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Genetic characterization of human-derived hydatid fluid based on mitochondrial gene sequencing in individuals from northern and western China

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Abstract

This study investigated *Echinococcus* genotypes in patients with hydatidosis that reside in Inner Mongolia, Tibet or Qinghai Province by partially sequencing the cytochrome *c* oxidase subunit 1 (*cox1*) and NADH dehydrogenase 1 (*nad1*) genes. Cyst fluids were collected from 23 patients with hydatidosis and DNA was extracted. Portions of the *cox1* and *nad1* genes were amplified and subsequently sequenced. Sequencing analysis determined that one of the isolates contained *Echinococcus multilocularis*, and the other 22 isolates contained *E. granulosus sensu lato*. The isolates were then further classified based on genotype, and *E. granulosus sensu stricto* (*s.s.*) G1 (n = 20), *E. granulosus s.s.* G3 (n = 1) and *E. canadensis* G6/7 (n = 1) were identified. Additionally, the sequences were concatenated (pcox1 + pnad1) and 11 haplotypes were identified among the *E. granulosus s.s.* isolates (G1 and G3), with a shared common haplotype (H1) identified. Overall, these findings provide further understanding of the genetic patterns of *Echinococcus* in western and northern China.

Introduction

Echinococcosis, which is listed by the World Health Organization (WHO) as a neglected tropical disease, is a zoonotic infection caused by several cestode species from the genus *Echinococcus* (Eckert and Deplazes, 2004; Brunetti *et al.*, 2010). Adult worms inhabit the small intestine of a wild or domestic species of Canidae, which serves as a definitive host. Intermediate hosts (ungulates) and accidental hosts (humans) become infected after ingesting parasitic eggs, which subsequently develop into hydatid cysts in the liver, lungs or other organs. Furthermore, humans can also acquire the infection by direct contact with dogs (Eckert *et al.*, 2001). Alveolar echinococcosis (AE) and cystic echinococcosis (CE) are regarded as neglected zoonoses, with a higher overall disease burden associated with CE due to its global distribution and high regional prevalence, while a higher pathogenicity and case fatality rate is associated with AE, especially in Asia (Eckert and Deplazes, 2004; Deplazes *et al.*, 2017).

Echinococcus granulosus sensu lato (*s.l.*) was taxonomically defined based on sequence comparisons of portions of the mitochondrial genes cytochrome *c* oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) (Bowles *et al.*, 1992; Bowles and McManus, 1993; McManus, 2006; Rostami Nejad *et al.*, 2012; Alvarez Rojas *et al.*, 2014). Moreover, *E. granulosus s.l.* can be further divided into *E. granulosus sensu stricto* (*s.s.*) G1 (identified in sheep), G2 (identified in Tasmanian sheep), or G3 (identified in buffalo); *E. equinus* G4; *E. ortleppi* G5; *E. canadensis* (G6–G10); and *E. felidis* (lion strain) (Kedra *et al.*, 1999; Nakao *et al.*, 2013a). Variations between *E. granulosus* species include differences in infectivity within different intermediate hosts, the survival rate within a definitive host, antigenicity, transmission dynamics, and pathology (Eckert *et al.*, 1993; Thompson, 2008; Soriano *et al.*, 2010; Siracusano *et al.*, 2012; Alvarez Rojas *et al.*, 2013; Cucher *et al.*, 2013; McManus, 2013; Chaâbane-Banaoues *et al.*, 2016). Thus, molecular epidemiological surveys utilizing *E. granulosus* isolates are fundamental to understanding the spatio-temporal epidemiology in endemic areas and will aid in disease control and prevention (McManus, 2006, 2013; Thompson, 2008).

So far, human CE cases reported in China have been associated with the G1, G3, G6, G7 and G10 genotypes, with most cases caused by G1. Furthermore, in China, disease cases are most often reported in the western, north-western and northern regions (Zhang *et al.*, 1998, 2014; Cardona and Carmena, 2013; Yan *et al.*, 2013; Wang *et al.*, 2014; Yang *et al.*, 2015; Deplazes *et al.*, 2017; Ito and Budke, 2017). To further investigate the population genetic patterns in these regions, surgically obtained hydatid fluids were examined and *Echinococcus cox1* and *nad1* genes were sequenced and analysed.

