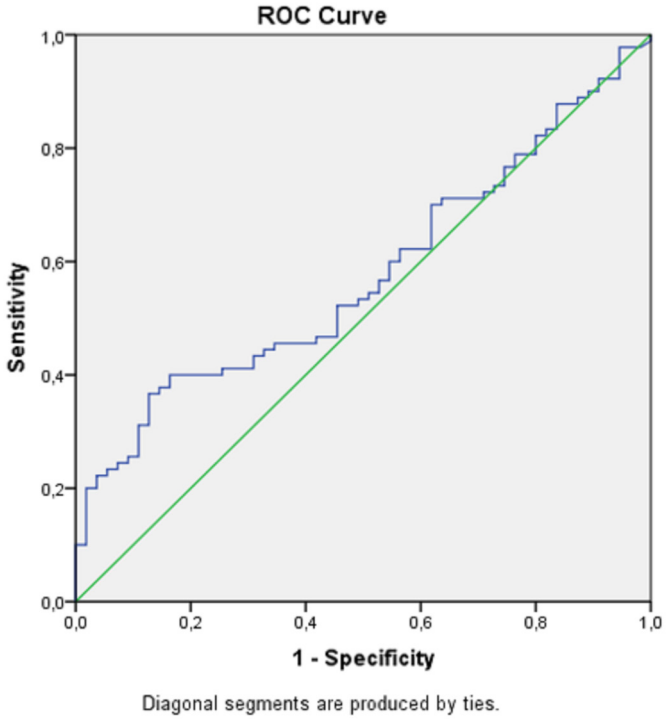

Figure 2. Area under curve

International Journal of Echinococcoses 2022;1(2):38-45
DOI: 10.5455/IJE.2022.01.01

Biliary fistula management

Biliary fistula is also defined as intrabiliary rupture and is one of the most common complications of CE. In the present study, we managed patients with cystobiliary communication applying an algorithm developed in our clinic based on patient data by Öztürk, Yildirgan (18). Accordingly, patients with CE who had jaundice and cholangitis in the preoperative period primarily underwent sphincterotomy with endoscopic retrograde cholangiopancreatography (ERCP). When ERCP failed, it was repeated or emergency surgery was performed. Elective surgery was performed in those who underwent sphincteromy with ERCP. In patients with postoperative biliary fistula, if there was bilioma, the collection was evacuated by percutaneous drainage and the patient was followed-up. In patients with bile leakage from the drain due to biliary fistula, sphincterotomy with ERCP was performed if the drainage did not fall below 100 mL/day on the postoperative 7th day. If drainage continued, a stent was inserted. The stent and drain were removed after the biliary fistula closed. If a major bile duct opened into the cyst and a biliary fistula developed, irrigation and drainage were performed in the cyst with a nasobiliary stent. When the amount of drainage fell to <10 mL/day and there was no collection in the radiological imaging, the drain and nasobiliary drainage (NBD) were removed. If endoscopic methods were unsuccessful and sepsis developed, surgery was performed.

Statistical analyses

SPSS (version 22) statistical software was used for all analyses. Multivariate linear regression was used to examine the effects of PNI, NLR, and PLR on the recovery times of biliary fistula. $P < 0.05$ with a 95% confidence interval was considered significant for the regression model and meant that at least one variable was effective. Receiver operating characteristic (ROC) analysis was then performed to determine the cutoff value of the significant factors. Binary logistic regression was used to predict recovery times exceeding 10 days by dividing PNI, NLR, and PLR into high and low categories according to the cutoff values determined. Furthermore, the chi-squared test was used to analyze the differences between low-grade and high-grade complications according to the Clavien–Dindo classification in the high and low PNI, NLR, and PLR categories. $P < 0.05$ was accepted as statistically significant in all analyses.

RESULTS

The general characteristics of the patients are shown in Table 1. Of the 145 patients with cystobiliary fistula, 131 (90.3%) were of the occult type and 14 (9.7%) were of the frank type. Of the patients with occult type biliary fistula, 23(%17.6) were CE1, 52(%39.7) CE2, 28 (%21.4) CE3A, (%14.5)19 CE3B and 9 (%6.9) CE4 cysts. Also, 82 (56.6%) patients were female and 63 (43.4%) were male. The mean age of the patients was 40.78 ± 15.48 years (20–83 years). The mean recovery time of postoperative biliary fistula was 13.97 ± 7.33 days. No mortality was observed in the patients. The mean PNI, NLR, and PLR values were 48.68 ± 8.46, 3.09 ± 2.61, and 149.34 ± 86.17, respectively. The cysts were in the right lobe in 95 (65.5%) of the cases and in the left lobe in 17 (11.7%) of the cases. The cysts were bilobed in 10 (6.9%) of the cases and centrally located in 21 (14.5%) of the cases. Two (1.4%) patients had a cyst located in the caudate lobe. Surgical treatment was applied to CE1, CE2, CE3A, CE3B, and CE4 cysts according to the WHO classification. The most common cystobiliary fistula was CE2 cyst with 56 (38.6%) cases. Among the patients who developed cystobiliary fistula, 27 (1.6%) were CE1, 30 (20.7%) were CE3A, 22 (15.2%) were CE3B, and 10 (6.9%) were CE4 hydatid cysts. Conservative surgical treatment was applied to all patients. Cystotomy was applied to 68 (46.9%) patients, and it was the most common surgical technique. Intraoperative biliary fistula repair was performed in 123 (84.8%) patients owing to cystobiliary fistula. No intraoperative repair was performed in 22 (15.2%) of the patients. Percutaneous intervention was performed in 13 patients (9%) because of cavity abscess. Surgical drainage was performed in six (4.1%) patients owing to cavity abscess. Long-term hepaticojejunostomy was performed in one (0.7%) patient for biliary stricture. While postoperative endoscopic intervention was performed in 37 (25.5%) patients, preoperative endoscopic intervention for cystobiliary fistula was performed in 14 (9.7%) patients.

To examine the effects of categorical variables on the recovery times of biliary fistula, each variable was analyzed using the chi-squared test. Pearson chi-squared value was evaluated in cases with a large enough sample size, and Fisher's exact value was calculated in cases with an insufficient sample size. Those with $P < 0.05$ were considered significant. Accordingly, it was found that only endoscopic interventions had a positive and significant effect on the recovery time of biliary fistula (Fisher's exact $P = 0.001$).

Multivariate linear regression analysis was performed to determine the effects of PNI, NLR, and PLR on the recovery time of postoperative biliary fistula in patients with liver hydatid cysts. Regression analysis revealed that the independent variables explained 4.5% of the variance in the dependent variable ($F (3, 141) = 3.27$, $P < 0.023$, $R2adjusted = 0.045$). According to the regression analysis, PLR significantly affected the recovery times ($\beta = 0.25$, $t (141) = 2.17$, $P < 0.03$, $pr2 = 0.03$). An increase in PLR meant a prolonged recovery period. PNI and NLR had no significant effect on the recovery times of biliary fistula (P

International Journal of Echinococcoses 2022;1(2):38-45
DOI: 10.5455/IJE.2022.01.01

= 0.66 and 0.67, respectively; Table 2).

ROC analysis was used to determine a cutoff value for PLR in predicting the recovery time (Figure 2). The cutoff value for PLR was determined as 216 with 20% sensitivity and 98% specificity (AUC = 0.576, lower bound = 0,483, upper bound = 0.668, LR = 11). Patients were classified into high and low PLR groups according to the cutoff value (PLR ≥ 216 and PLR < 216). Patients were also classified into two groups according to recovery time (long and short according to recovery exceeding 10 days). Binary logistic regression analysis was performed for these groups. This analysis showed that high PLR according to the cutoff value could predict recovery time exceeding 10 days, and a one unit increase in PLR prolonged the recovery time by 0.15 units (B = −1.89, exp (B) = 0.15, P = 0.014; Table 3).

According to the cutoff value obtained in the ROC analysis, patients were classified into two groups as those with PLR > 216 and those with PLR < 216. These two groups were compared with respect to high-grade and low-grade complication groups according to the Clavien–Dindo classification, and the effect of high PLR on complications was analyzed via the chi-squared test. No significant difference was found between the groups (Pearson's chi square P = 0.204). Thus, it was determined that high PLR had no effect on complications.

DISCUSSION

Cystobiliary communication manifests itself as an occult type in the postoperative period at a rate of 10%–37%. (19). This type of biliary fistula is bile leakage in minor biliary tract determined in the intraoperative procedure. Flank type biliary fistula is determined preoperative period for CE with using laboratory tests and radiological imagine tecniques. Bile leakage developing after conservative surgical procedures for CE is one of the main causes of morbidity and mortality (8, 9). It is mostly observed after conservative surgical methods(20). In the patient series examined in this study, bile leakage was not detected in the postoperative period in any of the patients who underwent radical surgery. However, bile leakage was detected in 30% of the patients who underwent conservative surgery. This high rate is likely due to the fact that our center is the tertiary treatment center in our region and that complicated cases are referred to us from other centers.

Prophylactic sphincterotomy provides shorter hospital stay and drain removal time after partial cystectomy (19). So it can be said that endoscopic sphincterotomy performed in the preoperative period in patients with frank-type cystobiliary communication has a positive effect on the recovery times of biliary fistula. In the present study, only endoscopic interventions had a positive effect on the recovery time of biliary fistula (P < 0.001). In patients with cystobiliary communication, preoperative endoscopic interventions accelerate the flow of bile into the duodenum, thereby reducing the pressure on the small bile ducts in the cyst. Thus, the accumulation of bile in the cyst decreases and the closure of the minor bile ducts of the cystobiliary communication is ensured.

It is more difficult to manage patients without cholangitis in the preoperative period and whose laboratory parameters do not indicate a cystobiliary communication but are detected to have a cystobiliary communication in the intraoperative period. In this patient group, determining the location of the cystobiliary communication and ligation with primary suture can reduce bile leakage. However, it is not always easy to locate the cystobiliary communication. There are several techniques for this as well. By placing white gauze in the cyst cavity and waiting for a few minutes, the location of the leak can be determined by stains on the gauze. In another technique, cholecystectomy is performed and the cystic duct is catheterized, and the location of the leak can then be determined with pressurized isotonic solution.(21,22) Cholangiography can also be performed in conjunction with this method to understand whether the leaking duct is a major or minor duct. Thus, a more careful repair can avoid complications such as biliary stricture. If the bile leakage is from a major duct, it is safer not to ligate to avoid possible complications. In the present study, intraoperative cystobiliary communication ligation was performed in 123 (84.8%) patients with biliary fistula. Cholecystectomy was performed in 38 (26.2%) patients, and the cystic duct was removed by cysticostomy in 11 (7.5%) patients using a feeding catheter. The biliary tract had no statistically significant effect on the recovery time in these patients (P = 0.11). Accordingly, if the communication point is not ideal for repair and if it is not a minor branch, we do not recommend repair because of possible complications. In fact, Roux-en-Y hepaticojejunostomy was performed in one of our patients owing to the development of stricture during long-term follow-up.

