Identification of potential 'hot spots' of cystic echinococcosis transmission in the province of Río Negro, Argentina

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Highlights

•Deworming of definitive hosts of *E. granulosus* with Praziquantel reduce *E. granulosus* transmission

•Was observed a statistically significant reduction in the prevalence of canine echinococcosis and in the CE in schoolchildren

•CoproELISA test and US showed statistically significant differences between the initial and subsequent studies that can be seen on hot spot maps

•Control programme was effective even in a difficult, remote environment in the Patagonia region

ABSTRACT

Cystic echinococcosis (CE) is a parasitic zoonosis caused by *Echinococcus granulosus*. The control program of CE of Rio Negro province, Argentina, involves annual surveillance using ultrasound (US) screening in school children, and five-year cross-sectional surveys to detect livestock farms with parasitized dogs by coproELISA with confirmation tests (Western Blot or PCR). Control program is based on deworming of dogs with praziquantel and the aim is to identify areas at risk of Cystic echinococcosis transmission to humans, using all available data sources. The information was spatially distributed in 13 program areas and, at a smaller geographical scale, in 80 Primary Health Care Centers. CoproELISA surveys involved three randomized sampling periods (2003-05, 2009-10, 2017-18), with 1790 canine fecal samples. The US surveys were conducted in 2003-08, 2009-16 and 2017-18 in 34515 children. Heat maps were created at the smallest geographic scale with QGIS 3.4.6. For the consecutive sampling periods, prevalence of positive canine fecal samples from livestock farms were 14.7, 12.1 and 7.8%, respectively, and children prevalence was 0.4, 0.2 and 0.1%, respectively. The study has been developed on a scale according to which the temporal-spatial distribution of CE allows to adjust control strategies in those areas of potential transmission of the zoonosis to humans.

Introduction

Cystic echinococcosis (CE) or hydatidosis is a parasitic zoonosis caused by *Echinococcus granulosus* (Cestoda: Taeniidae)*.* This parasite requires two mammal hosts to complete its life cycle, the definitive host where it develops into the strobilar adult stage, and the intermediate host where it develops into the larval stage (or metacestode). In South America, the main definitive and intermediate hosts are the dogs and sheep, respectively, but goats, pigs and cattle can also become infected (Craig et al., 2017).

CE is widely distributed in South America, where many control efforts have been made (Pavletic et al., 2017; Larrieu and Zanini, 2012). In Río Negro province, Argentina, it is endemic and the most important zoonosis from an economic and social point of view (Larrieu et al., 2000; Bingham et al., 2016).

Risk factors for high prevalence of CE in the region include poor knowledge of general sanitary practices, a rural economy based on rural subsistence smallholder production of sheep, an inadequate urban infrastructure without minimum sanitary standards for slaughter of adult sheep for human consumption, slaughter at livestock farms which allows dogs to feed on infected viscera, and a large number of domestic dogs.

Since 1980, the Zoonosis Department of the Ministry of Health has launched a CE control program in Rio Negro, which achieved a significant decrease in the prevalence in dogs and humans. However, CE transmission is still sustained by the endemic level of infection of dogs and sheep, leading to new cases in children (Perez et al., 2006; Larrieu et al., 2011).

The objective of this study was to identify, using the current surveillance system, potential risk areas of CE transmission in Rio Negro province, where control activities need to be strengthened. The analysis was conducted to the lowest possible geographical level and based on all available data sources, including the detection of *E. granulosus* in canine fecal samples from livestock farms by coproELISA and recent transmission to school children by ultrasound (US) surveys.

Materials and methods

Study Area

Río Negro province is located in northern Patagonia. The landscape is characterized by shrub-like vegetation. Annual rainfall ranges between 100 and 300 mm and mean annual temperature ranges between 8 and 15°C, with seasonal variations (higher temperatures in summer which drop to near freezing in winter). In the western mountain region, the vegetation is dominated by small trees, annual rainfall ranges between 200 and 1200 mm and mean annual temperature is 9°C, with occasional snowstorms in winter.

The CE endemic area is located in the south-west of the province, in an area of 120013 km^2 with a population density of 0.88 inhabitants/km^2. The program area has 13 hospitals and a network of 80 Primary Health Care Centers (PHCCs); their staff are only a sanitary agent or nurse in rural areas, and a general practitioner could be present in PHCCs urban areas (Figure 1).

