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Molecular Epidemiology and the Control and Prevention of Cystic Echinococcosis in China: What is Known from Current Research

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Abstract

As a zoonotic parasitic disease, echinococcosis is a severe global public health issue caused by the larvae of *Echinococcus* spp. Not only does echinococcosis threaten human health, but echinococcosis also causes enormous economic losses. China ranks first in the range of echinococcosis endemic areas, the number of infected patients, and the number of threatened populations worldwide, hence the most severe echinococcosis epidemic currently exists in China. Cystic echinococcosis (CE) is the most important form of echinococcosis. Accounting for nearly 80% of all echinococcosis cases, CE is the most important cause of the echinococcosis disease burden. *Echinococcus granulosus sensu lato* (*s.l.*) is the causative agent of CE and is considered a multi-genotype complex. The different genotypes of *E. granulosus s.l.* exhibit differences in morphology, transmission route, and epidemic characteristics. The corresponding clinical characteristics, clinical treatment, and vaccine responses also differ between the genotypes of *E. granulosus s.l.* During the past two decades, China has implemented echinococcosis control and prevention programs in endemic areas with impressive results. Specifically, the prevalence of echinococcosis has decreased. With such extraordinary achievements, precise control and prevention of the genotypes of *E. granulosus s.l.* have never been more important. Nevertheless, insufficient attention has been devoted to molecular epidemiology in the current control programs, and the lack of genotype data from humans and animal hosts exacerbates the situation. Hence, based on the ongoing control and surveillance programs, collecting additional molecular epidemiologic data and geographic information from humans and animals, as well as monitoring the clinical manifestations and drug and vaccine responses of the different genotypes, are invaluable for establishing a molecular epidemiologic database, which in turn can enhance the precise control and prevention of echinococcosis.

Key words: *Echinococcus granulosus s.l.*, control and prevention, echinococcosis, genotype, molecular epidemiology

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BACKGROUND

Echinococcosis is a zoonotic parasitic disease with a global distribution that threatens human health and adds to the enormous economic burden on society, thus making echinococcosis a serious public health issue. Echinococcosis is caused by *Echinococcus* spp. larvae. The World Health Organization lists echinococcosis as one of 17 neglected tropical diseases, and the Food and Agriculture Organization of the United Nations lists echinococcosis as the second most serious foodborne parasitic disease worldwide [1,2]. Echinococcosis is endemic in 370 counties (western pastoral and semi-pastoral areas) in China, thus China is the leading country worldwide for endemic echinococcosis [3]. Indeed, China is ranked first with respect to the scope, number of patients, and the population at risk for echinococcosis.

Echinococcosis refers to two severe zoonotic diseases in China (cystic echinococcosis [CE] and alveolar echinococcosis [AE]); *Echinococcus granulosus sensu lato* (*s.l.*) and *Echinococcus multilocularis* are the causative agents for CE and AE, respectively. CE is the most prevalent form of echinococcosis, accounting for approximately 80% of all cases in China [3,4]. Given the magnitude of the echinococcosis epidemic in China, a large-scale control and prevention program has been implemented nationwide over the past two decades, and the government has invested a substantial amount of funds to reduce the echinococcosis epidemic [5,6]. Indeed, remarkable advances have been made in controlling and preventing the spread of echinococcosis. Most notably, the prevalence of echinococcosis has been significantly reduced and reached a low prevalence in some geographic areas with epidemics. As a result of scientific research focusing on echinococcosis, considerable progress has been made, especially in the molecular epidemiology of echinococcosis. *E. granulosus s.l*, the causative agent of CE, is thought to be a genotype complex and is recognized as 10 strains/genotypes (G1-G10) based on mitochondrial DNA sequencing. At present, *E. granulosus s.l.* is demarcated into five species, with each species exhibiting differences in pathology, mode of transmission, host preference, and responses to drugs and vaccines [4,7,8]. In the current echinococcosis epidemic, it is critical to carry out precise control and prevention for each genotype. In addition, an increasing number of *E. granulosus s.l.* genotypes and haploids from different regions or hosts have been reported over the years, even in non-endemic areas [9]. An emphasis on genotypes is not part of the current national control and prevention program, and surveys regarding the various genotypes from humans and animal hosts have not received sufficient attention. Therefore, data on the molecular epidemiology of echinococcosis is limited.