PLR is a novel marker of systemic inflammation. An increase in the severity of inflammation has been shown to be associated with an increase in platelet activity. (23) It has been shown that inflammation risk and inflammation may increase in high PLR levels even without thrombocytosis.(24, 25) In addition to being a marker of systemic inflammation, it has been shown to be a predictor of prognosis and response to treatment in various malignancies.(26, 27) In the present study, we attempted to determine the predictive power of prognostic markers on recovery times of biliary fistula after CE surgery. The results showed that only PLR can be used as a prognostic marker for this purpose. Accordingly, recovery times may be prolonged in patients with high PLR values. This, in

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DOI: 10.5455/IJE.2022.01.01

turn, will prolong the length of hospital stay and increase the frequency of rehospitalization. Therefore, the patient should be followed-up and treated more carefully if the PLR value is higher than the cutoff value. In particular, in patients with minimally elevated cholestasis enzyme levels and who are hesitant to undergo preoperative endoscopic intervention, PLR can be used as a marker and can be considered in decision-making. As shown in the present study, endoscopic interventions have positive effects on the recovery times. However, they should be performed with caution as they are invasive. In such cases, PLR may contribute as a secondary marker in decision-making in addition to cholestasis enzyme levels. Furthermore, PLR had no effect on complications.

Although PNI and NLR are other inflammation and prognostic markers, our analyses could not demonstrate any effect on the recovery time of biliary fistula in patients with CE. However, as prognostic markers are also inflammation and immunity indicators, further studies can be conducted on their effects on CE. We believe that this study will act as a guide for such future studies.

CONCLUSION

We concluded that high PLR value prolongs the biliary fistula recovery time. In patients with a high PLR value, caution should be exercised in terms of postoperative biliary fistula. PLR can be used as a marker if the cyst is to be intervened in the preoperative period.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: Ethical Committee for Clinical Research of Ataturk University School of Medicine (24.02.2022-Approval number 2-7)

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DOI: 10.5455/IJE.2022.01.01

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International Journal of Echinococcoses 2022;1(2):46-8
DOI: 10.5455/IJE.2022.02.02

International Journal of Echinococcoses

Journal homepage: www.echinococcoses.org

Case Report

Alveolar Echinococcosis Located in the Liver: Report of a Case

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ARTICLE INFO

Received: Feb 22, 2022
Accepted: Apr 16, 2022
Available online: May 20, 2022

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CITATION

Ozsoy MS, Buyuker F, Baysal H, Alimoglu O. Alveolar Echinococcosis Located in the Liver: Report of a Case. International Journal of Echinococcoses 2022;1(2):46-8. DOI: 10.5455/IJE.2022.02.02

The journal is the official publication of The Turkish Association of Hydatidology

ABSTRACT

Echinococcosis is a parasitic infection caused by larval and cyst stages of *Echinococcus granulosus* living in dogs in humans. The definitive host of the parasite is dog, intermediate hosts are sheep, cattle and humans. The causative pathogen is mostly *Echinococcus granulosus*. *Echinococcus multilocularis* (alveolaris) and other agents are less common. Only 3% of hepatic hydatid cysts are alveolar hydatid cysts. In this study, we aimed to present a case of Alveolar echinococcosis located in the right lobe of the liver due to Ecinococcus alveolaris, together with the operation images.

We evaluated the preoperative, peroperative and postoperative course of a patient who was examined with abdominal pain in our clinic and in radiological and serological examinations, we found an alveolar hydatid cyst located in the liver and underwent surgery.

Our patient was a 31-year-old female. She applied for abdominal pain. Ultrasonography revealed a cystic mass of approximately 17 cm in diameter in the right lobe of the liver. In laboratory examinations, the hydatid hemagglutination test was positive at a titer of 1/2560. Hematological and biochemical parameters were normal. MRI showed a 171*125 mm sized, lobulated, locally calcified cystic mass in the right lobe of the liver. After evaluating the relationship of the lesion defined in CT angiography imaging with vascular structures, the decision to operate was made with the diagnosis of Alveolar echinococcosis.. Nonanatomical segmental right hepatectomy and total cyst excision operation was performed in our patient who was thought to have Alveolar echinococcosis based on clinical, serological and radiological findings. The patient, who had an uneventful postoperative period, was discharged on the 7th postoperative day with full recovery.

In cases of echinococcosis, the treatment is determined according to the type of the agent, the stage and localization of the lesion. Medical treatment, PAIR and surgery are the treatment options that can be applied. Total excision of the mass is recommended in cases of Alveolar echinococcosis.

Keywords: Alveolar Echinococcosis, Cystectomy, Hepatectomy

International Journal of Echinococcoses 2022;1(2):46-8
DOI: 10.5455/IJE.2022.02.02

INTRODUCTION

In this study, we aimed to present a case who presented with abdominal pain and was found to have an alveolar echinococcosis located in the right lobe of the liver with the operative and radiological images.

CASE REPORT

The patient was a 31-years-old female. She admitted to hospital with intermittent abdominal pain. Abdominal ultrasonography revealed a cystic mass of approximately 17 cm in diameter in the right lobe of the liver. In laboratory examinations, the hydatid hemagglutination test was positive at a titer of 1/2560. Hematological and biochemical parameters were normal. MRI showed a 171*125 mm sized, lobulated, locally calcified cystic mass in the right lobe of the liver (Figure 1) and multiple stones in the gallbladder (Figure 2) . A diagnosis of alveolar echinococcosis was made based on clinical, serological and radiological findings. A diagnosis of alveolar echinococcosis was made in the presence of clinical, serological and radiological findings. The patient underwent surgery and right hepatectomy and cholecystectomy with total cyst excision were performed (Figure 3, 4). The histopathologic findings were found to be compatible with alveolar echinococcosis. The patient was discharged on the 7th postoperative day uneventfully.

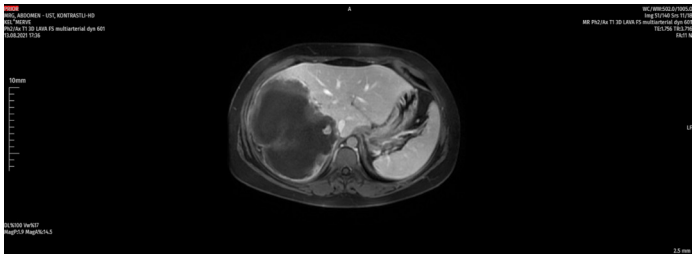

Figure 1. Appearance of a lobular, local calcified cystic mass in the right lobe of the liver in upper abdominal MRI

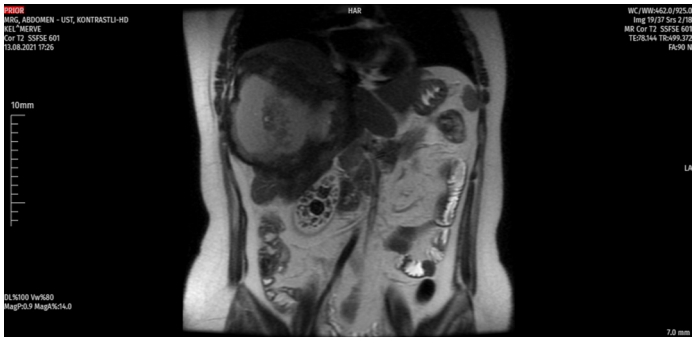

Figure 2. Upper abdomen MRI coronal section view of multipl gallbladder stones

Figure 3. View of right hepatectomy

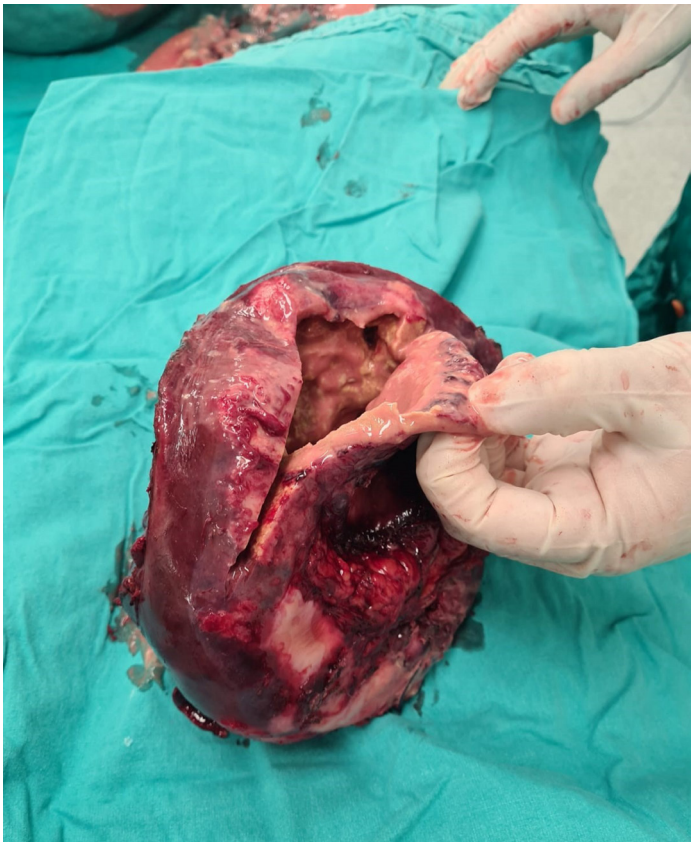

Figure 4. View of total cystectomy material

International Journal of Echinococcoses 2022;1(2):46-8
DOI: 10.5455/IJE.2022.02.02

DISCUSSION

Considerable gaps of knowledge exist in pediatric Cystic echinococcosis (CE) has a worldwide distribution, while Alveolar echinococcosis (AE) is endemic in the Northern hemisphere, including North America and several Asian and European countries, like France, Germany and Austria *(1)*. The disease is predominantly found in China and remains a major public health problem in Western China.

Recent studies in Europe and Asia have shown that the endemic area of *Echinococcus multilocularis* is larger than previously known and spread regionally from rural to urban areas *(2)*.

Humans acquire the infection by accidental ingestion of eggs released into the environment by infected definitive hosts. A cyst located in the liver can cause symptoms when it reaches more than about 10 cm in diameter or when more than 70% of the organ volume is affected. Cystic mass can cause damage and compression to the hepatic and portal veins or bile ducts *(3)*.

Alveolar echinococcosis causes liver tumors that result in infiltrative growth and distant metastases. Differential diagnosis of metastases from any other tumor should be made *(4)*.

Clinically, AE behaves like a malignant tumor and the prognosis is generally poor. Alveolar echinococcosis is a serious disease with a greater than 90% mortality rate in untreated patients *(5)*.

Hepatic resection is considered safe and the only curative treatment for AE when the lesion is able to be removed completely. It may be necessary to perform extrahepatic procedures like choledochejunostomy, diaphragm resection, adrenalectomy, nephrectomy, lung lobectomy, splenectomy to achieve radical resection, if there was adjacent organ invasion *(6)*. Even if resection is noncurative for AE, good long-term survival and stabilization of the disease can be achieved with benzimidazole therapy if the lesion volume is reduced by 90% *(7)*.

Studies on liver transplantation in patients with AE are limited, due to the excellent outcome of patients undergoing complete resection of the alveolar equinoccus and favorable results with conservative treatment. However, WHO recommends that transplantation may be considered in the presence of severe hepatic failure or recurrent life-threatening cholangitis and in the absence of extrahepatic disease and if not suitable for radical liver resection *(8)*.

