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RESEARCH ARTICLE

Advances in the study of molecular identification technology of *Echinococcus* species

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ABSTRACT

The larvae of *Echinococcus* (hydatidcyst) can parasitize humans and animals, causing a serious zoonotic disease-echinococcosis. The life history of *Echinococcus* is complicated, and as the disease progresses slowly after infection, early diagnosis is difficult to establish. Due to the limitations of imaging and immunological diagnosis in this respect, domestic and foreign scholars have established a variety of molecular detection techniques for the pathogen *Echinococcus* over recent years, mainly including nested polymerase chain reaction (PCR), multiplex PCR, real-time quantitative PCR, and nucleic acid isothermal amplification technology. In this article, the research progress of molecular detection technology for *Echinococcus* infection currently was reviewed and the significance of these methods in the detection and diagnosis of hydatid and hydatid diseases was also discussed.

Keywords: *Echinococcus*; echinococcosis; molecular detection technology.

INTRODUCTION

The larvae of *Echinococcus* can parasitize humans, multiple livestock, and other animals, leading to echinococcosis, hydatid disease, and hydatidosis. The disease is widely distributed worldwide, and it continuously spreads with the development of animal husbandry, becoming a major global public health and economic problem (Agudelo Higuita *et al*., 2016).

Currently, there are five globally accepted types of *Echinococcus*, i.e., *Echinococcus granulosus*, *E. multilocularis*, *E. vogeli*, *E. oligarthrus* and *E. shiquicus*, among which *E. granulosus* and *E. multilocularis* are the most important (Cerda *et al*., 2018; Wen *et al*., 2019). *E. granulosus* is distributed worldwide, predominantly in pastoral and rural areas of Africa, the Middle East, the Mediterranean, Europe, Central Asia, South America and Western China (Deplazes *et al*., 2017). *E. multilocularis* is mainly distributed in the northern hemisphere, and the main epidemic area is the Tibetan Plateau. *E. shiquicus* is a new type that was recently discovered only in the Tibetan Plateau of China (Jin, 2020; Zhu *et al*., 2020). The remaining two are rarely found in China.

After *Echinococcus* parasitizes humans or animals, the parasite's growth exerts mechanical pressure on the host tissue, leading to tissue atrophy and dysfunction, while its metabolites can also cause inflammation and allergic reactions. It can parasitize any organ of humans. In the liver and lung, the organ parenchyma is compressed and highly atrophied, and can result in death (Yang, 2018). The infection of *Echinococcus* in cattle, sheep, and other livestock can cause emaciation, weakness, decreased milk yield, and other symptoms (Li, 2020). The study estimated that there are about 18,235 new cases of alveolar echinococcosis (AE) per year globally, and the global burden of AE was about 666,433 disability adjusted life years (DALYs), of which roughly 91% (16,629) and 95% of DALYs occur in China. Russia, located in Asia, has the second highest incidence rate after China with about 1180 cases per year, while the remaining cases are mainly distributed in Turkey, Central Asia and Europe (Torgerson *et al*., 2010). The estimated global number of new cases of cystic echinococcosis (CE) per year is 188,079, and the global disease burden is 18,353 DALYs (Torgerson *et al*., 2015). From the above results, the human health burden of CE was much lower than that of AE, the impact of CE on the livestock industry and socioeconomics was much higher than that of AE (Deplazes *et al*., 2017).

The threat of human AE and CE in Iran, Turkey, and Pakistan was underestimated, and the large number of asymptomatic or undetected cases results in higher true prevalence and incidence than reported, with an annual monetary burden of $232 million from

CE for humans and animals in Iran, $89 million from CE for livestock in Turkey, and high economic losses from CE for livestock in Pakistan (Borhani *et al*., 2021). Europe is limited by past diagnostic methods and case statistics of echinococcosis at low incidence, the true epidemic area and prevalence should be much higher than expected, and the pressure of AE and CE infection in Europe and the number of reported cases have increased rapidly in recent years and will continue to rise in the coming decades (Gottstein *et al*., 2015; Vuitton *et al*., 2015; Casulli *et al*., 2022). In North America, AE and CE have been reported more frequently in Alaska and Canada, with Alaska being more prevalent and only one case of human AE reported in the U.S. mainland, Minnesota (Cerda *et al*., 2018). Argentina, Chile, Peru, Uruguay and Brazil in South America are the endemic regions for CE (Pavletic *et al*., 2017).