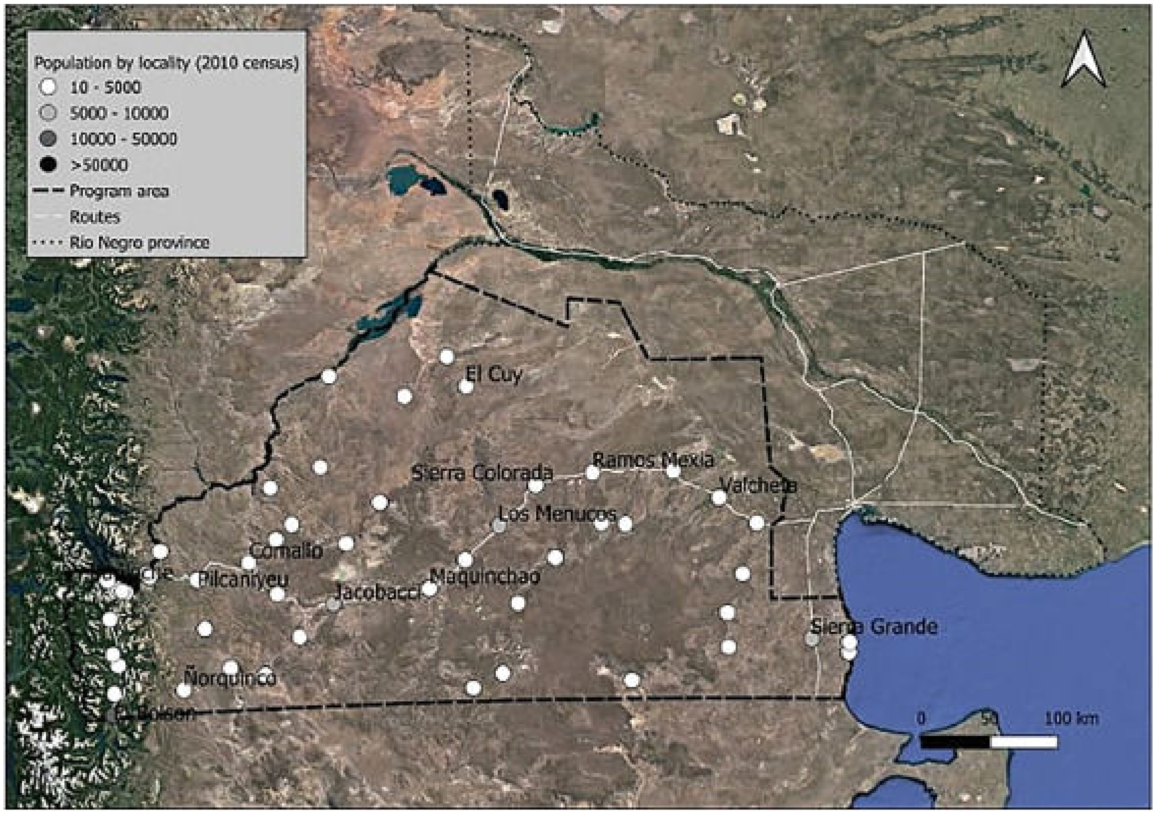


FIGURE 1. Work area, Rio Negro province, Argentina. 2003-2018

The program area is situated along national route 23 running across the Patagonian plateau from east (Atlantic Ocean) to west (San Carlos de Bariloche City), crossing localities of Valcheta (geographical coordinates -41.3308100/-695457600), El Cuy (-39.9250400/-68.3436400), Ingeniero Jacobacci (-41.3308100/-69.5457600), El Cuy (-39.9250400/-68.3436400) and El Bolson (-41.9655300/-71.5341200). The main agricultural activity in the region is sheep and goat farming.

General features of the CE control program

Twelve veterinarians provided scientific and technical support to the program involving dog surveillance systems, and 65 rural health assistants were responsible for sanitary education and for deworming dogs with praziquantel **(**PZQ**)** at a dose of 5 mg/kg four times a year, that usually was supplied to the dog's owner, for him to give it to the dog (Larrieu et al., 2000) and more recently by dosing of the tablets smeared with liver pate by rural health assistants .
In 2006, vaccination with EG95 was started as a control method for lambs in selected risk areas with a high level of CE prevalence in sheep (Larrieu et al., 2019a).

Human diagnosis and treatment are part of the control program. New cases detected in all public hospitals were recorded and patients underwent follow-up. In addition, surveys were conducted in the community in a framework of health care based on strong cooperation among veterinarians, general physicians from rural areas and surgery services (Larrieu et al., 2000, 2011, 2019b).

The control program and surveillance activities have the financial support of the Ministry of Health of Rio Negro Province, Argentina.

CE surveillance in livestock farms

Three cross-sectional studies were carried out in the endemic area in the periods 2003-2005 (Perez et al., 2006), 2009-2010 and 2017-2018. The livestock farms were selected according to simple random sampling (the number of each livestock farm is provided by the national animal health service and is used to randomize, even more a single livestock farm can be selected for more than one transversal study). Sample size was determined at 95% confidence interval, with an absolute error of 4%.

One stool sample for every two dogs present in each livestock farm was collected. from the ground close to the house, avoiding external contamination and placed separately (with any preservative addition) in clean and dry plastic containers with screw caps. They were immediately sent to the Laboratory of Environmental Health in San Carlos de Bariloche or to the laboratory of the Faculty of Veterinary Medicine, National University of Rio Negro, and stored at -40°C until processing for coproantigen extraction (Guarnera et al., 2000; Perez et al., 2006).

Finally, stool samples had been analyzed using coproELISA (Guarnera et al., 2000) at the National Institute of Microbiology ANLIS/MALBRAN or at the Laboratory of Environmental Health in San Carlos de Bariloche. Positive samples were confirmed by Western blot (WB) in the first two study periods and by PCR (Cabrera et al., 2002) in the last one.

The sampling design precluded identification of individual dogs (different single sample could not necessarily be assigned to a given dog). Therefore, we used livestock farm as the surveillance unit, which was considered as positive when it showed at least one positive sample by coproELISA confirmed by WB or PCR. Results are expressed as a proportion of positive livestock farms/ total evaluated livestock farms.

CE surveillance in children between 6-14 years old

Well-trained general physicians (Del Carpio et al., 2012) conducted annual US screening in school children between 6-14 years old in the period 2003-2018 (Larrieu et al., 2019b). The studies are carried out annually in all schools of the risk area as a strategy for early diagnosis and timely treatment, with previous informed consent of the parents (Larrieu et al., 2019b). The US screening is also a surveillance system that evaluates the impact of the program on human populations. Based on this, schoolchildren can be evaluated several times during the work period (Larrieu et al., 2019b). Results were expressed as proportion of new cases/total evaluated schoolchildren.

All cases were analyzed from an epidemiological perspective to determine the probable exposure site with a survey to identify contact with risk sites (for example, relationship with livestock identifiers) that was completed by parents (Larrieu et al., 2011; 2019b).

In addition, they were grouped into the periods 2003-2008, 2009-2016 and 2017-2018 so that the first year of these periods matched with the first year of the surveillance in livestock farms -see the section above- and the last year of each period is the one before the beginning of the following cross-sectional study in livestock farms. In this way, it was possible to observe the temporal and spatial relationship of cases in children, with those geographic areas which showed the highest proportion of livestock with positive dogs.

Statistical Analysis and georeferencing

Sampling size for studies in livestock farms and randomization to select the farms were estimated and the statistical analysis of results was made using EPIDAT 3.1. Proportions of positive farm and proportions of positive schoolchildren were calculated and their confidence intervals were set at 95%.

The prevalence obtained for the different periods were compared by the Chi-square linear trend test. Significant differences were set at p = 0.05, and odds ratio (OR) with IC95% were estimated.

The livestock farms were georeferenced during fecal sampling, while children with CE cysts were georeferenced according to their most likely exposure site determined in the risk survey.

The livestock farms were associated with the nearest PHCCs. With that information, heat maps were constructed, based on an algorithm that creates a density raster from a layer of vector points using the kernel density estimation. Density was calculated based on the number of points in a location. Heat maps were created in QGIS3 by selecting a radius of 30 km (bada kernel width) and weighted by proportion. Similar maps were constructed for cases in children but associated to the geolocation of each case with the nearest PHCCs (Kernel density is only to visualize the number of cases, the more cases the more density).