CONCLUSION

Surgery is the first choice for alveolar echinococcosis of the liver. Transplantation should also be considered as an option in selected cases.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Patient informed consent: Patient consent was obtained for the publication of this article.

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International Journal of Echinococcoses 2022;1(2):49-53
DOI: 10.5455/IJE.2022.02.03

International Journal of Echinococcoses

Review Article

International Journal of Echinococcoses

Journal homepage: www.echinococcoses.org

Cystic Echinococcosis in Children: Addressing Research Needs, Filling Knowledge Gaps

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ARTICLE INFO

Received: Feb 22, 2022
Accepted: Apr 29, 2022
Available online: May 21, 2022

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CITATION

Borhani M, Bazargan N, Eslami S, Fathi S, Harandi MF. Cystic Echinococcosis in Children: Addressing Research Needs, Filling Knowledge Gaps. International Journal of Echinococcoses 2022;1(2):49-53. DOI: 10.5455/IJE.2022.02.03

The journal is the official publication of The Turkish Association of Hydatidology

ABSTRACT

Cystic Echinococcosis (CE) is a common neglected disease in many communities around the world, causing substantial costs on the endemic communities. CE has been considered a disease of adulthood, mostly affecting age groups within 20-59 years. However, CE can be seen in early life even in patients younger than 5 years. While the disease is frequently reported from children in the endemic areas across the globe, children CE is even more neglected and under-represented in the literature. Here we present an overview on this topic, summarizing main features of pediatric CE, current findings, existing knowledge and information gaps and research needs on this issue.

In adults the lungs are involved in less than 30% of CE cases, however in children, pulmonary hydatid disease has been reported to be up to 67%. Different patterns of hydatid cyst and multiple organ involvement have been frequently observed in children, probably due to the immature filtering mechanisms of the liver and lungs during infancy and childhood and the role of intestinal lymphatic channels in dissemination of the parasite. It is believed that children immune system is less responsive to hydatid cysts, therefore serological assays of CE in children are likely to result in more false negative outcomes. As the proportion of internal organs occupied by the cyst is much greater in children than the adult patients, CE in children is more likely to be symptomatic than adults.

Another peculiar aspect of pediatric CE is that the disease has been more frequently observed in boys than girls. According to the data recently published by the European Register of Cystic Echinococcosis the contribution of male patients is more than the females in children and adolescents. While males constitute 65% of the CE patients in 0-9 years age group, the proportion decreased to 50% and 45% in 30-39 and 70-79 years age groups, respectively.

Considerable gaps of knowledge exist in pediatric echinococcosis and further investigations are required on this topic. Major information gaps in children CE include the lack of age-specific data, natural history, detailed clinical picture, clinical trials of non-surgical approaches, genotype data, specific guidelines for diagnosis and treatment and WHO ultrasound classification.

Keywords: Pediatric hydatid disease, Children hydatid cyst, Adolescents, Knowledge gaps

International Journal of Echinococcoses 2022;1(2):49-53
DOI: 10.5455/IJE.2022.02.03

INTRODUCTION

Cystic Echinococcosis (CE) is a common zoonotic infection in many communities around the world. CE is caused by larval stages of different genotypes within *Echinococcus granulosus* sensu lato. Adult worms of E. granulosus develop in dogs and other carnivores as the definitive hosts excreting eggs in feces to infect sheep and other herbivores as the intermediate hosts. Humans are accidentally infected by food and water contaminated with the parasite eggs. Basically, the parasites reach the liver and lungs through the small intestine and portal veins (1).

The disease is distributed worldwide extending from South America to North Africa, Mediterranean basin, the Middle East, Central Asia and Western China. CE imposes substantial animal and human costs, estimated at 0.01-0.04% of the gross domestic products in different endemic countries (2,3). According to WHO, it is estimated that one million people around the world are suffering from CE. Patients undergoing treatment will have a lower quality of life, with an estimated annual burden of 871000 disability-adjusted life-years with an approximate cost of US$ 3 billion associated with CE including monetary burden for treating patients and livestock-related losses.

Surgery and chemotherapy are the main modalities of CE treatment. However, 6.5% of patients exhibits a relapse of disease after surgery, and 2.2% post-operative death (4,5). Several retrospective studies have been conducted on the recurrence of CE. Findings of recent studies demonstrated the relapse rates of CE ranges from 0-22%. Current evidence indicates that CE recurrence is rather a technical/clinical issue and is not linked to epidemiological variables. A number of factors including incomplete peri-cystectomy, previous complications of the cyst, inadequate treatment, minute spillage of the cyst, a diameter greater than 7 cm, and extrahepatic location are the most important determinants of CE recurrence. CE surgery is not only complicated and should be performed in centers familiar with hepato-thoracic surgery, but also the management of patients with recurrent disease is difficult (6,7).

Cystic echinococcosis in children

CE has been considered a disease of adulthood, mostly affecting age groups within 20-59 years (4). However, CE can be seen in early life even in patients younger than 5 years. The disease is frequently reported from children in the endemic areas around the globe. Nevertheless with the recent influx of immigrants from the Middle East and North Africa to the Europe, CE is being frequently diagnosed in children originating from endemic areas (8,9). Therefore even in European pediatric centers, CE should be suspected in the children with space-occupying lesions in thoracoabdominal cavity.

The incubation period is believed to be at least one year to more than 5 to 15 years (4,5). Therefore, it has been assumed that many people get infected with E. granulosus eggs early in the childhood and the infection usually remains asymptomatic for a long period of time depending on the cyst size and location and may be accidentally diagnosed during routine clinical / paraclinical workups (10).

Specific features of CE in Children

Liver and lungs are the most common organs affected by the parasite, but it should be noted that hydatid cysts can be located in virtually every organ of the body. In adults 60-70% of the cysts are hepatic, and the lungs are involved in less than 30% of CE cases, however in children, pulmonary hydatid disease has been reported to be up to 67% (5,11). In addition many unusual or atypical presentations of CE in children have been published in the literature (12).

Different patterns of hydatid cyst and multiple organ involvement have been frequently observed in children, probably due to the immature filtering mechanisms of the liver and lungs during infancy and childhood, the role of intestinal lymphatic channels in dissemination of the parasite and different epidemiological features of childhood echinococcosis in various endemic countries (12,13). CE growth is believed to be faster in younger individuals, i.e., children and adolescents, and slower in the elderly (5,14). The growth rate of lung CE has been estimated to be at least five-fold higher than liver CE. It is worth noting that, negative pressure, compressible nature and vascularization are reasons for faster growth of the cyst in children lungs (15-18).

It is believed that children immune system is less responsive to hydatid cysts and antibody production is assumed to be lower in children than adults (4). Therefore serological assays of CE in children are likely to result in more false negative outcomes. Although several sero-epidemiological studies have been performed for diagnosis of CE, most serological methods are unreliable due to poor sensitivity and specificity (19). Furthermore, observations indicate that serology demonstrates less sensitivity in pulmonary CE than in liver CE (20).

CE in children is more likely to be symptomatic than adults as the proportion of internal organs occupied by the cyst is much greater in children than the adult patients. This is especially true for pulmonary hydatid cysts in which children present more severe manifestations and higher risk of cyst rupture (11). Therefore in the pediatric age group, low resistance of the lungs to hydatid cysts may result in the expansion and subsequent tension pneumothorax following rapid growth of the cyst. In such cases, to avoid

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DOI: 10.5455/IJE.2022.02.03

further complications early surgical removal of the cyst is advocated (11). Nevertheless, randomized controlled trials are required on different treatment modalities of CE in children as very few studies are available on the surgical and chemotherapeutic management of CE in pediatric settings (21).

Allergic manifestations and anaphylactic reactions are usually observed in the ruptured hydatid cysts. More than 30% of patients with pulmonary CE present with ruptured cysts (11,22,23). Several factors can lead to the rupture by destroying the cyst membranes, e.g., age, anthelmintic treatment, chemical reactions, cyst size, and host immune system (17). The bronchopleural fistula, pneumothorax, pleural thickening, lung collapse, large residual cavity, and empyema are among the complications of pulmonary hydatid cysts ruptured into the pleural or pericardial cavity (24). Running hydatid fluid and cyst membranes into the bronchial tree, can cause suffocation and anaphylactic shock following cyst rupture. Age has been considered as a risk factor for anaphylactic shock in CE, however this warrants further investigations (25).

Another peculiar aspect of pediatric CE is that the disease has been more frequently observed in boys than girls. This is in contrast to the findings of several studies in adult population indicating a slight gender difference towards females (5,26). According to the data recently published by the European Register of Cystic Echinococcosis the contribution of male patients is more than females in children and adolescents and it is gradually declined towards adulthood and elderly (27). While 65% of the patients in 0-9 years age group were boys, the rate decreased to 50% and 45% in 30-39 and 70-79 years age groups, respectively. This is partly because of the fact that in CE endemic countries boys spend more time in outdoor environments and have more chance of contact with dogs and soil, while adult females are more at risk of infection due to the daily activities of food and vegetable preparation and handling livestock. However our knowledge on the nature of echinococcosis in children and adolescents is poor.

Knowledge gaps and research needs

Considerable gaps of knowledge exist in pediatric echinococcosis and further investigations are required on this topic. Figure 1 demonstrated specific features and major research and information gaps regarding CE in children. Unfortunately the literature suffers from shortcomings in reporting CE data in humans. One major defect is the lack of age-specific data on many aspects of human CE. For example in most hospital CE studies, the cyst location has not been specified according to different age groups. Therefore the precise distribution of the organs involved in pediatric CE is not known. Details of the clinical picture of CE in children have not been provided in many studies. Natural history of childhood hydatid cysts is not clear and reporting hydatid cyst stage according to WHO ultrasound classification of cystic echinococcosis in children and adolescents is almost absent in the literature.

