Parasitol Res
DOI 10.1007/s00436-017-5555-2

SHORT COMMUNICATION

Genetic characterization of human hydatid cysts shows coinfection by *Echinococcus canadensis* G7 and *Echinococcus granulosus sensu stricto* G1 in Argentina

María Florencia Debiaggi[1,2] · Silvia Viviana Soriano[1] · Nora Beatriz Pierangeli[1] · Lorena Evelina Lazzarini[1] · Luis Alfredo Pianciola[3] · Melina Leonor Mazzeo[3] · Sergio Moguillansky[4] · Juan Angel Basualdo Farjat[5]

Received: 24 April 2017 / Accepted: 5 July 2017

Abstract Human cystic echinococcosis caused by the larval stage of *Echinococcus granulosus sensu lato* (*s.l.*) is a highly endemic disease in the province of Neuquén, Patagonia, Argentina. Human infections with *E. granulosus sensu stricto* (*s.s.*) G1 and *Echinococcus canadensis* G6 were reported in Neuquén in previous studies, whereas four genotypes were identified in livestock: G1, G3, G6, and G7. The aim of this study was to identify the genotypes of *E. granulosus s.l.* isolates from humans of Neuquén province, Patagonia, Argentina, through the 2005–2014 period. Twenty six hydatid cysts were obtained from 21 patients. The most frequent locations were the liver and lungs. Single cysts were observed in 81.0% of patients, and combined infection of liver and lungs was detected in 9.5% of cases. Partial sequencing of mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) genes identified the presence of *E. granulosus s.s.* G1 (n = 11; 42.3%) including three different partial sequences; *E. canadensis* G6 (n = 14; 53.8%) and *E. canadensis* G7 (n = 1; 3.9%). Coinfection with G1 and G7 genotypes was detected in one patient who harbored three liver cysts. Most of the liver cysts corresponded to G1 and G6 genotypes. This study presents the first report in the Americas of a human infection with *E. canadensis* G7 and the second worldwide report of a coinfection with two different species and genotypes of *E. granulosus s.l* in humans. The molecular diversity of this parasite should be considered to redesign or improve the control program strategies in endemic regions.

Keywords *Echinococcus canadensis* · *Echinococcus granulosus* · G7 genotype · Coinfection · Human echinococcosis

Introduction

Cystic echinococcosis (CE) caused by the larval stage of the tapeworm *Echinococcus granulosus* sensu *lato* (*E. granulosus s.l.*), produces significant economic losses and health problems in both rural and urban population (Eckert et al. 2001; Budke et al. 2006). CE is an important worldwide zoonotic disease that affects more than 100 countries, particularly affecting pastoral and poor rural communities (Alvarez Rojas et al. 2014). In South America, the disease is endemic or hyperendemic in Argentina, Uruguay, Chile, Southern Brazil, and mountain regions of Perú and Bolivia (Cucher et al. 2016). Neuquén is one of the provinces with higher hydatid disease endemicity in Argentina, despite the fact that provincial sanitary authorities carry out a control program of CE since 1970. The mean annual incidence (MAI) of

✉ María Florencia Debiaggi
fdebiaggi@yahoo.com

[1] Cátedra de Microbiología y Parasitología, Facultad de Ciencias Médicas, Universidad Nacional del Comahue, Neuquén, Argentina

[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Neuquén, Argentina

[3] Laboratorio Central, Subsecretaría de Salud de Neuquén, Neuquén, Argentina

[4] Cátedra de Anatomía e Imágenes Normales, Facultad de Ciencias Médicas, Universidad Nacional del Comahue, Neuquén, Argentina

[5] Centro Universitario de Estudios Microbiológicos y Parasitológicos (CUDEMyP) sede Cátedra de Microbiología y Parasitología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina

Published online: 18 July 2017

human CE in Neuquén, based on official records of confirmed cases, was 24.4 per 100,000 inhabitants and 9.7 per 100,000 in children aged 0–14 years between 1995 and 2004 (Pierangeli et al. 2007). Fifty-nine new cases of human CE were confirmed in the province in 2015 (MAI: 8.9 per 100,000) (National System of Health Surveillance 2017).