In China, more than 50 million cattle and sheep suffer from echinococcosis every year, while the direct economic loss caused by the echinococcosis induced death, wasting of the liver and lung, and other organs in livestock is more than 3 billion Yuan every year (Wu, 2017; Robertson *et al*., 2018; Guan *et al*., 2019). If the single-phase extinction strategy would be adopted, the related cost of deworming for (pastoral) dogs in western China could amount to 200 million Yuan. If the two-phase pathogenic cycle chain cutting strategy is used and dog deworming and sheep vaccination are simultaneously carried out, the vaccine cost is expected to reach some 1.6 billion Yuan (Zhang *et al*., 2017). The annual prevalence of CE in China is 0.73 cases per 10,000 people, and the annual prevalence of AE is 0.26 cases per 10,000 people. The prevalence of AE has been declining and is at a low level under the prevention of the One Health strategies (Wang *et al*., 2020a, 2021).

The diagnostic methods for echinococcosis mainly include epidemiological survey, clinical signs examination, pathogen detection, molecular detection, immunology, and imaging diagnosis. Among these, imaging is the first option. Echinococcosis develops slowly in humans and being extremely small in the early stage, it is challenging to find the lesion in early infection, which often leads to a missed diagnosis. Immunological detection is common for screening parasites. However, the antibody level in vivo is low or the sensitivity and specificity of diagnostic reagents are not high in early infection, which may result in false-positive or false-negative (Dinkel *et al*., 1998). Etiological detection is the "gold standard" for diagnosing and defining parasitic diseases (Lass *et al*., 2017). However, due to the slow onset of echinococcosis, the difficulty of early diagnosis, and the complicated process of infection inspection in the final host canine, it is challenging to apply for the detection of early infection as well as animal infection for time-consuming, complicated operation, and high requirements for operators. Due to the limitations of immunological and imaging diagnostic techniques in the early diagnosis of echinococcosis (Wang *et al*., 2019), it is necessary to develop detection methods with high sensitivity and specificity that could improve the accuracy of early diagnosis.

Following the development of science and technology, molecular detection techniques have become increasingly used in the diagnosis and detection of parasites and parasitic diseases on account of the various advantages, such as small sample size, high sensitivity and specificity, and fast response. There were mainly 7 species molecular methods being used in echinococcosis detection and diagnosis, including conventional PCR, nested PCR, multiple-PCR, restriction fragment length polymorphism (RFLP-PCR), quantitative real-time PCR, loop-mediated isothermal amplification (LAMP), as well as recombinaseC aided isothermal amplification assay (RAA). Polymerase chain reaction (PCR) simulates DNA synthesis in vitro and specifically amplifies the target gene fragment under appropriate conditions using a pair of primers, which is widely used in nucleic acid detection due to high sensitivity, specificity, and detection speed (Garibyan & Avashia, 2013; Wittwer & Makrigiorgos, 2018). In Nested PCR Two pairs of primers are applied to amplify fragments. The first PCR reaction is similar to the conventional PCR reaction, moreover, the second pair is known as nested primers that are combined in the first PCR product, making the second PCR amplification fragment shorter than the first one (Yu *et al*., 2015; Green & Sambrook, 2019). Multiplex PCR can simultaneously amplify multiple nucleic acid fragments by adding multiple pairs of primers into the same PCR reaction system, which is a common method for diagnosing and detecting parasitic infection (Markoulatos *et al*., 2002; Wittwer & Makrigiorgos, 2018). Restriction fragment length polymorphism (RFLP)-PCR technology is a method based on RFLP and PCR. The product of the target gene by PCR amplification is digested by restriction endonuclease to form a fragment with a specific size that can be directly analyzed by gel electrophoresis with bands of different sizes (Sharma & Changotra, 2017; Furtado *et al*., 2018). Compared with conventional PCR, RT q-PCR measures the real-time fluorescence signal during PCR amplification. The number of PCR products in the exponential amplification period is proportional to the number of templates, during which period the data acquired can be quantitatively analyzed (Smith & Osborn, 2009). Nucleic acid isothermal amplification technology can be used for nucleic acid amplification at constant temperature and simple conditions. In addition to loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), nucleic acid sequence-based amplification (NASBA), and rolling circle amplification (RCA) are common nucleic acid isothermal amplification techniques (Piepenburg *et al*., 2006; Deng *et al*., 2017; Zhu *et al*., 2019; Chen *et al*., 2021; Ju *et al*., 2021).

