

Molecular characterization and B-cell epitope analysis of the TSP11 gene in *Echinococcus* infection strains from Yunnan Province

Research Article

Cite this article: Xu Q *et al* (2024). Molecular characterization and B-cell epitope analysis of the TSP11 gene in *Echinococcus* infection strains from Yunnan Province. *Parasitology* **151**, 1108–1117. <https://doi.org/10.1017/S0031182024000726>


Received: 8 January 2024
Revised: 30 March 2024
Accepted: 24 May 2024
First published online: 12 November 2024

Keywords:

B-cell epitope; *Echinococcus*; *E. granulosus*; genotype; TSP11 gene; Yunnan

Corresponding author:

Ying Dong;
Email: luxidongying@126.com;
Yaming Yang;
Email: yangym@126.com

Qian Xu , Zhengqing Wang, Jinrong Zi, Xuan Cai, Fangwei Wu, Benfu Li, Jia Peng, Jianxiong Li, Xinliu Yan, Ying Dong and Yaming Yang

Yunnan Institute of Parasitic Diseases, Puer, 665000, China

Abstract

This study investigates the molecular intricacies of the transmembrane protein TSP11 gene in *Echinococcus* strains isolated from livestock and patients in Yunnan Province afflicted with *Echinococcus granulosus* (*E. granulosus*) between 2016 and 2020. Gene typing analysis of the ND1 gene revealed the presence of the G1 type, G5 type and untyped strains, constituting 52.4, 38.1 and 9.5%, respectively. The analysis of 42 DNA sequences has revealed 24 novel single nucleotide polymorphic sites, delineating 11 haplotypes, all of which were of the mutant type. Importantly, there were no variations observed in mutation sites or haplotypes in any of the hosts. The total length of the TSP11 gene's 4 exons is 762 bp, encoding 254 amino acids. Our analysis posits the existence of 6 potential B-cell antigenic epitopes within TSP11, specifically at positions 49-KSN-51, 139-GKRG-142, 162-DNG-164, 169-NGS-171, 185-DS-186 and 231-PPRFTN-236. Notably, these epitopes exhibit consistent presence among various intermediate hosts and haplotypes. However, further validation is imperative to ascertain their viability as diagnostic antigens for *E. granulosus* in the Yunnan Province.

Introduction

Echinococcosis, also known as hydatid disease, is a persistent cystic zoonotic parasitic disease that affects humans and both domestic and wild odd-toed ungulates. It is caused by the larval stage of the dog tapeworm, a member of the *Echinococcus*, family Taeniidae, specifically *E. granulosus* (Chi, 2015).

Designated by the World Health Organization as one of the 20 easily neglected tropical diseases, *E. granulosus* is regionally endemic throughout Europe, North and East Africa, Central Asia, the Middle East, Central and South America and Australia (World Health Organization, 2021; Hogeia *et al.*, 2024), it currently affects approximately 3 million patients with this disease, leading to an estimated annual economic loss of 760 million USD. In the livestock industry, the infection of domestic animals by the larval stage of *Echinococcus* results in economic losses exceeding 3 billion USD annually due to reduced weight and lower fertility (Budke *et al.*, 2006; Otero-Abad and Torgerson, 2013; Oian *et al.*, 2017).

China bears one of the highest incidences of hydatid disease, with primary endemic areas including Xinjiang, Sichuan, Qinghai, Tibet, Gansu, Ningxia, Inner Mongolia and semi-agricultural and semi-pastoral regions (Sivalingam and Shepherd, 2012). Incomplete statistics indicate that by the end of 2016, over 368 counties (districts) in China had been affected by hydatid disease, with an incidence rate among the population ranging from 0.6 to 4.5%, totaling approximately 170 000 patients (Kolaskar and Tongaonkar, 1990; Jiang, 2002). Hydatid disease constitutes 40% of the global burden (Wu, 2017). In Yunnan Province, *Echinococcus* endemic areas are concentrated primarily west of 25°N latitude, in regions highly affected by hydatid disease, including Ganzi in Sichuan, Changdu in Tibet and others (Qiu *et al.*, 2000; Huang *et al.*, 2012; Lei and Wang, 2012). Notably, counties (districts) such as Deqen and Dali exhibit an incidence rate of 0.06% among the population, with all infections identified as *E. granulosus*. However, the Diqing region also demonstrates high infection rates among animal hosts, indicating a potential risk of natural focal transmission (Li *et al.*, 2019; Li *et al.*, 2020).

Mitigating the disease burden of human echinococcosis involves both effective patient treatment and accurate diagnoses. Presently, the primary treatment for hydatid disease involves the surgical excision of lesion tissue and chemotherapy (Wen *et al.*, 2015). While surgical intervention can promptly alleviate the harm caused by hydatid disease, it is associated with high recurrence rates and numerous postoperative complications (Guo *et al.*, 2019), necessitating adjunctive drug therapy (Aghayev, 2016). Very few adverse events have been reported by treatment with albendazole, however, even a single dose treatment (for empirical or seasonal use) of albendazole (400 mg) could cause acute liver toxicity in adult patients (Chai *et al.*, 2021).

Conversely, diagnostic methods reliant on imaging technology are not conducive to clear diagnoses of early-stage, non-cystic patients with echinococcosis (Craig *et al.*, 2007). Consequently, immunodiagnostic tools have gained widespread use in recent years for

© The Author(s), 2024. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

epidemiological screening and early diagnosis of echinococcosis (Li and Gao, 2017). Nevertheless, the antigen employed for enzyme-linked immunosorbent assay (ELISA) detection plates typically comprises echinococcosis cyst fluid. The complexity of protein components in the cyst fluid compromises the accuracy of detecting *Echinococcus* due to cross-reactivity with other tapeworms of the family Diplostriaenidae. Therefore, selecting specific and highly sensitive coating antigens has become a focal point of current research (Chow *et al.*, 2004; Liu *et al.*, 2015; Xu *et al.*, 2018). Notably, the 4-transmembrane protein (TSP11) on the surface of *Echinococcus*, identified for its pivotal role in stimulating the host's acquired immune response, serves as a specific marker for echinococcosis infection (Wang *et al.*, 2017). This current study aimed to analyse the polymorphism of the TSP11 gene distributed in Yunnan and its surrounding areas and investigation of B antigenic determinant cluster of this protein for use as a diagnostic antigen.

Materials and methods

Study sample

Throughout the implementation of the 'Yunnan Province Hydatid Disease Monitoring Program' from 2016 to 2020, post-slaughter inspections and parasitic infection checks were carried out on the organs of livestock (hosts) including cattle, pigs and sheep, which displayed cysts, cystic formations or nodules. Following slaughter, we collected organ tissues with cystic masses and fluid from lesions for parasitic infection examination. The samples encompassed single-cystic, multi-vesicular and collapsed internal cyst types of hepatic hydatid disease with an average cyst diameter >5 cm. Additionally, various types of hepatic hydatid with an average cyst diameter <5 cm, situated in the first or second porta hepatis and likely to cause severe complications (such as obstructive jaundice, portal hypertension, Budd–Chiari syndrome) were included. We also considered various types of hepatic hydatid disease where drug adverse reactions were significant (hepatorenal dysfunction and other detrimental side effects), or patients struggled to adhere to medication, or the cyst continued to enlarge after more than 6 months of drug treatment. In these cases, tissues from surgically removed cystic masses and fluid from lesions were gathered for parasitic infection examination (National Health Commission of the People's Republic of China, 2017).