Humans become infected with *E. granulosus s.l.* by the ingestion of eggs released from infected dogs or other canids through direct contact with the animals or consumption of water and/or vegetables contaminated with infected canid feces (Thompson and McManus 2001). After ingestion, eggs hatch, and each onchosphere can develop an unilocular hydatid cyst, located in different organs. The liver is the most common involved organ followed by the lungs, but the cysts can develop less frequently in other locations like the kidneys, spleen, heart, bone and brain (Moro and Schantz 2009).

E. granulosus s.l. is considered a species complex with a high genetic and phenotypic variation, and its taxonomy is still controversial (Romig et al. 2015). In this work, we followed the nomenclature proposed by Nakao et al. (2013) for practical reasons. To date ten different genotypes (G1–G10) have been described within this complex using molecular tools, comprising: *E. granulosus* sensu stricto (*s.s.*) (genotypes G1–G3); *Echinococcus equinus* (G4); *Echinococcus ortleppi* (G5) and *Echinococcus canadensis* (G6–G10) (Bowles et al. 1992; Lavikainen et al. 2003; Nakao et al. 2013). Recently, the "lion strain" (*E. felidis*) has been proposed as another valid species, as it is evidently phylogenetically sister to *E. granulosus s.s* (Hüttner et al. 2008).

Up to the present, four genotypes have been found in human CE isolates in Argentina: G1, G2, G5, and G6, as well as six genotypes in livestock: G1, G2, G3, G5, G6, and G7 (Cucher et al. 2016; Avila et al. 2017). Among the G1 isolates of Argentina, eight different partial sequences have been identified: the original G1 sequence described by Bowles et al. (1992), which we named G1nqnA in this work, and seven microvariants of this genotype (Kamenetzky et al. 2002; Soriano et al. 2010, 2015).

Human infections with *E. granulosus s.s.* G1 and *E. canadensis* G6 were reported in Neuquén in previous studies, whereas four genotypes were identified in livestock: G1 (goats and sheep), G3 (sheep), G6 (goats and cattle), and G7 (pigs) (Kamenetzky et al. 2002; Guarnera et al. 2004; Soriano et al. 2010, 2015). The diversity of genotypes in livestock is greater than that found to date in humans in Neuquén, suggesting that more genotypes could also cause human CE in this region. The aim of this study was to identify the genotypes of *E. granulosus s.l.* isolates from humans of Neuquén province, Patagonia, Argentina through the 2005–2014 period.

Materials and methods

A prospective, observational, and descriptive study was conducted between September 2005 and March 2014 in the province of Neuquén. This province is located in the north of the Argentine Patagonia region, comprising an area of 94,078 km^2 extending from 36° 39′ to 41° 01′ S latitude and 68° 00′ to 71° 58′ W longitude. The total population was 565,242 inhabitants in 2010. According to the last official records, 684,777 goats; 164,161 sheep; 186,873 cattle; and 16,927 pigs were reported in the province in 2008 (Provincial Direction of Statistics and Census 2017). The main economic activity in rural areas is raising livestock for subsistence (goats, sheep, and cattle), under a transhumant model of production. This fact leads to a close contact among humans, livestock, and shepherd dogs. Pigs are mostly raised in periurban areas, near to the largest cities.

Samples of hydatid cysts were collected from clinically and/or imaging diagnosed CE patients who underwent surgery for the disease in different public and private hospitals from Neuquén. The samples were pieces of germinal layer and/or hydatid fluid obtained from each cyst, by the surgeons, immediately after the surgery. CE materials were sent refrigerated to our laboratory in a lapse of no more than 4 h. Data about age and gender of patients as well as cysts location were recorded. Cysts were not pooled as each cyst was considered a single sample. The protoscoleces (PSC) or pieces of germinal layer were preserved in 70% ethanol at 4 °C.