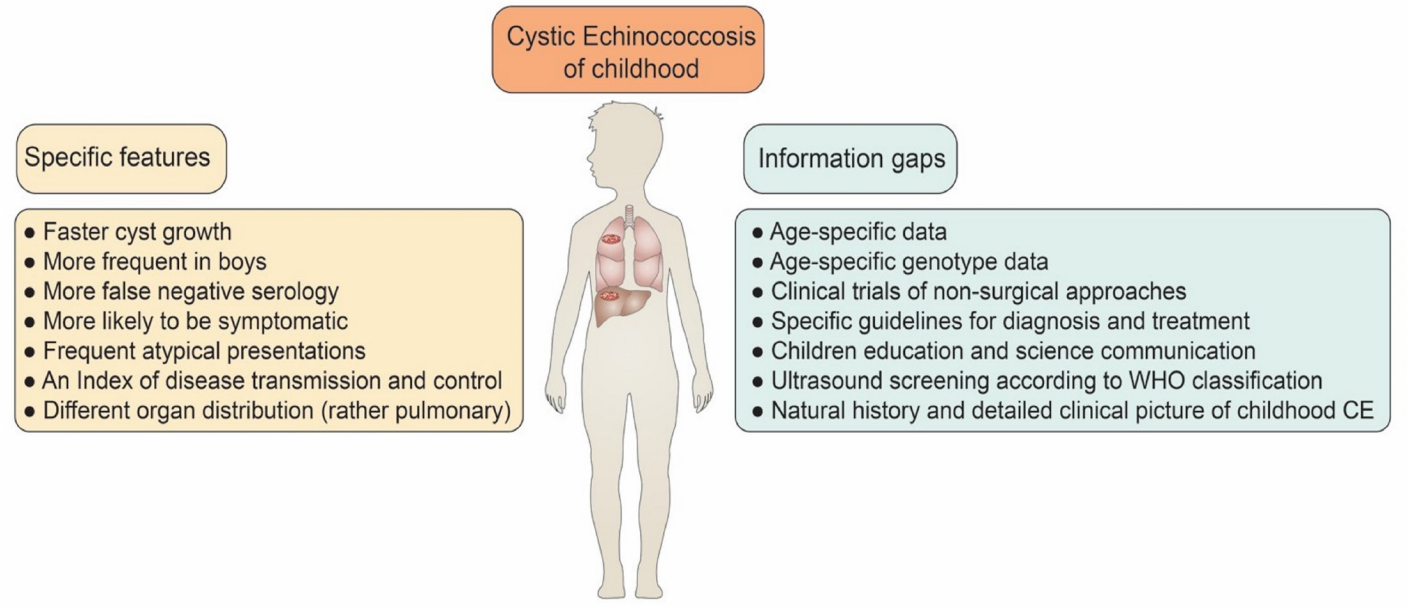

Figure 1. Cystic Echinococcosis of childhood: specific features and major research and information gaps

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DOI: 10.5455/IJE.2022.02.03

Unfortunately dozens of research papers on the molecular epidemiology of CE did not report age-specific genotype data, therefore understanding differential contribution of E. granulosus genotypes in the adults and children CE is not possible. Specific guidelines for the diagnosis and management of CE in children have not been developed, consequently in the absence of relevant clinical trials, the outcomes of particular interventions as well as the pros and cons of various diagnostic and therapeutic approaches have not been clearly demonstrated.

Regarding the long incubation period and the slow growth of hydatid cysts in human, occurrence of CE in adults can indicate an infection acquired in the distant past, while the incidence of CE in children is indicative of active parasite transmission, and this can be epidemiologically important (28). Therefore the incidence of cystic echinococcosis in children is an important indication of active recent disease transmission in the endemic communities. Frequency of CE in children reflects the recent changes of disease transmission in endemic regions and is an essential indicator for measuring the success of CE control programs. (29).

CE is a neglected tropical disease (NTD) and its control clearly deserves further attention in the endemic regions. Thereby, the need for elaboration of specific strategies and measures for CE control is becoming apparent in endemic regions (30). Dog deworming, livestock vaccination and public/professional education are the main tools of CE control. Children as a target population, play a pivotal role in improving public awareness and hand hygiene of the endemic communities. An effective public education program using mass media communication with available digital / non-digital tools can be achieved via both families and the schools (29).

Lack of detailed epidemiological data on children CE is a pitfall for control programs. To resolve this issue, CE registry systems, reliable national surveillance and community-based ultrasound screening are of paramount importance for providing reliable data regarding children CE (27). A successful control plan needs commitment and coordination of national bodies under a One Health / One Medicine approach.

CONCLUSION

Cystic echinococcosis is a common but neglected disease in pediatric settings in CE endemic countries. The disease is frequently reported from children in the endemic areas around the world. Substantial gaps of knowledge exist in pediatric echinococcosis. Lack of age-specific data, detailed clinical picture of CE in children, lack of age-specific genotype data, unclear natural history of childhood hydatid cysts and under reporting WHO ultrasound classification of cystic echinococcosis in children are among the major information gaps for understanding CE features in children. As CE in children is an important indication of disease transmission dynamics and endemicity in endemic regions, further specific studies on children CE and increased pediatric research activities are required for implementing a successful control program.

Acknowledgements: The authors wish to thank Dr Hashem Khanbabaei for his valuable technical assistance.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

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International Journal of Echinococcoses 2022;1(2):54-71
DOI: 10.5455/IJE.2021.11.07

International Journal of Echinococcoses

Journal homepage: www.echinococcoses.org

Review Article

Past and Future Methods for Controlling *Echinococcus granulosus* in South America

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ARTICLE INFO

Received: November 22, 2021
Accepted: May 21, 2022
Available online: May 21, 2022

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CITATION

Poggio TV, Jensen O, Saravia TC, Nuñez AP, Boado LA, Gómez JM, Heath D. Past and Future Methods for controlling *Echinococcus Granulosus* in South America. International Journal of Echinococcoses 2022;1(2):54-70. DOI: 10.5455/IJE.2021.11.07

The journal is the official publication of The Turkish Association of Hydatidology

ABSTRACT

The various Countries of South America (Peru, Brazil, Chile, Uruguay, Argentina) all have problems with *Echinococcus granulosus s.l* in humans. Control of the disease in dogs and grazing animals began in Uruguay in 1879, and continues in all countries from various beginnings until 2022. Our objective is to describe the new vaccine to prevent grazing animals from acquiring *E.granulosus s.l.*, and to predict the possible high degree of control using the addition of the vaccine to the normal control procedures even when programmes address many practical difficulties.

The recombinant vaccine was used under field conditions using the same protocol in sheep, goats and llamas older than 2 months and up to 6 years: Two injections, one month apart and annual booster. The baseline and the final evaluation were carried out by necropsy in control programmes included in Argentina (Chubut, 2007-2013; Río Negro 2009-2017) and in Chile (Alto Biobio, 2016-2020; Aysen 2020-2022).

Elimination of echinococcosis have been successful only in insular countries. In consequence, to validate a model supporting the One Health approach that might be reproducible successfully in different regions of South America is required. Including the socio-cultural understanding and the environmental context is mandatory to optimize the use of the vaccine under these operational conditions.

The EG95 vaccine, made in Argentina, has been tested, and continues to be tested, in Argentina and Chile, and more recently in Peru. Furthermore, the vaccine, now available, is being made in large quantities in Argentina and China, and appears to be an additional control technology that may allow elimination of *E.granulosus s.l.* from South America. The best control strategies appear to be dog treatments and regular vaccination of sheep and goats for 10 years until all old sheep have been removed. If dogs or grazing animals enter from outside the controlled environment, treatments will need to be continued. The vaccine also seems to reduce *E.granulosus s.l.* cysts reaching infectivity for dogs, and has some effect against Fasciola hepatica.

Keywords: *Echinococcus granulosus s.l.*, grazing animals, Vaccine, South America, Control

International Journal of Echinococcoses 2022;1(2):54-71
DOI: 10.5455/IJE.2021.11.07

INTRODUCTION

Cystic echinococcosis (CE) is a parasitic infection caused by *Echinococcus granulosus sensu lato (E granulosus s.l.*), belonging to the family Taeniidae. The parasite causes zoonotic disease in humans predominantly from pastoral communities. The adult tapeworm is found in the small intestine of dogs and other canids, while the larval stage is located in the viscera of ungulates, especially sheep, goats and cattle. Transmission of *E. granulosus s.l.* between domestic livestock and dogs is increased by uncontrolled slaughter practices whereby dogs can access to contaminated offal (1).

Echinococcus Control Programmes in South America: the history of their control policy.

Elimination of CE as a public health problem was possible in insular countries including Iceland, New Zealand, Tasmania, Cyprus and the Falkland Islands (Malvinas) (2-6). A detailed evaluation of each control programme has been reviewed (7-15).

Since the 1970s control programmes have been developed based on new tools and approaches such as dog-targeted interventions including anthelmintic treatments; use of ultrasonography for human screening, a highly effective vaccine (EG95) to prevent CE in animal intermediate hosts; laboratory-based tests; computer-based modelling of cost-benefit for interventions; and transmission dynamics and predictive modelling for intervention combinations (2).

The impact of CE control programmes in South America during 1974–2010 was extensively reported (16). The Regional CE Initiative has been successful in promoting and developing strategies and action plans for control the disease in South America. Pavletic et al. have reported annual incidence of CE in humans, the occurrence in ovine and bovine and the prevalence in dogs in Argentina, Brazil, Chile, Peru and Uruguay in 2009–2014. Results did not show considerable progress towards control, except for in Uruguay and localized successes elsewhere (17).

We describe the history of the control policy in Argentina Uruguay, South Brazil, Chile and Peru, from its outset to the present and summarize the most important topics in Table 1.

Argentina

The legal framework of the Echinococcosis control measures and features of actions developed by the National Programme for the Control of Zoonotic Diseases (Ministry of Health), and the National Service of Health and Agro-Food quality (SENASA) were summarized in Table 1 (18,19).

Due to each province is autonomous in planning and executing its programmes, a National Framework Plan for the CE control was created based on different strategies described in Table 1 (20).

In South America, the first structured programme was developed by the Province of Neuquén between 1970 and 1985 and even today is in force (Table 1).

Decreasing CE dog prevalence (%) from 1970 to 2004 has been described in Table 1 (21).

In 2005, the validation and implementation of coproantigen ELISA test (CAg) for surveillance of CE in dogs was started (22). Since 2011, detection has been improved due to greater sensitivity of the method. During the period 2013-2018, CE dog prevalence have remained stable (11.66%), demonstrating the importance to improve control and surveillance measures (Table 1) (23).

The overall mean annual human incidences showed a significant decrease from 1995 to 1999 and remained stable from 2000 to 2004 (20,24). The Provincial Hydatid Disease Programme in 2013 implements ultrasound screening in children from endemic areas. Annual human incidences were recorded from 2013 to 2021 and ranged from 8.19 to 5.8/100.000 proving the success of the programme (Table 1). However, it is remarkable that children accounted 12,3% of cases (24).

Human infections with *E. granulosus sensu stricto (s.s.)* G1 and *Echinococcus canadensis* G6 were reported in Neuquén, whereas four genotypes were identified in livestock: G1, G3, G6, and G7. Coinfection with G1 and G7 genotypes was detected in one patient who carried hepatic cysts (25).

In the period 1975-1982, the Provincial Public Health Services extended the control programmes, based on dog deworming, to all the Patagonian Provinces (Rio Negro, Chubut, Santa Cruz and Tierra del Fuego).

The CE control programme in the Province of Tierra del Fuego was launched in 1976 and consisted of oral deworming twice a year to 100% of dogs by a veterinarian responsible.

The construction of closed slaughterhouses and kennels to decrease reinfection among the dogs was the main priority (16). The insular programme has been maintained over time and it is to be close to reaching the control and elimination of CE.

The Rio Negro CE Programme has operated continuously since 1980. The "One Health" strategy including methods and activities for control of CE and their results has been described in detail by Mujica et al, 2021 (26).

In 1980 the pilot plan for CE control was launched in the Department of Cushamen, endemic area from Chubut

Province. Human serology (DD5) and registration of positive cases were included. The spreading to the entire province started in 1984 (24,27).

CE control and surveillance measures and its impact have been detailed in Table 1.

Results of the inclusion of the EG95 vaccine for sheep in control programmes from Rio Negro and Chubut will be exposed in the next section.

