Prevalence of Human Cystic Echinococcosis in Rio Negro Province, Argentina and Direct Risk Factors for Infection

Leonardo Uchiumi
Health Ministry of Rio Negro Province

Guillermo Mujica
Centro de Investigaciones y Transferencia Río Negro: Centro de Investigaciones y Transferencia Río Negro

Daniel Araya
Health Ministry of Rio Negro

Juan Carlos Salvitti
Hospital Bernardo Carrillo, Bariloche

Mariano Sobrino
Hospital Bernardo Carrillo, Bariloche

Sergio Moguillansky
Universidad Nacional del Comahue

Alejandro Solari
Hospital Valcheta

Patricia Blanco
Health Ministry of Rio Negro

Fabiana Barrera
Hospital de Ramos Mexia

janeL Lamuniere
Hospital Rural Ñorquinco

Marcos Arezo
Health Ministry of Rio Negro

Marcos Seleiman
Health Ministry of Rio Negro

ZaidE Yadon
Academia Nacional de Medicina

Francesca Tamarozzi
Istituto Superiore di Sanità: Istituto Superiore Di Sanita

Adriano Casulli
Istituto Superiore di Sanità: Istituto Superiore Di Sanita
Research

Keywords: cystic echinococcosis, Echinococcus granulosus sensu lato, ultrasound screening, epidemiology, risk factors.

DOI: https://doi.org/10.21203/rs.3.rs-260421/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

**Background:** Cystic echinococcosis (CE) is a parasitic zoonosis caused by infection with the larval stage of *Echinococcus granulosus sensu lato* This study investigated the prevalence and potential risk factors associated with human CE in the towns and rural areas of Ñorquinco and Ramos Mexia, Rio Negro province, Argentina.

**Methods:** In order to detect abdominal CE cysts, we screened 892 volunteers by ultrasound and investigated potential risk factors for CE using a standardized questionnaire. Bivariate and multivariate analyses were used to estimate the Prevalence Ratio (PR) and their 95% CIs of the association between CE and the factors investigated.

**Results:** Abdominal CE was detected in 42/892 screened volunteers (4.7%, CI 3.2-6.1), only two of who being under 15 years of age. Thirteen CE (30.9%) cases had 25 cysts in active stages (CE1, CE2, CE3) The most relevant risk factors identified in the bivariate analysis included: live in rural area (p=0.003), age >40 years (p=0.000), drinking always water of natural source (p=0.007), residing in rural areas during first five years of life (p=0.000) and live more than 20 years at your current address (p=0.013). In the multivariate model, statistically significant risk factors were: frequently touch dogs (p=0.012), residing in rural areas during first five years of life (p=0.004), smoking (p=0.000), age > 60 years (p=0.002) and live in rural areas (p=0.017).

**Conclusions:** our results point toward infection with CE being acquired since childhood and with constant exposure throughout life, especially in rural areas with a general environmental contamination

**Background**

Cystic echinococcosis (CE) is a parasitic zoonosis, mainly prevalent in rural areas, caused by infection with the larval stage (metacestode) of *Echinococcus granulosus sensu lato* (Eg). The transmission cycle of the parasite requires two mammalian hosts, a definitive canid host (usually the dog) for the development of the adult tapeworm, and an intermediate host (usually livestock, mainly sheep), where the parasitic larval stage develops as a cyst in internal organs [1,2]. The parasite transmission cycle is fostered by human practices of feeding dogs with raw offal containing infective Eg cysts after home slaughtering [3].

Humans behave as accidental dead-end intermediate hosts for the cestode, without participating in its biological cycle [4]. Pathways of transmission such as food, contaminated water, direct contact or playing with dogs, are classically mentioned as sources of human infection and biologically plausible potential risk factors [4]. However, there are no rigorously gathered data on the contaminated matrices and pathways of transmission of infection to humans and their relative contribution in different transmission areas.
In South America, CE is a public health problem, particularly in Argentina, Brazil, Chile, Uruguay, and Peru [2,5]. In Argentina, in the Rio Negro province, CE burdens the health system with high costs for patients care[6]. In this province, the CE Control Program, launched in 1980 by the Ministry of Health, is based on Primary Health Care and One Health strategies that include deworming of dogs with praziquantel, sheep vaccination, health education, early diagnosis in humans by means of regular ultrasound (US) screenings, and medical and/or surgical treatment of infected individuals [5,7,8]. Since 1997, the CE Control Program has used abdominal US screening in the asymptomatic school children population[5], and although not routinely, also in adults[9,10].

In 2019, the CE Control Program, in partnership with the Universidad Nacional de Río Negro and the Italian Istituto Superiore di Sanità (project coordinator), participated in the collaborative, multicenter study “Molecular-Epidemiological Studies on Pathways of Transmission and Long Lasting Capacity Building to Prevent Cystic Echinococcosis” (PERITAS), funded by the European Union through the EU-LAC Health project. Other project’s partners included the Consejo Superior de Investigaciones Científicas (Spain), the Instituto de Salud Carlos III (Spain), the Universidad Austral de Chile (Chile), and the Universidad Peruana Cayetano Heredia (Peru). PERITAS aims to i) conduct abdominal US surveys to assess CE prevalence and identify clusters of infection in all-age population of selected areas of Argentina, Chile, and Peru, ii) carry out environmental sampling for the detection of Eg eggs and iii) identify the potential risk factors associated with the transmission of Eg to humans.