Genomic DNA was extracted from each sample using the QIAamp DNA mini kit (QIAGEN, Germany) according to the manufacter's instructions. DNA was then stored at −20 °C until PCR amplification. For molecular identification of *E. granulosus s.l.* genotypes, a 450 bp fragment of the *cox1* gene was amplified from each isolate using previously published primers (Bowles et al. 1992). To confirm the differentiation between the G6 and G7 genotypes, a 500 bp fragment of the *nad1* gene was also amplified from each *E. canadensis* isolate using published primer sets (Bowles and McManus 1993). PCR conditions were the same as described by Soriano et al. (2010). PCR products were purified by ethanol precipitation and sequenced in both directions. Sequences were aligned and compared with reference sequences of *E. granulosus s.l.* downloaded from GenBank (GenBank accession numbers: U50464 (G1), M84662 (G2), M84663 (G3), M84664 (G4), M84665 (G5), M84666 (G6), M84667 (G7), AB235848 (G8), and AF525457 (G10), using ClustalX v2.1 and Bioedit v7.2.5 softwares for Windows.

Results and discussion

Twenty-six hydatid cysts were obtained from 21 patients aged 4 to 80 years, and 13.6% of the patients were children

between 0 and 14 years. Male accounted for 12/21 of cases (57.1%). The most frequent location was the liver, followed by the lungs (Table 1). Cyst location analysis showed that in 90.5% of cases, only one organ was affected. This result is in accordance with the 95.8% described previously in 938 patients of Neuquén between 1995 and 2004 (Pierangeli et al. 2007). Simultaneous infection in the liver and lungs was detected in 9.5% of patients (including children and adults). This finding is similar to the proportion of 10.0% reported in a study about CE patients in Mar del Plata, Argentina (ages ranged from 15 to 84 years) but higher than the 1.8% of combined liver and lung involvement described previously in patients from 7 to 93 years in Neuquén (Dopchiz et al. 2007; Pierangeli et al. 2007). In contrast, the 19.3% of combined liver and lung involvement was described in patients from 2 to 86 years in Turkey (Kurul et al. 2002).

Hepatic cysts were more frequent than pulmonary cysts at a ratio of 2.5:1, in accordance with reports from other authors based on surgical and/or symptomatic cases (Larrieu and Frider 2001; Djuricic et al. 2010). The factors that determine the final anatomic location of cysts are still unknown; however, the liver is the organ most frequently infected because the oncospheres penetrate the intestinal wall and tend to disseminate to the liver via the portal vein. Variation in cyst location frequencies observed in diverse studies throughout the world could be due to infection with different genotypes of *E. granulosus s.l.*, which could have an affinity for different organs (Pierangeli et al. 2007).

Sequence analysis revealed the presence of two species and three genotypes of *E. granulosus s.l.* in Neuquén human isolates: *E. granulosus s.s.* G1 ($n = 11$; 42.3%), *E. canadensis* G6 ($n = 14$; 53.8%), and *E. canadensis* G7 ($n = 1$; 3.9%). Among the G1 isolates, we identified three different partial sequences including the original G1 sequence described by Bowles et al. (1992) which we named G1nqnA, and two microvariants of this sequence: G1nqnC (shows a change of C x T in position 73 compared with the reference sequence) and G1nqnD (shows a change of G x A in position 136 compared with the reference sequence). The sequence of the G1nqnC microvariant showed 100% identity with a sequence previously described in humans in Algeria, in cattle in Brazil, and in dogs in Neuquén, whereas the G1nqnD microvariant was detected, to date, only in Neuquén (Soriano et al. 2010; Laurimäe et al. 2016; Zait et al. 2016; authors' unpublished data). The original G1 sequence (G1nqnA) was already found in Neuquén in sheep, cattle, and dogs isolates; G1nqnC microvariant was previously detected in cattle and in one dog of the study area whereas the presence of G1nqnD microvariant was also characterized in sheep and cattle isolates from Neuquén (Kamenetzky et al. 2002; Soriano et al. 2010; authors' unpublished data). The fact that the two G1 microvariants identified in humans were also present in other local intermediate hosts as well as in dogs suggests that they are not casual findings. On the contrary, active lifecycles of each of them seems to coexist in sympatry. The GenBank accession numbers for the nucleotide sequences characterized in this study as well as the distribution of genotypes in relation to the anatomic location of cysts is shown in Table 2. Our results confirm previous findings of Kamenetzky et al. (2002) about the presence of *E. granulosus s.l.* G1 and G6 in human isolates but also demonstrate that other species/genotypes/microvariants are responsible of local human cystic echinococcosis.

