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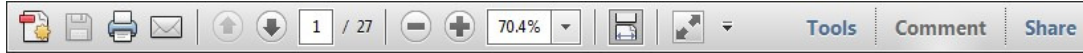
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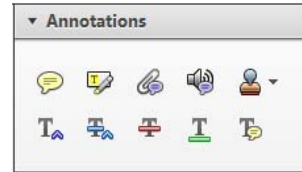
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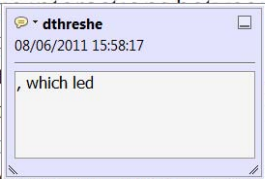


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standard framework for the analysis of microeconomic behavior. Nevertheless, it also led to the development of a new paradigm of strategic behavior. The number of competitors in the industry is that the structure of the industry is a key determinant of the main components of the industry. At the industry level, are externalities important? (M. Henceforth) we open the 'black b



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there is no room for extra profits as mark-ups are zero and the number of firms (net) values are not determined by market structure. Blanchard ~~and Kiyotaki~~ (1987), perfect competition in general equilibrium. The effects of aggregate demand and supply shocks in the classical framework assuming monopoly. When an exogenous number of firms

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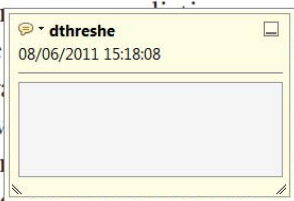


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and supply shocks. Most of the literature on the effects of monetary policy on the number of firms in the industry is that the structure of the sector



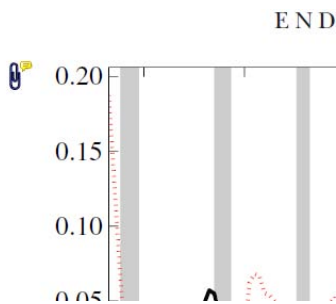
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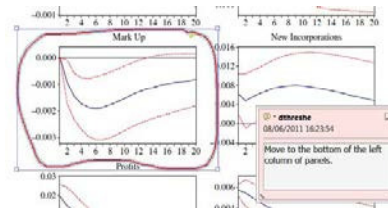


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Systematic Review

Cystic echinococcosis in South America: systematic review of species and genotypes of *Echinococcus granulosus sensu lato* in humans and natural domestic hosts

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Abstract

OBJECTIVE To systematically review publications on *Echinococcus granulosus sensu lato* species/genotypes reported in domestic intermediate and definitive hosts in South America and in human cases worldwide, taking into account those articles where DNA sequencing was performed; and to analyse the density of each type of livestock that can act as intermediate host, and features of medical importance such as cyst organ location.

METHODS Literature search in numerous databases. We included only articles where samples were genotyped by sequencing since to date it is the most accurate method to unambiguously identify all *E. granulosus* s. l. genotypes. Also, we report new *E. granulosus* s. l. samples from Argentina and Uruguay analysed by sequencing of cox1 gene.

RESULTS In South America, five countries have cystic echinococcosis cases for which sequencing data are available: Argentina, Brazil, Chile, Peru and Uruguay, adding up 1534 cases. *E. granulosus* s. s. (G1) accounts for most of the global burden of human and livestock cases. Also, *E. canadensis* (G6) plays a significant role in human cystic echinococcosis. Likewise, worldwide analysis of human cases showed that 72.9% are caused by *E. granulosus* s. s. (G1) and 12.2% and 9.6% by *E. canadensis* G6 and G7, respectively.

CONCLUSIONS *E. granulosus* s. s. (G1) accounts for most of the global burden followed by *E. canadensis* (G6 and G7) in South America and worldwide. This information should be taken into account to suit local cystic echinococcosis control and prevention programmes according to each molecular epidemiological situation.

keywords *Echinococcus granulosus sensu lato*, genotypes, cystic echinococcosis, South America, neglected disease

Introduction

The larval stage of the cestode parasite *Echinococcus granulosus sensu lato* (s. l.) causes cystic echinococcosis or cystic hydatid disease, a chronic parasitic zoonosis that affects humans as well as domestic and wild mammals. Associated with poverty and poor hygiene practices,

particularly in livestock-raising communities [1], this affliction is essentially preventable and considered a neglected disease by WHO. It has been estimated that 1–3.6 million DALYs are lost worldwide because of human cystic echinococcosis [2] and that up to \$2 billion are lost annually in the livestock industry [3]. This zoonosis is distributed worldwide and is endemic or

hyperendemic in South America, especially in Argentina, southern Brazil, Uruguay, Chile and mountainous regions of Peru and Bolivia.