Uruguay

The implementation of government policies related to the study and prophylaxis of CE started in 1939 (Table 1).

In 1965 Uruguay (28) introduced the dog patent, allowing departmental governments to collect the tax to fight against zoonoses and vector-borne diseases.

The first structured programme was developed by the Department of Flores in 1973 (29). It was based on dosification with arecoline hydrobromide provided for the owners to treat dogs every six weeks. Regrettably, the prevalence in livestock was not decreased because the owner may have not given anthelmintic to their dogs properly (30).

In 1991 a new national control programme was started and all dogs are treated with praziquantel monthly. The programme schedules for control included planning and diagnosis phase (phase 1: 1972-1992); attack phase (phase 2: 1992-2009) and consolidation phase (phase 3: 2009-to present) (31).

Since 2005, the National Commission for Zoonoses (NCZ) obliges by Law No. 17,930, all dog owners to register them and comply with their obligation to pay the tax. The money raised is used to combat zoonoses such as CE, rabies, Chagas disease and Leishmaniasis, providing the canine population with the infrastructure to carry out deworming (32). The NCZ has so far conducted preventive and control programmes profiling CE.

The current control programme, implemented since 2006, has employed strategies described in Table 1. Based in the published results, the programme has been successful, suggesting that Uruguay is on the right track to control CE (Table 1) (33).

Chile

An unique national CE control programme including dog treatment was launched in 1982 by the Servicio Agrícola Ganadero, (SAG, official phytosanitary institution of Chile) in Regions XI and XII (Aysen and Magallanes respectively). The CE incidence rates in humans dropped leading E. granulosus s.l. to a probable state of elimination (Table 1) Unfortunately, this vertical programme was dismantled in 1998 (16,35).

One of the most relevant and successful local control programmes was developed in Magallanes region between 1979–2004. The main strategy of the control programme was the use of praziquantel together with register of dogs. Other measures that have contributed to sustain the programme and its impact have been described in table 1 (36).

During the period 2008–2015 the O'Higgins region launched a programme based on educational activities in eight municipalities (36).

At the moment there is no official national programme for the CE control and statistical data are based on CE cases discharged only from hospitals indicating an important level of underreporting of the disease in Chile (17).

Since 2015 four different control programmes have been initiated in Coquimbo, Biobio, Araucania and Aysen. Results of the inclusion of the EG95 vaccine for sheep in control programmes from Biobio and Aysen will be exposed in the next section.

Peru

In Peru, within the framework of the Agrarian Reform, the creation of agricultural societies of social interest (SAIS) was implemented from 1975 to 1984. SAIS Túpac Amaru and Pachacutec (Department of Junín) considered carry out periodic dosages to dogs and health education. The programme demonstrated the feasibility for control reducing the infection rate in dogs and the human seroprevalence. The latter indicator tripled during 1994-1995, the increased human seroprevalence is explained by the high percentages of infected sheep (89%) and dogs (33%), the two most important hosts to maintain the biological cycle of the parasite (38) (Table 1).

The programme did not achieve continuity and human CE remains as a not notifiable disease (39).

In 2015 Peru has started a pilot CE control programme only in five regions that include 17 rural communities (31). In 2019, the Permanent Multisectoral Commission for the prevention and control of Zoonotic Diseases was created with the aim of evaluating the strategies implemented in the pilot CE control programme: canine deworming with praziquantel alone, sheep vaccination alone or combined with dosification, and sheep deworming with oxfendazole plus dog deworming. Health education and control of slaughterhouse and home slaughter, besides of active surveillance through coproELISA in dog feces and sheep serology were included. The cost/benefit analysis and the required time to achieve control is being evaluated and it could provide information on the effectiveness and feasibility of each strategy (40).

Brazil

In Brazil, an attempt to implement a control programme

was carried out in the city of Santana do Livramento in Rio Grande do Sul in the 1990s, based on treatment of dogs (41). Despite causing a decrease in the number of infected dogs, the project failed to raise awareness in the rural population, in relation to the prophylaxis of the disease, not being able to establish a permanent survey. It was finished in 1999 by the Health Secretary.

Human CE is a notifiable disease in only one state of Brazil (Rio Grande do Sul) (Table1).

It is noteworthy that, the National Reference Laboratory in Hydatidosis of Brazil (LRNH-IOC/Fiocruz) receives samples countrywide for serological diagnosis using an "in-house" IgG anti-Echinococcus sp immunoblotting (42).

EG95 Vaccine Development as a new control tool

New Zealand & Australia's development of a vaccine against hydatid disease (1960 – 1993) has now been used in China for the past 4 years in grazing animals. Two injections, one month apart, provide protection to animals for up to a year. A third injection, 6-12 months after the second, provides protection for up to 5 years (43).

Chonqing Auleon Biologicals Co. Ltd has produced 40-45 million vaccine doses each year (2016 – 2019) for the Chinese Ministry of Agriculture (US$ 0.28/dose).

Since 2010, praziquantel has been used in the endemic and hyperendemic areas of China. Transmission by dogs has been reduced, from 8% in 2010 (95% CI: 6.0–10.2) to 1.7% in 2018 (95% CI: 0.5–3.6) and a high heterogeneity between region and province subgroups has been found, thus the public health situation remains serious (44).

From 2016 – 2019, Central Government launched comprehensive compulsory vaccination of sheep and goats in the hyper-endemic areas of Western China (45). The praziquantel programme continues as before. After 4 years, slaughter examination of sheep and goats evidenced a reduction in prevalence from 5 to 1% and from 44 to 20%, the findings of cysts in liver and lung respectively (46).

Results from the use in sheep of the EG95 oil-based inclusion body vaccine (approximately 50% pure) commercially manufactured by Tecnovax S.A., Buenos Aires, Argentina (Providean Hidatil EG95®) demonstrated 84.2% and 94.7% of reduction in fertile cysts with 3 and 4 vaccinations on day 0-30-465 and 1440 respectively after experimental challenge (47).

In 2017 Tecnovax S.A. launched a new lyophilized formulation called Providean Hidatec EG95® which is bioequivalent to the Australian vaccine. The company has produced 30-50,000 doses each year (2017– 2020) for the SAG included in the Echinococcus control programmes from Rio Negro in Argentina and from Alto Biobio, and also, the Aysen region in Chile.

Lyophilization process is an expensive technology thus increase the cost of the vaccine and low demand due to the few ongoing programmes in South America, makes the cost go up. Currently, 40-50 million doses are made each year in China for these reasons the price get close to one fifth of that reached by the Argentine vaccine.

An EG95 vaccine plus 10 Clostridial antigens for use in sheep is now in progress and the registration by Tecnovax company is expected in 2022.

CONTROL PROGRAMMES IN SOUTH AMERICA INCLUDING VACCINATION

We described each participating country and their regions to provide a complete report to estimate the degree of control from each programme reducing the CE infection.

Patagonia region, shared by Argentina and Chile, is inhabited by Mapuche, Tehuelche and Pehuenche native communities, whose religious-political leader is the "Lonco" (cacique). Native communities live in a common property land without subdivisions. The region has no road infrastructure and the extreme cold climatic conditions during autumn and winter limits the accessibility to the farms in remote communities with poor animals handling facilities and poor communication technologies.

Veterinary and technician teams involved in control programmes reported an adequate knowledge of non-verbal language. Furthermore, a relative knowledge of the culture and of the customs and rites of the inhabitants were useful to cement the bases of trust in the community (48).

There, CE is endemic in sheep and goats and therefore all strategies for control and surveillance must consider not only the technical and epidemiological study, but also the social-cultural context, not usually addressed in conventional animal health criteria.

In Argentina, mediation between healthcare team and indigenous was found through Mapuche or Tehuelche health worker residing in the place, who communicate to the community by public radio the schedule and the precise tasks that veterinarians and technicians will carry out in the area.

In Chile, mediation between healthcare team and Pehuenche communities was reached by means of the training of indigenous technicians as a way of inducing trust between farmers and healthcare team involved in vaccination activities. The training was carried out through a living-learning experience.

Argentina Chubut Province, El Chalia Colony (2007-2013)

From 1984 to 2015 the colony called El Chalia, was inhabited by 20 families of Tehuelche indigenous origin. Description of the detailed programme is shown in Table 2.

From 1984 to 2007 the control programmme (49), located in the South West of the Province of Chubut (including El Chalia) had planned deworming of dogs 8 times a year with 2 tablets 50mg/each one of praziquantel and health education. However, an average of four visits could be completed annually by the same health agent (also belonging to a Tehuelche family) submitted praziquantel dog dosing "to the owners", who gave the pill to their dogs in each farm.

From 2007 to 2013 treatment of dogs was carried out each season (4 times/ year), only on request of farmers and health education activities were covered.

Vaccination schedule was done as follows: Lambs and goats received 2 doses of EG95 vaccine 1 month apart produced by the University of Melbourne and AgResearch containing 50µg EG95 plus 1 mg Quil A/dose (43) (2007-2011) and the bioequivalent by Tecnovax S.A (47) (2012-2013) injected subcutaneously into the rib-cage area of the animal, and an annual booster for all mature animals during each of the 7 years of the control programme.

In December, the first dose of the vaccine was administered to lambs and goats from one to two months of age, and the second dose was received in February. Vaccination was carried out by the Ministry of Health staff and technicians from SENASA.

During seven years of the programme, 32,000 doses were applied. However, 60-70% of livestock received vaccination out of 70% of farms (14/20); later, 95% of dwellings had joined to the programme (19/20).

The inconvenience to gather the livestock made harder the vaccination procedure having no facilities. In every season, for different reasons, some farmers could not gather the livestock. At other times, lambs and goats that received primo-vaccination were often sold at the time of second dose. Sometimes, bad climatic conditions did not contribute to support the two doses schedule. When the animals are gathered, not all the animals in an establishment could be collected at shearing in December. At the February vaccination, which coincided with the mandatory bath, we had the maximum number of animals and better facilities to work with.

As a consequence of a poor reproductive management system, lambing occurred at any time of year. Because of the extreme climatic conditions, not all offsprings were born alive.

In the first two seasons' lambs and goats were vaccinated. From the third-year pregnant livestock were added to the programme promoting adequate colostral immunity to breeds.

Each family, dwelling or farm was equivalent to one Epidemiological Unit (UE) including livestock and many dogs sharing the same habitat with children, and having its assigned place to herd their sheep or goats and the tradition of home slaughter.

The vaccination programme was not mandatory, but it could be carried out with the willing of owners. The farmers accepted the vaccine (because in every family there was someone carrying CE), as well as other aspects of the control programme (dog deworming and education activities) that have continued to be implemented.

In 2013 the programme has ended because it was planned for seven years.

Evidence for E.granulosus s.l. worms was monitored before and after vaccination programme by arecoline test in 100% of dogs and by necropsy in old livestock. Serology and ultrasound screening in humans have also performed.

In 1984, the prevalence of E.granulosus s.l. infection in dogs was found to be 55% by arecoline test, and 73% of the households had parasitized dogs.