E. granulosus s.s. G1 is the most frequent genotype associated with human CE worldwide, being responsible for 72.9% of the global burden of the disease, whereas G6 genotype is considered poorly infective to human accounting for 12.2% of human cases (Alvarez Rojas et al. 2014; Cucher et al. 2016). However, *E. canadensis* G6 was the major causative agent of CE in Neuquén patients (53.8%), followed by *E. granulosus s.s.* G1 and *E. canadensis* G7 in the present work. There are reports of human infection with *E. canadensis* G6 in other provinces of Argentina and other South American countries

Table 1 Frequency of cyst location in confirmed human cystic echinococcosis cases from Neuquén between 2005 and 2014

Location	No. of cases	% of total
Liver	11	52.4
Lungs	3	14.2
Liver + lungs	2	9.5
Brain	1	4.8
Spleen	1	4.8
Kidney	1	4.8
Peritoneum	1	4.8
Retroperitoneum	1	4.8
Total	21	100.0

Table 2 Distribution of genotypes of *E. granulosus sensu lato* according to anatomic location

Location	G1nqnA[a]	G1nqnC[a]	G1nqnD[a]	G6[a,b]	G7[a,b]	Total
Liver	4	1	2	7	1	15
Lung	2	–	–	4	–	6
Brain	–	–	–	1	–	1
Peritoneum	1	–	–	–	–	1
Retroperitoneum	1	–	–	–	–	1
Kidney	–	–	–	1	–	1
Spleen	–	–	–	1	–	1
Total	8	1	2	14	1	26

[a] Accession number for *cox1*: JN176931 (G1nqnA), KT719395 (G1nqnC), JN176930 (G1nqnD), JN176934 (G6), and JN176935 (G7)

[b] Accession number for *nad1*: KT749868 (G6) and KT868832 (G7)

(Chile and Perú) that are close to Argentina, but in all these cases *E. granulosus s.s* G1 is still the most frequent genotype involved in human CE (Cucher et al. 2016; Avila et al. 2017). The predominance of *E. canadensis* G6 in humans could be related to particular socio-economic and cultural characteristics that differentiate Neuquén from other regions. Most rural population in Neuquén raise goats under a model of production with great migrating movements every year (transhumance), from small areas near to their homes in the winter to fertile lands in the mountains in the summer. Transhumance involves a close contact between the families, livestock, and shepherd dogs, and this fact could increase the risk of human infection by *E. canadensis* G6, as goats are the major reservoir of this species/genotype in Neuquén (Soriano et al. 2010). More studies concerning human CE molecular epidemiology in Neuquén, involving a higher number of cases, are needed to confirm or refute this finding.