Echinococcus granulosus s. l. requires two mammalian hosts to complete its life cycle: a definitive host (usually dogs or other canids) and an intermediate host (wild or livestock mammals). Humans act as accidental hosts. The strobilar adult stage develops as a flatworm in the gut of the definitive host and produces eggs with oncospheres by sexual reproduction. The infective eggs are released with the host faeces into the environment, where they are ingested by the intermediate host. Upon ingestion, the oncospheres are released, migrate through the intestinal wall and spread through the bloodstream to various organs, most commonly the liver and lung. Each oncosphere has the potential to develop into a metacestode (hydatid cyst). Within the hydatid cyst, protoscoleces, the next larval stage, are produced by asexual multiplication. The hydatid cysts with protoscoleces are called fertile cysts. Protoscoleces are able to develop into adult parasites after hydatid cyst ingestion by the definitive host. Also, if content leakage from a fertile hydatid cyst occurs within the intermediate host, protoscoleces have the ability to develop into new hydatid cysts (secondary cystic echinococcosis).

Species and genotypes of *Echinococcus granulosus sensu lato*

Echinococcus granulosus s. l. is composed of numerous variants initially identified by J.D. Smyth and Z. Davies (1974) [4] who called them 'physiological strains'. This term was due to the observation of radical differences in the *in vitro* development of protoscoleces isolated from sheep and horse cysts [4, 5]. Since then, several works have shown that the strains differ in many features such as protein profile [6], carbohydrate and lipid repertoires [7], hooks morphology [8–10], metabolic requirements (reviewed in [7]), fertile cyst development in natural infections (reviewed in [11]), cyst development in experimental infections [12], intermediate host specificity, prepatent period [13, 14], antigenicity [15] and infectivity and pathogenicity in humans [11, 16].

Later on, molecular biology techniques allowed the identification of DNA polymorphisms in mitochondrial genes of *E. granulosus* s. l. Those polymorphisms were detected by DNA sequencing [17, 18] and PCR-RFLP [19] and correlated with the strains described until then. This allowed to assign a genotype to each strain. Since then, several molecular tools have been applied to determine *Echinococcus* spp. genetic variability, such as Southern blot [20], PCR-SSCP [21], RAPD [22], multiplex PCR

[23], LAMP [24] and HRM [25] among others. To date, ten genotypes have been described (G1–G10) [26].

In the last years, mitochondrial phylogenetic analysis allowed to classify most of the genotypes as new species [11, 27]. The new classification infers that *Echinococcus granulosus sensu stricto* (s. s.) groups the G1, G2 and G3 genotypes. Particularly, the G1 genotype is the most frequently found worldwide, produces fertile hydatid cysts mainly in sheep and is frequently isolated from humans. Recently, *E. granulosus* s. s. (G1) has been identified in cats; however, the epidemiological importance of the cat as intermediate host could be considered marginal due to the few cases reported since the first case was described [28, 29].

Echinococcus equinus (G4 genotype) has remarkable morphological and developmental differences with the G1 genotype and has only been found in horses and other equines, and no human cases have been reported to date [11]. *Echinococcus ortleppi* (G5 genotype) produces fertile cysts mainly in cattle and has been described in few human cases [30]. *Echinococcus canadensis* includes the G6, G7, G8 and G10 genotypes since phylogenetic analysis grouped them as a monophyletic group [27]. Camels and goats are the main intermediate hosts for the G6 genotype, pigs for the G7 genotype and cervids for the G8 and G10 genotypes. All these genotypes have been isolated from humans. Finally, the G9 genotype human cases described by Scott *et al.* (1997) [31] are now considered to have belonged to the G7 genotype [32]. In summary, the *E. granulosus* s. l. complex groups include the following: *E. granulosus* s. s. (G1/G2/G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6/G7/G8/G10).

In this work, we provide new data and perform a comprehensive review of the circulating *E. granulosus* s. l. genotypes in domestic animals and human cases in South America identified by DNA sequencing. The information obtained on human echinococcosis was further integrated with data reported worldwide to compare the South American situation with the global scene.