We present the results of the study carried out to estimate the prevalence of abdominal CE and to identify specific risk factors associated with infection transmission in Rio Negro, Argentina.

**Methods**

**Study design**

We conducted a cross-sectional, community-based study on volunteer participants by means of abdominal US. Participants were also interviewed using a structured and standardized questionnaire focused on potential risk factors and habits, including frequencies of their acting, which may favor infection (Supplementary material Annex I).

**Work area**

The study areas were the towns of Ñorquinco and Ramos Mexia as well as their surrounding rural areas in the Rio Negro Province (Figure 1), with a population estimated in nearly 3,200 people (1,800 in Ñorquinco and 1,400 in Ramos Mexia). Since the population census is more than 10 years old, the population was estimated by the primary health care service of the local hospitals, based on records arising from home visits by rural health workers. The majority of the population lives in urban areas. The rural population resides in small clusters (27 inhabitants in Treneta) or dispersed settlements around Ñorquinco (511 people).
Both towns have a small rural hospital staffed by two general practitioners, who coordinate the rural Primary Health Care Centres (PHCC) and the medical care posts (Figure 1). This health care network is the only health care provider in each area.

Ñorquinco area is located in the western mountain region of the province, extending over approximately 5,706 km\(^2\), with ideal conditions of humidity, temperature and vegetation for the survival of Eg eggs. Ramos Mexia area extends over approximately 9,680 km\(^2\), and is located in the east of the province, in the Patagonian steppe, where the very dry and hot summer could limits the survival of Eg eggs. The study areas have the conditions for the maintenance of the parasite life cycle: a high proportion of population with a low socioeconomic status, high number of families that own several dogs, a predominance of sheep farming for wool production, and the practice of home slaughtering sheep or goats and feeding dogs with raw viscera [8,11].

Both areas are targeted by the CE Control Program since 1980 in Ñorquinco and 1986 in Ramos Mexia; rural health workers are responsible for health education during house-to-house visits and for the distribution of praziquantel for dogs deworming four times a year. Deworming is usually demanded to the dog's owner. Veterinary teams are responsible for the surveillance systems of the infection in dogs (originally with arecoline test and currently with coproELISA) and in sheep (by necropsy). Human surveillance includes doing regularly US screenings in schoolchildren performed by general practitioners and the systematic registration of cases identified [5,7,8]. As a result of program activities during the period 1980-1996, 1,720 new cases were identified in the province (an average of 101 cases per year), while in 2006-2016 period there were registered 478 cases (an average of 43 cases per year) [5].

Ñorquinco always presented higher prevalence rates than Ramos Mexia. For example, in 2003 the percentage of sheep farms with infected dogs (tested with coproELISA test / PCR) was 11.8% in Ñorquinco and 0% in Ramos Mexia, while the prevalence rate in schoolchildren (detected with US) was 1.0% in Ñorquinco and 0.3% in Ramos Mexia [5]. In both areas, prevalence shows a decreasing trend [8,12].

**Population screening**

Health workers from each rural hospital made house-to-house visits to explain the aim of the study and invite the people to participate as volunteer. US screening was conducted on a convenience sample of all volunteers including of all ages and both sexes, who agreed to participate. Each adult participant, or a parent or legal representative in case of minors, signed the informed consent form and filled the questionnaire (Supplementary material, Annex 1). Two US examination machines were installed in the hospitals (one or two US machines) and the others were rotated through the rural PHCC and schools. In total, 5 and 6 machines were available in Ramos Mexia and Ñorquinco, respectively.

The Focused Assessment with Sonography for Echinococcosis (FASE) protocol[13] was used for the abdominal US screenings. CE diagnosis and cyst staging was carried out according to the WHO Informal Working Group on Echinococcosis (WHO-IWGE) expert consensus [14]. CE case definition and clinical
management were applied according to the Provincial Norms of Diagnosis and Treatment of Cystic Echinococcosis, approved by the Resolution 2624-2018 of the Ministry of Health, Rio Negro Province[5,13]. Briefly, in this document, a CE case is defined as the presence of pathognomonic features on imaging, or macro- or microscopic visualization or identification of any component of the CE cyst in a specimen, or morphologic changes of the cyst or seroconversion after medical treatment. A suspected CE case is defined by the presence of only one serological positive test (different than Western blot) or in the presence of a cyst without pathognomonic features on the image. In case of uncertain diagnosis, the participants were referred to the hospitals for further advanced imaging testing by US, CT, or MRI, as needed to finally classify them as confirmed or not, and then, the epidemiological information was processed according to the final diagnosis. All CE identified and confirmed cases were entered in the CE Control Program database and medical records were checked to verify whether the case was new or had a previous diagnosis of CE.

Data collection

The epidemiological information was collected using a standardized questionnaire (Supplementary material Annex 1), before the abdominal US scan was done.