In 2007, arecoline test demonstrated that the prevalence of CE had reached 25% in dogs and 72% of the houses had dogs infected with the *E granulosus s.l.* tapeworm (Table 2). Furthermore, arecoline tests carried out every three years (from 1984 to 2007) never detected a dog prevalence lower than 25%. In other words, previous 23 years of regular treatment of dogs does not appear to be highly effective. However, in three surrounding rural towns, Aldea Beleiro, Ricardo Rojas and Lago Blanco, which had similar characteristics, the prevalence of E.granulosus s.l. infection in dogs was found to be 44% , 25% and 15% respectively, in 1984. After 23 years of Control Programme including health education and deworming, the canine prevalence decreased to minimum values ranging from 0 to 3 % in these three locations. It is noteworthy, that these areas were visited by the same health agent than El Chalia. In other words, the control programme worked in rural areas where most people live in small villages (towns), but not in El Chalia when praziquantel is given to the owners.

After the fourth year of vaccination, no cysts were found in 100 animals slaughtered for human consumption, or were small non-fertile cysts (<10mm) (Table 2).

Surveillance using arecoline test carried out 2 years after the end of the vaccination programme (2015) demonstrated that 2 dogs from 83 dosed (2.4%) were

found with *E granulosus s.l.* The dogs belonged to 2 dwellings, decreasing to 11% the proportion of homes with parasitized dogs. Workers from these dwellings also worked on other farms away from this project.

Even so 18 dogs belonging to 7 dwellings presented tapeworm infection caused by *Taenia hydatigena*. This finding was an indicator of ingestion of untreated viscera and non-dog dosing with praziquantel.

In 2007 and 2011, human cases notified in this area were 4.6/100.000 and 0.6/100.000.

From 2014 to 2021, no human cases of CE have been reported in El Chalia colony (data obtained from the National Health Surveillance System) (Table 2). No place in Chubut province, the prevalence returned to its initial level.

In El Chalia area, the Provincial Government continues the development of the CE programme through dog treatment with PZQ (4 times a year), until it decides to use the vaccine on a wide area for a long time.

Argentina, Rio Negro Province (2009-2017)

Rio Negro has introduced the vaccine as an additional control tool to treat dogs in some areas of the province since December 2009. Features of this vaccination programme and its control area have been described in Table 2 (50).

For the duration of the trial, other CE control activities (praziquantel treatment of dogs every 3 months) continued throughout the regions comprising the vaccination areas and control areas.

Evaluation of vaccine effectiveness and its impact under operational conditions was undertaken using necropsy of sheep and serological methods.

The decreasing prevalence of E. granulosus s.l. infection in adult grazing animals and the reduced percentage of UE with at least one sheep positive to necropsy have been described from 2009 to 2015 after 6 years of control programme including vaccination (Table 2).

In addition, CE prevalence in 6-year-old sheep after necropsy and percentage of farms having infected sheep comparing the same period have been shown in Table 2.

It is noteworthy that the cysts found in older sheep after vaccination were quite small (Table 2) (50).

In 2011, the specific EG95 antibody response could be detected for at least a year after two immunizations in lambs and long lasting following the annual booster (Table 2) (51).

In 2009 4.3% of the dogs were positive for E. granulosus s.l. infection using the arecoline test, 9.6% of dog feces gave positive result by copro-ELISA demonstrating the higher sensitivity of the immunotest. Besides, 20.3% of the farms had at least one infected dog.

In 2017, 4.5% of dogs were found positive for E. granulosus s.l. by arecoline purgation, 3.7% of feces were positive using copro-ELISA and 8.9% of farms have a positive dog (Table 2) (52).

Ultrasonography screening showed no symptomatic cases in the period. Only one asymptomatic case of E. granulosus s.l. infection having a lung cyst was diagnosed among 84 school children (1.1%; ranging 6-14 years old) (52).

Chile, Alto Biobio Region (2016-2020)

In the 8th Region of Alto Biobío-Chile (53), the vaccination of 8137 sheep from 454 small farmers mainly Pehuenches was carried out by SAG. The control programme started in November 2016 with the first administration (V1) (Tecnovax S.A.) to all sheep flocks (2-3 and 5-9 months of age). An attempt was made to administer a second vaccine 1 month after the first, but given the difficult land work, a 12-month delay in the second vaccinations (V2) were recorded (November 2017). Annual boosters were applied in November 2018 (V3), and V4 in December 2019. Over November 2016 and December 2019, 90% of animals received the full dose vaccination schedule. The programme included local Pehuenche workers as vaccinators who were trained by SAG`s veterinarians.

A non-vaccinated control group was not available for comparison with the vaccinated one.

A baseline was established in 2016 and the presence of cysts in viscera (liver and lungs) was analyzed on a sample of 224 sheep (62.5% of them were 2–4-year-old).

In 2020 SAG decided that necropsies should be carried out to show how Echinococcus cyst can perhaps be reduced after 3 or 4 years, even though most dogs will still be shedding Echinococcus eggs for all that time. In 2020, necropsy was done in 200 animals that were vaccinated being lambs in their first year of life.

After vaccination with 3-4 doses, 200 livers of vaccinated sheep were analyzed and it was observed that only a few numbers of liver presented damage, injury, inflammation, scarring and thickening of the bile ducts consistent with the infestation by *Fasciola hepatica*, compared with those necropsied in 2016 (data not shown).

Features of vaccination schedule and necropsy results (Table 2) showed that similar frequency of cysts was obtained by sheep during the 4 years of vaccination (55%) to those seen before the trial began (46.8%). However, the vaccinated sheep had small cysts after 4 years (and very few of the cysts had protoscoleces) (53).

International Journal of Echinococcoses 2022;1(2):54-71
DOI: 10.5455/IJE.2021.11.07

Table 1: Echinococcus Control Programmes in Argentina, Uruguay, Chile, Peru and Brazil: the history of their control policy and impact of actions taken to date

Please add the following sentences: ⋆ canine CE through arecoline purgation. ⋆⋆ CE through CoproElisa. ⋆⋆⋆ Dogs examined were obtained from scattered rural areas, small towns with risk characteristics and suburban areas of critical socio-economic context. © Prevalence of E. granulosus s.s. using polymerase chain reaction in environmental dog faecal samples (37).

Country	Regions	Actions	Impact of actions (Prevalence-Incidence)		
Argentina	Nationwide	1941. A National Law established that CE must be confronted by the State through a control programme (18) 1948. The Ministry of Agriculture began control of E. granulosus s.l. by treating dogs against the parasite, and health education in Buenos Aires, Corrientes, Rio Negro and Chubut provinces (19). 1960. Mandatory notification through the National Surveillance System for Health (SNVS) and the National Surveillance System from Laboratories (SIVILA) National Programme for the Control of Zoonotic Diseases (Ministry of Health) provides dog and human anti-parasitic drugs. It carries out canine surveillance and ultrasound screening of schoolchildren in control areas based on risk criteria. It supplies training in ultrasonographic assessments to CE national field staff. SIGICA system from SENASA gathers the data loaded by the slaughterhouses (seizures, the animal identification and traceability, and georeferenced farms) 2015- to present. Due to each province is autonomous in planning and executing its programmes, a National Framework Plan for the CE control was created based on different strategies: - Periodic deworming of definitive hosts - Vaccination of intermediate hosts - Control of slaughter and notification through SIGICA - Control of the orchard - Responsible dog ownership - Health education of the exposed population - Surveillance in canines (coproELISA analysis, PCR assay) - Considering the role of regional wildlife in the disease cycle			
	Neuquen Province (1970- to present)	1970-1977. Dog deworming with Arecoline hydrobromide (4 mg/kg) every six weeks 1978- to present. Praziquantel 5mg/kg twice/year (non-endemic area) and every 45 days in high-risk zone 1978-1986. X-Ray and serologic cadastral 1984-to present. Ultrasound screening in children from endemic areas. 1970-2004 Surveillance of canine CE through arecoline purgation ⋆ (Sensitivity 73.3%; Specificity 89.9%) 2005- to present. CoproElisa ⋆⋆ (Sensitivity 93.6%; Specificity 88.5%). Surveillance of livestock through postmortem inspection at official slaughterhouses (SENASA) Health education		Human (100.000)	Dog (%)
			1970	54.6	28.2⋆
			1985	-	1.0⋆
			1995	43.9	1.4⋆
			2000	15.9	0.5⋆
			2004	15.5	0.85⋆
			2005	-	10.7⋆⋆
			2007	-	8.7⋆⋆
			2009	-	12.5⋆⋆
			2011	-	5.6⋆⋆
			2013	8.19	2013-18 1.66 (22)
			2016	6.36	
			2017	9.72	
			2018	6.96	
			2019	7.32	
			2020	5.12	
			2021	5.80(23)	

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Argentina

Rio Negro Province (1980-to present)

1980- to present Control of tapeworm in dog populations and farms with Praziquantel 5mg/kg 4 times/ year was carried out by sanitary agent. Health education
1980–2003 the arecoline test* was performed in dogs for surveillance purposes
CoproElisa** and confirmation of positive dogs by Western blot analysis and PCR assay 2003-to present)
1980-1997 Search for asymptomatic carriers through serology (DD5)
1984 First use of ultrasonography (US)
1997 Cadastral as method of choice for chidren diagnosis
2009- to present Vaccination of sheep in endemic areas (Mapuche community)
Surveillance of livestock offal at official slaughterhouses (SENASA)

	Dog (%)	Sheep (%)	(16,25) (%)
1980	41.5 *		61.0
1981	6.1*		-
1982	4.5*		-
1998	2.9*		18.0
2003-05	4.8 *-17**		-
2009-10	18.2 **		-
2017-18	8.2 **		-

Children 0–16 years of age (25) (%)

	DD5	ELISA	US
1980	2.05	-	-
1984	0.41	1.70	-
1986	-	-	5.6
1993	-	1.0	-
1999	-	-	1.2
2005	-	-	0.3
2018	-	-	0.2

Chubut Province (1975-to present)

1980. Started a pilot plan for CE control in the Department of Cushamen (endemic area) based on human serology by DD5 (progremme justification)
1984 CE Control Progamme including active searching by DD5 serology and registration of positive cases throughout the entire province.
1987-2000, relaunched from 2005 -to present) Search for asymptomatic carriers through ELISA serology and cadastral ultrasonography and pharmacologic treatment.
1980 Control of tapeworm in dog populations and farms with Praziquantel 5mg/kg 4 times/year in endemic region and twice in non-endemic areas carried out by sanitary agent
2007-2013 Vaccination of sheep and goat in endemic areas
Surveillance of livestock offal at official slaughterhouses (SENASA)

	Dog (%)	Sheep (%)	Human (100.000)
1984	70	25-60	84
2008	3-6	0-10	15
2010	-	-	12.75

Tierra del Fuego (1982-to present

CE Control Programme focused on canine deworming twice/year in rural area (with coverage of 97%), elimination of viscera in farms, identification of dog owners, epidemiological surveillance in hosts, information to the community and a legal framework of reference.