Methods

Literature search

The literature search was conducted mainly using PubMed database (Figure S1) using three terms: echinococcus, echinococcosis or hydatid disease, in the period 1992–2014. Records obtained were further filtered by combining related keywords using Boolean operators. The keywords used were genotype, strain, species, sequence, molecular marker and gene in Title/Abstract. After removing duplicated reports, full articles in English or Spanish

1 were retrieved by an in-house bash script. We further con-
2 sidered those articles that reported *E. granulosus* s. l.
3 genotype identification by DNA sequencing and analysed
4 samples from domestic definitive and natural intermediate
5 hosts from South American countries together with
6 reports from human cases worldwide (inclusion criteria).
7 We excluded cystic echinococcosis reports with ambigu-
8 ous genotype description, such as cryptic sequence analy-
9 sis. Finally, complementary searches were performed in
10 four databases: Science Direct, Journal of Citation
11 Reports, Scopus and Google Scholar and relevant articles
12 were included in the final review if they met the inclusion
13 criteria. Moreover, samples reported in more than one
14 article were taken into consideration once. The combina-
15 tion of the different criteria aimed to retrieve as many rele-
16 vant publications as possible but at the same time tried to
17 narrow the amount of results only to those articles which
18 employed an accurate method to identify *E. granulosus* s.
19 l. genotypes. The aim of the present review is to highlight
20 only results from sequenced samples since to date it is the
21 most accurate method to unambiguously identify all
22 *E. granulosus* s. l. genotypes. We would like to mention
23 that it is possible that relevant papers, which did not con-
24 tain in their titles or abstracts the keywords used in our
25 search, may have been overlooked.

26 27 28 **New *Echinococcus granulosus sensu lato* samples 29 identified by DNA sequencing in Argentina**

30 Total protoscolex DNA was prepared from fresh, frozen
31 in liquid nitrogen or 70% ethanol preserved isolates of
32 *E. granulosus* s. l. by conventional techniques. In the case
33 of samples with PCR inhibitors present (i.e. cyst layers),
34 the DNeasy Blood & Tissue Kit (QIAGEN) was used
35 [21]. Analysis of the mitochondrial *cox1* gene was made
36 by PCR as described [12], the product obtained was
37 sequenced and aligned with published sequences for all
38 species/genotypes from *Echinococcus* reported. A total of
39 131 samples were obtained from Argentina and Uruguay
40 and were added to the reviewed data. Detailed informa-
41 tion of genotype, host, geographical origin, number and
42 reference from all the *E. granulosus* s. l. samples identi-
43 fied in South America until 2014 are shown in Table S1.

44 45 46 **Results**

47 48 ***Echinococcus granulosus* s. l. genotypes isolated from 49 natural intermediate and definitive hosts from South 50 America**

51 In South America, five countries have cystic echinococco-
52 sis cases identified in natural intermediate and definitive

hosts for which sequencing data are available: Argentina,
Brazil, Chile, Peru and Uruguay (Figure 1). A total of six
genotypes have been found in the region: G1, G2, G3,
G5, G6 and G7. The types of livestock affected are
sheep, cattle, pig, goat and llama (Figure 1). The majority
of the cases are caused by *E. granulosus* s. s. (genotypes
G1/G2/G3). The G1 genotype shows the widest distribu-
tion and is the most frequently found in the species of
livestock analysed as well as in the domestic definitive
host (836/1379). The second most frequent genotype is
G5 (348/1379) mainly isolated from Brazilian cattle
(Table 1). Detailed information of genotype, host, geo-
graphical origin, number and reference from all the
E. granulosus s. l. samples identified by DNA sequencing
in South America until 2014 is described in Table S1.
The genotypes circulating in Brazilian livestock are G1
(58.5%), G5 (41.0%) and G7 (0.5%) being cattle the
principal hosts described (810/815). In Peru, the genotype
G1 (89.6%) was found in alpacas, sheep, cattle, goats
and pigs; G6 (3.1%) in goats; and G7 (7.3%) in pigs.
The range of host species involved in maintaining the life
cycle in Peru is the widest in the region since five species
of livestock were found to have hydatid cysts (Figure 1,
Table S1). With respect to the situation in Argentina,
here we provide new *cox1* sequencing data according to
[12]. One hundred and eighteen *E. granulosus* s. l. iso-
lates from livestock and dogs from different provinces
were integrated with data from existing reports tallying
up 373 samples (Table S1). A total of six genotypes are
circulating in livestock in Argentina (Figure 1): G1
(49.3%) in sheep, cattle, goat and pig; G2 (1.7%) in
sheep and cattle; G3 (0.3%) in sheep; G5 (2.6%) in cat-
tle; G6 (8.4%) in goats and cattle; and G7 (37.7%) in
pigs (Table S1). At least two different genotypes were iso-
lated from each species of intermediate host, being the
G1 genotype present in all of them (Figure 2a). Cattle
and sheep are the main reservoirs of the G1 genotype.
However, goats and pigs are the main reservoirs of the
G6 and G7 genotypes, respectively (Figure 2a). Regarding
the molecular epidemiological scenario in definitive hosts,
a total of four genotypes (G1, G5, G6 and G7) were
found in dogs (Figure 2a). These data show that at least
the most widely distributed and frequent genotypes are
developing the complete life cycle in our country. It is
worth mentioning that in one case, a co-infection of the
G1 and G6 genotypes was found [21]. Finally, there is
still not enough information from Uruguay and Chile to
draw conclusions (Table S1).