The information collected demographic data (age, sex, place and time of residence, place of living during the first 5 years of life) health information (having a relative with CE in the house), behaviors associated with probable ingestion of Eg eggs (own and touch dogs, grow and eat raw and unwashed vegetables, source of drinking water, habits related to hand wash, smoke, use toothpick, and nail bite).

Analysis of data

The database was created in Microsoft Excel® 2.0 (Redmond, USA). A descriptive analysis of the variables was performed with EPIDAT 3.2™ (Xunta de Galicia, Spain) estimating the CE prevalence according to age, (sex, place of residence, and proportion of newly diagnosed and known and registered cases, with corresponding 95% confidence interval (CI). Place of residence during the first 5 years of life was classified for data processing in urban or rural areas and the years living in the area of the current address was categorized as less than 5 years, 5 to 10 years, 11 to 20 years and more the 20 years.

Bivariate analyses and multivariate analysis using STATATM 12.0 were used to estimate the Prevalence Rate Ratio (PR) with 95% CI of the association between CE and the variables studied.

The variable eat raw unwashed vegetables, was not included in the results because cases stated they cook the vegetables or always wash them.

A complete case approach for the multivariate analysis was used. A multivariate analysis binomial regression model was conducted starting with all factors that had a p-value =< 0.25 in the bivariate analysis and those risk factors that we decided a priori, based on existing published evidence model (dog ownership in the past 5 years and touching dogs). A manual stepwise backward selection was used to
the final model. We used the lowest BIC (Bayesian information criterion) to identify the best-fitting models given the data collected. Only variables yielding a two-tailed p-value <0.05 was considered significant and presented in the final model

Results

Population screening

A total of 892 volunteers participated in the survey, representing 28% of 3,200 total population living in the study areas. Of them, 42/892 (4.7%, CI 3.2-6.1) were positive for abdominal CE. Of them 13 CE cases (30.9%) have 25 cysts in active stages (CE1, CE2, CE3).

Based on place of residence, 309 of 1,800 (17.2%) inhabitants were examined in Ñorquinco with 13 CE cases detected (4.2%, CI 1.8-6.6) in volunteers of age in the range of 10-83 years. Out of them, 6 were living in urban areas (6/231; 2.6%, CI 0.95-5.5) and 7 in rural areas (7/78; 9.0%, CI 3.6-17.6). In Ramos Mexia, 583 of 1,400 inhabitants were examined (41.6%) with 29 subjects US positive for CE (4.9%, age in the range of 12-81 years, CI 3.1-6.8). Out of them, 18 were living in urban areas (18/451; 4.0%, CI 2.4-6.3) and 11 in rural areas (11/132; 8.3%, CI 4.2-14.4) (Table 1). 78% of the cases were born before the start of the control program.

The prevalence of abdominal CE between Ñorquinco and Ramos Mexia were similar (p= 0.60), however the prevalence rate was significantly higher in rural areas than in urban areas (p= 0.003) (Table 1). Figure 2 shows the prevalence of CE by place of residence.

The majority of the study participants were women (58% women, 42% men). The proportion of positive cases was higher in men (24/42; 57%, 6.3%, CI 3.7-8.9) than in women (18/42; 43%, 3.5%, CI 1.8-5.1), being the difference between prevalence statistically significant (p= 0.047) (Table 1).

The mean age of the study population was 34 ± 21 years. The age was significantly different (p< 0.0001) between volunteers with abdominal CE (mean 57 ± 19 years) and without CE (mean 33 ±20 years). The analysis of the prevalence by age group (categorized in 0-19, 20-39, 40-59, >60 years) shows an increase of CE with age (p< 0.00001) (Table 1).

Table 2 shows the number of individuals with abdominal CE cyst stages by number of years living in the rural areas. All individuals with the CE1 cysts stage, independently of length of living in the study rural areas, had been living in rural areas during their first five years of life.