CONTROL AND PREVENTION OF ECHINOCOCCOSIS IN CHINA

Echinococcus have a complex life cycle that involves two hosts (definitive and intermediate hosts). The primary definitive hosts are canids, which harbor adult worms in the small intestines. The adult worms mature to produce eggs that are released in canid feces into the environment. Humans and herbivores, specifically sheep, are the intermediate hosts for this parasite. The intermediate hosts are infected after ingesting the eggs released in the feces of the definitive hosts. The eggs hatch in the intestine to release oncospheres that pass through the portal and lymphatic vessels and reach the liver, lung, or other organs, where the oncospheres usually settle and develop into metacestodes (hydatid cysts). Protoscoleces produced by the metacestodes are subsequently released into hydatid fluid. When ingested by the definitive host, protoscoleces adhere to the intestinal wall and develop into mature, egg-producing adult worms. Domestic dogs are the primary definitive host for *E. granulosus s.l*, while livestock, such as sheep, goats, cattle, and pigs, act as the intermediate hosts. Dogs, as definitive hosts, have a pivotal role in the transmission of echinococcosis. Of note, humans are typically not involved in the transmission of CE [4,10].

The complex life cycle of *Echinococcus* requires a multi-level, complex project for the control and prevention of echinococcosis, as well as participation by the entire society. A nationwide echinococcosis control and prevention program was launched in China in 2005. The program had a substantial amount of dedicated funds, and several targeted measures have been implemented to control echinococcosis. The strategy for the control and prevention of echinococcosis in China is based on controlling the source of infection and combining the control and prevention of intermediate hosts with patient screening and treatment. Specific control and prevention measures include monthly deworming of dogs, safe slaughter of livestock, screening of patients, and free treatment to prevent the transmission of *Echinococcus* from dogs-to-humans and from intermediate hosts-to-dogs [5,6].

To identify the endemic areas and endemic status of echinococcosis in China, a nationwide survey on echinococcosis was carried out during 2012-2016. According to the results, a total of 370 counties distributed in 9 provinces and autonomous regions (Inner Mongolia, Sichuan, Tibet, Gansu, Qinghai, Ningxia, Yunnan, Shaanxi, and Xinjiang ([including Xinjiang Production and Construction Corps]), were identified as endemic for echinococcosis, as illustrated in Fig 1. Echinococcosis was shown to be highly endemic in pasture and semi-pasture areas of northwestern China, where the prevalence of echinococcosis in humans, livestock, and dogs was as high as 0.28%, 4.7%, and 4.3%, respectively. With > 0.16 million patients and > 50 million individuals at risk, the echinococcosis endemic is more severe in China than elsewhere globally [3]. As shown in Fig 1, the Qinghai-Tibet Plateau area is the most severely affected region in China, where the prevalence in humans (1.28%) is significantly higher than the non-Qinghai-Tibet Plateau area (0.13%) [3,11]. These survey results clarified the scope and level of the echinococcosis epidemic in China and provided robust
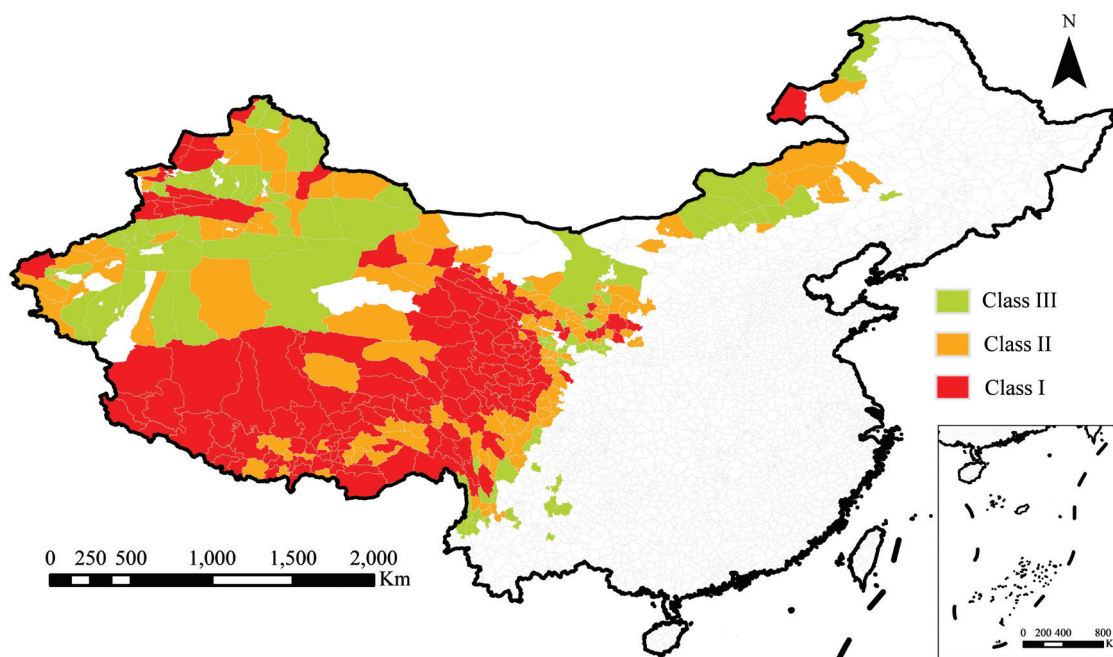
FIGURE 1 | Epidemiologic map of echinococcosis at the county level in China based on the results of the national epidemiologic survey of echinococcosis from 2012-2016 (Tibet Autonomous Region based on the survey results of 2016), cited from the *Technical Plan for Echinococcosis Control* (2019 version).
Notes: Class I represents the prevalence in humans ≥ 1% or infection rate of dogs ≥ 5%; Class II represents 0.1% ≤ prevalence in humans < 1% or 1% ≤ infection rate of dogs < 5%; Class III represents 0 < prevalence in humans < 0.1% or 0 < infection rate of dogs < 1%.