Taking into consideration all the available data from
Argentina, a deeper analysis was performed. As shown in
Figure 2a, 36.1% (30/83) of the human cases in Argen-
tina are caused by the G6 genotype. Most of the reported

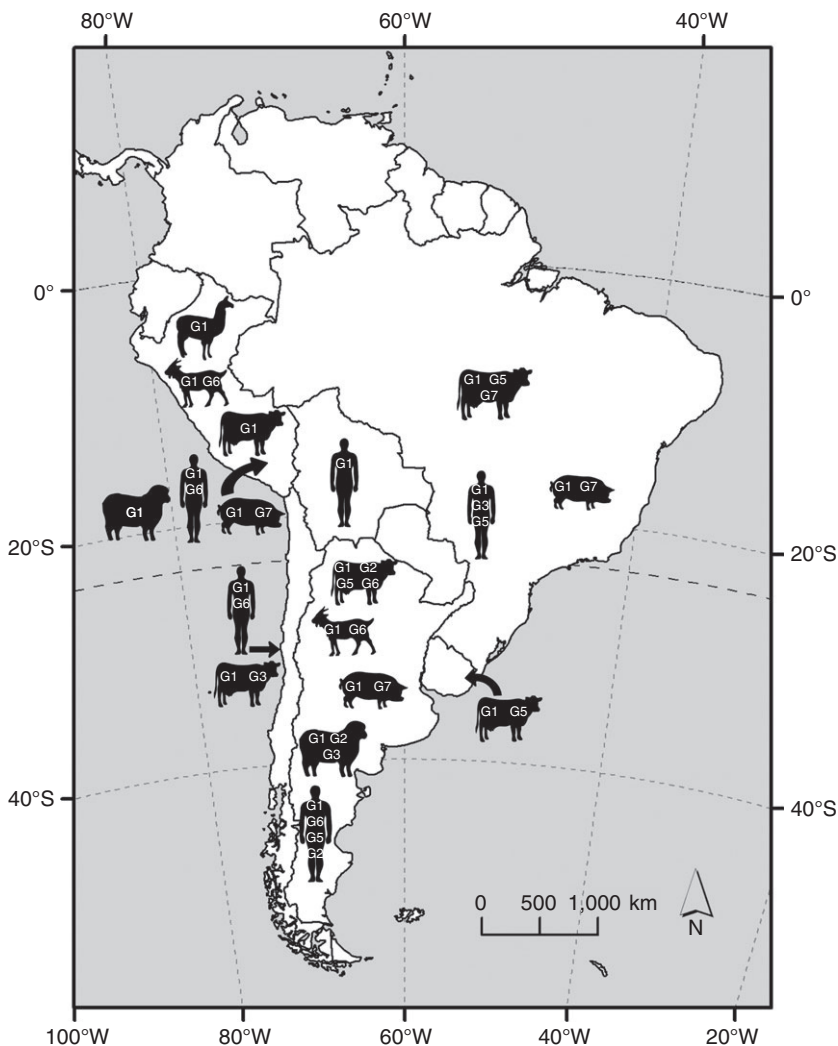


Figure 1 Schematic representation of the geographical and intermediate host distribution of *Echinococcus granulosus sensu lato* genotypes isolated in South America and identified by DNA sequencing. The position of the hosts figures is not indicative of the state/province/department location within a country.