Table 1

Demographic and risk factors for abdominal cystic echinococcosis in Ñorquinco and Ramos Mexia, Rio Negro, Argentina, 2019.
| Variables                          | Obs | US+ (n=42) | US- (n=850) | RR (95%CI) | P-value |
|-----------------------------------|-----|------------|-------------|------------|---------|
| N N % | N N % |
| Geographical residence           |     |            |             |            |         |
| Norquinco                        | 309 | 13 30.9    | 296 34.8    | 1          |         |
| Ramos Mexia                      | 583 | 29 69.1    | 554 65.2    | 1.18 (0.62-2.24) | 0.608 |
| Total                            | 892 | 42     | 850         | 1          |         |
| Place of living                  |     |            |             |            |         |
| Urban                            | 682 | 24 57.1    | 658 77.4    | 1          |         |
| Rural                            | 210 | 18 42.9    | 192 22.6    | 2.43 (1.34-4.40) | 0.003 |
| Total                            | 892 | 42     | 850         | 1          |         |
| Age Groups                       |     |            |             |            |         |
| 0-19                             | 317 | 3 7.1      | 314 37.0    | 1          |         |
| 20-39                            | 219 | 6 14.3     | 213 25.1    | 2.89 (0.73-11.46) | 0.130 |
| 40-59                            | 244 | 14 33.3    | 230 27.2    | 6.0 (1.7-20.8) | 0.004 |
| >60                              | 110 | 19 45.3    | 91 10.7     | 18.2 (5.5-60.5) | 0.000 |
| Total                            | 890 | 42     | 848         | 1          |         |
| Sex                              |     |            |             |            |         |
| Female                           | 514 | 18 42.9    | 496 58.4    | 1          |         |
| Male                             | 378 | 24 57.1    | 354 41.6    | 1.81 (0.99-2.9) | 0.051 |
| Total                            | 892 | 42     | 850         | 1          |         |
| Dog ownership in the past 5 years|     |            |             |            |         |
| No                               | 70  | 2 5.1      | 68 9.4      | 1          |         |
| Yes                              | 692 | 37 94.9    | 655 90.6    | 1.87 (0.46-7.60) | 0.381 |
| Total                            | 762 | 39     | 723         | 1          |         |
| Touch dogs                       |     |            |             |            |         |
|                | Never | Rarely | Frequently | Total |
|----------------|-------|--------|------------|-------|
| Never          | 175   | 6      | 169        | 20.8  | 1      |
| Rarely         | 146   | 9      | 137        | 16.7  | 1.79 (0.65-4.93) | 0.255 |
| Frequently     | 535   | 25     | 510        | 62.5  | 1.36 (0.56-3.26) | 0.488 |
| Total          | 856   | 40     | 816        |       |        |
| **Eat vegetables grown in a kitchen garden** | | | | |
| Never          | 510   | 23     | 487        | 59.5  | 1      |
| Rarely         | 213   | 11     | 202        | 24.7  | 1.14 (0.56-2.30) | 0.705 |
| Frequently     | 136   | 7      | 129        | 15.8  | 1.14 (0.50-2.60) | 0.754 |
| Total          | 859   | 41     | 818        |       |        |
| **Nail biting** | | | | |
| No             | 654   | 36     | 618        | 75.0  | 1      |
| Yes            | 212   | 6      | 206        | 25.0  | 0.51 (0.21-1.20) | 0.125 |
| Total          | 866   | 42     | 824        |       |        |
| **Smoke**      | | | | |
| No             | 691   | 33     | 658        | 88.4  | 1      |
| Yes            | 95    | 9      | 86         | 11.6  | 1.98 (0.97-4.01) | 0.057 |
| Total          | 786   | 42     | 744        |       |        |
| **Use toothpicks or chew blade of grass** | | | | |
| No             | 581   | 26     | 555        | 73.5  | 1      |
| Yes            | 215   | 15     | 200        | 26.5  | 1.55 (0.84-2.88) | 0.158 |
| Total          | 796   | 41     | 755        |       |        |
| **Handwash before Cooking** | | | | |
| Never          | 795   | 39     | 756        | 94.5  | 1      |
| Every time     | 47    | 3      | 38         | 4.7   | 1.30 (0.41-4.05) | 0.650 |
| Type of drinking water                  |     |     |     |     |     |
|----------------------------------------|-----|-----|-----|-----|-----|
| Always public network or bottled       | 636 | 22  | 52.4| 614 | 73.3|
| Occasionally Non-potable               | 108 | 8   | 19.0| 100 | 11.9|
| Always Non-potable                     | 136 | 12  | 28.6| 124 | 14.8|
| Total                                  | 880 | 42  | 838 |

| Have relatives with CE in the house    |     |     |     |     |     |
|----------------------------------------|-----|-----|-----|-----|-----|
| No                                     | 559 | 29  | 76.3| 530 | 75.8|
| Yes                                    | 179 | 9   | 23.7| 170 | 24.2|
| Total                                  | 738 | 38  | 700 |

| Reside in rural areas during first five years of life |     |     |     |     |     |
|-------------------------------------------------------|-----|-----|-----|-----|-----|
| No                                                    | 418 | 8   | 20.5| 410 | 58.5|
| Yes                                                   | 322 | 31  | 79.5| 291 | 41.5|
| Total                                                 | 740 | 39  | 701 |

| Years that lived in the area of the current address   |     |     |     |     |     |
|-------------------------------------------------------|-----|-----|-----|-----|-----|
| Less than 5 years                                      | 133 | 1   | 2.5 | 132 | 17.0|
| 5 to 10 years                                          | 139 | 5   | 12.5| 134 | 17.0|
| 11 to 20 years                                         | 209 | 3   | 7.5 | 206 | 26.0|
| More than 20 years                                     | 346 | 32  | 77.5| 315 | 40.0|
| Total                                                 | 827 | 41  | 787 |

**Table 2.**

Abdominal CE cases stratified by years of living in the study areas by place of residence during the first five years of life.
Of the 42 abdominal CE positive cases, 31 were diagnosed during the project screening and classified as new cases (31/892; 3.5%, CI 2.2-4.7), while 11 (11/892; 1.2%, CI 0.4-2.0) had been previously diagnosed in a clinical setting or during the activities of the CE Control Program (Table 2). 83.3% (35/42) of the detected cases were born before the implementation of the CE control program.