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Materials and methods

Collection of patient samples

Hydatid fluids were collected from 23 clinically confirmed hydatidosis patients. Seven samples were collected from herdsmen in the Xilingol League Hospital, Inner Mongolia. The remaining 16 samples were obtained by the 302 Military Hospital during a one-year period (from December 2015 to November 2016), with two patients from Qinghai Province and 14 from the Tibet Autonomous Region. Approximately 1 ml of hydatid fluid was collected per patient and stored at −20°C. All patients provided informed written consent prior to surgery, but the hospital Medical Ethics Board deemed consent for molecular characterization of hydatid fluids exempt due to collection occurring post surgery and samples being examined anonymously. The study protocol was approved by the Medical Ethics Review Committee of Ordos Central Hospital, Inner Mongolia, China.

DNA extraction

Genomic DNA was extracted from the hydatid fluid using a blood/cell/tissue genomic DNA extraction kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. DNA concentrations were determined using a spectrophotometer (Implen, Munich, Germany) and samples were stored at −20°C until further use.

Mitochondrial polymerase chain reaction

Two mitochondrial DNA fragments within the *cox1* and *nad1* genes were amplified from each individual DNA sample using primer pairs designed by analysing *Echinococcus* mitochondrial gene sequences in GenBank. The *cox1* DNA fragments (pcox1) were amplified using COI-FOR (5′-GATCGTAATTTTT GTTCTGC-3′) and COI-REV (5′-ATAAGAACCTAACGACA TAACATA-3′) primers, with an expected amplicon size of 528 bp. *Echinococcus granulosus s.s nad1* DNA fragments (pnad1) were amplified using NDI-FOR1 (5′-GTGGTTGTTTTTGGG TTAGT-3′) and NDI-REV1 (5′-AGCAAACAGCCCTAAAA TCAA-3′) primers, with an expected amplicon size of 882 bp. *Echinococcus multilocularis* pnad1 was amplified using specific NDI-FOR2 (5′-TTGAGTTTGCGTCTCGATGA-3′) and NDI-REV2 (5′-CTAATTAACAATAACAAAAAATC-3′) primers, with an expected amplicon size of 1001 bp. *Echinococcus canadensis* pnad1 was amplified using NDI-FOR3 (5′-ATGGTTGTTTT GGGATTAAT-3′) and NDI-REV3 (5′-TACAAAAAATCCTAA AATCAA-3′) primers, with an expected amplicon size of 882 bp. Polymerase chain reactions (PCR) were carried out in a total volume of 50 µl [4 µl DNA (50–100 ng), 25 µl 2× Ultra-pfu Master Mix (0.1 U/µl Ultra-Pfu DNA Polymerase), 4 mM $MgSO_4$, and 0.4 mM of each dNTPs in 2× PCR reaction buffer and 20 pmol of each primer]. Negative controls, with sterilized water substituted for the DNA template, were included for each PCR experiment. PCR was performed in a thermocycler with the following programme: an initial denaturation step at 94°C for 3 minutes, then 35 cycles of denaturation at 94°C for 30 s, annealing at 46.5°C (for primer pairs COI-FOR/COI-REV, NDI-FOR1/NDI-REV1 or NDI-FOR3/NDI-REV3) or 50°C (for the NDI-FOR2/NDI-REV2 pair) for 40 s and extension at 72°C for 40 s, followed by a final extension at 72°C for 6 minutes. PCR products were evaluated via electrophoresis on a 1% agarose gel containing the nucleic acid stain Golden View (Biomed, Beijing, China).

DNA sequencing and phylogenetic analysis

The PCR products were purified using a TIANgel Midi Purification Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions and then subjected to bidirectional, automated sequencing (ABI 3730XL DNA Sequencer; Applied Biosystems, Foster City, CA, USA) using the same primers that were employed in the amplification process. Sequence comparisons were performed using two steps: (1) sequences were first queried against the BLAST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and (2) multiple sequence alignments were performed using the obtained amplicons and some reference sequences in Clustal W within the Lasergene software package (DNASTAR, Madison, WI, USA). Sequence editing and pcox1 and pnad1 concatenations were performed using the EditSeq program in DNASTAR. The concatenated sequences were then aligned using the Clustal W program in MEGA6 and a haplotype network was constructed using statistical parsimony in TCS1.21 (Clement *et al.*, 2000).