data to assist in the control and prevention of the disease. Based on the prevalence of echinococcosis in humans and the infection rate in dogs, all 370 counties endemic for echinococcosis were categorized into 3 classes (Fig 1), as follows: class I, prevalence ≥ 1% in humans or a dog infection rate ≥ 5%; class II, prevalence between 0.1% and 1% or 1% in humans or a dog infection rate between 5% and 1%; and class III, prevalence of <0.1% in humans or a dog infection rate < 1%. Class I is the most severe level. There were minimal differences in the number of patients screened, case investigations, and frequency of dog deworming in the control and prevention programs for the three classes of counties.

Considerable progress has been made in the continuous control and prevention program for echinococcosis epidemic areas, the effects of which can be determined from the echinococcosis surveillance data. Surveillance on the prevalence of humans, livestock, and dogs has been carried out in all endemic counties since 2016. Annual surveillance accurately evaluates the impact of control and prevention programs in addition to gaining a better understanding of the trend in echinococcosis prevalence. The surveillance data in recent years demonstrated that the prevalence of echinococcosis in humans, livestock, and dogs has significantly decreased in most endemic areas. Some areas have even achieved low endemic levels [12,13]. Nevertheless, there is a gradually increasing trend that warrants attention; specifically, echinococcosis patients have been identified in all non-endemic provinces [14,15]. An epidemiologic investigation revealed that some patients had a

history of living or traveling in endemic areas. It is challenging, however, to determine the route of infection in locally infected cases. The answers may lie in molecular epidemiologic investigations. An echinococcosis diagnosis was confirmed as part of the current control and surveillance programs via ultrasound and immunologic testing in humans, necropsy in livestock, and immunologic testing in dogs. Interestingly, the identification of *Echinococcus* genotypes is not mandatory and is only required for livestock surveillance, thus the molecular epidemiologic data for humans and animals are limited in the national control and surveillance programs. The current *Echinococcus* genotype data were all reported based on the academic literature for scientific research purposes.