Microscopic confirmation of echinococcosis infection and its genotyping

The collected tissues from affected organs and fluid from lesions underwent immediate optical microscopy (10–40×) to identify

Echinococcus cyst walls, daughter cysts, protoscoleces or small hooks (Zhu and Su, 2019).

For samples testing positive for *E. granulosus* infection, we further sequenced the ND1 gene to identify the genotype. PCR amplification of the ND1 gene for types 1 and 5 utilized primers designed with reference sequences MN199128.1 (<https://www.ncbi.nlm.nih.gov/nucleotide/MN199128.1>) and KY766908.1 (<https://www.ncbi.nlm.nih.gov/nucleotide/KY766908.1>) (Table 1) (Li *et al.*, 2023).

Design of primers for *Echinococcus* TSP11 gene

The primers for amplifying the *Echinococcus* TSP11 gene were designed based on the reference sequence (XP_024352489.1) (Huang *et al.*, 2017) (Table 1).

Echinococcus DNA extraction and nested PCR amplification of ND1 and TSP11 gene

Approximately 20 mg of tissue exhibiting cyst-like changes was excised from the affected organs and cut into 5–10 mm fragments. DNA extraction of *Echinococcus* was conducted following the guidelines of the DNA extraction kit, and the extracted DNA was stored at –20°C for subsequent use.

The nested PCR amplification for NAD1 and TSP11 genes involved 4 reaction systems, each comprising DNA template (2.5 µL), 2 × Taq enzyme (14.0 µL), primer forward and reverse (10 µmol/L each, 0.7 µL), and ddH₂O (7.1 µL). The PCR reaction conditions were set at 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 55°C for 45 sec and 72°C for 1 min 30 sec, concluding with 72°C for 10 min (Han and Gao, 2020). The second-round amplification products were visualized through 2% agarose gel electrophoresis, and the resulting products were sent to Guangzhou Tianyi Huiyuan Genetic Technology Co., Ltd., for bidirectional sequencing.

TSP11 gene polymorphism and evolutionary relationships of sequences from different host sources

The PCR product sequencing results were compiled using DNASTar 11.0 or BioEdit 7.2.5 software to generate DNA sequences for the NAD1 and TSP11 genes. The organized sequences of 2 genes were individually analysed using BLAST, comparing them against the reference sequences MN199128.1 and XP_024352489.1. The query coverage and identity were examined, and when both query coverage and identity exceeded 98%, it was indicative that the organized sequencing sequences represented the target sequences. A match of both coverage (Query cover) and similarity (Identifies) exceeding 98% confirmed the compiled sequences as the target sequences. The coding DNA sequences (CDS) for the 4 exons encoding the TSP11

Table 1. Primer sequence and other information

Gene name	Primer name	Sequence (5'-3')	Length of fragment/bp	Amplification region
TSP11	TSP11-F1	TGTTTTCTATTTTTACCCACTAGAT	1230	85 417–86 646
	TSP11-R1	TTTAAAGTTAATTAGGAGAAGGACG		
	TSP11-F2	TATTAATCCAGCTTTAAGGTCATTT	1021	
	TSP11-R2	TATTTGTCCAGATAGGATTTAATGG		
NAD1	NAD1-F1	TGTTACTTGTTACTCCATTGTCT	1486	6869–8354
	NAD1-R1	TATCAAAGTAACCTGCTATGCAG		
	NAD1-F2	TTTAAGTTGGGTGGTGATTA	1232	
	NAD1-R2	TTGAAGTTAACAGCATCACG		

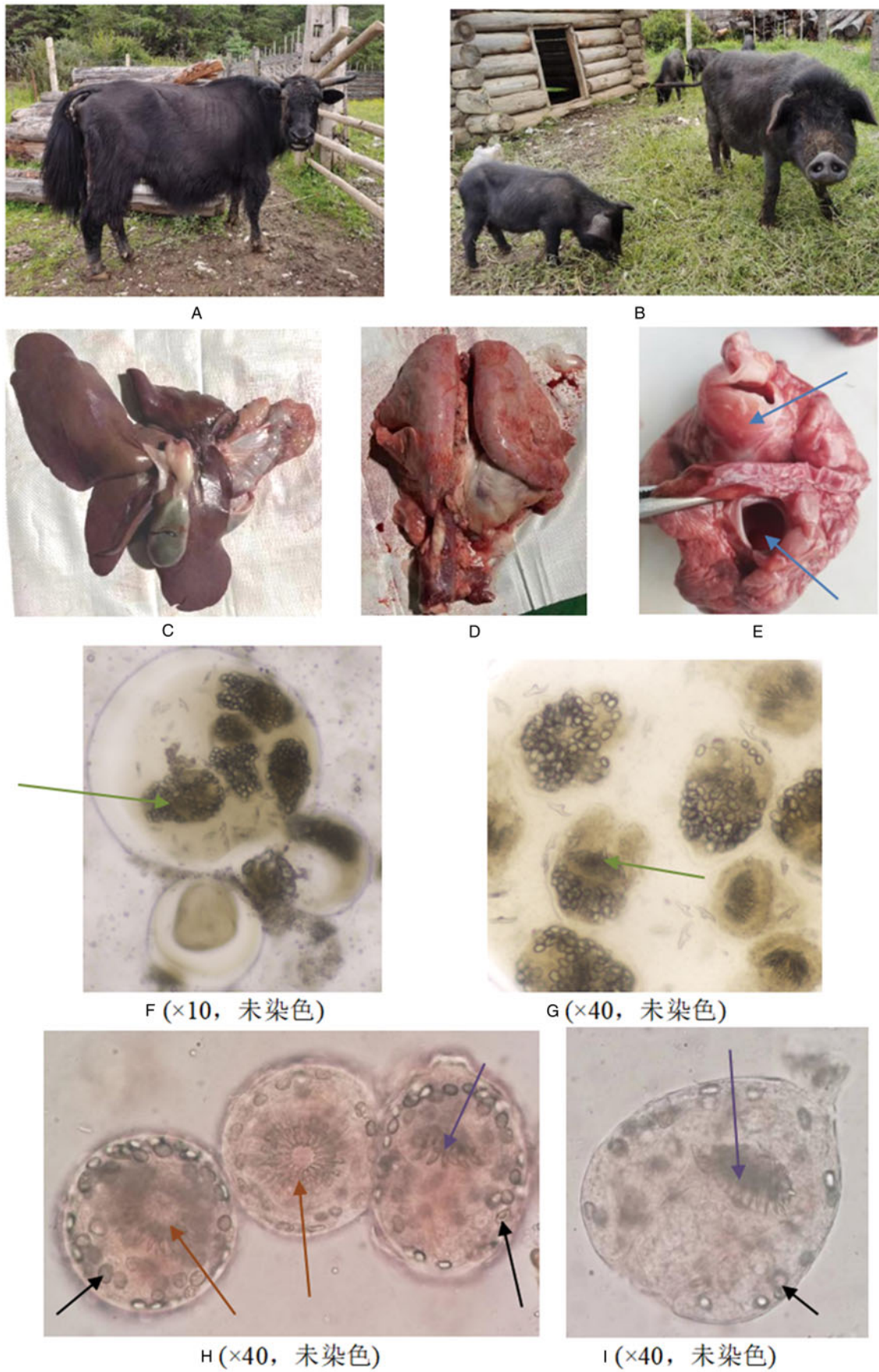


Figure 1. Intermediate host, diseased organ, protocephalic segment of *Echinococcus granulosus*