Results and discussion

When examining the causative agent in hydatid samples, it is necessary to obtain a molecular characterization of population genetic patterns to gain a better understanding of the distribution and diversity of *Echinococcus* globally (Nakao *et al.*, 2007). In the present study, etiological agents from 23 patients with hydatidosis that were located in epidemic areas were systematically examined by sequencing *Echinococcus cox1* and *nad1* genes. Two amplicons, pcox1 (528 bp) and pnad1 (822 bp), were successfully amplified from each specimen, with a single 1001 bp amplicon generated when using the NDI-FOR2/NDI-REV2 primers with the sample 'Qinghai1'. The 46 DNA fragments obtained were then bidirectionally sequenced.

BLAST results showed that the 1001-bp-long amplicon contains a truncated 894 bp region (at position 108 to 1001) encoding an *E. multilocularis nad1* gene. Furthermore, sequence alignments indicated that the truncated region exhibits a high sequence identity (99.6–99.9%) with representative reference *nad1* genes from *E. multilocularis* (GenBank accession nos AB018440 and AJ237639). The pcox1 gene fragment amplified from the same isolate also shared a high sequence identity (< 99.8%) with *E. multilocularis cox1* genes (GenBank accession nos AB018440 and M84668). These two sequences obtained from the 'Qinghai1' sample were submitted to the GenBank database under the accession numbers KY094609 (pnad1) and KY094608 (pcox1). These results suggest that the etiological agent in the 'Qinghai1' sample was *E. multilocularis*. Further detailed information about this isolate is available in our previous paper, where the isolate is designated as E6 (Li *et al.*, 2017).

While *E. canadensis* has been considered to have a low infectivity in humans (Eckert *et al.*, 2001), in recent years, reports from countries such as Mongolia, Austria, Poland, Argentina, USA and China have indicated that *E. canadensis* is an underestimated cause of human echinococcosis and may include variants with differing human pathogenicities (McManus *et al.*, 2002; Guarnera *et al.*, 2004; Schneider *et al.*, 2010; Jabbar *et al.*, 2011; Dybicz *et al.*, 2013; Zhang *et al.*, 2014). In one patient sample from Tibet ('Tibet14'), pcox1 exhibited a 99.4% identity with

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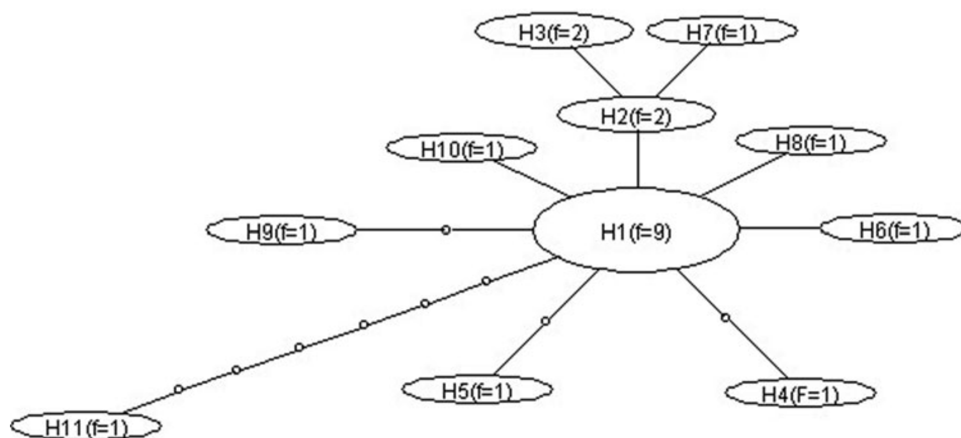

Fig. 1. Parsimonious haplotype network of mitochondrial DNA pcox1+pnad1 (1,410 bp) obtained from 21 *Echinococcus granulosus s.s.* samples. Haplotypes are represented by circles and labelled as haplotypes H1 to H11. The circle sizes approximately indicate the sample frequencies (indicated in parentheses) and each mutation event is indicated by a white circle.