Results

Canine echinococcosis surveillance in livestock farms

In the cross-sectional surveys conducted in 2003-2005, 2009-2010 and 2017-2018 we collected 706, 562 and 512 canine stool samples from 265, 272 and 243 livestock farms, respectively. The percentages of livestock farm with at least one dog with positive fecal samples by coproELISA were 32.0%, 32.9% and 15.6% and further confirmation by WB or PCR revealed the presence of at least one infected dog in 14.7% (CI95% 10.7-19.5), 12.1% (CI95% 8.1-16.2) and 7.4% (CI95% 4-7-11.9) livestock farms for the first, second and third surveys, respectively (Table 1, Figure 2). The chi-square trend suggests that there is a statistically significant linear decrease in the prevalence between periods ($p = 0.01$), with ORs of 1 for the first, 0.8 (IC95% 0.5-1.2) for the second and 0.5 (CI95% 0.29-0.85) for the third survey. Analyzed in pairs, there are no significant differences between the first and second period ($p = 0.37$) nor between the second and third period ($p = 0.07$) while there are significant differences between the first and third periods ($p = 0.009$).

Table 1. Epidemiological surveillance of Cystic Echinococcosis (CE) by coproELISA (WB/PCR) in sheep farms. CE Control Program of province of Río Negro, Argentina, 2003-2018

PERIOD AREA	2003-2005n° + %*	2009-2010n° + %*	2017-2018n° + %*
Bariloche	1 1 100	5 0 0.0	1 1 100
El Bolson	6 1 16.7	38 4 10.5	12 0 0.0
Comallo	17 2 11.8	12 1 8.3	4 1 25.0
El Cuy	27 4 14.8	47 7 14.9	41 5 12.2
Ing. Jacobacci	32 5 15.6	28 6 21.4	29 2 6.9
Maquinchao	24 1 4.2	12 4 33.3	20 0 0.0
Los Menucos	37 7 18.9	17 1 5.9	11 2 18.2
Ñorquinco	17 2 11.8	20 1 5.0	26 2 7.7
Pilcaniyeu	16 3 18.8	11 0 0.0	19 1 5.3
Ramos Mexia	11 0 0.0	6 0 0.0	14 1 7.1
Sierra Colorada	13 2 15.4	18 1 5.6	16 2 12.5
Sierra Grande	11 3 27.3	18 1 5.6	12 0 0.0
Valcheta	53 8 15.1	40 7 17.5	38 1 2.6
TOTAL	265 39 14.7 (10.7-19.5)	272 33 12.1 (12.1-16.6)	243 18 7.4 (4.7-11.9)

*

n°: number of sheep farms tested; +: number of sheep farms with at least one *E. granulosus*-positive dog fecal sample; %: prevalence of positive sheep farms (CI95%).

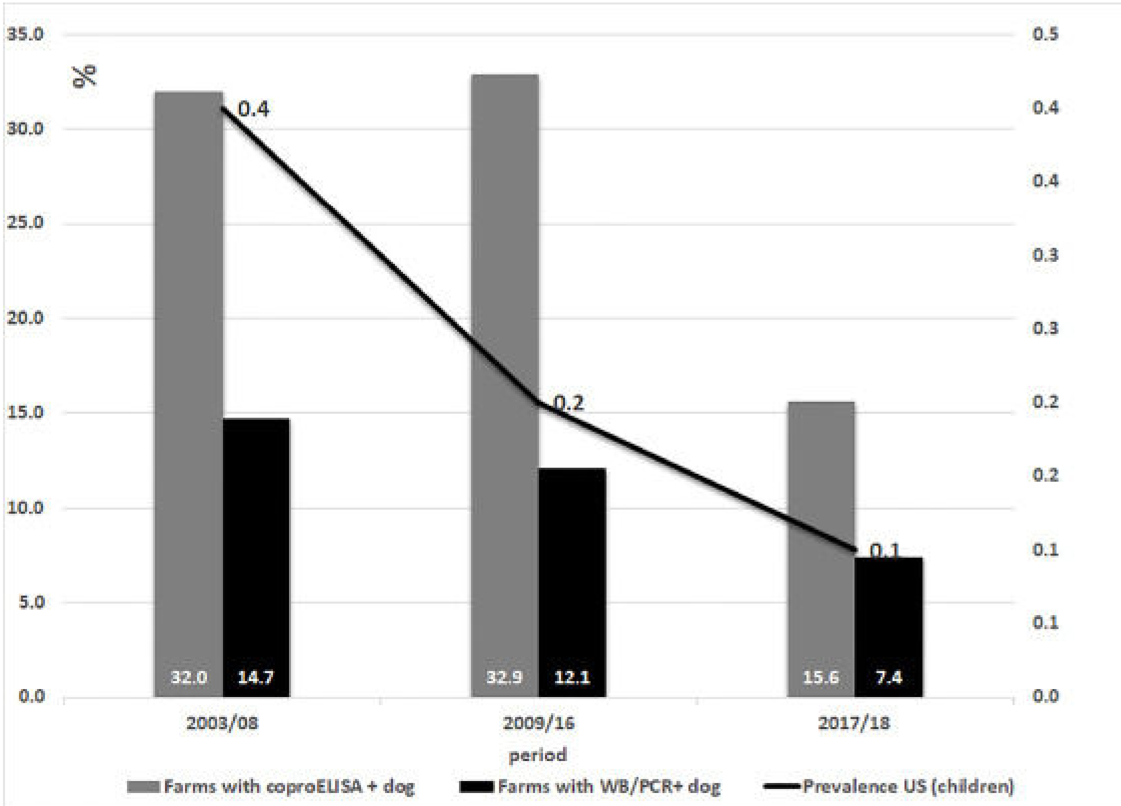
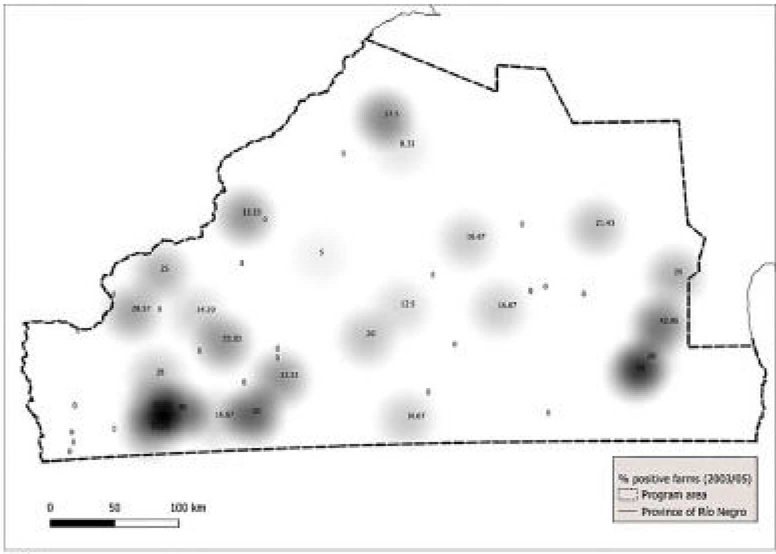