G6 cases in livestock were isolated in Neuquen province. Interestingly, concurrent geographical location of human and animal cases were found in this province, being goats the most frequent host harbouring the G6 genotype (Figure 2a and Table S1). It is imperative to analyse a larger number of samples from other provinces focusing on the main intermediate hosts described for this genotype, that is goats and camelids. Livestock density distribution in Argentina reveals the priority areas to analyse in the future: the provinces of Mendoza, Formosa, Chaco, Santiago del Estero, Salta, San Luis, La Rioja, Jujuy and Santa Cruz, where goat and camelid production is concentrated (Figure 2b) and for which the molecular epidemiological situation in livestock is scarce or unknown. It would also be useful to analyse whether South American camelids, such as alpacas, llamas and guanacos, play a role in maintaining the natural life cycle of *E. granulosus s. l.* in

Argentina. The G6 genotype displays features that are important for cystic echinococcosis diagnosis and control such as sequence variability in the diagnostic antigen B [33, 34] and the vaccine EG95 antigen [35]. Also, differences in anti-EG95 antibody response to infection between the G1 and G6 genotypes have been described [15]. *Echinococcus equinus* (G4 genotype) has not been identified in South America yet, since the cystic echinococcosis cases found in horses lack genotype determination [36]. Also, there are still no reports of *E. canadensis* (G8 and G10 genotypes) in the region.

Echinococcus granulosus s. l. genotypes identified in human cases from South America

In South America, 155 human cystic echinococcosis cases with sequencing information have been reported. The

Table 1 South American species and genotypes of *Echinococcus granulosus sensu lato* identified in domestic natural hosts (intermediate and definitive) and humans in South America by molecular markers sequencing

South American cases						
Species	Genotype (strain)	Intermediate natural host ^a	Definitive host ^b	Number of cases ^{a+b}	Human cases ^c (%)	References ^{a+b+c}
<i>E. granulosus sensu stricto</i> (s.s.)	G1 (sheep)	Sheep, camelids, cattle, goats, pigs, cats	Dog	836	112 (72.3)	This work, [20, 21, 28, 37, 38, 40–47]
	G2 (Tasmanian sheep)	Sheep, cattle	–	6	6 (3.9)	[20, 21, 38]
<i>E. ortleppi</i>	G3 (buffalo)	Sheep	Dog	3	1 (0.6)	[42, 44]
	G5 (cattle)	Cattle	Dog	348	3 (1.9)	This work, 21, 38, 40, 45, 48, 52.
<i>E. canadensis</i>	G6 (camel)	Goats, cattle	Dog	38	33 (21.3)	This work, [10, 20, 21, 41, 42, 49–52]
	G7 (pig)	Pigs, cattle Total	Dog	148 1379	– 155	This work, [20, 21, 40–42, 46]

The main intermediate host of each genotype in South America is highlighted in bold.

main *E. granulosus* s. l. genotypes infecting humans are G1 (72.3%) and G6 (21.3%) (Table 1). This information is of particular interest since these genotypes belong to two different species, *E. granulosus* s. s. and *E. canadensis*, respectively, according to the new classification of the complex. Human cases caused by the genotypes G2, G3 and G5 (Figure 1) have also been reported, but with lower frequencies (<4%) (Table 1). Particularly, in Chile and Peru, G1 and G6 are present in humans being G1 responsible for 96% (24/25) and 95% (38/40) of the cases, respectively. It is interesting to note that despite the high number of human cases identified in Peru, none was caused by the G7 genotype, even though this genotype has been isolated from pigs in this country. In spite of the fact that there are few human cystic echinococcosis cases with genotype information from Brazil ($N = 6$), three genotypes were found (G1, G3 and G5). The only human cystic echinococcosis case from Bolivia belonged to the G1 genotype (Table S1).

The most complete molecular epidemiological picture for human cystic echinococcosis corresponds to Argentina ($N = 83$). The genotypes identified in patients were G1 (54.2%), G2 (7.2%), G5 (2.4%) and G6 (36.1%) (Figure 2a, Table S1). In agreement with the situation in Peru, the data reviewed in this work strongly support the idea that in Argentina, pigs are the only reservoir of the G7 genotype (Figure 2a) and that this genotype seems not to be a substantial human health problem. For an accurate determination of human infection risk with the G7 genotype in Argentina, we integrated the information on genotypes from human echinococcosis cases with density (animals/ha) of swine livestock all over the country. The main producers of pigs are the provinces of Cordoba and Santa Fe, followed by a lower production in Buenos Aires, Chaco, Entre Rios, Misiones, Formosa and San Luis (Figure 2b). Unfortunately, there is little or no genotype information on human cases reported from these provinces (Table S1). As shown in Figure 2b, there is an intensive bovine livestock activity in Argentina. Cattle were found to harbour the genotypes G1, G2, G5 and G6, which are all infective to humans (Figure 2a). This highlights the fact that cattle play a crucial role in maintaining the life cycle of almost all circulating genotypes in Argentina and also in human transmission, especially taking into account the high reported median fertility rate of cattle cysts in this country (42%) [21, 37, 38].