Note: a: Yak from Weixi County, Diqing Prefecture; b: Diqing Tibetan pig; c: the liver; d: lungs; e: diseased part of the lung (arrow points to the diseased part); f, g: hydatid (40X); h, i: protoscolex (10X)

gene were concatenated from the 5' to 3' end (Xu *et al.*, 2020). Amino acid sequences were deduced using MEGA 7.0.26 software for TSP11 and the alignment file. DnaSP 5.10 software was employed to identify haplotypes, single-nucleotide polymorphism (SNP) sites, and their mutation types (synonymous/non-synonymous) for the 4 exons of the TSP11 gene. Expected heterozygosity (He) and nucleotide diversity (π) were calculated for each haplotype (Dong *et al.*, 2019 and Xu *et al.*, 2020). All base substitutions were verified by examining the sequencing peak charts. Network 10.0 software was used to create intermediate network evolutionary diagrams for each haplotype.

Prediction of B-cell antigenic determinants of different haplotypes of the TSP11 gene

B-cell epitopes of TSP11 were predicted using the IEDB online platform (<https://www.iedb.org/>) and the 'Protein' module of DNASTar 11.0. Comparative analysis of different hydrophilicity plots (Ponomarenko *et al.*, 2011), accessibility (Allcorn and Martin, 2002), flexibility (Li *et al.*, 2016), antigenicity (Resende *et al.*, 2012) and β -turn regions among the amino acid chains of different TSP11 haplotypes (Hu *et al.*, 2014) was conducted. Parameters such as hydrophilicity, antigenic index, flexibility and β -turn were assessed to identify common B-cell antigenic epitopes among different intermediate hosts and haplotypes. Points with the highest local average hydrophilicity were often situated at or adjacent to the antigenic determinant clusters (epitopes). Surface accessibility prediction considered the likelihood of amino acid residues in the antigen coming into contact with corresponding antibodies or solvent molecules. Polar amino acids, more likely to be exposed on the protein surface, were deemed probable components of antigenic epitopes. Amino acid residues with high activity represented flexible sites likely to form antigenic epitopes. The β -turn region and structurally loose, prominently exposed, deformable and twisted areas in irregularly coiled regions were identified as potential antigenic epitope regions exposed on the protein surface that could easily bind to antibodies.

Statistical analysis

Establishing a counting database within Excel software, we conducted chi-square tests to analyse the distribution variances among intermediate hosts exhibiting different genotypes of

Echinococcus, nucleotide peptides of the TSP11 gene, and disparities in haplotype detection rates across diverse intermediate hosts, maintaining a significance level of $\alpha = 0.05$.

Results

Sample collection sites and Echinococcus infection

Between 2016 and 2020, a comprehensive total of 42 samples of visceral tissues from *Echinococcus* were meticulously collected and subjected to rigorous testing. These samples comprised 13 of human origin (4 from Jianchuan County, 4 from Ganzi Prefecture, 3 from Yulong County and 2 from Weixi County), 13 sourced from pigs (5 from Dagan County, 3 from Eryuan County, 3 from Lushui City, 1 from Shangri-La and 1 from Weixi), 14 originating from cattle (as illustrated in Fig. 1a, b) (13 from Shangri-La and 1 from Weixi) and 2 derived from sheep (1 from Weixi and 1 from Honghe). These samples encompassed 37 instances of diseased liver tissues and 6 cases of diseased lung tissues (as depicted in Figs 1c–e) (refer to Supplementary Material 1 for further details).

Echinococcus was detected in all 42 samples of infected tissues (Fig. 1f–i). Under microscopic examination, structures with double-layered cyst walls enclosing fluid and sand-like bodies were observed. The inner layer of the cyst wall was pink, and the cyst fluid was either transparent or slightly turbid. Small hooks floated in the fluid, and internally, elliptical or circular structures resembling 'original heads' were observed, which were invaginated and contracted (Fig. 1f, g, green arrows). Inside the 'original heads,' brown elliptical structures were identified as calcareous corpuscles (Fig. 1h, i, black arrows). The wheel-shaped structure represented a sucker (Fig. 1h, brown arrows), and the bushy structure featured small hooks (Fig. 1h, i, purple arrows).

Sequencing of gene PCR amplification products

PCR amplification of TSP11 and ND1 genes produced target bands of approximately 1021 and 1232 bp respectively (Fig. 2).

42 ND1 Gene Sequences of the 42 ND1 genes exhibited greater than 98% similarity with the reference sequences MN199128.1 and KY766908.1. Among these, the ND1 gene DNA sequences of 22 samples were identical to MN199128.1, with a length of 894 bp, classifying them as Type 1. 16 samples exhibited ND1 gene DNA sequences identical to KY766908.1, also with a length

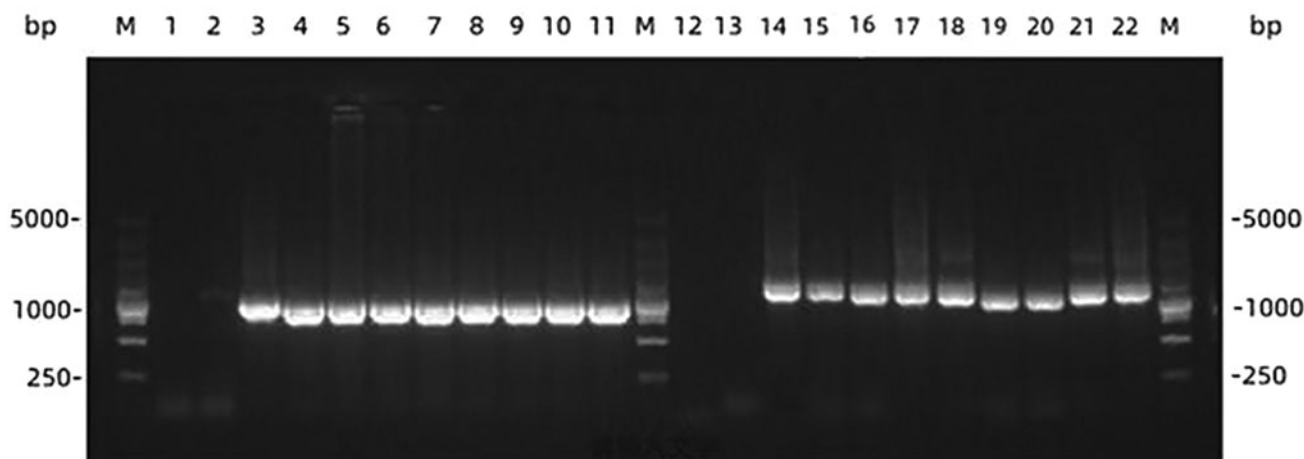


Figure 2. Electrophoresis Map of PCR Amplified Products of TSP11 Gene and ND1 Gene in the Pathological Organs of *Echinococcus granulosus*