Herein, we reviewed the progress and application of molecular detection for *Echinococcus* over recent years to provide a reference for the diagnosis of echinococcosis. A summary of the detection methods, type of Echinococcus, sample source and target genes is shown in Table 1.

DATA COLLECTION METHOD

The application of molecular detection for *Echinococcus* over recent years were summarized through the literature analysis. The academic papers, including research papers, reviews, and case reports, in the field of *Echinococcus* from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov), China Knowledge Network data platform (http://www.Cnki.net), Wanfang data platform (http://www.wanfangdata.com.cn), and Weipu data platform (http://vip.hbdlib.cn/) were searched with the keywords *Echinococcus*, echinococcosis, molecular detection technology. A total of 90 papers were retrieved, including 62 research papers, 19 reviews, 2 systematic review and meta-analysis, 5 books and documents, 1 comparative study and 1 case reports.

CONVENTIONAL PCR

Currently, the most commonly used genes for the detection and diagnosis of *Echinococcus* are mitochondrial genes (*cox*1, *nad*1, *nad*5, 12S rRNA), nuclear genes (18S rRNA, ITS), and highly repetitive sequences. PCR has been developed following the demand for molecular detection to diagnose *Echinococcus* in increasing application areas, such as hydatid disease detection of human and animal, epidemiological investigation, as well as genotyping of *Echinococcus*, etc. (Armua-Fernandez *et al*., 2011; Soares *et al*., 2013; Avcioglu *et al*., 2017; He *et al*., 2017; Bittencourt-Oliveira *et al*., 2018; Gorgani-Firouzjaee *et al*., 2019; Moradi *et al*., 2019; Ohiolei *et al*., 2019; Wan *et al*., 2020; Grimm *et al*., 2021).

NESTED PCR

Two pairs of primers are applied to amplify fragments in Nested PCR. The first PCR reaction is similar to the conventional PCR reaction, moreover, the second pair is known as nested primers that are combined in the first PCR product, making the second PCR amplification fragment shorter than the first one (Yu *et al*., 2015; Green & Sambrook, 2019). Mitochondrial 12S rRNA-specific primers for nested PCR amplification of mouse and horses cyst was

Table 1. Summary of Molecular Detection Techniques for *Echinococcus*

Detection methods	Type			Sample source			Target gene	Reference
	Echinococcus granulosus	*E. multilocularis*	Other	Cyst	Feces	Other		
Conventional		√				Livers tissue	12S rRNA	Avcioglu *et al*., 2017
PCR		√		√			12S rRNA	Grimm *et al*., 2021
	√			√		Serum	*cox*1, *nad*1	Moradi *et al*., 2019
	√			√			*nad*1, *nad*5	Ohiolei *et al*., 2019
			E. oligarthrus	√			*cox*1	Soares *et al*., 2013
	√	√	*E. vogeli*		√		*nad*1	Armua-Fernandez *et al*., 2011
			E. vogeli	√			*cox*1	Bittencourt-Oliveira *et al*., 2018
		√		√			18S rRNA	He *et al*., 2017
	√			√			ITS1, *cox*1	Gorgani-Firouzjaee *et al*., 2019
	√	√		√		Plasma	cf DNA	Wan *et al*., 2020
Nested PCR		√				Nodular lesions	12S rRNA	Tomczuk *et al*., 2020
		√		√			12S rRNA	Fukumoto *et al*., 2017
		√				Fruits and Vegetables	12S rRNA	Lass *et al*., 2017
	√	√			√		*nad*1, rrnS	Skrzypek *et al*., 2020
		√				Water source	12S rRNA	Lass *et al*., 2020
Multiplex	√			√			*ef*1*a cal*, *cox*1, *pold*, *elp*1	Tahiri *et al*., 2019
PCR	√	√			√		*nad*1, rrnS	Skrzypek *et al*., 2020
	√			√			*cox*1, *nad*1, e*lp*1, *cal*	Santolamazza *et al*., 2020
		√				Berry, eggs	12S rRNA	Temesgen *et al*., 2019
	√	√	*E. canadensis*			Liver tissue, blood	*cox*1, *gd*3, *mtb*2	Chen *et al*., 2019
q-PCR		√	*E. canadensis*		√		*nad*2, *cox*1	Santa *et al*., 2019
		√			√		12S rRNA	Maas *et al*., 2016
	√	√				Greens, berries, eggs	*nad*1, rrnS	Frey *et al*., 2019
	√			√			*cox*1	Mohaghegh *et al*., 2019
		√				Berry, eggs	12S rRNA	Temesgen *et al*., 2019
		√				Berry, eggs	*nad*1	Malkamɲki *et al*., 2019
	√	√	*E. equinus, E. ortleppi, E. canadensis*	√	√		*cox*1, *cox* 3, *nad*5	Maksimov *et al*., 2020
	√					Plasma	egr-miR-71, egr-let-7	Alizadeh *et al*., 2020