Human cystic echinococcosis worldwide

A total of 877 human cystic echinococcosis isolates have been sequenced and reported worldwide (Figure 3a and Table S2). *Echinococcus granulosus* s. s. is the aetiological

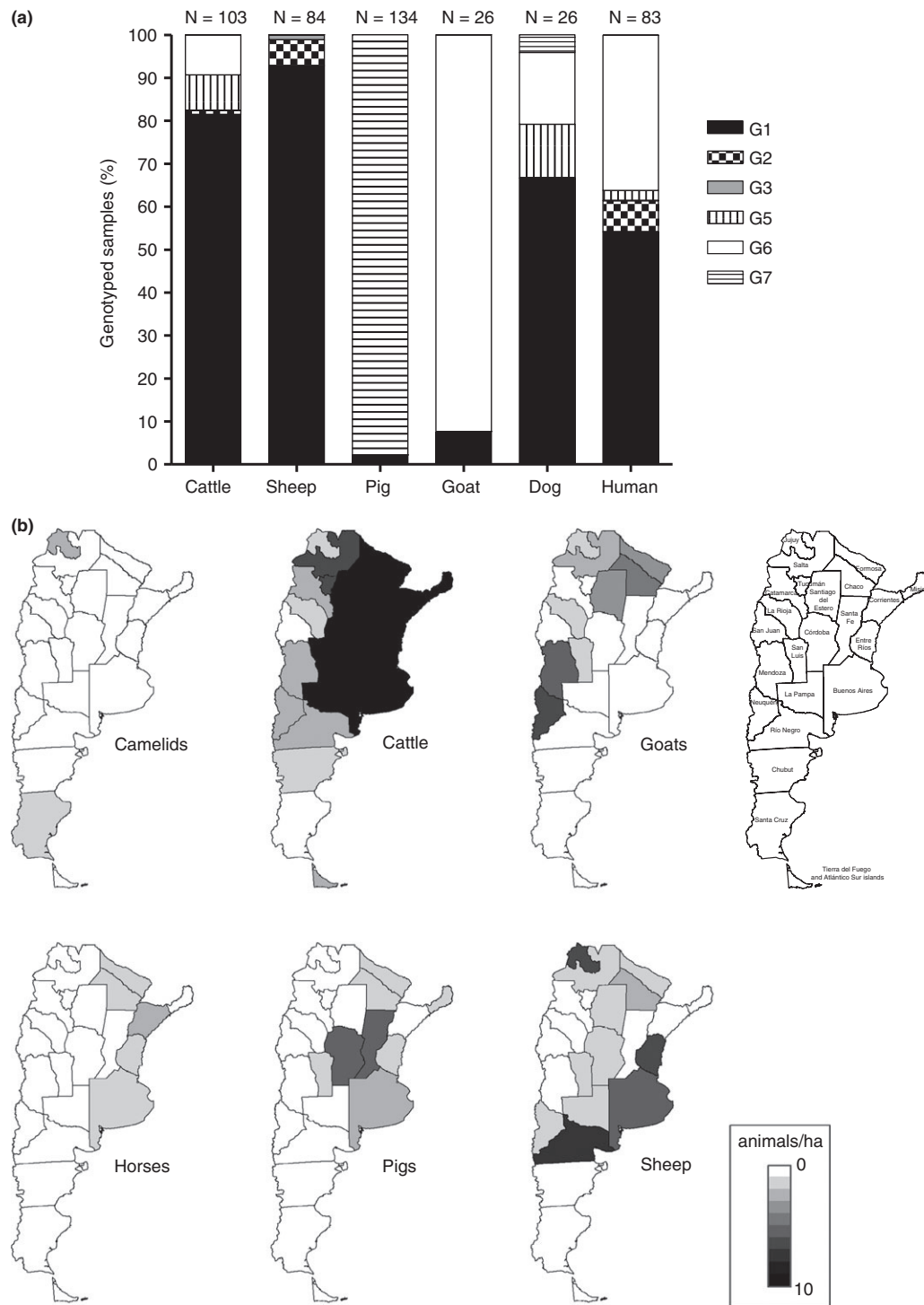


Figure 2 Molecular epidemiological situation of cystic echinococcosis in Argentina. (a) *Echinococcus granulosus sensu lato* genotypes harboured in definitive and intermediate hosts in Argentina. Samples with genotype identification by DNA sequencing are shown. (b) Livestock density (animals/ha) in Argentina. Data source: Ministerio de Agricultura, Ganadería y Pesca de la Nación, Secretaría de Agricultura, Ganadería y Pesca and Secretaría de Ambiente y Desarrollo Sustentable-INTA.