Note: M: DNA marker; 1, 2: Negative control of the first and second rounds of PCR of TSP11 gene; 3: TSP11 gene PCR positive control; 12, 13: Negative control of ND1 gene in the first and second rounds of PCR; 14: ND1 gene positive control; 4, 5, 6, 7, 8, 9, 10: amplification products of TSP11 gene; 16, 17, 18, 19, 20, 21, 22: amplification products of ND1 gene

Table 2. Interspecific difference of ND1 gene

Order	Somatotype (According to ND1)	intermediate host				p
		Person (n = 13)	Pig (n = 13)	Cow (n = 14)	Sheep (n = 2)	
1	G1 (n = 22, 52.4%)	10 (45.4)	4 (18.2)	8 (36.4)	0	0.045
2	G5 (n = 16, 38.1%)	2 (12.5)	8 (50.0)	5 (31.3)	1 (6.2)	0.083
3	Undefined type (n = 4, 9.5%)	1 (25.0)	1 (25.0)	1 (25.0)	1 (25.0)	0.0428

of 894 bp, categorizing them as Type 5. For the remaining 4 samples, the similarity to both MN199128.1 and KY766908.1 did not reach 100%, designating them as an undefined genotype (Table 2).

In all, 3 distinct genotypes were identified: Type 1, Type 5 and undefined, constituting 52.4, 38.1 and 9.5% of the total, respectively. While all 3 genotypes were detectable across intermediate hosts including humans, pigs, cattle and sheep, the discrepancies in their detection rates did not reach statistical significance ($P = 0.045$, $P = 0.083$ and $P = 0.0428$) (refer to Table 2).

Nucleotide diversity of TSP11 gene CDS sequences

Sequencing the TSP11 gene from the 42 *Echinococcus* samples resulted in complete coding DNA sequences (CDS), each comprising 4 exons with a length of 762 bp. These sequences exhibited

greater than 98% similarity with the reference sequence XP_024352489.1. The nucleotide diversity index (π) was calculated to be 0.00413. There were 24 polymorphic sites, with the most frequent biallelic site being c.127 (42.9%, 18/42), and the minor allele frequency (MAF) for c.192 was 26.2% (11/42). There were 8 singleton variable sites and 16 parsimony informative sites (2 variants). Among the 24 polymorphic sites, 66.7% (16/24) were located at the third base of amino acid codons, and only 6.25% (1/16) of base substitutions resulted in amino acid variations. The proportions for the second and first base positions were 8.3% (2/24) and 25.0% (6/24), respectively (Table 3). The detection rates of these 24 polymorphic sites in sequences from different intermediate hosts such as humans, pigs, cattle and sheep showed no statistically significant differences.

Table 3. Single nucleotide polymorphism in the TSP11 gene of *Echinococcus granulos*

Order	Loci	Alleles	Coding	Amino acid variation	Frequency No. of all CDSs (n = 42,%)	intermediate host				P
						Person (n = 13,%)	Pig (n = 13,%)	Cow (n = 14,%)	Sheep (n = 2,%)	
1	c.46	G > A	GCA/ACA	A16T	3 (7.1)	1 (7.7)	0	2 (14.2)	0	1.000
2	c.64	G > C	GGG/CGG	G22R	1 (2.4)	0	0	1 (7.1)	0	/
3	c.91	G > C	GTG/CTG	V31L	4 (9.5)	1 (7.7)	0	3 (21.4)	0	0.596
4	c.97	T > C	TTT/CTT	F33L	4 (9.5)	1 (7.7)	0	3 (21.4)	0	0.596
5	c.127	A > G	ATT/GTT	L43V	37 (88.1)	12	13 (100)	10 (71.4)	2 (100)	0.172
6	c.192	G > A	GGG/GGA	G64G	11 (26.2)	1 (7.7)	4 (30.8)	5 (11.9)	1 (50)	0.258
7	c.229	T > A	TTC/ATT	F77L	3 (7.1)	1 (7.7)	0	2 (14.2)	0	1.000
8	c.231	C > T	TTC/ATT	F77L	3 (7.1)	1 (7.7)	0	2 (14.2)	0	1.000
9	c.234	T > C	CTT/CTC	L78L	1 (2.4)	1 (7.7)	0	0	0	/
10	c.249	G > A	TCG/TCA	S83S	4 (9.5)	1 (7.7)	0	3 (21.4)	0	0.596
11	c.252	T > C	ATT/ATC	I84I	3 (7.1)	1 (7.7)	0	2 (14.2)	0	1.000
12	c.333	A > G	GCA/GTG	A111A	1 (2.4)	1 (7.7)	0	0	0	/
13	c.492	C > T	GGC/GGT	G164G	4 (9.5)	1 (7.7)	0	3 (21.4)	0	0.596
14	c.551	T > C	GTC/GCC	V184A	3 (7.1)	1 (7.7)	0	2 (14.2)	0	1.000
15	c.582	C > T	TTC/TTT	F194F	1 (2.4)	0	0	1 (7.1)	0	/
16	c.600	A > G	AAA/AAG	K200K	1 (2.4)	0	0	1 (7.1)	0	/
17	c.606	C > T	TGC/TGT	C202C	1 (2.4)	0	1 (7.7)	0	0	/
18	c.672	C > T	TGC/TGT	C224C	4 (9.5)	1 (7.7)	0	3 (21.4)	0	0.596
19	c.729	A > C	CTA/CTC	L243L	3 (7.1)	1 (7.7)	0	2 (14.2)	0	1.000
20	c.741	A > C	GTA/GTC	V247V	1 (2.4)	0	0	1 (7.1)	0	/
21	c.744	C > T	AGC/AGT	S248S	4 (9.5)	1 (7.7)	0	3 (21.4)	0	0.596
22	c.747	C > T	TCC/TCT	S249S	3 (7.1)	1 (7.7)	0	2 (14.2)	0	1.000
23	c.758	A > C	TAT/TCT	Y253S	1 (2.4)	0	0	1 (7.1)	0	/
24	c.759	T > C	TAT/TAC	Y253Y	4 (9.5)	1 (7.7)	0	3 (21.4)	0	0.596