The sequence analysis of PCR products allowed us to describe for the first time a case of human infection with *E. canadensis* G7 in Argentina and in the Americas, which confirms the role of this genotype as a causative agent of human infection. The sequence of the G7 genotype showed complete *cox1* and *nad1* identity with published sequences described in pigs of Neuquén (Soriano et al. 2010), in pigs and cattle of other provinces of Argentina and other countries (Kamenetzky et al. 2002; Beato et al. 2013; Konyaev et al. 2013; Monteiro et al. 2014), and in humans of China (Zhang et al. 2014). To date, there are no reports of human infection with *E. canadensis* G7 in the Americas (Alvarez Rojas et al. 2014; Cucher et al. 2016). Human cases due to genotype G7 were reported in central and eastern European countries, Asia, and in South Africa, associated with pig transmission (Dybicz et al. 2013; Alvarez Rojas et al. 2014; Zhang et al. 2014). The role of *E. canadensis* G7 as causative agent of human CE is generally underestimated. Schneider et al. (2010) suggested that the reasons for this underestimation could be that G7 metacestodes tend to be small, located in the liver, and may be asymptomatic for years or probably never detected. Our results seem to be in accordance with that suggestion, as the only *E. canadensis* G7 cyst detection was located in the liver, and the cyst's diameter was estimated by the surgeon as approximately 0.5 cm.

Coinfection with *E. granulosus s.s*. G1nqnD and *E. canadensis* G7 was detected in one patient who harbored three hydatid cysts in the liver. The patient was a woman of 43 years old from Andacollo, a small rural village in the north of Neuquén. This patient underwent two surgeries. In the first one, two cysts were removed and were genetically characterized as G1nqnD and G7, respectively. The second surgery was conducted 3 months later, to remove another hydatid cyst from the liver that was also characterized as G1nqnD microvariant.

There are few reports of mixed *E. granulosus s.l.* infections in intermediate hosts in the world. Coinfection has been reported in France and in the Republic of Moldova, where two different genotypes of *E. granulosus s.s*. were identified in the lungs and liver of sheep and cattle (Umhang et al. 2013, 2014). The presence of mixed infections could be the result of successive infections of the intermediate host, or by a single infection due to a definitive host harboring simultaneously adult worms of different species of *E. granulosus s.l.* (Umhang et al. 2014). Recently, Oudni-M'rad et al. (2016) reported for the first time a coinfection in humans, involving *E. granulosus* s.s. G1 and *E. canadensis* G6 in a child from Tunisia. Coincidently, in the present study, we were able to describe the first case of human mixed infection with two *E. granulosus s.l.* species: *E. granulosus s.s.* G1 (microvariant G1nqnD) and *E. canadensis* G7 in the Americas. The patient was from Sanitary Area III, an endemic rural area of Neuquén province with a MAI of 78.4 per 100,000 inhabitants (Pierangeli et al. 2007). These results demonstrate that human coinfection is a fact that should be taken into account, especially in endemic regions where different species and genotypes of *E. granulosus s.l.* are present with sympatric lifecycles.

The analysis of distribution of genotypes in relation to location of cysts showed that most of the liver cysts corresponded to *E. granulosus s.s*. G1 and *E. canadensis* G6 genotype (Table 2). Although we observed that most of G6 genotype cysts were located in the liver (50%), this genotype also infected other organs like the lungs, kidney, spleen, and brain. Interestingly, the location of *E. canadensis* G6 cysts in humans seems to be different from the location previously observed in goats from Neuquén, in which a clear tropism to the lungs has been demonstrated (Soriano et al. 2010). Despite the fact that *E. canadensis* G6 was the causative agent of the only brain cyst detected in this study, our results are not enough to confirm the hypothesis of Sadjjadi et al. (2013), who suggested that genotype G6 seems to have a higher affinity for the human brain, as only 7% (1/14) of G6 genotype cysts corresponded to this location. More studies of molecular characterization of *E. granulosus s.l.* metacestodes from different locations are needed to assess if the different species/genotypes are associated with preferential locations in humans.