LAMP	√				√	Tissue	*nad*5, 12s rRNA	Ni *et al*., 2014a
		√			√	Tissue	*nad*5, 12s rRNA	Ni *et al*., 2014b
	√					Nodule	*cyt*b	Hifumi *et al*., 2021
	√		*E. ortleppi, E. canadensis*	√	√		*cox*1	Avila *et al*., 2020
	√			√			*nad*1	Ahmed *et al*., 2016
RAA	√			√		Tissue		Zhou *et al*., 2020a
	√	√		√	√	Tissue		Zhou *et al*., 2020b
		√		√	√	Tissue		Zhou *et al*., 2020c
RFLP-PCR	√				√		12S rRNA	Alejandro *et al*., 2019
	√			√			ITS1, *cox*1	Gorgani-Firouzjaee *et al*., 2019
	√			√			*cox*1, *nad*1, e*lp*1, *cal*	Santolamazza e*t al*., 2020
			E. ortleppi, E. canadensis	√	√		*nad*1, *cox*1	Fbab *et al*., 2020
	√	√	*E. shiquicus*	√	√		*nad*1	Chaabane-Banaoues *et al*., 2016
Other	√					Plasma	cf DNA	Wan *et al*., 2020
molecular	√	√				Plasma	cf DNA	Wang *et al*., 2020b
detection	√	√				Plasma	cf DNA	Fan *et al*., 2021
method	√	√				Plasma, serum, urine	cf DNA	Zhao *et al*., 2021
	√		√				microsatellite	M'Rad *et al*., 2020
		√			√		12S rRNA (droplet digital PCR)	Bagɳ *et al*., 2021

analyzed, with the band size and sequence of the product were consistent with *E. multilocularis* (Fukumoto *et al*., 2017; Tomczuk *et al*., 2020). In addition to detecting *Echinococcus* infection in the surrounding environment, 104 samples of fruits, vegetables, and mushrooms from different sources in a non-endemic area of Poland were detected by nested PCR based on mitochondrial 12S rRNA gene. *E. multilocularis* DNA was detected in 6.7% of the samples, indicating that the environment in the area has been polluted, thus posing an infection threat to human (Lass *et al*., 2017). The authors also analyzed wastewater samples from different sources in the Qinghai Tibet Plateau of China by nested PCR, fluorescence real-time (RT) quantitative PCR (q-PCR), and LAMP, revealing that the three methods had high consistency and effectiveness in detecting parasitic DNA in environmental materials, and indicating the role of wastewater in the transmission of *E. multilocularis* and the risk of polluting water sources (Lass *et al*., 2020).