The number of detected CE cysts was 58 (mean 1.4 cysts per case): 52 (89.7%) in the liver, 5 (8.6%) in the spleen, and 1 (1.7%) in the kidney. According to the WHO-IWGE classification, 16 (27.6%) cysts were in the stage CE1, 1 (1.7%) CE2, 8 (13.8%) CE3a, 14 (24.1%) CE4, and 19 (32.8%) CE5. Cases with cysts in the CE1 stage were in the range of 32 to 80 years of age; 31.5% of cysts in the CE1 stage, and 51.5% of cysts in the CE4 and the CE5 stages were in the >60 years age group (Table 2, Figure 3). Of 234 screened children with equal or less than 15 years of age, two cases (one new and the other already identified), had abdominal CE (4.8% of all cases), being the prevalence of CE among children 0.9% (CI 0.1-3.0). (Table 3).

Table 3

CE cases and cyst stages according to age groups. Ñorquinco and Ramos Mexia, Rio Negro, Argentina, 2019.

| Years lived in the area of the current address | Resided in rural areas during the first five years of life | No data available |
|-----------------------------------------------|----------------------------------------------------------|-------------------|
|                                               | No (cases with CE1)                                      | Yes (cases with CE1) |
| 0 to 5                                        | 0                                                        | 1 (0)             |
| 6 to 10                                       | 1 (0)                                                    | 3 (2)             |
| 11 to 20                                      | 0                                                        | 3 (2)             |
| More than 20                                  | 7 (0)                                                    | 23 (2)            |
| No data available                             | 1 (1)                                                    |                   |
| Total CE-positives (CE1)                      | 8 (0)                                                    | 31 (7)            |


### Age groups / type cases

| Age groups / type cases | Cases n° (%) | CE1 (%) | CE2 (%) | CE3a (%) | CE4 (%) | CE5 (%) | Total cysts n° (%) |
|-------------------------|--------------|---------|---------|----------|---------|---------|--------------------|
| <=15 years              |              |         |         |          |         |         |                    |
| new                     | 1            | 1       | 1       |          |         |         | 1                  |
| old                     | 1            | 1       |         |          |         |         | 1                  |
| 16-30 old               | 2            | 1       | 1       |          |         |         | 2                  |
| 16-30 new               | 0            | 1       | 1       |          |         |         | 0                  |
| 31-45 new               | 9            | 4       | 2       | 5        | 1       |         | 12                 |
| 31-45 old               | 1            | 1       |         |          |         |         | 1                  |
| 46-60 new               | 7            | 4       | 1       | 1        | 5       |         | 11                 |
| 46-60 old               | 3            | 3       | 2       | 1        |         |         | 6                  |
| 61-75 new               | 10           | 3       |         | 2        | 7       |         | 12                 |
| 61-75 old               | 4            | 0       | 2       | 2        | 3       |         | 7                  |
| 76-90 new               | 4            | 2       |         | 1        | 2       |         | 5                  |
| 76-90 old               | 0            |         |         |          |         |         |                    |
| Total new               | 31 (73.8)    | 13      | 1       | 3        | 9       | 15      | 41 (70.7)          |
| Total old               | 11 (26.2)    | 3       | 5       | 5        | 4       |         | 17 (29.3)          |
| Total                   | 42 (100)     | 16 (27.6)| 1 (1.7)| 8 (13.8) | 14 (24.1)| 19 (32.8)| 58 (100)          |

### Risk factors analysis

The demographic characteristic and risk factors associated of the 892 subjects included in the study are shown in Table 1. Potential risk factors significantly associated in the bivariate analysis were being ≥40 years old, have had lived in rural area during the first five years of life, living in rural area, drinking non potable water, touch frequently dogs, and smoking.

Some cases can be particularly analyzed by their relationship with the place of birth and the years lived in the same address: a 63-year-old case with less than 5 years lived in her current home (Treneta), came from another endemic rural area (Yaminue). Five cases with less than 10 years spent in their current domicile, lived their first five years of life in endemic rural areas. Only one male CE aged 10-year-old has lived all his life in an urban area (Rio Chico) but visited frequently the family farm in a nearby rural area. Table 4 shows the variables that remained in the final model with correspondent PR and 95 % CI.
Table 4.

Binomial logistic regression model of risk factors for abdominal cystic echinococcosis in Ñorquinco and Ramos Mexia, Rio Negro 2019.

| Variables                                             | RR (95%CI)     | P-values |
|-------------------------------------------------------|----------------|----------|
| Age Group                                             |                |          |
| 0-19                                                  | 1              |          |
| 20-39                                                 | 0.37 (0.31-5.33) | 0.713    |
| 40-59                                                 | 1.27 (0.65-7.87) | 0.197    |
| >60                                                   | 3.12 (2.01-23.4) | 0.002    |
| Place of residence                                    |                |          |
| Urban                                                 | 1              |          |
| Rural                                                 | 2.28 (1.1-2.8)  | 0.017    |
| Touch dog                                             |                |          |
| Never                                                 | 1              |          |
| Rarely                                                | 1.76 (0.88-9.99) | 0.078    |
| Frequently                                            | 2.50 (1.30-9.29) | 0.012    |
| Resided in rural areas during the first five years of life |            |          |
| No                                                    | 1              |          |
| Yes                                                   | 2.89 (1.47-3.87) | 0.004    |
| Smoke                                                 |                |          |
| No                                                    | 1              |          |
| Yes                                                   | 4.36 (1.67-3.99) | 0.000    |

n=692

Discussion
The US abdominal screening resulted in 4.7% overall prevalence of asymptomatic cystic echinococcosis in the Departments of Ñorquinco and Ramos Mexia, Rio Negro Province with a 1.5% prevalence of CE active stages. Thus, despite control efforts made in the province since 1980, the overall prevalence of CE, is still relatively high indicating that CE remains as a public health problem yet.