The occurrence of cases in children indicates that there is an active disease transmission, although an echinococcosis control program has been carried out in Neuquén province for several decades. Moreover, our results showed that a diversity of species and genotypes of *E. granulosus s.l.* can infect humans in Neuquén. Molecular diversity of *E. granulosus s.l.* can affect life cycle patterns such as host specificity, growth and development rates in the definitive hosts, infectivity to intermediate hosts, pathogenicity, and antigenicity (Soriano et al. 2016; Avila et al. 2017). Therefore,

the diversity of species and microvariants found in different hosts including humans in Neuquén, could have important consequences in immunological diagnosis, response to therapy and vaccines, as well as in the control and surveillance of CE. For instance, the control program in Neuquén is based on periodic anthelminthic treatment of dogs every 6 weeks, taking into account the prepatent period of *E. granulosus s.s*. G1. However, in dogs infected with *E. canadensis* G6 and G7, the prepatent periods are shorter (about 40 and 35 days, respectively) (Soriano et al. 2016). The increase of knowledge of the molecular epidemiology and the diversity of *E. granulosus s.l.* could contribute to improve or redesign the control program in Neuquén.

Further studies concerning human CE molecular epidemiology are needed to assess the possible associations between the different species/genotypes/microvariants and biological aspects of human infection like clinical and immunological features of the disease, tropism, biochemistry, and fertility and viability of cysts, among others.

Acknowledgements The authors are grateful with the surgery department of public and private hospitals of Neuquén province.

Compliance with ethical standards

Funding This study was funded by grant 04/N018 from the Secretaría de Ciencia y Técnica de la Universidad Nacional del Comahue.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This research study was approved by the Science and Technology Secretary of the National University of Comahue according to Permission No. 0991/05 Record No. 02327/05 dated on September 27, 2005. All adult subjects and parents/tutors of the children gave their written informed consent for surgery. The information of patients has been anonymised so that they cannot be identified.

References

Alvarez Rojas CA, Romig T, Lightowlers MW (2014) *Echinococcus granulosus* sensu lato genotypes infecting humans—review of current knowledge. Int J Parasitol 44:9–18

Avila HG, Santos GB, Cucher MA, Macchiaroli N, Pérez MG, Baldi G, Jensen O, Pérez V, López R, Negro P, Scialfa E, Zaha A, Ferreira HB, Rosenzvit M, Kamenetzky L (2017) Implementation of new tools in molecular epidemiology studies of *Echinococcus granulosus* sensu lato in South America. Parasitol Int 66:250–257

Beato S, Parreira R, Roque C, Gonçalves M, Silva L, Maurelli MP, Cringoli G, Grácio MA (2013) *Echinococcus granulosus* in Portugal: the first report of the G7 genotype in cattle. Vet Parasitol 198:235–239

Bowles J, McManus DP (1993) NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. Int J Parasitol 23:969–972

Bowles J, Blair D, McManus DP (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol Biochem Parasitol 54:165–174

Budke CM, Deplazes P, Torgerson PR (2006) Global socioeconomic impact of cystic echinococcosis. Emerg Infect Dis 12:296–303

Cucher MA, Macchiaroli N, Baldi G, Camicia F, Prada L, Maldonado L, Avila HG, Fox G, Gutiérrez A, Negro P, López R, Jensen O, Rosenzvit M, Kamenetzky L (2016) Cystic echnococcosis in South America: systematic review of species and genotypes of *Echinococcus granulosus* sensu lato in humans and natural domestic hosts. Tropical Med Int Health 21:166–175

Djuricic SM, Grebeldinger S, Kafka DI, Djan I, Vukadin M, Vasiljevic ZM (2010) Cystic echinococcosis in children—the seventeen-year experience of two large medical centers in Serbia. Parasitol Int 59: 257–261

Dopchiz MC, Elissondo MC, Rossin MA, Denegri G (2007) Hydatidosis cases in one of Mar del Plata hospitals, Buenos Aires, Argentina. Rev Soc Bras Med Trop 40:635–639