GENOTYPES OF *E. GRANULOSUS SENSU LATO* IN CHINA

E. granulosus s.l. causes CE and is the primary species infecting humans. CE is distributed worldwide and accounts for the majority of patients with echinococcosis. According to morphologic, taxonomic, epidemiologic, and molecular biological data, *E. granulosus s.l.* is a multi-genotype complex that includes 10 genotypes and lion strains. Advances in genetics and sequencing technology have facilitated the classification of *E. granulosus s.l.* At present, *E. granulosus s.l.* is split into 5 species: *E. granulosus sensu stricto (s.s.)* [G1-G3 genotypes]; *E. equinus* [G4 genotype]; *E. ortleppi* [G5 genotype]; *E. canadensis* group

[G6–G10 genotypes]; and *E. felidis* [lion strain]. The species have distinct morphologies, transmission modes, and epidemic characteristics that correspond to the immune diagnosis, clinical response, and vaccine response [16,17].

E. granulosus s.s. comprises the G1, G2, and G3 genotypes corresponding to the sheep, Tasmanian sheep, and buffalo strains, which were originally named based on the epidemiologic characteristics. Furthermore, the G1, G2, and G3 genotypes of *E. granulosus s.s.* are the most commonly encountered pathogens causing CE and can infect humans and various animals. Numerous domestic animals, including yaks [18], cattle, buffalo [19], pigs [20], horses [21], and camels [22], can be infected as intermediate hosts. The most frequent transmission routes are from sheep-to-dogs and cattle-to-dogs. *E. granulosus s.s.* is distributed globally, with G1 the predominant genotype. The human CE infection rate caused by G1 exceeds 88% worldwide [23], while the G2 and G3 genotypes also infect humans, but the number of reported cases is limited.

E. equinus was first detected in horses. The morphology of *E. equinus* is different from the sheep strain. *E. equinus* corresponds to the G4 genotype and is primarily transmitted between horses and dogs. Moreover, *E. equinus* is the only species that has not been shown to infect humans. In addition, *E. equinus* is host-specific and can be detected in horses, donkeys, mules, and zebra. *E. equinus* was once thought to only infect equines and was not pathogenic to humans, but *E. equinus* has been detected in lemurs, confirming a possible zoonosis, but more evidence is required to draw a more definitive conclusion [24,25].

E. ortleppi was first demonstrated in cattle and was dubbed the cattle strain, corresponding to the G5 genotype. The morphology of *E. ortleppi* is different from *E. granulosus s.s.* The principal intermediate host of *E. ortleppi* is cattle, and infections in cattle are common. The most prevalent genotype causing cattle infection is the G1 genotype of *E. granulosus s.s.* The characteristics of infection caused by G1 and G5 are different. The cysts formed by G1 infection are mostly infertile cysts, whereas the cysts caused by G5 are mostly infectious fertile cysts, thus the latter is more contagious than the former for the transmission of CE [26]. In addition to cattle, *E. ortleppi* can also infect buffalo [20], goats [27], camels [28], and other domestic animals. Lastly, *E. ortleppi* can also infect humans, but reported cases are rare.

E. canadensis is currently considered to consist of G6–G10, but this remains controversial; G9 has been hypothesized to be a variant of G7 [29]. Furthermore, attempts have been made to split *E. canadensis* into three groups (*E. intermedius*, composed of G6 and G7; *E. borealis*, composed of G8 and G10; and *E. canadensis*) [30]. Therefore, additional biological, morphologic, and genetic data are needed to develop a universally accepted taxonomic theory.

The G6 and G7 genotypes of *E. canadensis* are distributed globally. Infection with the G6 and G7 genotypes of *E. canadensis* is common in domestic animals, among which the main intermediate hosts are camels and pigs.

The prevalence of human CE caused by G6 and G7 infection is second only to G1, and the human infection rates are 7.34% and 3.7%, respectively. Thus, the influence on the global echinococcosis disease burden cannot be overlooked [23]. Notably, G6 and G7 are dominant genotypes or even the only genotypes found in some countries and regions, hence damage caused by these genotypes may be underestimated [24].

The G8 and G10 genotypes of *E. canadensis* are principally transmitted among wolves and deer, but to date the G8 and G10 genotypes of *E. canadensis* are only found and reported in the northern hemisphere [24]. As one of the pathogens that can lead to human CE, the number of reported cases caused by the G8 and G10 genotypes is limited.