The surveillance strategy of the control program in Rio Negro Province is based on US screenings in children and case notification. Surveillance data in Rio Negro Province show a sustained decrease of human CE cases associated with the implementation of control measures [5,8]. In accordance, in our survey the number of cases in children under 15 years of age was extremely low.

In general population, studies conducted in other geographical areas of Rio Negro province the prevalence of CE reported was significantly lower in the Departments of Ñorquinco and Ramos Mexia: overall prevalence 8.3% in Pilcaniyeu and Comallo in 1984[10] (p=0.00001) and prevalence 7.1% in Ingeniero Jacobacci in 2009 [9].

The risk of CE was significantly enhanced by factors related to geodemographic characteristics and hand to mouth transmission (frequently dog contact and smoking). The final model shows that there was a significantly increasing risk of CE with increasing age, living in rural area during the first five years of live, living in rural areas, frequently touching dogs, and smoking.

The higher prevalence rate of abdominal CE in subjects born before the implementation of the control measure, particularly those who resided during their first five year of life in rural areas suggest that the transmission may occurs during the first 15 years of life and consistent with previous finding in the provinces [5,11].

The finding of a high percentage of CE1 cyst stages (27.6%) in adults may arguably reflect a recent transmission in adults. Nevertheless, it may also be the result of the stability in CE1 stage of an infection acquired in the past. This is consistent with previous results reported in Kenya and Morocco with 87.3% of cyst remaining in the same class [15], and the province of Rio Negro, where 18.8% of CE1 and CE3 cyst stages in children remained unaltered even after 10 years of follow-up [5].

Ñorquinco and Ramos Mexia presented similar prevalence rate, but it was significantly higher in rural (8.57%) than urban areas (3.51% (p= 0.003). This result is consistent with CE being a rural zoonosis and overall, with the results of other studies carried out in the area [9,24].

The increased risk of CE associated with frequently touching dog is consistent with the facts that in the study area dogs have access to parasitized viscera and human co-habit and interact with dogs from birth and drink non potable water that may be contaminated. Thus, the supply of Eg eggs for them occurs early in life and abundant (in the present study 79.5% of the cases and 100% of the cases with CE1 cystic was in this conditions). The literature review shows studies with compatible results[16–18], but there are others that are not supporting our finding [20–22]. It is possible, that different cultural habits may explain these differences.
Smoking, a novel finding of our study, was found to be associated with CE, plausibly transmitted through a “hand-to-mouth” mechanism. The subjects that responded as smokers were => 17 years old.

With the exception of frequently touching dogs and smoking no other variables that we studies related to specific behavior of putting hands in mouth were associated with CE.

Source of drinking water were associated with CE only in the bivariate. On the contrary, food-related variables were not found associated with risk of infection. The use of non-drinking water in South America has been shown to be a significant risk factor for CE in Peru and Uruguay ([22,23] and in Rio Negro (only in bivariate analyses) [24], while in Europe it has been suggested not to be significant [4]. While also results from a recent systematic review and meta-analysis supported the hypothesis that dog contact and drinking contaminated water (and to a less extent contaminated food) would be major pathways of transmission for CE [25], currently virtual no experimental evidence exists on the contamination of these matrices[4], which is therefore needed to support these circumstantial findings.

The strengths of this study are that a large number of subjects volunteered to be screened (28% of the population). The study also used the routine district health services for its implementation a considerable number of machines were available to do the US screening. Differently from what generally is done in other studies of this type, we included variables to evaluate behaviors and their frequency, that potentially can induce the ingestion of Eg eggs, rather than questions related merely to the perpetuation of the parasite cycle.

As was recently highlighted [26], risk factors evaluation for chronic diseases like CE, which have long latency and no acute symptoms that can induce the patient to consultation, have limitations particularly when using cross sectional design to asses analyze behavior over a period of time

The study was carry out in an area where CE Control Program has been implemented and health education campaigns was carried out regularly in schools and through the media during the control program activities what may have influenced not only changes in the actual people behaviors, but also induce them to give as an answer that the researcher wants to hear, probably leading to bias.

Selection bias also may arise because the screen sample was self-selected due to the voluntary nature of their participation. The difficulties of mobilizing the community from remote rural areas to the US screening post may also contributed to selection bias influencing the result in either direction.

The selection of the appropriate professionally trained interviewers is desirable, when possible, however, in this study, this option was not possible and the interviewers were non-professional health workers and trained only short time what can have introduce some problem with the recollection of the information leading to missing data for some variables (from 1-17%) thus preventing the availability of the full data set to be included for the multivariate analysis.