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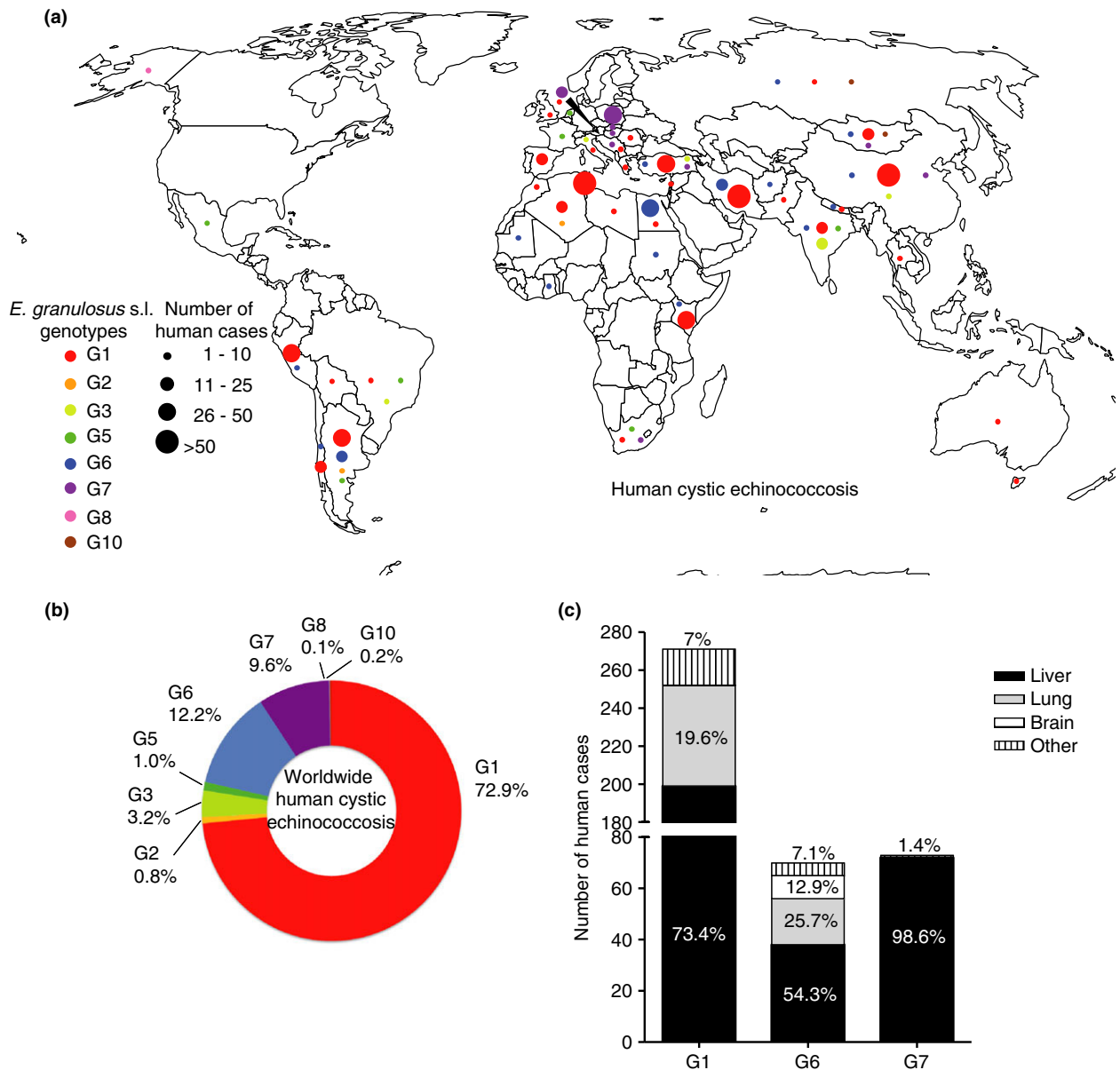