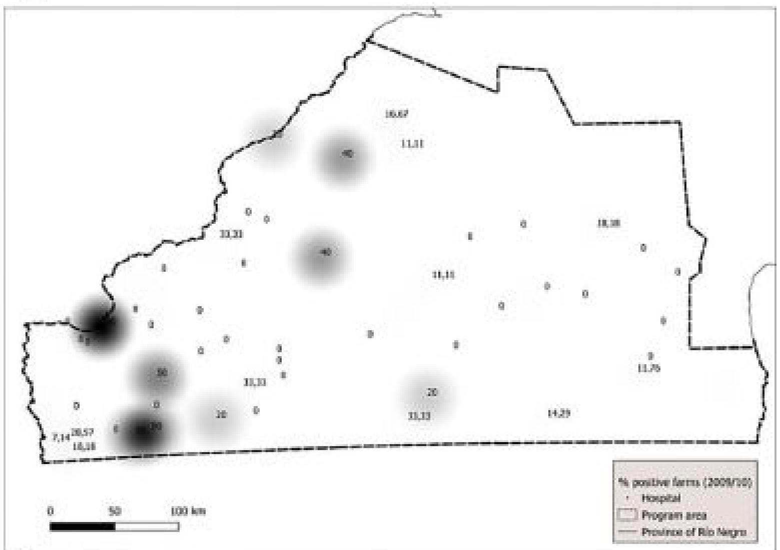
FIGURE 2. Evolution of percentage of samples positive to coproELISA in dogs, farms with positive dogs and positive schoolchildren with US. Rio Negro province, 2003-2018

The highest prevalence values were recorded in livestock farms from the Andean and Pre-Andean regions (Program Areas of Ñorquinco, Comallo and Pilcaniyeu) to the central plateau of the province (Program Areas of Ingeniero Jacobacci, Los Menucos and Maquinchao) and to the east (Program Areas of Valcheta and Ramos Mexia). In the last survey, proportion of positive farms were associated only with PHCCs in the Andean and Pre-Andean regions (Program Areas of Bariloche, Pilcaniyeu and Ñorquinco and in the center (Program Areas of Ingeniero Jacobacci) (Figures 3a, 3b, 3c).



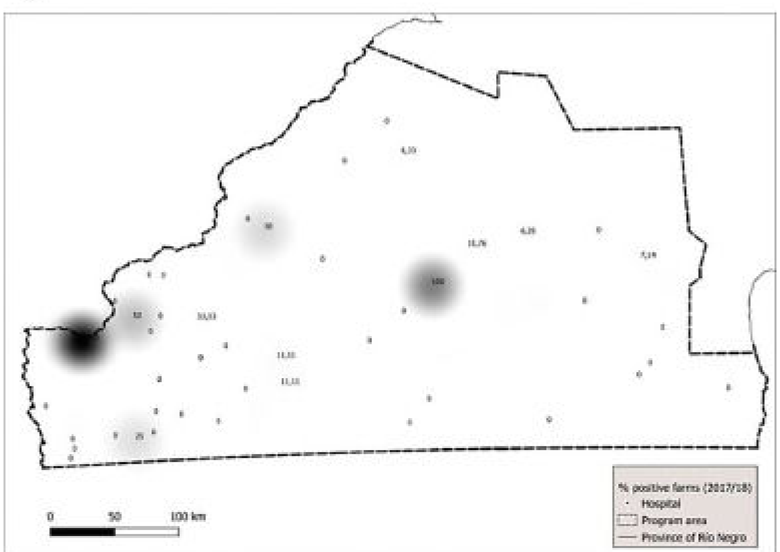
% positive farms (2003/05)
Program area
Province of Río Negro
0
50
100 km

a



% positive farms (2009/10)
Hospital
Program area
Province of Río Negro
0
50
100 km

b



% positive farms (2017/18)
Hospital
Program area
Province of Río Negro
0
50
100 km

c

FIGURE 3. 'hot spots' of cystic echinococcosis transmission in farm in the province of Río Negro, Argentina 3a 2003-2005, 3b 2009-2010, 3c 2017-2018

3.2. CE surveillance in children

In the period 2003-2018, 95 asymptomatic cases with hepatic CE were diagnosed in children under 15 years old with US.

According to the available registries, a total of 13002, 17018 and 4495 US were performed in children between 6 and 14 years old in the periods 2003-2008, 2009-2016 and 2017-2018, with prevalences of 0.4% (CI95% 0.3-0.6), 0.2% (CI95% 0.1-0.3) and 0.1% (CI95% 0.05-0.3), respectively (Table 2, Figure 2). The chi-square trend suggests that there is a statistically significant linear decrease in prevalence between periods ($p = 0.0001$), with ORs of 1 for the first, 0.4 (IC95% 0.3-0.7) for the second and 0.3 (CI95% 0.1-0.7) for the third survey. Analyzed in pairs, there are statistical differences between the first and second period ($p = 0.0004$) while there are no significant statistical differences between the second and third periods ($p = 0.35$).

Table 2. Epidemiological surveillance of Cystic Echinococcosis (CE) using ultrasound screening (US) in school children from 6 to 14 years old.

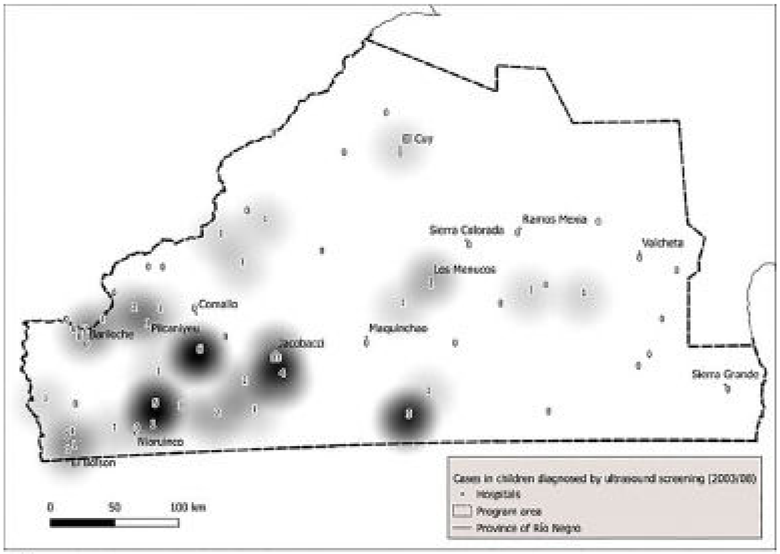
PERIOD	**2003-2008**			**2009-2016**			**2017-2018**		
AREA	n°	+	%	n°	+	%	n°	+	%
Bariloche	989	3	0.3	2084	0	0.0	528	0	0.0
El Bolson	1225	4	0.3	2960	2	0.1	1416	0	0.0
Comallo	829	5	0.6	1037	5	0.5	291	2	0.7
El Cuy	1831	2	0.1	1003	1	0.1	299	0	0.0
Ing. Jacobacci	1731	15	0.9	2359	3	0.2	398	0	0.0
Maquinchao	669	8	1.2	499	2	0.4	271	0	0.0
Los Menucos	1262	3	0.2	1584	6	0.4	289	0	0.0
Ñorquinco	669	7	1.0	895	4	0.4	91	0	0.0
Pilcaniyeu	1000	5	0.5	934	5	0.5	101	1	1.0