Multiple mutations and evolutionary analysis of TSP11 gene CDS sequences

The alignment of the 42 TSP11 gene CDS sequences with the reference sequence (XP_024352489.1) revealed the presence of 11 haplotypes. Haplotype Hap_1 perfectly matched the reference sequence, with the remaining 10 haplotypes (Hap_2 to Hap_11) representing mutated forms of the reference sequence (XP_024352489.1), resulting in expected heterozygosity (He) of 0.6622. Among them, Hap_2 had the highest frequency (57%, 24/42), followed by Hap_3 (19%, 8/42) and Hap_8 (4.8%, 2/42), with the remaining haplotypes each accounting for (2.4% 1/42) (Table 4). The mildest mutations were observed in Hap_2 (57%, 24/42) and Hap_9 (2.4%, 1/42), while the most intense mutation was observed in Hap_8 (4.8%, 2/42). Hap_4, Hap_5 and Hap_11 were exclusive to human samples, Hap_6 was only found in pig samples, and Hap_7, Hap_8, Hap_9, Hap_10 and Hap_12 were solely present in cattle samples. Both Hap_2 and Hap_3 were detected in samples from humans, pigs, cattle and sheep, with no statistically significant differences among the 4 groups ($P = 0.197$ and $P = 0.387$) (Table 4).

The network diagram illustrated that the 11 haplotypes (Hap_2 to Hap_12) evolved from the reference sequence (XP_024352489.1) (Hap_1) through 1 mutation (Hap_2, Hap_9), progressing to 2 mutations (Hap_3, Hap_4, Hap_5, Hap_12), 3 mutations (Hap_6), 6 mutations (Hap_7), 8 mutations (Hap_10), 12 mutations (Hap_11) and 13 mutations (Hap_8). Notably, Hap_8 and Hap_11 shared the c.229 and c.231 positions, representing the same protein F77L, counted as 1 mutation in Table 4. Additionally, Hap_11 had 2 mutations at position c.747, reverting to the wild-type allele S (S249), making the joint mutation multiplicity higher in Hap_8 and Hap_11 than indicated in Table 4 (Fig. 3).

Prediction of B-cell antigen precursors in the amino acid chain of the TSP11 gene

The translation of the 11 haplotypes (Hap_2 to Hap_12) of the TSP11 gene CDS into amino acid chains, followed by prediction

using IEBD and DNA Star software, revealed 5 and 10 B-cell antigenic determinant clusters, respectively (Table 5). These clusters had lengths ranging from 3 to 40 amino acids, with an average hydrophilicity value of 0.460 (see Supplementary Material 2 for detailed results). The predicted regions, such as 49-KSN-51, 139-GKRG-142, 162-DNG-164, 169-NGS-171, 185-DS-186 and 231-PPRFTN-236, were identified as 6 conserved B-cell antigenic epitopes among the 11 amino acid chains (Table 5).

Discussion

This study builds upon both morphological and genetic confirmation of *E. granulosus* sensu lato, focusing on the identification of B-cell antigenic epitopes in the TSP11 protein of the protoscolex of Yunnan Province and its surrounding regions. The diversity of the ND1 gene sequence presently allows the classification of *E. granulosus* into 10 genotypes (Yang *et al.*, 2015) and 5 strains. Specifically, G1–G3 represent *E. granulosus* strains (*Echinococcus granulosus* sensu stricto), with G1 for the sheep strain, G2 for the Tasmanian sheep strain (this genotype is not currently recognized as valid) and G3 for the water buffalo strain (Omadang *et al.*, 2024). The remaining strains, G4–G10, include the horse strain (G4 – *Echinococcus equinus*), Ortlepp's strain (G5 – *Echinococcus ortleppi*) and the Canadian strain (G6–G10) comprising the camel strain (G6), pig strain (G7), deer strain (G8), Poland strain (G9) (this genotype is not currently recognized as valid) and elk strain (G10) (Nakao *et al.*, 2007; 2013; Wassermann *et al.*, 2024). The predominant genotypes observed among the *E. granulosus* samples in this study were G1 and G5, with G1 being the most prevalent. However, the differences in detection rates of G1 and G5 genotypes across various intermediate hosts such as humans, pigs, cattle and sheep did not attain statistical significance, indicating an absence of bias in host sources. However, its prevalence was slightly lower than the previously reported detection rates of 98.1 and 97.9% in Chinese populations and animals (Alvarez *et al.*, 2014; Zhang *et al.*, 2014). This discrepancy may be attributed to the high sensitivity

Table 4. Different haplotypes having mutations in TSP11 gene

Haplotype	Multiple mutants of different degrees		Frequency No. of all CDSs (n = 42, 100%)	intermediate host				p
	Type	Multiplicity		person (n = 13, 31.0%)	pig (n = 13, 31.0%)	cow (n = 14, 33.3%)	sheep (n = 2, 4.8%)	
Hap_2	L43V	1	24 (57)	9 (69.2)	9 (69.2)	5 (35.7)	1 (50)	0.197
Hap_9	G64G	1	1 (2.4)	0	0	1 (7.1)	0	/
Hap_3	L43V/G64G	2	8 (19)	1 (7.7)	3 (23.1)	3 (21.4)	1 (50)	0.387
Hap_4	L43V/L78L	2	1 (2.4)	1 (7.7)	0	0	0	/
Hap_5	L43V/A111A	2	1 (2.4)	1 (7.7)	0	0	0	/
Hap_12	L43V/K200K	2	1 (2.4)	0	0	1 (7.1)	0	/
Hap_6	L43V/G64G/C202C	3	1 (2.4)	0	1 (7.7)	0	0	/
Hap_7	G22R/L43V/F194F/V247V/Y253S	6	1 (2.4)	0	0	1 (7.1)	0	/
Hap_10	V31L/F33L/S83S/G164G/ C224C/S248S/S249S/Y253Y	8	1 (2.4)	0	0	1 (7.1)	0	/
Hap_11	A16T/V31L/F33L/F77L/S83S/ I84I/G164G/V184A/C224C/ L243L/S248S/Y253Y	12	1 (2.4)	1 (7.7)	0	0	0	/
Hap_8	A16T/V31L/F33L/F77L/S83S/ I84I/G164G/V184A/C224C/ L243L/S248S/S249S/Y254Y	13	2 (4.8)	0	0	2 (14.3)	0	/



Figure 3. Evolutionary network of haplotype of TSP11 gene in the diseased organs of *Echinococcus granulosus*.
 Note: The size of the circle is proportional to the number of isolates showing a particular haplotype; lines represent evolutionary steps connecting haplotypes.

of the ND1 full-gene sequence alignment utilized for genotyping in this study, resulting in 9.5% of the sample sequences being unclassified according to the published reference sequences.