Molecular epidemiology data [31] indicate that all genotypes of *E. granulosus s.l*, with the exception of G4, have been reported in China. As anticipated, G1 is also the dominant genotype in China, and the majority of reported cases are concentrated in the Qinghai–Tibet Plateau, where the epidemic is most severe. The geographic distribution of different genotypes from human CE reported in China is delineated in Fig 2 and Table 1 [9,31,32]. Human CE cases caused by G1, G3, and G6 have been reported in echinococcosis endemic provinces (Xinjiang, Qinghai, Sichuan, Gansu, Ningxia, Tibet, and Inner Mongolia), whereas human CE cases caused by G1, G5, G7, and G10 have been reported in echinococcosis non-endemic provinces (Heilongjiang, Liaoning, Guangxi, and Guizhou). Although the number of cases is far lower in non-endemic provinces than in endemic provinces, the causative agents in non-endemic provinces are more diverse. From 2004–2020, the number of echinococcosis cases exceeded 66,000 in China, of which 98.9% were concentrated in endemic
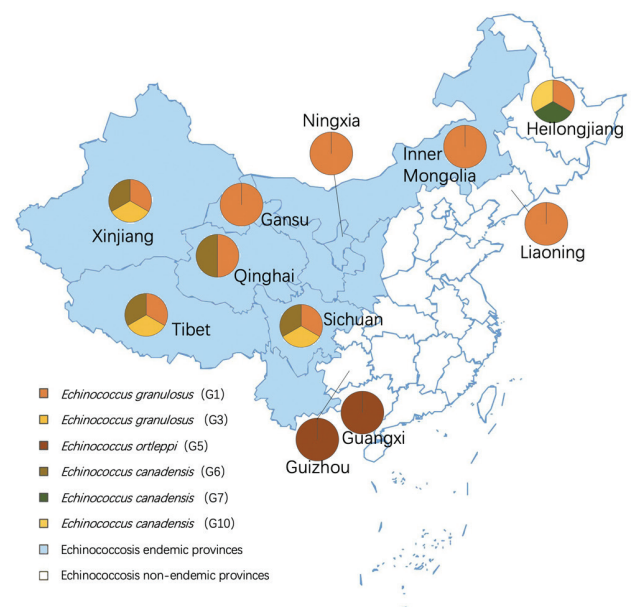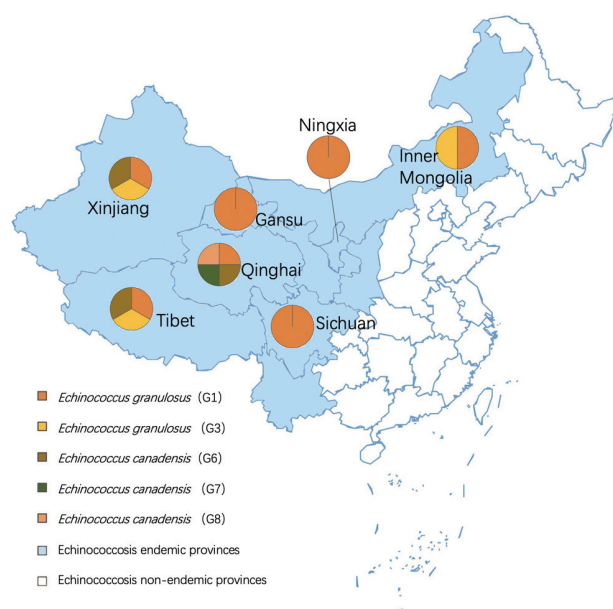

FIGURE 2 | Distribution of *E. granulosus s.l.* genotypes from humans in China.

TABLE 1 | Genotypes of *E. granulosus s.l.* in China.