Dybicz M, Gierczak A, Dąbrowska J, Rdzanek Ł, Michałowicz B (2013) Molecular diagnosis of cystic echinococcosis in humans from central Poland. Parasitol Int 62:364–367

Eckert J, Schantz PM, Gasser RB, Torgerson PR, Bessonov AS, Movessian SO, Thakur A, Grim F, Nikogossian MA (2001) Geographic distribution and prevalence. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski Z (eds) WHO/OIE manual on echinococcosis in humans and animals. WHO/OIE, Paris, pp 100–142

Guarnera EA, Parra A, Kamenetzky L, García G, Gutiérrez A (2004) Cystic echinococcosis in Argentina: evolution of metacestode and clinical expression in various *Echinococcus granulosus* strains. Acta Trop 92:153–159

Hüttner M, Nakao M, Wassermann T, Siefert L, Boomker JD, Dinkel A, Sako Y, Mackenstedt U, Romig T, Ito A (2008) Genetic characterization and phylogenetic position of *Echinococcus felidis* (Cestoda: Taeniidae) from the African lion. Int J Parasitol 38:861–868

Kamenetzky L, Gutierrez AM, Canova SG, Haag KL, Guarnera EA, Parra A, Rosenzvit MC (2002) Several strains of *Echinococcus granulosus* infect livestock and humans in Argentina. Infect Genet Evol 2:129–136

Konyaev SV, Yanagida T, Nakao M, Ingovatova GM, Shoykhet YN, Bondarev AY, Odnokurtsev VA, Loskutova KS, Lukmanova GI, Dokuchaev NE, Spiridonov S, Alshinecky MV, Sivkova TN, Andreyanov ON, Abramov SA, Krivopalov AV, Karpenko SV, Lopatina NV, Dupal TA, Sako Y, Ito A (2013) Genetic diversity of *Echinococcus* spp. in Russia. Parasitology 140:1637–1647

Kurul IC, Topcu S, Altinok T, Yazucu U, Tastepe I, Kaya S, Cetin G (2002) One-stage operation for hydatid disease of lung and liver: principles of treatment. J Thorac Cardiovasc Surg 124:1212–1215

Larrieu EJ, Frider B (2001) Human cystic echinococcosis: contributions to the natural history of the disease. Am J Trop Med Parasitol 95: 679–687

Laurimäe T, Kinkar L, Andresiuk V, Haag KL, Ponce-Gordo F, Acosta-Jamett G, Garate T, Gonzàlez LM, Saarma U (2016) Genetic diversity and phylogeography of highly zoonotic *Echinococcus granulosus* genotype G1 in the Americas (Argentina, Brazil, Chile and Mexico) based on 8279bp of mtDNA. Infect Genet Evol 45: 290–296

Lavikainen A, Lehtinen MJ, Meri T, Hirvelä-Koski V, Meri S (2003) Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of *Echinococcus granulosus*. Parasitology 127:207–215

Monteiro DU, Botton SA, Tonin AA, Azevedo MI, Graichen DA, Noal CB, de la Rue ML (2014) *Echinococcus canadensis* (G7) and

Echinococcus granulosus sensu stricto (G1) in swine of southern Brazil. Vet Parasitol 202:335–338

Moro P, Schantz PM (2009) Echinococcosis: a review. Int J Infect Dis 13: 125–133

Nakao M, Lavikainen A, Yanagida T, Ito A (2013) Phylogenetic systematics of the genus *Echinococcus* (Cestoda: Taeniidae). Int J Parasitol 43:1017–1029

National System of Health Surveillance (2017) http://www.msal.gob.ar/index.php/home/boletin-integrado-de-vigilancia. Accessed 15 April 2017