MULTIPLEX-PCR

Multiplex PCR can detect multiple target genes in different templates or different regions of a template by adding more than two pairs of specific primers in a reaction system (Yu *et al*., 2015; Green & Sambrook, 2019). Multiplex PCR is widely used in the differential detection of *Echinococcus*. It can simultaneously detect multiple species of *Echinococcus* in human or various animals infected samples, which makes this method fast, efficient and economical (Chen *et al*., 2019; Shang *et al*., 2019). Multiplex PCR allows species identification from cysts or stool specimens. For instance cysts from CE patients, performed multiplex PCR using primers of the *EF1A CAL*, *cox*1, *POLD*, and *ELP1* gene, and verified the positive results by PCR amplification of *cox*1. (Tahiri *et al*., 2019). Cysts from patients and stools from other host can be detected by this method as well as be applied in species identification and genotyped. For instance cysts from CE patients, performed multiplex PCR using primers of the EF1A CAL, *cox*1, POLD, and ELP1 gene, and verified the positive results by PCR amplification of *cox*1. (Tahiri *et al*., 2019). The efficiency of two DNA extraction kits with nested PCR and multiplex PCR was compared in detecting *E. multilocularis* in fox feces. Presence of inhibitory components in animal feces directly affected the quality of isolated DNA and subsequent PCR performance, leading to false-negative results (Skrzypek *et al*., 2020). Meanwhile, the degradation of genetic materials in the process of field acquisition, freezing, and thawing also affected the PCR result, which is also an urgent problem for various parasite detection methods. When it comes to the species identification, simultaneous detection of *E. granulosus*, *E. multilocularis*, and *E. Canadensis* (*E. granulosus* subtype) based on multiplex PCR, reporting that the detection threshold for *E. granulosus* and *Echinococcus canadensis* was 0.32 pg and 1.6 pg for *E*. *multilocularis*. In addition to the weak cross reaction with *E. shiquicus*, the specificity for the other 18 parasites was 100% (Chen *et al*., 2019). In the genotyping research, multiplex PCR was used to identify *E. granulosus* species except G1 and G3 genotypes, which has been developed and verified in the European Union Parasite Reference Laboratory (EURLP) (Santolamazza *et al*., 2020).

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP-PCR)

Alejandro *et al*. (2019) combined PCR and RFLP to establish a method for identifying *E. granulosus* and taenia in infected dog feces. The target gene was ribosomal 12S rRNA and digested by the *SspI* restriction enzyme. The product of *E. granulosus* was 160 bp complete fragment, and the product of *Taenia* had two fragments with 62 bp and 98 bp. The method has good repeatability and specificity, with the sensitivity as low as single egg (Alejandro *et al*., 2019). Moreover, genotyping of *E. granulosus* in feces of animals, dogs, and cysts of cattle, revealing that cattle was very likely to become the intermediate host of *Echinococcus*, and dog get infection from accident ingestion of viscera of slaughtered cattle. Pigs in Zambia were also prone to infect dogs due to their great freedom of movement (Fbab *et al*., 2020). Species identification of *E. granulosus* using *nad*1 gene product (1071bp) with HaeIII and HinfI double restriction enzymes, the differences between *E. granulosus*, *E. multilocularis* and *E. shiquicus* were analyzed with close homology, showing that PCR-RFLP could clearly distinguish these three parasites (Chaabane-Banaoues *et al*., 2016). Thus, PCR-RFLP is suitable for large-scale diagnosis in developing countries, and can also be applied to multiple *Echinococcus* species occurring in the same area.

QUANTITATIVE REAL-TIME PCR

Recently, the use of this technique has been reported for the detection of cysts *Echinococcus* species and *Echinococcus* eggs (Frey *et al*., 2019; Malkamäki *et al*., 2019). RT q-PCR was applied with melting curve analysis to detect the presence of *E. multilocularis, E. granulosus* and *Taenia* spp. and cercariae in agricultural products, reporting that the detection sensitivity of this method was 0.01 ng and 1 pg for *E. granulosus* and *E. multilocularis,* respectively. The specificity was verified by sequencing the product (Frey *et al*., 2019). A method for detecting the 12S ribosomal RNA of *E. multilocularis* in animal feces was also established by using DNA magnetic bead extraction combined with fluorescence q-PCR. The combination has been shown to have higher sensitivity and specificity than that of phenol-chloroform DNA extraction combined with single-tube nested PCR. Its sensitivity was also higher than the sedimentation and counting technique (SCT) and intestinal scraping technique. Also, there was no need for exposure to dangerous chemicals. However, the results of some samples could be affected by inhibitors (Maas *et al*., 2016). Moreover, according to Mass *et al*.'s research, Santa *et al*. (2019) applied the automatic DNA extraction kit. The sensitivity and specificity for the detection of *E. multilocularis* in feces are equivalent to that of SCT, which overcomes the influence of PCR inhibitors (Santa *et al*., 2019). Its advantages in detection efficiency and cost can be used as a pre-screening test before SCT.