	Children	Dog	Sheep (16) (%)
1980	-	41.1	52.0
1996	0.0	2.5	1.5
2001	0.2	2.5	2.5
2008-10	0.0	-	0.3
2013	0.0	0.9	2.0
2016-17	0.0	6.0	0.85

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<td>Uruguay</td>
<td>Nationwide (1972-to present)</td>
<td>1939. The creation of the Center for the Study and Prophylaxis of Hydatidosis, was legitimized. The mandatory reporting of human and animal echinococcosis cases, the ban on feeding dogs with offals, surveillance of slaughterhouses, the limitation of the number of dogs, and health education in rural areas were stablished.
1965. Introduction of dog patent and tax collection (28)
1970– 1992. First CE control programme was based on dosing dogs with arecoline hydrobromide every six weeks (30)
1991 A National control programme was started and all dogs were treated with praziquantel monthly.
2005 to the present. The NCZ has obliged the dog registration and payment of the tax to owners and the money has been conducted to CE preventive and control programmes employing the following strategies (32) (33):
2007 A voluntary and free surgical castration for owned dogs was introduced.
2008 Treatment of dogs with praziquantel 5 mg/kg/bw (monthly) or broad-spectrum anthelmintics (pyrantel pamoate + PZQ + febantel) administered once a
year to all registered dogs and three times per year in areas with other parasitic zoonoses, such as toxocariasis and ancylostomiasis
- Health education conducted by the Working Days on Health (WDH) programme, at Public Health Centres (as one of the most important tools for the control and prevention of CE): Verbal, visual and graphic methods were employed Surveillance measures:
- CoproElisa for canine diagnosis -.
- Dog population control: spaying of dogs of both sexes
- Human Diagnosis by ultrasonography
- Surveillance in livestock by the Ministry of Livestock, Agriculture and Fisheries
- A strengthened community participation.</td>
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<table>
<tr><th></th><th>Dog</th><th>Human (%)</th><th>Sheep (%)</th><th>Cattle (33) (rate per 1000)</th></tr>
<tr><td>2004</td><td>6.4</td><td>-</td><td>7.85</td><td>11</td></tr>
<tr><td>2006</td><td>-</td><td>1-2</td><td>-</td><td>-</td></tr>
<tr><td>2008</td><td>10.2</td><td>6.5</td><td>-</td><td>-</td></tr>
<tr><td>2009</td><td>4.4</td><td>3.8</td><td>5.5</td><td>7.2</td></tr>
<tr><td>2013</td><td>1.6</td><td>2.8</td><td>3.2</td><td>5.3</td></tr>
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<td>Chile</td>
<td>Nationwide

Intermittent local control plans</td>
<td>1951. Mandatory notification system (34)
E granulosus s.l. is the second most important cause of condemnation of viscera in livestock (after Fasciola hepatica). The most affected regions are Los Lagos, Araucania, Los Rios, Aysen and Magallanes (35)

1982-1998 A national CE control programme deal with by SAG including
-Deworming of dogs with praziquantel every 45 days for 15 years in Magallanes and Aysen regions (16)

1979–2004 Magallanes region: a programme including PZQ treatment in dogs with register of dogs, surveillance (dogs and sheep infection), sanitary control,building of diagnostic laboratories and training in diagnostic techniques, and creation of new laws and regulations (36)
2008–2015 A CE programme based on educational activities was launched in eight municipalities from O'Higgins region In total, 8,909 students from primary and secondary schools have received instructions in prevention of CE by the team of the Epidemiology Department (36).

Since 2015 Control programmes have been initiated in Coquimbo, Biobio, Araucania and Aysen, including health education, canine deparasitation, spaying of dog and the pilot sheep vaccination in some of these.</td>
<td>
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<tr><th></th><th>Magallanes Human (100.000)</th><th>Aysen (16)</th></tr>
<tr><td>1982</td><td>38</td><td>80.7</td></tr>
<tr><td>1998</td><td>6</td><td>44.8</td></tr>
<tr><td>2001-2005</td><td>9.2</td><td>38</td></tr>
<tr><td>2008-2017</td><td>7.8</td><td>40.1</td></tr>
<tr><td>2019</td><td>-</td><td>38.4</td></tr>
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<tr><th></th><th>Magallanes Dog (%)</th><th>Region Sheep</th></tr>
<tr><td>1979</td><td>70</td><td>60</td></tr>
<tr><td>2004</td><td>0.5</td><td>0.73</td></tr>
<tr><td>2015</td><td>-</td><td>2.9</td></tr>
<tr><td>2021</td><td>18© (37)</td><td></td></tr>
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Peru	Junin Five regions- 17 rural communities	Human CE is not a notifiable disease (39) 1975-1984 SAIS Túpac Amaru and Pachacutec The Pilot programme for CE control considered periodic dosages to dogs and health education (38) 2015-2019 Pilot CE control programme: canine deworming with praziquantel alone, sheep vaccination alone or combined with dosification, and sheep deworming with oxfendazole plus dog deworming. Health education and control of slaughterhouse and home slaughter, besides of active surveillance through coproELISA in dog feces and sheep serology were included.effectiveness and feasibility of each strategy are still being evaluated (31, 40)	SAIS Tupac Amaru (Junín) Dog / Human (%) / Sheep 1974 11.6 0.1[1] - 1984 2.6 - - 1989 - 1.9[2] - 1994-1995 33 6.4 89 1 Isoelectric focusing (IEF) 2 DD5-Immunoblot
Brazil	Local	Human CE is not a notifiable disease in only one state of Brazil (Rio Grande do Sul) (17) 1990-1999 Santana do Livramento - Rio Grande do Sul, based on treatment of dogs (41). Brazil remains without a national coordinated effort, though there are incipient initiatives towards enhanced data collection and coordination (17) (e.g. 2010 – 2021 Serological diagnosis by immunoblotting countrywide (LRNH-IOC/Fiocruz National Reference Laboratory in Hydatidosis of Brazil) (42)	Annual human cases reported[a] / Sheep (%) / Bovine (17) (%) 2009 11 8.76 0.37 2010 12 12.04 0.45 2011 30 11.15 0.44 2012 15 8.65 0.35 2013 12 3.12 0.29 2014 11 4.64 0.27 [a] At the National Surveillance System

Table 2: Description of Control Programmes for CE including vaccination in Argentina and Chile

Country Province Region	Duration of the Programme	Area	Population and species	Control tool	Initial Prevalence of E.granulosus s.l. infection	Final Prevalence of E.granulosus s.l. infection
Argentina Chubut El Chalia Colony (49)	2007-2013	Southwest of the Senguer Department 30.000 hectares	20 farms, 90 people (between 1 and 10 per farm), 100 dogs, 1500 goats, 10000 sheep Health staff : 2 teams (3 people each one onboard two vans) Vaccination of Complete rodeo took 5 days Registration of all inhabitants, and their dogs and livestock	Dog treatment "upon request" (4times/year) EG95 Vaccination of sheep & goats (University of Melbourne & AgResearch (2007-2011) and Tecnovax (2012-2013): Two injections, one month apart and annual booster with 60-70 % average for fully sheep vaccinated along the area Health education	2007 Dog Prevalence 25% *E. granulosus s.l.* (Arecoline test). 72% of farms with at least 1 infected dog Human Incidence: 4.6/100.000	2015 Dog Prevalence 2% *E. granulosus s.l.* (Arecoline test). These dogs also worked on farms outside the test area. 11% of housing had at least 1 parasitized dog 35% of dwellings presented infection by *Taenia hydatigena* Sheep prevalence: 0% No cysts were found in 100 sheep slaughtered for human consumption (or its were smaller than 10mm). Human Incidence: 0.6/100.000 (Ultrasonography) No new human cases were detected from 2014 to 2021 The programme is in force and include health education and dog deworming

Argentina Rio Negro (Anecon Grande, Rio Chico Abajo, Nahuel Pan, Manuel Choique, Blancura Centro, Lipetren) (50,51,52)	2009-2017	1054 km^2	Vaccinated Group: 79 farms, 8443 sheep. Control Group: 71 farms, 2255 sheep and 2096 lambs which received no vaccinations. 309 dogs belonging to both areas. Goats were not included in the vaccination trial Health staff : 3 teams (4 people each one onboard three vans) Vaccination of the complete rodeo took 3 days	Dog Treatment: Praziquantel 5mg/kg 4 times/ year in control and vaccination areas. EG95 Vaccination of sheep (no goats were vaccinated) (University of Melbourne & AgResearch (2009-2017) Two injections, one month apart and annual booster with 57.3% average for fully sheep vaccinated along the area	2009 Dog Prevalence 4.3% (arecoline test) Dog Prevalence 9.6% (CoproELISA) 20.3% of farms with at least 1 infected dog Sheep Prevelence 56.3% 94.7% of had at least 1 sheep positive 56.3% of 6-year-old sheep showed CE and 84.2% of the farms had at least 1 infected sheep (necropsy).	2011 EG95 antibody response: Testing in lambs, and 1 to 6 years old vaccinated sheep and non-vaccinated control. Specific antiodies could be detected for at least a year after two immunizations in lambs and long lasting following the annual booster 2015 Sheep prevalence 21.1% 23.5% of farmers have least 1 sheep positive. 21.6% of sheep older than 6 years were positive. 20.2% of the farms showed to be infected (necropsy) Vaccinated group: 4 sheep showed 2 cysts in liver (only one was fertile) and 6 cysts in lung (1 × 1.3–0.2 × 0.2 cm in diameter) Average: 0.3 cysts/sheep. Control group: 13 sheep showed 47 cysts (some >5 cm in diameter) Average: 1.4 cysts/sheep (p = 0.02). 2017 Dog Prevalence 4.5% E. granulosus s.l. (Arecoline test). Dog Prevalence 3.7% (CoproELISA). 8.9% of farms have at least 1 positive dog. Human Incidence: 1 case (1,1% ranging 6-14 y.o) (Ultrasonography) No symptomatic cases in the eriod. The programme is in force and Tecnovax vaccine has been included since 2018.

Chile Biobio (Alto Biobio Communities: Butalelbún, Trapa-Trapa, Malla-Malla, Cauñicu and Pitril, Guallalí, El Barco, Ralco-Lepoy, El Avellano, Los Guindos and Quepuca-Ralco)	2016-2020	2.125 km² (10% grassland)	8137 sheep 454 farms Average animals: 18 Sheep/ flock 1 Dog/farm	No deworming of dogs EG95 Vaccination of only sheep >2-3 month of age (Tecnovax S.A) V1 with Providean HidatilEG95® (Oiled adjuvanted vaccine) V2 (delayed by 12 month), and annual bosters (V3andV4) with Providean Hidatec EG95® (Lyophilized)	2016 Frequency (95% CI) of infection in 223necropsied sheep (2-4 y.o sheep) 46.8% (38.4–55.2) Frequency of Fertile Cysts 28.1% Size: All > 5 mm	2020 Frequency (95% CI) of infection in 200 necropsied sheep (Lambs vaccinated during 1st year of life receiving 3-4 doses) 55% (48.0–62.0) Frequency of Fertile Cysts 8 % Size: 64.74% < 5 mm
Chile Aysen (Capitan Prat Province : Chile Chico, Mallín Grande, Puerto Guadal and Puerto Bertrand towns)	2020-2022	29796 km²	16,000 sheep in 206 herds	Canine deworming (4 times a year, in the mouth of the dog.) EG95 Vaccination includes pregnant sheep (one dose) and lambs (two doses, one month apart) and annual booster V1: September 2020 V2: December/ january 2020 V3: June 2021	2020 - Baseline survey -Registration and ID of vaccinated sheep and dogs associated with herds -Slaughter control using containers for offals. -EG95 serology 0-30-60 days post vaccination -Necropsy and cyst count in vaccinated sheep (baseline) Dog prevalence CoproAg - 2018 (IC90%) 19% (15%-22%)	2022 -Endline survey -Slaughterhouse control results. -EG95 serology after annual booster -Necropsy and cyst count in vaccinated sheep (endline) Dog prevalence CoproAg - 2022 (Data are not yet available)

Aysen Chile Control Programme (2020-2022)

The CE control programme in Aysen (54) promotes the repopulation of the regional sheep mass through the improvement of the productivity of the farms.