Oudni-M'rad M, M'rad S, Ksia A, Lamiri R, Mekki M, Nouri A, Mezhoud H, Babba H (2016) First molecular evidence of the simultaneous human infection with two species of *Echinococcus granulosus* sensu lato: *Echinococcus granulosus* sensu stricto and *Echinococcus canadensis*. Parasitol Res 115:1065–1069

Pierangeli NB, Soriano SV, Roccia I, Giménez J, Lazzarini LE, Grenóvero MS, Menestrina C, Basualdo JA (2007) Heterogeneous distribution of human cystic echinococcosis after a long-term control program in Neuquén, Patagonia Argentina. Parasitol Int 56:149–155

Provintial Direction of Statistic and Census (2017) Neuquén Province http://www.estadisticaneuquen.gob.ar/index.php?sec=aplicacion_neuquen_numeros. Accessed 15 April 2017

Romig T, Ebi D, Wassermann M (2015) Taxonomy and molecular epidemiology of *Echinococcus granulosus* sensu lato. Vet Parasitol 213:76–84

Sadjjadi SM, Mikaeili F, Karamian M, Maraghi S, Sadjjadi S, Shariat-Torbaghan S, Kia EB (2013) Evidence that the *Echinococcus granulosus* G6 genotype has an affinity for the brain in humans. Int J Parasitol 43:875–877

Schneider R, Gollackner B, Schindl M, Tucek G, Auer H (2010) *Echinococcus canadensis* G7 (pig strain): an underestimated cause of cystic echinococcosis in Austria. Am J Trop Med Hyg 82:871–874

Soriano SV, Pierangeli NB, Pianciola LA, Mazzeo M, Lazzarini LE, Saiz MS, Kossman AV, Bergagna HFJ, Chartier K, Basualdo JA (2010) Molecular characterization of *Echinococcus* isolates indicates goats as reservoir for *Echinococcus canadensis* G6 genotype in Neuquén, Patagonia Argentina. Parasitol Int 59:626–628

Soriano SV, Pierangeli NB, Pianciola LA, Mazzeo M, Lazzarini LE, Debiaggi MF, Bergagna HF, Basualdo JA (2015) The optimum cut-off value to differentiate *Echinococcus granulosus* sensu stricto from other species of *E. granulosus* sensu lato using larval rostellar hook morphometry. J Helminthol 89:1–8

Soriano SV, Debiaggi MF, Pierangeli NB, Pianciola LA, Bergagna HF, Lazzarini LE, Mazzeo M, Basualdo JA (2016) First study about the development of adult *Echinococcus canadensis* G6 genotype of goat origin in experimentally infected dogs. Vet Parasitol 228:6–12

Thompson RCA, McManus DP (2001) Aetiology: parasites and life-cycles. In: Eckert J, Gemmell A, Meslin FX, Pawlowski Z (eds) WHO/OIE manual on echinococcosis in humans and animals. WHO/OIE, Paris, pp 1–16

Umhang G, Richomme C, Boucher JM, Hormaz V, Boué F (2013) Prevalence survey and first molecular characterization of *Echinococcus granulosus* in France. Parasitol Res 112:1809–1812

Umhang G, Chihai O, Boué F (2014) Molecular characterization of *Echinococcus granulosus* in a hyperendemic European focus, the Republic of Moldova. Parasitol Res 113:4371–4376

Zait H, Kouidri M, Grenouillet FE, Umhang G, Millon L, Hamrioui B, Grenouillet F (2016) Molecular characterization of *Echinococcus granulosus* sensu stricto and *Echinococcus canadensis* in humans and livestock from Algeria. Parasitol Res 115:2423–2431

Zhang T, Yang D, Zeng Z, Zhao W, Liu A, Piao D, Jiang T, Cao J, Shen Y, Liu H, Zhang W (2014) Genetic characterization of human-derived hydatid cysts of *Echinococcus granulosus* sensu lato in Heilongjiang Province and the first report of G7 genotype of *E. canadensis* in humans in China. PLoS One 9:1–7 e109059