In the detection and genotyping research of fluorescence RT q-PCR with a high-resolution melting curve, the genotyping results of *E. granulosus* in 40 patient tissues showed 35 strains (87.5%), 4 strains (10%), and 1 strain (2.5%) categorized as G1, G3, and G6 genotypes, respectively. Among 1342 examined livestock, 39 (4%) sheep and 44 (12%) cattle were found with *E. granulosus* cysts, respectively (Mohaghegh *et al*., 2019). Moreover, a new multiplex RT q-PCR was established for the lack of standardized laboratory detection methods to simultaneously evaluate the presence of *E. multilocularis*, *T. gondii* and *Cyclospora cayetanensis* contamination on berries. In addition to the simultaneous detection of these three parasites, this approach has potential to be widely used for the detection of other fresh agricultural products, with good specificity, accuracy, and stability. The detection limit was estimated to be five *Echinococcus* oocysts per 30 g raspberry or blueberry (Temesgen *et al*., 2019). A semi-quantitative fluorescence RT PCR based on SYBR Green for the detection of *E. multilocularis* and *E. canadensis* DNA in fruits, vegetables, and other foods was established. The PCR primers were designed based on the mitochondrial *nad*1 gene and combined with the melting curve of PCR products. Besides having high specificity and sensitivity, this method was simple and rapid. Compared with the probe-based RT q-PCR, it could detect two species at the same time, at a low cost (Malkamäki *et al*., 2019). In terms of genotyping of *E. granulosus*, *cox*1, *cox*3, and *nad*5 genes for primer and TaqMan probe were designed, which can directly identify the four most important *E. granulosus* subspecies causing echinococcosis. The author simplified and improved existing diagnostic procedures from many different aspects, including improving sensitivity and specificity, the ability to quantify sample DNA, reducing test time, reducing sample processing and equipment costs (Maksimov *et al*., 2020).

Fluorescence q-PCR has also been used to study miRNA in *Echinococcus* and echinococcosis patients (Alizadeh *et al*., 2020; He *et al*., 2020). egr-mir-71 and egr-let-7 in the plasma of 30 patients with *E. granulosus* infection before and after cyst resection and the same number of healthy controls were detected. Their results showed that the levels of egr-mir-71 and egr-let-7 genes of all patients specifically increased, and the expression levels of these two miRNAs significantly decreased at three and six months postoperatively. It was speculated that the miRNAs in *Echinococcus* cyst, including egr-mir-71 and egr-let-7, have the potential to be used as a new biomarker for early diagnosis and monitoring of echinococcosis (Alizadeh *et al*., 2020).

NUCLEIC ACID ISOTHERMAL AMPLIFICATION TECHNIQUE

Loop-mediated isothermal amplification (LAMP)

In 2000, Notomi *et al*. (2000) published a nucleic acid isothermal amplification technique, also known as LAMP, which requires a set of four specific primers (a pair of special external primers and a pair of internal primers) to be designed. These in turn can identify six different sequences in the target gene and depend on high strand replacement active DNA polymerase. The amplification reaction is carried out at a constant temperature of 65°C after thermal denaturation and cooling of primers and target sequences. The PCR device can be replaced by a water bath. After about 15-60 min, 10^9 - 10^{10} times of nucleic acid amplification can be realized. The amplification reaction is the most efficient using 130-200 bp DNA as the target gene, and it is very low when exceeding 500 bp. This method is characterized by simple operation, high sensitivity, and high specificity (Notomi *et al*., 2000).