PERIOD	2003-2008			2009-2016			2017-2018		
Ramos Mexia	723	2	0.3	545	2	0.4	128	0	0.0
Sierra Colorada	562	0	0.0	847	0	0.0	192	0	0.0
Sierra Grande	44	0	0.0	571	4	0.7	41	0	0.0
Valcheta	1468	1	0.1	1700	1	0.1	450	3	0.7
TOTAL	13002	55	0.4 (0.3-0.6)	17018	34	0.2 (0.1-0.3)	4495	6	0.1 (0.05-0.3)

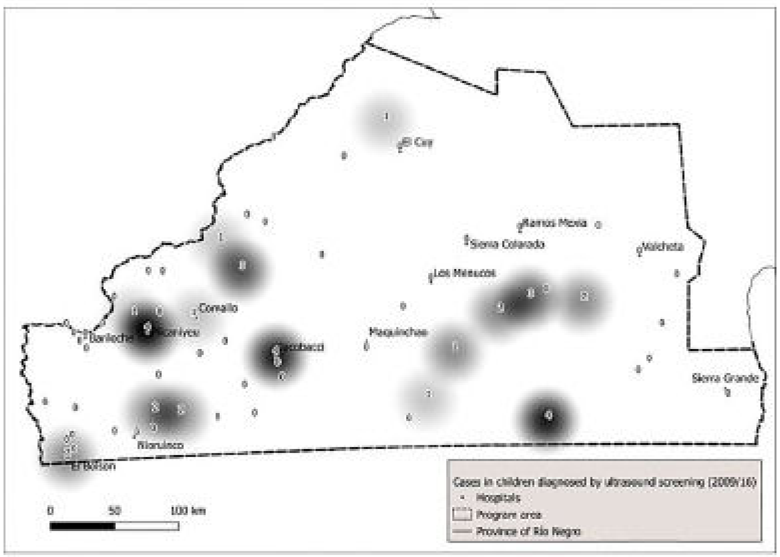
CE Control Program of province of Río Negro, Argentina, 2003-2018.

n°: number of school children tested. +: ultrasound-positive CE cases. %: prevalence of schoolchildren CE (CI95%)

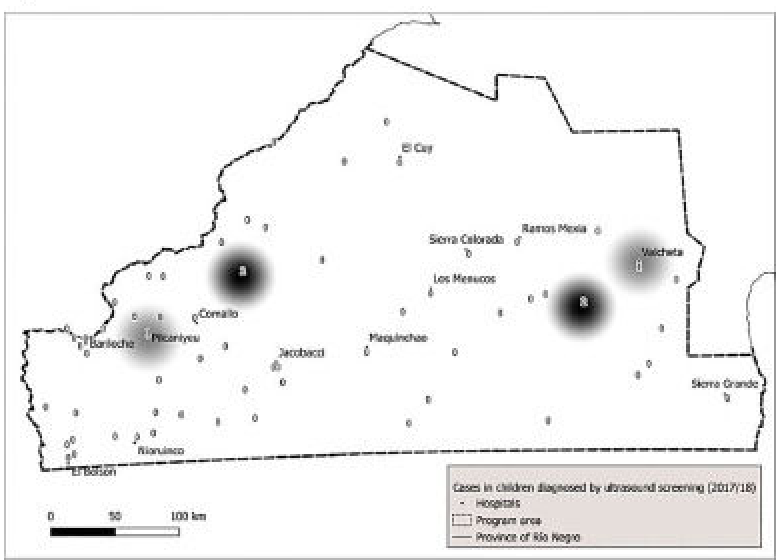
At a smaller spatial scale, the geographical distribution of CE cases seems to have shifted over time. In the period 2017-2018, cases were recorded in historic CE transmission areas (i.e. associated with the PHCCs of Laguna Blanca (Program Area of Comallo) and Pilcaniyeu (Program Area of Pilcaniyeu) in the Pre-Andean region to the west of the province and with the PHCCs of Yaminue (Program Area of Ramos Mexia) in the central plateau to the south-west of the province. However, the analysis also revealed CE transmission in locations where the disease had disappeared, which were associated with PHCCs located to the east of the province (Program Area of Valcheta (Figures 4a, 4b, 4c).


El Cuy
Sierra Colorada
Ramos Mexia
Valcheta
Los Menucos
Comallo
Bariloche
Pilcaniyeu
Jacobacci
Maquinchao
Sierra Grande
El Bolson
Cases in children diagnosed by ultrasound screening (2003/08)
Hospitals
Program area
Province of Río Negro
0
50
100 km

a


El Cuy
Ramos Mexia
Sierra Colorada
Valcheta
Los Menucos
Comallo
Bariloche
Jacobacci
Maquinchao
Sierra Grande
El Bolson
Cases in children diagnosed by ultrasound screening (2009/16)
Hospitals
Program area
Province of Río Negro
0
50
100 km

b


El Cuy
Sierra Colorado
Ramos Mexia
Valcheta
Los Menucos
Comallo
Bariloche
Pilcaniyeu
Jacobacci
Maquinchao
Sierra Grande
El Bolson
Cases in children diagnosed by ultrasound screening (2017/18)
Hospitals
Program area
Province of Río Negro
0
50
100 km

c

FIGURE 4. 'hot spots' of cystic echinococcosis transmission to schoolchildren in the province of Río Negro, Argentina 4a 2003-2008, 4b 2009-2016, 4c 2017-2018

Discussion

CE is a neglected zoonotic disease that causes heavy economic losses due to its impact on individual and public health associated with diagnosis, treatment, and follow-up, and on livestock through reduced productivity and viscera condemnation (Budke et al., 2006; Van Kesteren et al., 2017).