Primary prevention feasible measures aimed at avoiding the ingestion of Eg eggs can help reducing the burden of CE. The identification of the main sources of infection and habits which may increase the risk
of exposure may allow interventions that can be adjusted, including those relevant for health education messages[4,27].

**Conclusions**

The result of this study show that the prevalence of CE in Rio Negro is decreasing likely as the result of the Control Program measures. However, CE continues to be a relevant zoonosis in Río Negro. Although transmission to humans has been considerably reduced through canine deworming and health education strategies associated with the systematic search for asymptomatic carriers, especially in the school children population, cases continue to be detected in adults (some with cysts in active stages), and it remains to be determined whether these are recent or old infections.

Factors associated with higher risk of CE suggest that people acquire the infection with Eg in rural areas, a proxy of environmental contamination, over time while residing in such environment, rather than linked with very specific habit(s), together with direct or indirect contamination of hands more than ingesting eggs through contaminated food and water [28]. With respect to control strategies, it is important to identify risks which are susceptible to be reduced by feasible interventions.

**Declarations**

**Data availability**

The data used to support the results of this study are available from the corresponding author upon request.

**Competing Interest**

The authors declare that they have no competing interests.

**Acknowledgments:**

This work was supported by EU-LAC Health (http://eulachealth.eu/), the Argentine Ministry of Health and the Italian Ministry of Health – PERITAS project (Molecular epidemiological studies on pathways of transmission and long lasting capacity building to prevent cystic echinococcosis infection). The funding body had no involvement in the conception, preparation, and writing of the manuscript, in the viewpoints expressed, or in the decision to submit this article. We are grateful to the Directors of the Hospitals of Ramos Mexia, Lic. Mariana Comezaña, and Ñorquinco, Dr. Juan Manuel Montes and their health workers of Ñorquinco Aurelis Antilef, Hilda Fernandez, Lorena Luna, Juanita Pereyra, Analia Anabalon and of Ramos Mexia. Julio Bijarra, Maria Carriqueo, Evelin Garcia, Yanina Mortada and Marcela Huenumil. We are also thanks Diana Marin (Professor at the School of Medicine Universidad Pontificia Bolivariana, Medellín, Colombia) for statistical advice.

**Ethics approval and consent to participate**
The study was approved by the Bioethics Committee of the Ministry of Health of the Province of Rio Negro, Argentina, by Ministerial resolution N°5115/19 "MS". In addition, the directors of the hospitals of the investigated areas signed the authorization for the use of their facilities signed the authorization to conduct the study and to the use of their facilities.

**Authors’ contributions**

AC and FT conceived the study, produced the protocol and discussed the results. LU, JCS, MS, SM and AS performed the US and discussed the results. EL review and correct the database, carried out descriptive statistics and drafted the manuscript. ZY carried out formal analysis, review and edited the paper. GM, DA, PB, MS, FB and JL performed the interview, collected the data base and interpreted the results. MA collaborate in the statistical analyzes, in the elaboration of maps and in the general organization of the activities.

All authors read and approved the final manuscript.

**Funding**

EU-LAC Health, the Argentine Ministry of Health and the Italian Ministry of Health – PERITAS project (Molecular epidemiological studies on pathways of transmission and long lasting capacity building to prevent cystic echinococcosis infection).

**Consent for publication**

Not-applicable.

**References**

1. Otero-Abad B, Torgerson PR. A systematic review of the epidemiology of echinococcosis in domestic and wild animals. PLoS Negl Trop Dis. 2013;7:e2249.

2. Craig, P.S, Hegglin, D, Lightowlers, M.W, Torgerson, PR, Wang, Q. Echinococcosis: Control and Prevention. I. Echinococcus Echinococcosis. Thompson, R.C.A., Deplazes, P., Lymbery, A.J. 2017. p. 55–158.

3. Larrieu E, Gavidia CM, Lightowlers MW. Control of cystic echinococcosis: Background and prospects. Zoonoses Public Health. 2019;66:889–99.

4. Tamarozzi F, Akhan O, Cretu CM, Vutova K, Fabiani M, Orsten S, et al. Epidemiological factors associated with human cystic echinococcosis: a semi-structured questionnaire from a large population-based ultrasound cross-sectional study in eastern Europe and Turkey. Parasit Vectors [Internet]. 2019 [cited 2021 Feb 9];12. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6664724/

5. Bingham GM, Larrieu E, Uchiumi L, Mercapide C, Mujica G, Del Carpio M, et al. The Economic Impact of Cystic Echinococcosis in Rio Negro Province, Argentina. Am J Trop Med Hyg. 2016;94:615–25.
6. Larrieu E, Costa MT, Cantoni G, Labanchi JL, Bigatti R, Pérez A, et al. Control program of hydatid disease in the province of Río Negro Argentina. 1980-1997. Bol Chil Parasitol. 2000;55:49–53.

7. Arezo M, Mujica G, Uchiumi L, Santillán G, Herrero E, Labanchi JL, et al. Identification of potential “hot spots” of cystic echinococcosis transmission in the province of Río Negro, Argentina. Acta Trop. 2020;204:105341.

8. Bingham GM, Budke CM, Larrieu E, Del Carpio M, Mujica G, Slater MR, et al. A community-based study to examine the epidemiology of human cystic echinococcosis in Rio Negro Province, Argentina. Acta Trop. 2014;136:81–8.