Table 1. Frequency distributions for each haplotype among the 21 *E. granulosus s.s.* isolates from human CE patients in China.

Haplotype	Genotype	Total isolates	pcox1 accession no.	pnad1 accession no.	Sample IDs
H1	G1	9	MH050608	MH050620	IMG2, IMG4, IMG6, IMG7, Qinghai2, Tibet3, Tibet6, Tibet8 and Tibet11
H2	G1	2	MH050609	MH050621	Tibet10 and Tibet12
H3	G1	2	MH050610	MH050622	Tibet1 and Tibet2
H4	G1	1	MH050611	MH050623	IMG1
H5	G1	1	MH050612	MH050624	IMG3
H6	G1	1	MH050613	MH050625	IMG5
H7	G1	1	MH050614	MH050626	Tibet4
H8	G1	1	MH050615	MH050627	Tibet7
H9	G1	1	MH050616	MH050628	Tibet9
H10	G1	1	MH050617	MH050629	Tibet13
H11	G3	1	MH050618	MH050630	Tibet5

those from *E. canadensis* G6 strains (accession nos AB208063 and M84666; both from camels). However, for pnad1, a 100% identity with the *nad1* gene from *E. canadensis* G7 strain (accession no. AB235847; from a pig in Japan) was noted. The reference sequences for the *nad1* gene in the G6 (accession no. AJ237637) and G7 (accession no. AJ237638) strains are very similar and differ only at three nucleotide positions (positions 10, 333 and 438; Bowles and McManus, 1993). When examining the pnad1 sequence from the 'Tibet14' sample, two of the three nucleotides were shared with the G7 reference sequence (AJ237638), while only one of the three was identical to the G6 reference sequence (AJ237638). Thus, it was difficult to determine the specific genotype of the causative agent for this patient and this sample was instead determined to possess a G6/7 strain as previously described (Mogoye *et al.*, 2013; Nakao *et al.*, 2013b). The obtained sequences for the 'Tibet14' sample were submitted to the GenBank database under the accession numbers MH050619 (pcox1) and MH050631 (pnad1). Furthermore, the epidemiological data showed that this patient was a native Tibetan herdsman who had never lived in any camel-raising areas. Thus, we speculate that there may be a dog–goat or a dog–pig cycle that transmits the G6/7 strain in the Tibetan Plateau.

When examining pcox1 in the 'Tibet5' sample, a < 99.4% identity to some key reference sequences of *cox1* in *E. granulosus s.s.* genotype G3 (accession nos M84663 and KJ559023) was noted. Furthermore, pnad1 shared 100% identity with *nad1* genes from the G3 strain (accession nos AJ237634, from a buffalo in India, and KJ559023, from a patient in China). Therefore, the isolate was classified as *E. granulosus s.s.* G3 and the sequences were deposited in GenBank under accession numbers MH050618 (pcox1) and MH050630 (pnad1). As for all the remaining 20 isolates, their pcox1 and pnad1 gene fragments exhibited < 99.4% and < 99.7% identity with *E. granulosus s.s.* G1 mitochondrial complete genomes (accession nos AB786664 and AF297617).

In total, 11 haplotypes (labelled H1 to H11) were identified among the G1 and G3 isolates. All of the G1 isolates were classified as haplotypes H1 to H10, while only the G3 isolate was classified as H11. The constructed parsimony network showed a star-like expansion, with one common ancestral haplotype (H1)