CE and canine echinococcosis epidemiologic surveillance is of paramount importance for monitoring the disease situation in endemic areas and achieving a more efficient control scheme (Craig et al., 2015; 2017). Indeed, the surveillance of canine echinococcosis in dogs has proven to play a key role in CE control programs in the province of Río Negro (Larrieu et al., 2000; Perez et al., 2006).

The advantages and shortcomings of the arecoline test, which was originally used in the CE control program in the province of Río Negro, have been published elsewhere (Perez et al., 2006). Its main problems turned out to be a low predictive value at low levels of prevalence and operational difficulties concerning biosecurity and animal welfare (Perez et al., 2006; Torgerson and Deplazes, 2009; Craig et al., 2015).

In the present work, subsequent cross-sectional surveys using coproELISA allowed to determine canine echinococcosis distribution in the rural areas and the prevalence trend over time. Thus, coproELISA emerged as a reliable diagnostic method for use in canine echinococcosis surveillance and proved to be capable of detecting a decrease in transmission risk due to the control program.

Since its first description (Allan et al., 1992), coproELISA became the technique of choice for canine echinococcosis diagnosis in the definitive host and for epidemiological surveillance in control programs (Craig et al., 2015). It shows reasonable values of sensitivity (78%-100%) and specificity (85%) for a diagnostic situation (Craig et al., 2017) but it may give false negative results when parasite loads are low, and false positive results due to cross reactions with other cestodes, such as *Taenia hydatigena* (Allan and Craig 2006; Torgerson and Deplazes, 2009; Craig et al., 2017).

In the last years, many coproELISA methods have been standardized to be used in control programs in CE endemic areas worldwide (Guarnera et al., 2000: Pierangeli et al., 2010; Morel et al., 2013; Jara et al., 2019) and included in the surveillance systems in some countries, such as Peru, Argentina, Uruguay and Kyrgyzstan (Larrieu and Zanini 2012; Pavletic et al., 2017; Van Kesteren, et al., 2017). The specificity of coproELISA can be further improved using confirmation tests such as WB or PCR (Guarnera et al., 2000; Cabrera et al., 2002; Abbasi et al., 2003, Stefanic et al., 2004; Craig et al., 2015), which in turn provides more reliable prevalence estimations.

In our work, the prevalence of livestock farms with at least one sample positive to canine echinococcosis by coproELISA or coproELISA with confirmation tests showed a similar trend for the disease (figure 1). This suggests that coproELISA alone, when prevalences remain high, could be used for accurate diagnosis of canine echinococcosis during surveillance, with the advantage of being less expensive and more accessible than PCR in the context of the large number of samples that are processed.

Cestodes like *T. hydatigena* have the same life cycle as *E. granulosus* and are also susceptible to praziquantel. Therefore, despite their interspecific cross reaction, they contribute to reveal sanitary issues (negligent disposal of the viscera) and problems in the management of the CE control program which require immediate actions.

CoproELISA is a simple, inexpensive and, therefore, sustainable technique. However, the main disadvantage in comparison with the arecoline test is that results cannot be obtained immediately *in situ*. In Patagonia, where many livestock farms are located in areas of difficult access, a conflict arises with livestock producers who want to be informed of the results as soon as possible; moreover, this delay in the information precludes the program's taking immediate control measures, this creates a conflict with livestock producers because they want to be informed of the results as fast as possible and precludes to the program taking immediate control measures in canine echinococcosis positive farms.

In advanced stages of the program, likewise, confirmation of coproELISA positive samples with PCR or another confirmation system seem to be required. On the other hand, the CE surveillance in asymptomatic humans using systematic cross-sectional surveys has also been an important component of the control program in the province of Río Negro (Larrieu et al., 2000; 2011) to achieve an early diagnosis and prompt treatment, which represents a cost-effective strategy. Recently, results of the application of this strategy have been published related to the diagnosis and treatment in asymptomatic population (Larrieu et al., 2019b).

The US is currently the method of choice for abdominal CE screening in humans. This technique for abdominal CE, shows a high sensitivity (100% or close to 100%) and specificity (95%) (Del Carpio et al., 2000) in studies to identify abdominal cysts and is a valuable tool for epidemiological surveillance in control programs. For lung CE cysts US is not useful, unless the cysts are attached to parietal pleura or are huge. Moreover, when tested in school children between 6-14 years of age, it proved to detect *E. granulosus* transmission in the recent past (Frider et al., 2000; Larrieu et al., 2011, 2019b).

In this work, a decreasing trend of CE prevalence was found using both US in children and coproELISA in farms. Moreover, US provided information on changes in the geographic distribution of new cases, thus allowing the identification of areas associated with PHCCs where control actions should be strengthened.

The combination of different data sources has proven to be useful for CE surveillance in China (Cadavid Restrepo et al., 2018, Liu et al., 2018)). In the province of Rio Negro, active surveillance data of CE cases in humans and canine echinococcosis were integrated and showed similar temporal trend, they also seemed to show a delay in the results, which is consistent with their diagnostic capacity (present transmission for coproELISA vs recent transmission for US).

Surveillance of echinococcosis in dogs by coproELISA and for CE in children by US is valuable in planning a control program, with the additional advantage of using non-invasive tests (Frider et al., 2000; Guarnera et al., 2000; Perez et al., 2006; Van Kesteren et al., 2017).

Historically, the program used the data of surveillance systems obtained from geographic areas of wide extension, such as the reference area of a Hospital or a Department for adjusting the control strategies, placing more emphasis on areas with persistent infection. This led, for example, to the introduction of the EG95 vaccine in the Department of Ñorquinco (Larrieu et al., 2019a). This level of analysis was enough while the prevalence remained relatively high and homogeneously affecting the entire endemic area. The decrease in prevalence and areas without cases in children generated the need to use geographic analyses on a smaller scale, in order to identify more precisely areas where transmission is maintained, which requires special concentration on them of resources and actions.

The present work is a first step in this direction, with a geographical analysis based on PHCCs scale, and the entire surveillance system can be redefined with a design that associates in time and space coproELISA and US, as has been applied in China (Liu et al., 2018) lacking in the current system because was not applied in an integrated way, and was important limitation for interpretation of results.
In this paper the heat maps represent the density of points (each point is a PHCC) and they allow to represent which PHCC has the highest proportion of livestock farms with positive dogs and the highest number of cases in children. The selection of a radius of 30 km allows to limit the graphic representation in order to achieve a good visualization.