9. Salviti JC, Sobrino M, Del Carpio M, Mercaipide C, Uchiumi L, Moguilensky J, et al. [Hydatidosis: Ultrasonography screening in the Río Negro Province 25 years after the first screening]. Acta Gastroenterol Latinoam. 2015;45:51–5.

10. Larrieu E, Lester, R, Rodriguez Jauregui J, Odrozzola, M, Medina M, Agüero AM. Epidemiology of human hydatidosis in the Province of Rio Negro, Argentina. Acta Gastroenterol Latinoam. 1986;16:93–108.

11. Lawson A, Boaz R, Corberán-Vallet A, Arezo M, Larrieu E, Vigilato MA, et al. Integration of animal health and public health surveillance sources to exhaustively inform the risk of zoonosis: An application to echinococcosis in Rio Negro, Argentina. PLoS Negl Trop Dis. 2020;14:e0008545.

12. Del Carpio M, Mercaipide CH, Salvitti JC, Uchiumi L, Susteric J, Panomarenko H, et al. Early diagnosis, treatment and follow-up of cystic echinococcosis in remote rural areas in Patagonia: impact of ultrasound training of non-specialists. PLoS Negl Trop Dis. 2012;6:e1444.

13. Brunetti E, Kern P, Vuitton DA, Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop. 2010;114:1–16.

14. Solomon N, Kachani M, Zeyhle E, Macpherson CNL. The natural history of cystic echinococcosis in untreated and albendazole-treated patients. Acta Trop. 2017;171:52–7.

15. Ziaei Hezarjaribi H, Fakhar M, Rahimi Esboei B, Soosaraei M, Ghorbani A, Nabyan N, et al. Serological evidence of human cystic echinococcosis and associated risk factors among general population in Mazandaran Province, northern Iran. Ann Med Surg. 2017;18:1–5.

16. Acosta-Jamett G, Weitzel T, Boufana B, Adones C, Bahamonde A, Abarca K, et al. Prevalence and Risk Factors for Echinococcal Infection in a Rural Area of Northern Chile: A Household-Based Cross-Sectional Study. PLoS Negl Trop Dis [Internet]. 2014 [cited 2021 Feb 16];8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4148223/

17. Andrabi A, Tak H, Lone BA, Para BA. Seroprevalence of human cystic echinococcosis in South Kashmir, India. Parasite Epidemiol Control. 2020;11:e00172.

18. Xue J, Zartarian V, Moya J, Freeman N, Beamer P, Black K, et al. A meta-analysis of children’s hand-to-mouth frequency data for estimating nondietary ingestion exposure. Risk Anal Off Publ Soc Risk Anal. 2007;27:411–20.

19. Prince Antwi-Agyei, Adam Biran, Anne Peasey, Jane Bruce, Jeroen Ensink. A faecal exposure assessment of farm workers in Accra, Ghana: a cross sectional study. BMC Public Health.
2016;16:587.
20. Gorman Ng M, Davis A, van Tongeren M, Cowie H, Semple S. Inadvertent ingestion exposure: hand-
    and object-to-mouth behavior among workers. J Expo Sci Environ Epidemiol. 2016;26:9–16.
21. Cohen H, Paolillo E, Bonifacino R, Botta B, Parada L, Cabrera P, et al. Human cystic echinococcosis in
    a Uruguayan community: a sonographic, serologic, and epidemiologic study. Am J Trop Med Hyg.
    1998;59:620–7.
22. Moro PL, Cavero CA, Tambini M, Briceño Y, Jiménez R, Cabrera L. Identification of risk factors for
cystic echinococcosis in a peri-urban population of Peru. Trans R Soc Trop Med Hyg. 2008;102:75–8.
23. Larrieu EJ, Costa MT, del Carpio M, Moguillansky S, Bianchi G, Yadon ZE. A case-control study of the
    risk factors for cystic echinococcosis among the children of Rio Negro province, Argentina. Ann Trop
    Med Parasitol. 2002;96:43–52.
24. Torgerson PR, Robertson LJ, Enemark HL, Foehr J, Giessen JWB van der, Kapel CMO, et al. Source
    attribution of human echinococcosis: A systematic review and meta-analysis. PLoS Negl Trop Dis.
    Public Library of Science; 2020;14:e0008382.
25. Casulli A, Tamarozzi F. Tracing the source of infection of cystic and alveolar echinococcosis,
    neglected parasitic infections with long latency: The shaky road of “evidence” gathering. PLoS Negl
    Trop Dis [Internet]. 2021 [cited 2021 Feb 9];15. Available from:
    https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7819598/
26. Tamarozzi F, Deplazes P, Casulli A. Reinventing the Wheel of Echinococcus granulosus sensu lato
    Transmission to Humans. Trends Parasitol. 2020;36:427–34.
27. Possenti A, Manzano-Román R, Sánchez-Ovejero C, Boufana B, La Torre G, Siles-Lucas M, et al.
    Potential Risk Factors Associated with Human Cystic Echinococcosis: Systematic Review and Meta-
    analysis. PLoS Negl Trop Dis. 2016;10:e0005114.