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occupying the centre of the network. When examining only the H11 haplotype ('Tibet5', G3), seven mutations were found when comparing it to the common H1 haplotype, while only one or two mutations were detected between the other samples and their associated common haplotype (fig. 1). Following concatenation of the pcox1 and pnad1 sequences, a BLAST query of the 11 haplotypes against those previously deposited in GenBank was performed and showed that H1 shared 100% identity with many of the haplotypes for *E. granulosus s.s.* G1. Furthermore, the haplotypes were prevalent in many countries in both humans (accession nos MG672293 (Algeria), MG672257 (Kazakhstan), MG672254 (Mongolia) and MG672129 (Spain)) and animals (sheep in Iran (MG672243), Argentina (MG672253) and Italy (MG672280); cattle in Argentina (MG672252) and Turkey (MG672202); goats in Iran (MG672256) and Spain (MG672154); buffalo in India (MG672260); and a domestic pig in Mexico (MG672259)). For the remaining 10 haplotypes, no sequences with a high sequence identity were identified. Due to this study adopting new primer sets, the pcox1 and pnad1 amplicon lengths were longer than the classical reference sequences (Bowles *et al.*, 1992; Bowles and McManus, 1993). This increased length also contributed to increased sequence diversity, which may explain the difficulty in finding identical sequences for 10 of the haplotypes. Moreover, the parsimony network showed that the genetic relationships among the haplotypes were not associated with geographical divisions, as the haplotypes were seen across all of the examined regions (fig. 1, table 1). Furthermore, epidemiological examination showed that all of the patients were native herdsmen from Inner Mongolia, Tibet or Qinghai, who presumably remained local to their respective regions, without cross mobility occurring between these three regions. Moreover, these findings indicated that the regional populations of *E. granulosus s.s.* in the investigated areas were not fully differentiated from each other. Interestingly, a similar genetic structure was previously found for *E. granulosus* when examining patients in the Tibetan Plateau, eastern Tibet and Xinjiang regions (Nakao *et al.*, 2010; Yan *et al.*, 2013). While these results suggest that the population structure of *E. granulosus s.s.* may be highly uniform throughout China, a larger number of samples from both intermediate and definitive hosts in these regions should be collected and analysed for confirmation. The frequency distributions of each of the haplotypes within the 21 *E. granulosus s.s.* isolates and their relevant accession numbers are shown in table 1.

In summary, this study characterized the etiological agents in 23 hydatid patient samples from three provinces or autonomous regions. The 23 samples were found to possess *E. multilocularis* (n = 1), *E. canadensis* G6/7 (n = 1), *E. granulosus* G3 (n = 1) or *E. granulosus* G1 (n = 20). While this study provides further information about the genetic patterns of *Echinococcus* in western and northern China, future efforts will focus on the further surveillance of echinococcosis in these regions.

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Conflict of interest. None.

Ethical standards. The study protocol was approved by the Medical Ethics Review Committee of Ordos Central Hospital, Inner Mongolia, China.

References

Alvarez Rojas CA, Gauci CG and Lightowlers MW (2013) Antigenic differences between the EG95-related proteins from *Echinococcus granulosus* G1 and G6 genotypes: implications for vaccination. *Parasite Immunology* **35**, 99–102.

Alvarez Rojas CA, Romig T and Lightowlers MW (2014) *Echinococcus granulosus sensu lato* genotypes infecting humans--review of current knowledge. *International Journal for Parasitology* **44**, 9–18.

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

Bowles J, Blair D and McManus DP (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology* **54**, 165–173.

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

Cardona GA and Carmena D (2013) A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. *Veterinary Parasitology* **192**, 10–32.

Chaâbane-Banaoues R *et al.* (2016) A novel PCR-RFLP assay for molecular characterization of *Echinococcus granulosus sensu lato* and closely related species in developing countries. *Parasitology Research* **115**, 3817–3824.

Clement M, Posada D and Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657–1659.

Cucher M *et al.* (2013) *Echinococcus granulosus* pig strain (G7 genotype) protoscoleces did not develop secondary hydatid cysts in mice. *Veterinary Parasitology* **193**, 185–192.

Deplazes P *et al.* (2017) Global distribution of alveolar and cystic echinococcosis. *Advances in Parasitology* **95**, 315–493.

Dybicz M *et al.* (2013) Molecular diagnosis of cystic echinococcosis in humans from central Poland. *Parasitology International* **62**, 364–367.

Eckert J and Deplazes P (2004) Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clinical Microbiology Reviews* **17**, 107–135.

Eckert J *et al.* (1993) Further evidence for the occurrence of a distinct strain of *Echinococcus granulosus* in European pigs. *Parasitology Research* **79**, 42–48.

Eckert J *et al.* (2001) *WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern*. World Organization for Animal Health, Paris, France.

Guarnera EA *et al.* (2004) Cystic echinococcosis in Argentina: evolution of metacestode and clinical expression expression in various *Echinococcus granulosus* strains. *Acta Tropica* **92**, 153–159.