CE is very difficult to control or eliminate, especially in relatively remote continental regions where praziquantel dosing of dogs may be practically unfeasible (Craig and Larrieu 2006; Van Kesteren et al. 2017). In the province of Rio Negro, the access to livestock farms for anthelmintic treatment in dogs is limited by geographic, climatic and infrastructure constraints during a large part of the year (120013 km^2 with only 0.88 inhabitants/km^2 with rural roads and paths that are difficult to travel on, where the distance between the rural hospital and its PHCCs normally exceeds 80/100 km). Non-compliance with the treatment schedule results in the maintenance of some infection in dogs.
In the last years, the region has experienced rapid urban growth and many rural practices have been introduced into the city, such as domestic slaughter of small ruminants, as well as dogs going from the city to the countryside and back for livestock management, which resulted in the appearance of urban cases of CE in children. In the present work, urban foci have been identified and associated with the PHCCs in San Carlos de Bariloche and Valcheta, also identified by coproELISA. In this way, the urbanization process poses new epidemiological challenges to the control of CE, which is typically recognized as a rural zoonosis.

Despite some limitations, the CE program in the province of Rio Negro has continued uninterrupted since its launch in 1980. This is an important point, as well as the key for the decrease in the prevalence of echinococcosis in dogs and humans and it shows that the disease can be controlled by systematic and coordinated implementation of One Health strategies.

Authorship statements

Marcos Arezo. planned the experiments, analyzed the data and interpreted the results
Guillermo Mujica Field work in veterinary sciences, analyzed the data and interpreted the results
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Mariano Sobrino Salvitti Field work in US, analyzed the data and interpreted the results

Pablo Crowley analyzed the data and interpreted the results

Jose Daffner analyzed the data and interpreted the results

Edmundo Larrieu. planned the experiments, analyzed the data, wrote and reviewed the paper

Compliance with ethical standards

Ethical statement. Children included in the present study were examined with the ethical approval of Resolution 2624/18 of Ministry of Health of Río Negro, Argentina.

Declaration of Competing Interest

The authors report no conflict of interests.

References

Abbasi et al., 2003
I. Abbasi, A. Branzburg, M. Campos-Ponce, S.K. Abdel Hafez, F. Raoul, P.S. Craig, J. Hamburger**Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence**
Am. J. Trop. Med. Hyg, 69 (2003), pp. 324-330

Allan et al., 1992
J.C. Allan, P.S. Craig, J. Garcia Noval, F. Mencos, D. Liu, Y. Wang, H. Wen, P. Zhou, R. Stringer, M. Rogan**Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans**
Parasitology, 1104 (1992), pp. 347-356

Allan and C., 2006
J Allan, C. Craig, P.S.**Coproantigens in taeniasis and echinococcosis**
Parasitol. Int., 55 (2006), pp. S75-S80

Bingham et al., 2016
G.M. Bingham, E. Larrieu, L. Uchiumi, C.H. Mercapide, G. Mujica, M. Del Carpio, E. Herrero, J.C. Salvitti, B. Norby, C.M. Budke**The Economic Impact of Cystic Echinococcosis in Rio Negro Province, Argentina. infection in dogs by amplification of a newly identified repeated DNA sequence**
Am. J. Trop. Med. Hyg, 94 (3.) (2016), pp. 615-625

Budke et al., 2006
C.M. Budke, P. Deplazes, P.R. Torgerson**Global socioeconomic impact of cystic echinococcosis**
Emerg. Infect. Dis., 2 (2006), pp. 296-303

Cabrera et al., 2002
M. Cabrera, S. Canova, M. Rosenzvit, E. Guarnera**Identification of *Echinococcus granulosus* eggs. Diagn. Microbiol. Infect**
Dis, 44 (2002), pp. 29-34

Cadavid Restrepo et al., 2018
A.M. Cadavid
Restrepo, Y.R. Yang, D.P. McManus, D.J. Gray, T.S. Barnes, G.M. Williams, R.J. Soares Magalhães, A.C. Clements**Spatial prediction of the risk of exposure to *Echinococcus spp*. among schoolchildren and dogs in Ningxia Hui Autonomous Region, People's Republic of China**
Geospat. Health, 13 (1.) (2018), p. 644

Cavagión et al., 2005
L. Cavagión, A. Perez, G. Santillan, F. Zanini, O. Jensen, L. Saldıas, M. Diaz, G. Cantoni, E. Herrero, M.T. Costa, M. Volpe, D. Araya, N. Alvarez
Rubianes, C. Aguad, G. Meglia, E. Guarnera, E. Larrieu**Diagnosis of cystic echinococcosis on sheep farms in the south of Argentina: areas with a control program**
Vet. Parasitol., 128 (2005), pp. 73-81

Craig and Larrieu, 2006
P.S. Craig, E. Larrieu**Control of Cystic Echinococcosis-Hydatidosis: 1863-2002**
Adv. Parasitol., 61 (2006), pp. 443-508

Craig et al., 2017
Craig, P.S., Hegglin, D., Lightowlers, M.W., Torgerson, P.R., Wang, Q., 2017. Echinococcosis: Control and Prevention. In: Thompson RCA, Deplazes P, Lymbery A J,., Eds.., Echinococcus and Echinococcosis, Part B, pp. 55–158.

Craig et al., 2015
P. Craig, A. Mastin, F. van Kesteren, B. Boufana***Echinococcus granulosus*: Epidemiology and state-of-the-art of diagnostics in animals**
Vet. Parasitol., 13 (3-4) (2015), pp. 132-148

Del Carpio et al., 2000
M. Del
Carpio, S. Moguilansky, M.T. Costa, H. Panomarenko, G. Bianchi, S. Bendersky, M. Lazcano, B. Frider, E. Larrieu**Diagnosis of human hydatidosis: predictive value of the rural ultrasonographic survey in ann apparently health population**
Medicina (Bs As), 60 (2000), pp. 466-468

Del Carpio et al., 2012
M. Del
Carpio, C.H. Mercapide, J.C. Salvitti, L. Uchiumi, J. Sustercic, H. Panomarenko, J. Moguilensky, E. Herrero, G. Talmon, M. Volpe, D. Araya, G. Mujica, A. Calabro, S. Mancini, C. Chiosso