	Provinces	Species	Genotypes	Host species
Echinococcosis endemic provinces	Xinjiang	*E. granulosus s.s.*	G1	Human, sheep, cattle, pig, camel, dog
			G3	Human, sheep
		E. canadensis	G6	Human, cattle, camel, dog
	Qinghai	*E. granulosus s.s.*	G1	Human, sheep, yak, cattle, dog, goat
		E. canadensis	G6	Human, sheep, goat
			G7	Goat
			G8	Sheep
	Sichuan	*E. granulosus s.s.*	G1	Human, yak, sheep
			G3	Human
		E. canadensis	G6	Human
	Gansu	*E. granulosus s.s.*	G1	Human, sheep, yak
	Ningxia	*E. granulosus s.s.*	G1	Human, sheep, goat, squirrel
	Tibet	*E. granulosus s.s.*	G1	Human, sheep, yak, cattle
			G3	Human, yak
		E. canadensis	G6	Human, sheep, yak
	Inner Mongolia	*E. granulosus s.s.*	G1	Human, sheep
			G3	Sheep
Echinococcosis non-endemic provinces	Heilongjiang	*E. granulosus s.s.*	G1	Human
		E. canadensis	G7	Human
			G10	Human
	Liaoning	*E. granulosus s.s.*	G1	Human
	Guangxi	*E. ortleppi*	G5	Human
	Guizhou	*E. ortleppi*	G5	Human

provinces [14]. The majority of CE cases lack genotype information according to a systematic literature review. In fact, the *E. granulosus s.l.* genotypes have only been identified in 1240 human CE cases [31], which is clearly a huge gap in data. As mentioned above, the diagnosis of patients confirmed by ultrasound and immunologic detection in the control program and surveillance, and the genotype of pathogens was not mandatorily determined via molecular biological methods, which led to the lack of molecular epidemiologic data of cases in most epidemic areas. There are relatively few cases of echinococcosis in non–endemic provinces, thus warranting increased attention. More information, including the identification of pathogenic species, is needed to confirm the diagnosis. For all of the aforementioned reasons, more genotype data should be reported in non-epidemic areas.

The geographic distribution of genotypes from animal hosts in China is presented in Fig 3 and Table 1 [31,33]. Among animal hosts, G1 is also the dominant genotype with the widest distribution and the highest number of infected animal hosts, including sheep, cattle, pigs, camels,

FIGURE 3 | Distribution of *E. granulosus s.l.* genotypes from animal hosts in China.

yaks, goats, squirrels, and dogs, from Xinjiang, Qinghai, Sichuan, Ningxia, Gansu, Tibet and Inner Mongolia. In addition, G3 was detected in sheep and yaks, while G6 was detected in cattle, camels, sheep, goats, yaks, and dogs. Furthermore, G7 was detected in goats and G8 was detected in sheep. All genotype data from animal hosts were collected from reports of endemic provinces. Similar to the genotypes of human cases, there are various intermediate and definitive hosts detected in the control and surveillance programs. Animal infection could be verified by necropsy and immunologic detection. Given that it is not mandatory to determine the pathogenic species via molecular biological methods, data on the genotype information of animals are scarce; however, this does not imply that only the reported genotypes exist in animal hosts of endemic provinces, thus further investigations are required to complement these data.

As depicted in Figs 2 and 3, the geographic distribution of *E. granulosus s.l.* genotypes in humans are not completely consistent with the geographic distribution in animal hosts. This finding may be attributed to insufficient samples, including human and animal samples, for identifying genotypes in endemic provinces. In contrast, the essential feature behind this observation in non-endemic provinces may potentially be that the number of the collected samples, especially infected animal samples, was not sufficient to determine the infection route of locally infected cases. A greater number of genotypes identified in an area is a sign that more animal hosts may be involved in the transmission route, and thus more types of animal hosts should be included in the ongoing control and surveillance programs.

With improvements in molecular biology techniques, it is currently feasible to identify genotypes of *Echinococcus* by sequencing complete mitochondrial DNA or short genes (*cox1*, *nad1*, *nad2*, and *nad5*), as well as haplotypes, with more diversity and geographic specificity. Complete mitochondrial sequences offer superior phylogenetic resolution and accuracy to short genes. For technical and economic reasons, most studies have used short genes as phylogenetic markers. Among them, *cox1* is the short gene most extensively utilized. Fifty-one *E. granulosus s.l. cox1* haplotypes were shown to be endemic in China, including the most common haplotype (Hap_2) with the broadest geographic distributions and host range [31]. Comparing and analyzing haplotypes from different regions indicate the possible origin, differentiation time, and spatial diffusion pathway of *Echinococcus* [32,33], which have a decisive role in tracing the source of infection and formulating customized control strategies.