The programme proposes the creation of a high herd immunity against CE in Capitan Prat Province including small towns as Chile Chico, Mallín Grande, Puerto Guadal and Puerto Bertrand. In addition, the baseline survey about health education, registration and identification of vaccinated sheep and dogs associated with herds, canine deworming (4 times a year, in the mouth of the dog.), and slaughter control using containers for offals.

EG95 Vaccination includes pregnant sheep (one dose) and lambs (two doses, one month apart) comprising 90% of the reference population (16,000 sheep in 206 herds).

Between September 2020 and October 2021, 5.807 doses were applied in lambs according to dental chronometry and 17.662 doses in adult sheep that included also clostridial vaccination and its corresponding internal and external deworming.

The baseline survey showed a good knowledge of sheep-dog transmission, low level of knowledge of the dog-sheep contagion, confusion of hydatid disease with distomatosis due to liver fluke, and the use of viscera in feeding of dogs for economic reasons.

The ongoing programme includes EG95 antibody level detection and the lasting following 2 vaccinations, cyst count in vaccinated animals and, parasitological diagnosis in dogs (Table 2). Data will be published by SAG elsewhere.

DISCUSSION

Echinococcus remains a problem in South America

The most important CE programme topics in South America such as dosification using Praziquantel and health education (no feeding viscera to dogs) have proven to be successful.

However, holding a programme including dog dosification 12 times a year is an arduous, if not almost impossible, task in endemic areas with difficult environments and communities with entrenched customs (e.g. Chubut, Rio Negro Province or Alto Biobio, Chile).

At this time, 2022, although better anthelmintics have been discovered for treating dogs, *E granulosus s.l.* is still common in all these countries.

The schedules of dog-dosification have as main limitation the difficulty to achieve the effective coverage desired (higher to 80% of the existing dogs), in each round of deworming, due to the economic, geographical and climatic difficulties or to existing socio-cultural conditions in the areas under treatment.

Uruguay, with a monthly dog deworming process has moved to the Consolidation Phase, but it will take many years to achieve elimination. Interesting, the control programme that is currently implemented considered health education to be one of the most important tools for the control and prevention of CE.

According to Iriarte J (55), it is difficult to understand why two instructions, in theory, as simple as avoiding giving raw viscera to dogs and periodical dosification of dogs, are so difficult to implement in practice.

Socio-cultural patterns from Mapuche, Tehuelche and Pehuenche communities generally are not included in control programme criteria, demonstrating difficulties in the approach to these populations.

Mapuche and Tehuelche communities do not relate CE as a disease, but as a common suffering of parents, children, grandparents that responds to a cultural logic (56). Suarez Rojas describes (57) "CE is not just a disease; it is a social experience". In the Biobio (Chile) vaccination programme, Pehuenche community vaccinators acquired skill in the handling of the biological product, as a plus to their integrated knowledge about the cultural logic and the set of practices and symbolic aspects. Mapuche and Tehuelche sanitary agents in Chubut and Rio Negro Provinces respectively, living in the site, served as the link between Health Care team and the community.

This approach including the social-cultural context offers an effective and comprehensive response from the communities avoiding the damage of behavior patterns.

Assessment of the EG95 vaccine efficacy in different conditions in the setting of control programmes.

A. Impact of vaccination of intermediate hosts without treatment of dogs in control programmes

The new technology of a vaccine to prevent sheep, goats and llamas from being infected with E. granulosus s.l. could add the extra control required (43, 47). EG95 vaccine testing has shown that a high degree of protection against sheep, especially if given 2 doses about a month apart, and a third after 12 months (43). However, if eggs are eaten before the second dose of vaccine, some cysts will grow slowly (c.f. Alto Biobio). The vaccine is most useful when added to an Echinococcus normal control programme.

Because Biobio programme only included sheep vaccination and, no deworming treatment of the entire dog population were implemented in the commune prior to or during the vaccination trial, likely almost all areas could have had large numbers of E. granulosus s.l. eggs that young lambs might ingest, even at 2 months of age (53).

Thus, with no dog-dosing, sheep receiving the first vaccination and the second one long after birth and continuing 2 or 3 years, and if no controls of non-vaccinated group of sheep is included, it could be point out that the vaccine will not work correctly for Echinococcus control without other procedures, such as deworming. Vaccination for only 2 or 3 years are not long enough, especially without dog dosing because we do not know whether lambs had seen eggs later, while the vaccine was working.

After 4 years of programme, if vaccinated animals and some control animals were necropsied, perhaps the cysts from the vaccine group were not infective, compared to the controls (50). In this case, probably the vaccine would reduce markedly the number of cysts with protoscoleces, even though when sheep had been infected before vaccination (53).

Considering the 2 -3 and 4 year- naturally infected group, normally cysts should be 20 mm and with protoscoleces as was describes in New Zealand work without EG95 vaccine (58). In New Zealand trial, the size and the mean of the number of cysts were compiled in 40 sheep infected of which, 2 sheep were necropsied every 3 months for the 5 years.

Trials done in Chubut - Argentina including vaccinated sheep (43, 47, 59), necropsied 23 months after experimental challenge demonstrated that 50% of cysts in liver, and 15% of cysts in lung had smaller than 10-20 mm and were not infective. On the other hand, control non vaccinated sheep necropsied 9 and 14 months after challenge showed size of cysts higher than 20-30 mm carrying protoscoleces.

In addition, field evaluation of EG95 vaccine in sheep with no dog treatment carried out in Morocco (60) have shown that vaccinated animals had an average burden of viable cysts of 0.28, while in control animals the average burden was 9.10 ($p<0.001$), representing a reduction in the average burden of 97%. This means that vaccinated animals present far less viable cysts being an effective control option when 2 doses about a month apart, and a third after 12 months is performed.

Thus, if a CE control programme should be implemented, we recommend:

1. Two supervised annual deworming in the dog's mouth should be included in addition to vaccination.
2. Vaccination of young lambs and goats with V1 during shearing and V2 before winter (during obligatory bathing, eye peeling, broodstock release) including all adult females that could be pregnant during winter. It is very important to induce colostral immunity because lambs would be protected (43), until the first dose could be applied, by the antibodies that the mother passed to them through the milk. Lambs and goats start grazing after their third week of life. This possibility would be available from the second year of the vaccination programme.
3. Annual booster vaccination. It is noteworthy that, specific IgG responses to EG95 vaccination after the third or fourth vaccination induce an increased level of antibody greater than two immunizations and that this response is maintained longitudinally over time for at least 5 years. It would avoid infection of the sheep and/or development of fertile cysts during the period of its life span in the region (47, 51).

B. Prevalence of infected dogs with *E granulosus s.l.* even though vaccination of livestock was carried out in the programme

In programmes including intermediate host vaccination has been shown that infection in dogs decreased substantially (Table 2). However, in Rio Negro, after 8 years of vaccination and deworming programme, the infection in dogs was still detected with the arecoline and CoproELISA tests, indicating that dogs could have had access to viscera from non-vaccinated livestock and/or the ability of the dogs to feed outside of the vaccination area (although dogs have received 4 annual deworming). It should be noted that, Rio Negro trial had difficulty in that (a) Dogs were infected when tested, even though dogs were treated with praziquantel every 3 months, and (b) Dogs and sheep belonged to both areas – Vaccinated and Controls (50).

In El Chalia colony, surveillance using arecoline test carried out 2 years after the end of the vaccination programme demonstrated that *E granulosus s.l.* was found in dogs that also worked on other farms away from the project.

On de other hand, Rio Negro and Chubut programmes showed a significant compliance of sheep vaccination rate (57.3% and 60-70% respectively) receiving the full threedose schedule over their first year of life even under unfavorable conditions (although no goats were vaccinated in Rio Negro programme). It has been demonstrated that the effectiveness of the vaccination programme is seriously affected by the inability to deliver all three doses to approximately 40% of the livestock (31).

C. Additional findings associated to vaccination of intermediate hosts

With regard to Biobio trial, few findings consistent with the infestation by *Fasciola hepatica* were detected in vaccinated sheep that received 3-4 doses compared with those belong to the baseline. The EG95 is a fusion protein with glutathione S-transferase (GST) and GST constitute approximately 4% of the total soluble protein content of Fasciola hepatica. They have been extensively studied as

candidates for sheep vaccines from 1990 (61, 62, 63).

These results suggest that an immunogenicity and challenge test with metacercariae in target species (sheep) should be carried out in order to confirm the possibility to extend the EG95 vaccine register for use in the prevention of fasciolosis.

The duration of the vaccination programme

Since the vaccine does not affect established echinococcal cysts in animals, the vaccination schedule must be maintained until the replacement of all the grazing animals from the farm.

This livestock replacement is different according to specie, the productive system, sales season, climatic conditions and access to pastures which differs by year.

Along the Patagonian plateau, sheep often do not live beyond seven years of age. The productive systems make use of dental chronometry. Especially in El Chalia, hard grasses that concentrate silica correlate wearing with durability of the denture. At four years of age, sheep complete their 8 teeth ("full mouth") which then gradually suffer wear until they reach "half a tooth" indicating the end of their useful life (64).

CONCLUDING REMARKS

"One Health Programmes" attempting to eliminate CE using both old and new technology -knowing that even with the use of the vaccine, probably 10 years will be required.

Once the vaccination programme is over, health education and deworming should be continued.

A vaccination programme is not recommended where the disease prevalence is low and there are no children carrying CE.

The CE could quickly (or slowly) return to the existing previous time if:

1. The health education and dog dosification has no continuity or it is done for a short time

2. The programme do not manage to clean the environment of *E granulosus s.l.* eggs, to prevent the lambs born becoming infested or to develop the habit of not feeding the dogs with untreated viscera

The vaccine, now available, is being made in large quantities in Argentina and China, and appears to be the extra control technology to perhaps eliminate E.granulosus from South America.

The best procedure seems to be some dog treatments and regular vaccine to sheep and goats for 10 years including a comprehensive approach avoiding the damage of the community social matrix. If dogs or grazing animals enter from outside the controlled environment, treatments will need to be continued.

Acknowledgments: We thank all the workers from CE Control Programmes: Edmundo Larrieu, Guillermo Mujica, José Luis Labanchi, Patricia Blanco, Romina García, Claudia Grizmado and Guido Merino Rubilar.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: Proyectos Unidades Ejecutoras PUE 22920180100014CO ICT - MILSTEIN.

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