The Tetraspanin (TSP) family is a crucial component of the tetraspanin-enriched membrane microdomain (TEM) superfamily, comprising 4 main subfamilies: the CD family (CD9, CD81 and CD151), the slow retinal degeneration (RDS) family (RDS-ROM), the uroplakin family (UPK1A/1B) and the CD63 family (CD63 and TSPAN31). These proteins, ranging from 200 to 350 amino acids (Hu et al., 2015; Xian et al., 2021), exhibit a structural composition that includes 2 extracellular domains known as the small extracellular loop (EC1) and large extracellular loop (EC2/LEL), an intracellular loop (transitioning from structural

domain 2–3), and N- and C-terminal tails. Among the 4 highly conserved transmembrane domains (TM1–4) of TSP, the EC2/LEL structural domain, referred to as the ‘Tetraspanin Structural Web,’ serves as a binding site for numerous ligand proteins and stands out as an area of concentration for anti-TSP antibodies (Piratae et al., 2012; Graham et al., 2020; Ahmed et al., 2021).

TSP11 has long been recognized as a promising candidate protein for vaccine development targeting various diseases, including schistosomiasis (Tran et al., 2006; Cardoso et al., 2008; Jiang et al., 2010; Zhang et al., 2011), clonorchiasis (Kim et al., 2012; Chaiyadet et al., 2017), opisthorchiasis (Piratae et al., 2012; Tomii et al., 2019; Phumrattanaprapin et al., 2021), Manson’s schistosomiasis (Pearson et al., 2012; Cheng et al., 2013; Curti

introduces 24 novel nucleotide peptide sites and uncovers 11 haplotypes for the TSP11 transmembrane protein in Yunnan, all representing mutated forms. Proposing 6 potential B-cell antigenic epitopes in TSP11, the study maintains consistency among different intermediate hosts and haplotypes, laying the groundwork for considering TSP11 protein as a candidate antigen for diagnosing different species of *E. granulosus* protoscoleces, offering theoretical support for the diagnosis of *E. granulosus*.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0031182024000726>.

Data availability statement. Availability of Data: The materials and data related to this study are currently unavailable.

Acknowledgements. We express our gratitude to the disease prevention and control centers in the provinces and cities of Diqing, Dali, Nujiang, and Lijiang in Yunnan for their wholehearted support.

Author contributions. Xu Qian: Conducted PCR amplification experiments, data analysis, and manuscript writing. Dong Ying: Formulated the experimental plan, provided technical guidance, and ensured the quality control of the paper. Wang Zhengqing: Participated in experimental operations. Yang Yaming, Zi Jinrong, Cai Xuan, Wu Fangwei, Li Benfu, Peng Jia, Li Jianxiang, Yan Xinliu: Involved in sample collection and participated in pathogen diagnostics.

Financial support. This project received support from the Science and Technology Talent Platform Plan (Academician Workstation), with project number 202305AF15067, and the Key Laboratory for Research on Prevention and Treatment of Hydatid Disease of the National Health Commission, with funding number 202103.

Competing interests. The authors declare no conflicts of interest.

Ethical standards. The samples were obtained from discarded tissues generated during the surgical treatment of echinococcosis patients and the exploration of the pathogenic nature of deceased animal diseases. The research did not involve the personal privacy of the research subjects during the pathogenic research.

References

- Aghayev RM (2016) Liver echinococcosis complicated with lesions of bile ducts in Azerbaijan. *Euroasian Journal of Hepato-Gastroenterology* **6**, 125–130.
- Ahmed W, Neelakanta G and Sultana H (2021) Tetraspanins as potential therapeutic candidates for targeting flaviviruses. *Frontiers in Immunology* **12**, 630571.
- Allcorn LC and Martin ACR (2002) SACS--self-maintaining database of antibody crystal structure information. *Bioinformatics (Oxford, England)* **18**, 175–181.
- Alvarez RCA, Romig T and Lightowlers MW (2014) Echinococcus gralunosus sensu lato genotypes infecting humans--review of current knowledge. *International Journal for Parasitology* **44**, 9–18.
- Budke CM, Deplazes P and Torgerson PR (2006) Global socioeconomic impact of cystic echinococcosis. *Emerging Infectious Diseases* **12**, 296–303.
- Cardoso FC, Macedo GC, Gava E, Kitten GT, Mati VL, de Melo AL, Caliri MV, Almeida GT, Venancio TM, Verjovski-Almeida S and Oliveira SC (2008) *Schistosoma mansoni* tegument protein Sm29 is able to induce a Th1-type of immune response and protection against parasite infection. *PLoS Neglected Tropical Diseases* **2**, e308.
- Chai JY, Jung BK and Hong SJ (2021) Albendazole and mebendazole as anti-parasitic and anti-cancer agents: an update. *The Korean Journal of Parasitology* **59**, 189–225.