A LAMP technique for detecting *Egranulosus* positive dog feces collected in the field based on *nad*5 gene and compared the differences between the LAMP method, conventional PCR and copro-ELISA. The sensitivity of detecting *E. granulosus* DNA in canine host intestinal tissue was 100 times higher than that of conventional PCR, with detection limits of 10 pg DNA and 1 ng DNA, respectively. The positive results were detected by LAMP on the 22nd day of infection, and by conventional PCR and copro-ELISA on the 26th and 25th day, respectively, indicating that LAMP was the most sensitive. The detection limit of *E. granulosus* eggs in feces detected by the LAMP was 5 eggs/g of feces. In the detection of dog fecal samples, using conventional PCR as a reference, the specificity of the LAMP was 88.8% with a sensitivity of 100%. It was suggested that the LAMP was a diagnostic tool with low cost, simple operation, high sensitivity, and specificity, which could be widely used in the field detection of *E. granulosus* infection and for monitoring of *Echinococcus* infection in canine hosts, especially combined with copro-ELISA (Ni *et al*., 2014a). The author also applied this technique to identify *E. multilocularis* infection, reporting a higher detection rate and sensitivity compared with conventional PCR (Ni *et al*., 2014b). A new copro-LAMP detection that simultaneously detected *E. granulosus*, *E. oligonodus,* and *E. canadensis* in the final host was established. This method referred to the species-specific LAMP with high analytical sensitivity (10 fg-100 fg DNA) and revealed no cross reaction with host DNA or other parasite DNA. Accordingly, it could be used as a potential diagnostic tool for canine *Echinococcus* infection and for the control of CE in rural areas without precise equipment (Avila *et al*., 2020). RT-LAMP was also applied to rapidly detect CE. The sensitivity of this method to detect parasitic DNA could reach 10 fg. When gradient dilution was performed on the DNA extracted from the cyst, the detection result of the LAMP was completely consistent with that of nested PCR. In terms of specificity, it had no cross reaction with other parasites, including bovine cysticercus, *Clonorchis sinensis*, and *Schistosoma* (Ahmed *et al*., 2016). Moreover, there was also the development of a LAMP assay targeting the mitochondrial cytochrome b gene for the rapid detection of alveolar echinococcosis in hepatic nodules of horses (Hifumi *et al*., 2021). Compared with other methods, the DNA detection method of *E. multilocularis* and *E. granulosus* based on the LAMP can be used for rapid and early diagnosis of parasitic infection in a large range, with low cost and high popularization significance.

RecombinaseC aided isothermal amplification assay (RAA)

RecombinaseC aided isothermal amplification assay (RAA) and recombinase polymerase amplification (RPA) uses recombinase, single-stranded DNA binding protein (SSB), and DNA polymerase established, respectively. However, there is difference between the both, including UVS X recombinase of phage T4 for RPA, and recombinase from bacteria or fungi for RAA (Lu *et al*., 2010). They form a complex by pairing recombinase and primers. With the assistance of SSB, template DNA is unchained and paired with a primer. Then, under the action of DNA polymerase, the amplification like the PCR reaction is carried out. The suitable conditions of the used enzymes are between 37°C and 42°C, and no high temperature is required to denature the template. The reaction time is short, and the amplified fragment can be detected in 15~30 min. Except for LAMP, other nucleic acid isothermal amplification techniques have not yet been used for the detection and diagnosis of *Echinococcus* outside of China, while RPA/RAA technology has gradually gained popularity in China over recent years, as some scholars have started to establish a detection method for *Echinococcus* based on RAA technology. Nevertheless, other methods are still rarely applied. Currently, RAA method for the detection of *E. granulosus* and *E. multilocularis* have been established, respectively (Zhou *et al*., 2020a, 2000b), as well as a recombinase-mediated multiple nucleic acid isothermal amplification method (mRAA method) for the simultaneous detection of two species of *E. granulosus*. The minimum detection limit for DNA was 10 pg, and that for the recombinant plasmid of *E. granulosus* was 10^4 copies, while for the recombinant plasmid of *E. multilocularis,* it was 10^4 copies. Also, the results of positive samples were consistent with those of PCR. The minimum detection limit of mRAA for *Echinococcus* DNA was 2 pg/μL, and that of two *Echinococcus* recombinant plasmids was 200 copies/μL. The results of positive samples were consistent with those of the mPCR method, also with good specificity (Zhou *et al*., 2020a, 2020b, 2020c). On account of its time effectiveness, simplicity, high sensitivity and good specificity, RAA provides a new direction for identifying *Echinococcus* species and the gene diagnosis of echinococcosis.