, i J.L. Labanch, R. Saad, S. Goblirsch, E. Brunetti, E. Larrieu**Early diagnosis, treatment and follow-up of cystic echinococcosis in remote rural areas in Patagonia: impact of ultrasound training of non-specialists with a focused approach on CE**
Plos Negl. Tropl. Dis., 6 (1.) (2012), p. e1444

Frider et al., 2000
B. Frider, J. Moguilensky, J.C. Salvitti, M. Odriozola, G. Cantoni, E. Larrieu**Epidemiological surveillance of human hydatidosis by means of ultrasonography: its contributions to the evaluation of control programs**
Acta Trop, 79 (2000), pp. 219-223

Guarnera et al., 2000
E. Guarnera, G. Santillan, R. Botinelli, A. Franco**Canine echinococcosis: an alternative for surveillance epidemiology**
Vet. Parasitol., 88 (2000), pp. 131-134

L.M. Jara, M. Rodriguez, F. Altamirano, A. Herrera, M. Verastegui, L.G. Gímenez-Lirola, R.H. Gilman, C.M. Gavidia**Development and Validation of a Copro-Enzyme-Linked Immunosorbent Assay Sandwich for Detection of *Echinococcus granulosus*-Soluble Membrane Antigens in Dogs**
Am. J. Trop. Med. Hyg, 100 (2) (2019), pp. 330-335

E. Larrieu, M. Costa, G. Cantoni, J.L. Labanchi, R. Bigatti, A. Perez, D. Araya, S. Mancini, E. Herrero, G. Talmon, S. Romeo, A. Thakur**Control program of hydatid disease in the Province of Río Negro, Argentina, 1980-1997**
Bol. Chi. Parasitol., 55 (2000), pp. 49-53

Larrieu et al., 2011
E. Larrieu, M. Del Carpio, C.H. Mercapide, J.C. Salvitti, J. Sustercic, J. Moguilensky, H. Panomarenko, L. Uchiumi, E. Herrero, G. Talmon, M. Volpe, D. Araya, G. Mujica, S. Mancini, J.L. Labanchi, M. Odriozola Programme for ultrasound diagnoses and treatment with albendazole of cystic echinococcosis in asymptomatic carriers: 10 years of follow-up of cases
Acta Trop, 117 (2011), pp. 1-5

E. Larrieu, F. Zanini**Critical analysis of the strategies to control cystic echinococcosis and the use of praziquantel in South America: 1980 – 2009**
Rev. Panam. Salud Publica, 31 (2012), pp. 81-87

E. Larrieu, G. Mujica, D. Araya, J.L. Labanchi, M. Arezo, E. Herrero, G. Santillán, K. Vizcaychipi, L. Uchiumi, J.C. Salvitti, C. Grizmado, A. Calabro, G. Talmon, L. Sepulveda, J.M. Galvan, M. Cabrera, M. Seleiman, P. Crowley, G. Cespedes, M. García Cachau, L. Gino, L. Molina, J. Daffner, C.G. Gauci, M. Donadeu, M.W. Lightowlers**Pilot field**

trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: 8 years of work
Acta Trop, 191 (2019 a), pp. 1-7

E. Larrieu, L. Uchiumi, J.C. Salvitti, M. Sobrino, O. Panomarenko, H. Tissot, C.H. Mercapide, J. Sustercic, M. Arezo, G. Mujica, E. Herrero, J.L. Labanchi, C. Grizmado, D. Araya, G. Talmon, J.M. Galvan, L. Sepulveda, M. Seleiman, T. Cornejo, H. Echenique, M. Del Carpio**Epidemiology, diagnosis, treatment and follow up of cystic echinococcosis in asymptomatic carriers**
Trans. Roy. Soc. Trop. Med. Hyg, 113 (2.) (2019 b), pp. 74-80

C.N. Liu, Y.Y. Xu, A.M. Cadavid-Restrepo, Z.Z. Lou, H.B. Yan, L. Li, B.Q. Fu, D.J. Gray, A.A. Clements, T.S. Barnes, G.M. Williams, W.Z. Jia, D.P. McManus, Y.R. Yang**Estimating the prevalence of Echinococcus in domestic dogs in highly endemic for echinococcosis**
Infect. Dis. Poverty 9, 7 (1) (2018), p. 77

N. Morel, G. Lassabe, S. Elola, M. Bondad, S. Herrera, C. Marí, J.A. Last, O. Jensen, G. Gonzalez-Sapienza**A monoclonal antibody-based copro-ELISA kit for canine echinococcosis to support the PAHO effort for hydatid disease control in South America Plos Negl**
Tropl. Dis., 7 (2013), p. e1967

C.F. Pavletic, E. Larrieu, E.A. Guarnera, N. Casas, P. Irabedra, C. Ferreira, J. Sayes, C. M. Gavidia, E. Caldas, M. Zini Lise, M. Maxwell, M. Arezo, A.M. Navarro, M. Vigilato, O. Cosivi, M. Espinal, V.J. Del Rio Vilas**Cystic echinococcosis in South America: a call for action**
Rev. Panam. Salud Publica, 21 (41) (2017), p. e42

A. Perez, M.T. Costa, G. Cantoni, S. Mancini, C. Mercapide, E. Herrero, M. Volpe, D. Araya, G. Talmon, C. Chiosso, G. Vazquez, M. Del Carpio, G. Santillan, E. Larrieu**Vigilancia epidemiológica de la equinococcosis quística en perros, establecimientos ganaderos y poblaciones humanas en la Provincia de Río Negro**
Medicina (Bs As), 66 (2006), pp. 193-200

N.D. Pierangeli, S.V. Soriano, I. Roccia, H.F. Bergagna, L.E. Lazzarini, A. Celescinco, A. V. Kossman, M.S. Saiz, J.A. Basualdo**Usefulness and validation of a coproantigen test for dog echinococcosis screening in the consolidation phase of hydatid control in Neuquén, Argentina**
Parasitol. Int., 59 (2010), pp. 394-399

S. Stefanić, B.S. Shaikenov, P. Deplazes, A. Dinkel, P.R. Torgerson, A. Mathis**Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* "sheep strain". in naturally infected dogs**

Parasitol. Res, 92 (2004), pp. 347-351

P.R. Torgerson, P. Deplazes**Echinococcosis: diagnosis and diagnostic interpretation in population studies**
Trends Parasitol, 25 (2009), pp. 164-170

F. Van Kesteren, A. Mastin, P.R. Torgerson, B. Mytynova, P.S. Craig**Evaluation of the impact of 2 years of a dosing intervention on canine echinococcosis in the Alay Valley, Kyrgyzstan**
Parasitology, 144 (2017), pp. 1328-1337