Ito A and Budke CM (2017) The echinococcoses in Asia: the present situation. *Acta Tropica* **176**, 11–21.

Jabbar A *et al.* (2011) A first insight into the genotypes of *Echinococcus granulosus* from humans in Mongolia. *Molecular and Cellular Probes* **25**, 49–54.

Kedra A *et al.* (1999) Genetic analysis of *Echinococcus granulosus* from humans and pigs in Poland, Slovakia and Ukraine. A multicenter study. *Acta Parasitologica* **44**, 248–254.

Li W, Gao JL and Wang HB (2017) Molecular taxonomy of a hydatid disease specimen based on mitochondrial COI, NDI and 12S rRNA gene sequences. *Chinese Journal of Health Laboratory Technology* **27**, 1983–1986. (in Chinese)

McManus DP (2006) Molecular discrimination of taeniid cestodes. *Parasitology Internaltional* **55** Suppl, 31–37.

McManus DP (2013) Current status of the genetics and molecular taxonomy of *Echinococcus* species. *Parasitology* **140**, 1617–1623.

https://doi.org/10.1017/S0022149X18000883 Published online by Cambridge University Press

McManus DP *et al.* (2002) Short report: Molecular genetic characterization of an unusually severe case of hydatid disease in Alaska caused by the cervid strain of *Echinococcus granulosus*. *The American Journal of Tropical Medicine and Hygiene* **67**, 296–298.

Mogoye BK *et al.* (2013) First insights into species and genotypes of *Echinococcus* in South Africa. *Veterinary Parasitology* **196**, 427–432.

Nakao M *et al.* (2007) A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitology* **134**, 713–722.

Nakao M *et al.* (2010) Genetic polymorphisms of *Echinococcus* tapeworms in China as determined by mitochondrial and nuclear DNA sequences. *International Journal for Parasitology* **40**, 379–385.

Nakao M *et al.* (2013a) Phylogenetic systematics of the genus *Echinococcus* (Cestoda: taeniidae). *International Journal for Parasitology* **43**, 1017–1029.

Nakao M *et al.* (2013b) Mitochondrial phylogeny of the genus *Echinococcus* (Cestoda: Taeniidae) with emphasis on relationships among *Echinococcus Canadensis* genotypes. *Parasitology* **140**, 1625–1636.

Rostami Nejad M *et al.* (2012) Molecular identification of animal isolates of *Echinococcus granulosus* from Iran using four mitochondrial genes. *Journal of Helminthology* **86**, 485–492.

Schneider R *et al.* (2010) *Echinococcus canadensis* G7 (pig strain): an underestimated cause of cystic Echinococcosis in Austria. *The American Journal of Tropical Medicine and Hygiene* **82**, 871–874.

Siracusano A *et al.* (2012) Host–parasite relationship in cystic echinococcosis: an evolving story. *Clinical & Developmental Immunology* **2012**, 639362.

Soriano SV *et al.* (2010) Molecular characterization of *Echinococcus* isolates indicates goats as reservoir for *Echinococcus canadensis* G6 genotype in Neuquén, Patagonia Argentina. *Parasitology International* **59**, 626–628.

Thompson RC (2008) The taxonomy, phylogeny and transmission of *Echinococcus*. *Experimental Parasitology* **119**, 439–446.

Wang J *et al.* (2014) Genetic diversity of *Echinococcus granulosus* in southwest China determined by the mitochondrial NADH dehydrogenase subunit 2 gene. *Scientific World Journal* **2014**, 867839.

Yan N *et al.* (2013) Genetic variability of *Echinococcus granulosus* from the Tibetan Plateau inferred by mitochondrial DNA sequences. *Veterinary Parasitology* **196**, 179–183.

Yang D *et al.* (2015) The first report of human-derived G10 genotype of *Echinococcus canadensis* in China and possible sources and routes of transmission. *Parasitology International* **64**, 330–333.

Zhang LH *et al.* (1998) Mitochondrial genomic markers confirm the presence of the camel strain (G6 genotype) of *Echinococcus granulosus* in northwestern China. *Parasitology* **116**, 29–33.

Zhang T *et al.* (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**(10), e109059.

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