OTHER MOLECULAR DETECTION METHODS

Except for the above techniques, many other molecular detection techniques have been applied to detect *Echinococcus* or diagnose echinococcosis. For instances, microsatellite, circulating free DNA as well as droplet digital PCR were applied. M'Rad *et al*. (2020) applied microsatellite for the diagnosis of *Echinococcus* infection (M'Rad *et al*., 2020). Cell-free DNA (cfDNA) sequence was also screened, which has a great potential for diagnosing human echinococcosis as well as could be further explored in other areas of echinococcosis management, such as drug therapy monitoring or the effectiveness of surgical treatment. Its implications about the strong potential for using such a "liquid-biopsy" method for the ongoing monitoring of disease status in post-intervention echinococcosis patients (Wan *et al*., 2020; Wang *et al*., 2020b; Fan *et al*., 2021; Zhao *et al*., 2021). Moreover, droplet digital PCR was applied in the *E. multilocularis* from faeces, which shows strong potential as a high throughput method for diagnosing *E. multilocularis* prevalence in diverse canid populations as well as infection intensities of individual animals, giving valuable epidemiological insights of the distribution amongst wild canids as an alternative to conventional qPCR or macroscopic methods (Bagó *et al*., 2021).

CONCLUSION

Echinococcosis is a chronic parasitic disease that causes a serious burden to patients. Early stages of echinococcosis remain asymptomatic for 10-15 years, until the cysts grow up in different organs causing symptoms (Kern *et al*., 2017; Wen *et al*., 2019). Clinical symptoms and potentially life-threatening complications of physical compression and damage to organs caused by cysts, when cysts rupture at any position, fever, urticaria, and anaphylactic shock will follow (Kern *et al*., 2017; Siles-Lucas *et al*., 2017; Wen *et al*., 2019). Echinococcosis is mainly treated by surgical inactivation, removal or destruction of parasitic tissues and in combination with benzimidazoles (albendazole or mebendazole) (Kern *et al*., 2017; Qian *et al*., 2017).

For a long time, many countries have actively taken comprehensive prevention, and control strategies and measures to control the impact of echinococcosis on human health and the development of animal husbandry. In China, a national program for echinococcosis control was launched in 2005, a series of policies such as health education, sanitation improving, ultrasound screening of the human population, surgical interventions and drug therapy, including registration, sheltering and deworming of domestic and stray dogs (Yu *et al*., 2020; Wang *et al*., 2022). Through these measures, human echinococcosis prevalence rate decreases from 1.08% in 2004 to 0.24% in 2012, dog echinococcosis prevalence rate decreases from 7.3% in 2016 to 1.7% in 2019 (Yu *et al*., 2020; Wang *et al*., 2022). Japan uses praziquantel to deworm foxes in small public places, reducing the egg-positive to close to zero to prevent human infection with *E. multilocularis* tapeworms in the region (Uraguchi *et al*., 2022). Echinococcus is controlled by praziquantel in most endemic regions of the world and has achieved good results in livestock, the problem of how to ensure that wild animals take medicine at a dose and prevent the recurrence of positive eggs after drug withdrawal remains unresolved (Craig *et al*., 2017).

Development of echinococcosis research and molecular biology detection technology, as well as nucleic acid isothermal amplification technology, provide opportunities for the diagnosis technology of echinococcosis, breakthroughs in early diagnosis of echinococcosis, and environmental pollution monitoring of *Echinococcus* (Sun *et al*., 2020). The emergence and optimization of these new technologies are expected to have an important role in the detection of intermediate and terminal host infection (including feces), environmental monitoring, and the prevention, control, and diagnosis of echinococcosis. Using molecular detection technology to promote the development of the early diagnosis, as well as more sensitive and specific methods to timely and effectively monitor the host and environment may accelerate the process of control and elimination of echinococcosis.

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Conflicts of interest

The authors declare no conflicts of interest with regards to this study or the manuscript prepared for publication.

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