- Chaiyadet S, Krueajampa W, Hipkao W, Plosan Y, Piratae S, Sotillo J, Smout M, Sripa B, Brindley PJ, Loukas A and Laha T (2017) Suppression of mRNAs encoding CD63 family tetraspanins from the carcinogenic liver fluke *Opisthorchis viverrini* results in distinct tegument phenotypes. *Scientific Reports* **7**, 14342.
- Cheng WQ, Curti E, Rezende WC, Kwityn C, Zhan B, Gillespie P, Plieskatt J, Joshi SB, Volkin DB, Hotez PJ, Middaugh CR and Bottazzi ME (2013) Biophysical and formulation studies of the *Schistosoma mansoni* TSP-2 extracellular domain recombinant protein, a lead vaccine candidate antigen for intestinal schistosomiasis. *Human Vaccines & Immunotherapeutics* **9**, 2351–2361.
- Chi ZC (2015) *Practical Clinical Hepatology*, 2Edn. Beijing: People's Military Medical Publisher.
- Chow C, Gauci CG, Cowman AF and Lightowlers MW (2004) Echinococcus gralunosus: oncosphere-specific transcription of genes encoding a host-protective antigen. *Experimental Parasitology* **106**, 183–186.
- Craig PS, Chabalgoity JA, Gavidia CM, Gilman RH, Gonzalez AE, Lorca M, Naquira C and Schantz PM (2007) Prevention and control of cystic echinococcosis. *The Lancet Infectious* **7**, 385–394.
- Curti E, Kwityn C, Zhan B, Gillespie P, Brelsford J, Deumic V, Plieskatt J, Rezende WC, Tsao E, Kalampanayil B, Hotez PJ and Bottazzi ME (2013) Expression at a 20L scale and purification of the extracellular domain of the *Schistosoma mansoni* TSP-2 recombinant protein: a vaccine candidate for human intestinal schistosomiasis. *Human Vaccines & Immunotherapeutics* **9**, 2342–2350.
- Dakshinamoorthy G, Munirathinam G, Stoicescu K, Reddy MV and Kalyanasundaram R (2013) Large extracellular loop of tetraspanin as a potential vaccine candidate for filariasis. *PLoS One* **8**, e77394.
- Dang ZS, Watanabe J, Kajino K, Oku YB, Matsumoto J, Yagi KP, Kouguchi H and Sugimoto C (2009a) Molecular cloning and characterization of a T24-like protein in *Echinococcus multilocularis*. *Molecular and Biochemical Parasitology* **168**, 117–119.
- Dang ZS, Yagi KP, Oku YB, Kouguchi H, Kajino K, Watanabe J, Matsumoto J, Nakao R, Wakaguri H, Toyoda A and Sugimoto C (2009b) Evaluation of *Echinococcus multilocularis* tetraspanins as vaccine candidates against primary alveolar echinococcosis. *Vaccine* **27**, 7339–7345.
- Dang ZS, Yagi KP, Oku YB, Kouguchi H, Kajino K, Matsumoto J, Nakao R, Wakaguri H, Toyoda A, Yin H and Sugimoto C (2012a) A pilot study on developing mucosal vaccine against alveolar echinococcosis (AE) using recombinant tetraspanin 3: vaccine efficacy and immunology. *PLoS Neglected Tropical Diseases* **6**, e1570.
- Dang ZS, Feng JC, Yagi KP, Sugimoto C, Li W and Oku Y (2012b) Mucosal adjuvanticity of fibronectin-binding peptide (FBP) fused with *Echinococcus multilocularis* tetraspanin 3: systemic and local antibody responses. *PLoS Neglected Tropical Diseases* **6**, e1842.
- Dong Y, Liu SP, Xu YC, Liu Y, Deng Y and Chen MN (2019) Mutations and predicted structure change of G6PD isolated from a patient with primaquine-induced hemolysis in Yunnan Province. *Chinese Journal of Parasitology and Parasitic Diseases* **37**, 399–405.
- Gong FL (2016) *Medical Immunology*, 3rd Edn. Beijing: Science Press.
- Graham JB, Sunryd JC, Mathavan K, Weir E, Larsen ISB, Halim A, Clausen H, Cousin H, Alfandari D and Hebert DN (2020) Endoplasmic reticulum transmembrane protein TMTC3 contributes to O-mannosylation of E-cadherin, cellular adherence, and embryonic gastrulation. *Molecular Biology of the Cell* **31**, 167–183.
- Guo M, Tuerganeli AJ, Ran B, Wen H and SHAO YM (2019) Analysis on the quality of life of hydatid patients in a hospital of xinjiang. *Xinjiang Medical Journal* **49**, 671–675.
- Han J and Gao GQ (2020) *Medical Molecular Biology Techniques*, 4rd Edn. Beijing: People's Medical Publishing House.
- Hancock K, Patabhi S, Whitfield FW, Yushak ML, Lane WS, Garcia HH, Gonzalez AE, Gilman RH and Tsang VC (2006) Characterization and cloning of T24, a *Taenia solium* antigen diagnostic for cysticercosis. *Molecular and Biochemical Parasitology* **147**, 107–117.
- Hogea MO, Ciomaga BF, Muntean MM, Muntean AA, Popa MI and Popa GL (2024) Cystic Echinococcosis in the early 2020s: a review. *Tropical Medicine and Infectious Disease* **9**, 36.
- Hu YJ, Lin SC, Lin YL, Lin KH and You SN (2014) A meta-learning approach for B-cell conformational epitope prediction. *BMC Bioinformatics* **15**, 378.
- Hu DD, Song XJ, Xie Y, Zhong XQ, Wang N, Zheng Y, Gu XB, Wang T, Peng XR and Yang GY (2015) Molecular insights into a tetraspanin in the hydatid tapeworm *Echinococcus gralunosus*. *Department of Parasitology* **8**, 311.
- Huang Y, Wang Q, Yi DY, Huang L, Yu WJ, Qiu DC, Xiao N, Xu KJ, Xu GF, Qi YF, Qin SC and Li SC (2012) Prevalence investigation and evaluation of human echinococcosis in sichuan province. *Journal of Preventive Medicine Information* **28**, 594–598.
- Huang LH, Wang YQ, Liang S, Deng HM, Lai JB, Gu J and Zhang XJ (2017) *Molecular Biology Techniques: Foundation and Enhancement*, 4rd Edn. Beijing: Science Press.