Figure 3 Human cystic echinococcosis cases in the world with genotype identification by DNA sequencing. (a) Worldwide distribution of *Echinococcus granulosus sensu lato* genotypes isolated from humans. (b) Percentage of human cases caused by each *E. granulosus* s. l. genotype (c) Human hydatid cysts organ location of *E. granulosus* s. s. (G1 genotype) and *E. canadensis* (G6 and G7 genotypes). The category 'Other' includes organ location with fewer than three cases reported. The position of the dots shows the geographical country distribution independently of the state/province/department location.

agent of most human cases worldwide (Figure 3a) being G1 the most frequent genotype (72.9%) (Figure 3a and B), as observed in South America. Therefore, this genotype has the greatest epidemiological relevance due to its wide distribution. Moreover, the G6 and G7 genotypes historically considered poorly infective to humans

account for 12.2% and 9.6% of human cases worldwide, respectively (Figure 3b). The geographical distribution of human cystic echinococcosis caused by these two genotypes clearly differs (Figure 3a). The G6 genotype is present in human cases from America, Asia and Africa, while the G7 genotype cases have been detected in several

European countries. The remaining genotypes were rarely found in patients (<3.3%). No G4 human cases have been reported to date (Figure 3b and Table S2), reinforcing the hypothesis that this genotype is unable to establish infection in humans [32]. Our results are in accordance with those recently reported by [32], who also surveyed human echinococcosis cases worldwide. In spite of the fact that these authors included in their analysis all the cases reported independently of the methodology used for genotype determination, the relative abundance of each *E. granulosus* s. l. species/genotypes to the global burden of human cystic echinococcosis is similar.

For 429 (48.9%) of the reviewed human cystic echinococcosis cases, the corresponding cyst anatomical location (Table S2) was reported. Due to the fact that the G1, G6 and G7 genotypes are the most relevant from a sanitary aspect, we focused on their organ tropism regardless sex, age or ethnic background of the hosts. As can be observed in Figure 3c, liver is the most affected organ by the three genotypes. Interestingly, the G7 genotype seems to infect almost exclusively the liver (98.6%), while the G1 genotype also develops in lungs in a high proportion of the cases (19.6%). G6 genotype was found in liver (54.3%), lung (25.7%), brain (12.9%) and other organs (7.1%) although it has been described to have a brain location preference [39].

Conclusions

Echinococcus granulosus s. l. is composed of four species: *E. granulosus* s. s. (G1/G2/G3 genotypes), *E. equinus* (G4 genotype), *E. ortleppi* (G5 genotype) and *E. canadensis* (G6/G7/G8/G10 genotypes). The different species/genotypes display distinctive features of biological and epidemiological significance, which emphasises the need of studies concerning molecular characterisation and distribution in endemic areas. In this work, we provided new data and reviewed all those articles with sequencing identification of species/genotypes reported in natural intermediate and definitive hosts in South America as well as in human cases worldwide.

In South America, the countries with a larger number of analysed samples display a greater genetic complexity, for example Argentina, Brazil and Peru. *Echinococcus granulosus* s. s. (G1 genotype) account for most of the global burden of human and livestock cases in South America. Also, *E. canadensis* (G6 genotype) plays a significant role in South American human cystic echinococcosis. Taking into account the molecular epidemiological situation, it is relevant to evaluate the pathogenicity, diagnosis performance and response to

chemotherapy of the G6 genotype since antigenic differences between the EG95-related proteins from the G1 and G6 genotypes were observed [15]. Additionally, for an accurate determination of the public health relevance of *E. canadensis* (G7 genotype) in South America, a molecular epidemiological survey should be carried out in those regions where swine breeding is concentrated.

With respect to the worldwide situation of human cystic echinococcosis, *E. granulosus* s. s. (G1 genotype) accounts for most of the global burden followed by *E. canadensis* (G6 and G7 genotypes). No human cases of cystic echinococcosis caused by *E. equinus* (G4 genotype) have been detected yet. This could be due to the fact that this genotype is not infective for humans or that a higher number of samples from horse breeding regions should be tested.

The data reviewed in this work provide useful information that should be taken into account to suit local cystic echinococcosis control and prevention programmes according to each molecular epidemiological situation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow chart of articles selection process.

Table S1 Genotype, host, geographic origin, number and reference from all the *Echinococcus granulosus sensu lato* samples identified by sequencing of molecular markers in South America until 2014.

Table S2 Human cystic echinococcosis cases in the world with genotype determined by sequencing of molecular markers. Country, genotype (number of cases), molecular marker, organ and reference are shown.

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