Screening and treatment of patients and intermediate hosts, and definitive host control are the three fundamental aspects of echinococcosis control and prevention. Echinococcosis patients fulfilling the criteria for surgical intervention can be treated by surgery, while the remaining patients can be prescribed and administered appropriate therapeutic drugs, which may not be ideal for some patients owing to the unsatisfactory efficacy. The characteristics of echinococcosis caused by different genotypes are different. For example, G6 might exhibit more brain tropism than G1 [34]. Notably, a previous study showed that G3 infections were large active CE1, and G6 infections were mostly transitional CE3 with smaller cysts [32]. These differences may be responsible for the ineffectiveness of drug treatment in some patients. The control of intermediate hosts involves vaccination of livestock to prevent infection in addition to safe slaughter to block transmission from livestock-to-dogs. At present, the EG95 vaccine is the only approved vaccine and exerts excellent preventive effects against the G1 genotype but may not be effective against the G6 genotype [35]. Regardless of the pathogenic species and genotype, deworming is the main approach for the control of the definitive host, and there are fewer reports on the genotype of the definitive host than the intermediate host. Dogs are significant sources of infection of echinococcosis, and information on the pathogenic genotype is particularly important to establish the transmission route. All of the above facts suggest that molecular epidemiologic data are crucial for the control of echinococcosis.

CONCLUSION AND FUTURE PROSPECTIVE

With the promotion of echinococcosis control and prevention programs, the prevalence of echinococcosis has been decreasing. In areas of low prevalence, precise control and prevention of echinococcosis is particularly important. New local infection cases continue to appear in non-endemic areas, and it is challenging to determine the transmission route through epidemiologic investigations. The unexpected intermediate hosts may upscale the challenge. Additional molecular epidemiologic data are needed to identify more host species by expanding the host range of the survey and increasing the sample size.

Regarding the importance of molecular epidemiology in the control and prevention of echinococcosis, ongoing control program and surveillance systems should be supplemented with data on pathogenic species and genotypes. Molecular biological technology is a reliable method for the identification of pathogen species, genotypes, and haplotypes. Furthermore, provincial control and prevention departments, research institutions, and medical institutions can meet the technical and equipment requirements, which makes it feasible to carry out molecular epidemiologic investigations in echinococcosis epidemic and non-epidemic areas. Moreover, additional genotype data and geographic information, including human and animal information, should be collected. More importantly, molecular epidemiologic databases should be established. Monitoring the clinical manifestations caused by different genotypes, drug susceptibility, vaccine protective efficacy, and the effects of drug or surgical treatments are all essential to provide the basis for more accurate control and prevention of echinococcosis.

Some neighboring countries of China, such as Pakistan, Mongolia, India, and Nepal, are also endemic areas of echinococcosis [4]. Thus, the increasing frequency of personnel exchanges and animal trade may lead to the spread of pathogens and diseases. By analyzing pathogen genotype and haplotype information from domestic and overseas cases or from different regions in China, in conjunction with geographic information, possible transmission pathways can be deduced so that targeted measures can be implemented. Additionally, molecular epidemiologic research on the genotype and haplotype of *Echinococcus* can promote academic exchanges and cooperation between different countries, as well as joint control and prevention programs for echinococcosis in China and neighboring countries, which would be significant for the Belt and Road Initiative.

Understanding the genetic characteristics, transmission modes, hosts, and geographic distribution of the different *Echinococcus* is of great significance for the diagnosis, vaccine development, treatment, and precise control and prevention of echinococcosis. At present, there are numerous gaps in the molecular epidemiologic data of echinococcosis, but this situation can be improved by the promotion of the echinococcosis control programs and placing emphasis on molecular epidemiology.

In addition, because *Echinococcus* is a multi-host parasitic worm with a complex life cycle, it has a special niche in the ecologic environment, and its transmission chain is influenced by human, animal, and environmental factors. Therefore, irrespective of research on traditional epidemiology or molecular epidemiology, the One Health approach could be used to provide support for the control and prevention of echinococcosis. Based on molecular biological information, the interrelationship between humans, animals, and their shared environment can be fully recognized, and better public health results can be achieved through communication and cooperation among multiple departments.

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CONFLICTS OF INTEREST

The authors declare no competing interests.

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