- Jia XY, Schulte L, Loukas A, Pickering D, Pearson M, Mobli M, Jones A, Rosengren KJ, Daly NL, Gobert GN, Jones MK, Craik DJ and Mulvenna J (2014) Solution structure, membrane interactions, and protein binding partners of the tetraspanin Sm-TSP-2, a vaccine antigen from the human blood fluke *Schistosoma mansoni*. *The Journal of Biological Chemistry* **289**, 7151–7163.
- Jiang CP (2002) Recent prevalence of hydatid disease in China. *Notification of Disease Prevention and Control* **17**, 77–79.
- Jiang N, Cai PF, Yin JG, Hao LL, Lu HJ, Wang XR, Wang H and Chen QJ (2010) Characterization of antibody responses to the Sj23 antigen of *Schistosoma japonicum* after infection and immunization. *Acta Tropica* **116**, 9–14.
- Kim TY, Chung EJ, Sohn WM, Hong SH and Yong TS (2012) Molecular characterization of *Clonorchis sinensis* tetraspanin 2 extracellular loop 2. *Parasitology Research* **110**, 707–711.
- Kolaskar AS and Tongaonkar PC (1990) A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Letters* **276**, 172–174.
- Lei ZL and Wang LY (2012) Control situation and primary task of key parasitic diseases in China. *Chinese Journal of Parasitology and Parasitic Diseases* **30**, 1–5.
- Li W and Gao JL (2017) Echinococcosis and its diagnostic techniques. *Chinese Journal of Health Inspection* **27**, 1974–1976.
- Li BQ, Zheng LL, Feng KY, Hu LL, Huang GH and Chen L (2016) Prediction of linear B-cell epitopes with mRMR feature selection and analysis. *Current Bioinformatics* **11**, 22–31.
- Li BF, Wu FW, Yan XL, Zi JR, Peng J, BAO XY, CAI X and Yang YM (2019) Epidemiological analysis of echinococcosis in Yunnan Province from 2012 to 2017. *Chinese Journal of Parasitology and Parasitic Diseases* **5**, 576–582.
- Li BF, He WS, Zi JR, Wu FW, Yan XL, Wang ZQ, Peng J, CAI X, Yan J and Yang YM (2020) Analysis of the prevalence of and control measures for echinococcosis in Shangri la, Yunnan Province. *Journal of Pathogen Biology* **15**, 1436–1441.
- Li BF, Wang ZQ, Xu Q, Zi JR, Yan XL, Peng J, Li JX, Cai X, Wu FW and Yang YM (2023) Cloning and sequence analysis of the partial nad1 gene within mitochondrial DNA of *Echinococcus granulosus*. *Chinese Journal of Parasitology and Parasitic Diseases* **41**, 306–311.
- Liu TL, MENG QL, QIAO J, Chen C, Ma Y, HU ZX, CAI XP and Chen CF (2015) Cloning and bioinformatics analysis of TSP1 and TSP6 gene of *Echinococcus granulosus*. *Acta Agriculturae Boreali-Sinica* **30**, 41–46.
- Moribe H and Mekada E (2013) Tetraspanins in lower eukaryotes. *Tetraspanins. Proteins and Cell Regulation* **9**, 187–201.
- Nakao M, McManus DP, Schantz PM, Craig PS and Ito A (2007) A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitology* **134**, 713–722.
- Nakao M, Yanagida T, Konyaev S, Lavikainen A, Odnokurtsev VA, Zaikov VA and Ito A (2013) Mitochondrial phylogeny of the genus *Echinococcus* (Cestoda: Taeniidae) with emphasis on relationships among *Echinococcus canadensis* genotypes. *Parasitology* **140**, 1625–1636.
- National Health Commission of the People's Republic of China (2017). Echinococcosis Diagnosis and Treatment Protocol. retrieved from National Health Commission of the People's Republic of China website: ###a <http://www.nhc.gov.cn/zzyzgj/s3594q/201706/242fa472d0a243d7a72bf0560c0fd316.shtml> (accessed 1 October 2023).
- Oian MB, Abela-Ridder B, Wu WP and Zhou XN (2017) Combating echinococcosis in China: strengthening the research and development. *Infectious Diseases of Poverty* **6**, 1443–1447.
- Omadang L, Chamai M, Ejobi F, Erume J, Oba P and Ocaido M (2024) Prevalence of cystic echinococcosis among livestock in pastoral and agro-pastoral areas in Uganda. *Parasitology* **151**, 68–76.
- Otero-Abad B and Torgerson PR (2013) A systematic review of the epidemiology of echinococcosis in domestic and wild animals. *PLoS Neglected Tropical Diseases* **7**, 2249.
- Pearson MS, Pickering DA, McSorley HJ, Bethony JM, Tribolet L, Dougall AM, Hotez PJ and Loukas A (2012) Enhanced protective efficacy of a chimeric form of the schistosomiasis vaccine antigen Sm-TSP-2. *PLoS Neglected Tropical Diseases* **6**, e1564.
- Phumrattanaprapin W, Chaiyadet S, Brindley PJ, Pearson M, Smout MJ, Loukas A and Laha T (2021) Orally administered bacillus spores expressing an extracellular vesicle-derived tetraspanin protect hamsters against challenge infection with carcinogenic human liver fluke. *The Journal of Infectious Diseases* **223**, 1445–1455.
- Piratae S, Tesana S, Jones MK, Brindley PJ, Loukas A, Lovas E, Eursitthichai V, Sripa B, Thanasuwan S and Laha T (2012) Molecular characterization of a tetraspanin from the human liver fluke. *Opisthorchis viverrini*. *PLoS Neglected Tropical Diseases* **6**, 1939.
- Ponomarenko J, Papangelopoulos N, Zajonc DM, Peters B, Sette A and Bourne PE (2011) IEDB-3D: structural data within the immune epitope database. *Nucleic Acids Research* **39**, D1164–D1170.
- Qiu JM, Liu FJ, Schantz P, Akira I, Carol D, He JG, Zhang Y and Chen XW (2000) Epidemiological study on human hydatidosis in Tibetan region of western Sichuan. *Chinese Journal of Zoonoses* **16**, 77–80.
- Resende DM, Rezende AM, Oliveira NJD, Batista ICA, Corrêa-Oliveira R, Reis AB and Ruiz JC (2012) An assessment on epitope prediction methods for protozoa genomes. *BMC Bioinformatics* **13**, 309.
- Sivalingam GN and Shepherd AJ (2012) An analysis of B-cell epitope discontinuity. *Molecular Immunology* **51**, 304–309.
- Tomii K, Santos HJ and Nozaki T (2019) Genome-Wide analysis of known and potential tetraspanins in *Entamoeba histolytica*. *Genes (Basel)* **10**, 885.
- Tran MH, Pearson MS, Bethony JM, Smyth DJ, Jones MK, Duke M, Don TA, McManus DP, Correa-Oliveira R and Loukas A (2006) Tetraspanins on the surface of *Schistosoma mansoni* are protective antigens against schistosomiasis. *Nature Medicine* **12**, 835–840.
- Wang ZR, Bo XW, Zhang YY, Ma X, Wang ZX and Lu PP (2017) Protein profile analyses of protoscoleces in *Echinococcus granulosus*. *Acta Veterinaria et Zootechnica Sinica* **48**, 1519–1528.
- Wang WY, Wang YF, Lu BY, Ma X, Zhang YY, Meng JM, Wang ZR and Bo XW (2020) Differential expression and bioinformatic analysis of the TSP11 gene of *Echinococcus granulosus* at different developmental stages. *Journal of Pathogen Biology* **1**, 25–31.
- Wassermann M, Addy F, Kokolova L, Okhlopov I, Leibrock S, Oberle J, Oksanen A and Romig T (2024) High genetic diversity of *Echinococcus canadensis* G10 in northeastern Asia: is it the region of origin? *Parasitology* **151**, 93–101.
- Wen H, Tuerghaneli AJ, Shao YM, Lin RY, Li HT, Tuerhongjiang TX, Lv GD and Zhang WB (2015) Research achievements and challenges for echinococcosis control. *Chinese Journal of Parasitology and Parasitic Diseases* **33**, 466–471.
- World Health Organization (2021) *Ending the Neglect to Attain the Sustainable Development Goals: a Road Map for Neglected Tropical Diseases 2021–2030*. Geneva, Switzerland: World Health Organization.
- Wu WP (2017) Prevalence and distribution of two types of echinococcosis in China. *China Animal Health* **7**, 7–9.
- Xian JW, Zhao PP, Wang N, Wang WY, Zhang YY, Meng JM, Ma X, Wang ZR and Bo XW (2021) Molecular characterization of a tetraspanin TSP11 gene in *Echinococcus granulosus* and evaluation its immunoprotection in model dogs. *Frontiers in Veterinary Science* **8**, 759283.
- Xu MF, Lu PP, Ma X, Zhang YY, Wang WY, Meng JM, Wang ZR and Bo XW (2018) Advances in *Echinococcus granulosus* proteomics. *Acta Veterinaria et Zootechnica Sinica* **49**, 466–476.
- Xu YC, Dong Y, Deng Y, Mao XH, Chen MN, Zhang CL and Jiang LB (2020) Polymorphisms of circumsporozoite protein gene and population structure analysis of *Plasmodium vivax* with different infection sources in Yunnan Province. *Chinese Journal of Parasitology and Parasitic Diseases* **38**, 67–73.
- Yang D, Liu AQ, Zhao W and Zhang WZ (2015) Research progress on the typing and classification of *Echinococcus granulosus*. *Journal of Tropical Medicine* **09**, 1296–1299.
- Zhang W, Li J, Duke M, Jones MK, Kuang L, Zhang JF, Blair D, Li YS and McManus DP (2011) Inconsistent protective efficacy and marked polymorphism limits the value of *Schistosoma japonicum* tetraspanin-2 as a vaccine target. *PLoS Neglected Tropical Diseases* **5**, e1166.
- Zhang TM, Yang D, Zeng ZL, Zhao W, Liu AQ, Piao DX, Jiang T, Cao JP, Shen YJ, Liu H and Zhang WZ (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**, e109059–e109059.
- Zhu XP and Su C (2019) *Echinococcus granulosus*. In Zhu XP and Su C (eds), *Human Parasitology*. Beijing: People's Medical Publishing House, pp. 145–148.