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Current status and future prospects of *Echinococcus multilocularis* vaccine candidates: A systematic review

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ABSTRACT

The larval stages of *Echinococcus multilocularis* (*E. multilocularis*) are what cause the zoonotic disease known as alveolar echinococcosis (AE). Identifying the antigens that trigger immune responses during infection is extremely important for the development of vaccines against ***Echinococcus*** infections. Several studies conducted in recent decades have described the specific traits of the protective antigens found in *E. multilocularis* and their role in immunizing different animal hosts. The objective of the current systematic review was to summarize the findings of relevant literature on this topic and unravel the most effective vaccine candidate antigens for future research. A comprehensive search was conducted across five databases, including ProQuest, PubMed, Scopus, ScienceDirect, and Web of Science, until March 1, 2023. Two reviewers autonomously conducted the screening and evaluation of data extraction and quality assessment. In the present study, a total of 41 papers matched the criteria for inclusion. The study findings indicate that the combination of Em14-3-3 and BCG is widely considered the most often employed antigens for *E. multilocularis* immunization. In addition, the study describes antigen delivery, measurement of immune responses, adjuvants, animal models, as well as routes and doses of vaccination. The research indicated that recombinant vaccines containing EMY162, EM95, and EmII/3-Em14-3-3 antigens and crude or purified antigens containing ribotan-formulated excretory/secretory antigens exhibited the most favorable outcomes and elicited protective immune responses.

1. Introduction

Echinococcus multilocularis (*E. multilocularis*) is a parasitic tapeworm that causes alveolar echinococcosis (AE), a potentially life-threatening disease in animals and humans (Robbins et al., 2022). The life cycle commences when the mature tapeworm takes up residence in the intestines of its definitive host, usually wild canids such as foxes and raccoon dogs. These hosts excrete the eggs of the parasite in their feces, contaminating the environment. Intermediate hosts acquire infection through the ingestion of parasite eggs, which can occur in small mammals or humans. Upon entering the intermediate host, the eggs undergo hatching within the intestines, releasing oncospheres that penetrate the intestinal wall and migrate to various organs. The oncospheres transform into metacestodes, which are vesicles filled with fluid. When a definitive host ingests an infected intermediate host, the metacestodes' scoleces are liberated into the digestive system, where they adhere to the intestinal wall and mature into adult tapeworms, therefore completing the life cycle (Algros et al., 2003; Deplazes et al., 2017). The infection in humans is referred to as AE, which is distinguished by the formation of cysts in the liver and other organs, including the lungs and brain. Untreated cysts can lead to significant tissue damage and organ failure. The symptoms may encompass stomach pain, jaundice, weight loss, and

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general malaise (Williams and Walzthoni, 2023; Zheng et al., 2015). The diagnosis of AE in humans involves a combination of clinical evaluation, imaging studies, and serologic tests. An ultrasound, computed tomography (CT) scan, or magnetic resonance imaging (MRI) scan can be used to see the lesions that *E. multilocularis* causes in the liver and other organs that it affects. These imaging studies can help identify the presence of cysts and assess the extent of the disease. Serologic tests, such as enzyme-linked immunosorbent assays (ELISA) and immunoblot tests, are commonly used to detect these antibodies, which can provide supportive evidence of the infection (Aoki et al., 2015; Graeter et al., 2016).

The treatment typically involves a combination of surgery and antiparasitic drugs such as albendazole or mebendazole, which may be used before or after surgery to help control the growth of the parasite and prevent recurrence (Chen et al., 2018). Since surgery is the only treatment for this disease and also because of the complex life cycle of *E. multilocularis* infection, it is important to develop efficient control strategies, including vaccinations, to reduce the impact of this zoonotic disease on animal and human populations. There is currently no vaccine available for the prevention of *E. multilocularis* infection. Research into vaccine development for this parasitic infection is ongoing, but as of now, there are no approved vaccines for use in animals or humans. Preliminary results have been encouraging and show that vaccinated animals have a higher level of immunity to infection than unvaccinated control animals. The vaccination of definitive hosts against *E. multilocularis* is an important strategy for controlling the spread of this parasite. Vaccination of definitive hosts can help reduce the shedding of *E. multilocularis* eggs into the environment, thereby decreasing the risk of human and animal infection (Craig et al., 2007; Nunnari et al., 2012; Siegert and Neumann, 2022). The most important advantage of the *E. multilocularis* vaccine is that it can help reduce the burden of human and animal disease and also reduce its prevalence. However, several challenges must be addressed for the vaccine to be truly effective. These include improving the production process of recombinant antigens, ensuring their long-term stability, and improving vaccine efficacy using combinations of antigens. Because no comprehensive study on *E. multilocularis* vaccine candidate antigens has been conducted to date, the current systematic review aimed to summarize the findings of relevant literature on this topic and identify the most effective vaccine candidate antigens for future research.

2. Materials and methods

2.1. Database search strategy and study selection

The points considered in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) statement were observed in this systematic review (Moher et al., 2009). Such English-language databases as PubMed, ProQuest, Scopus, Science Direct, and Web of Science were searched for published articles on *E. multilocularis* vaccine research with no time limit up to March 1, 2023. Moreover, the included paper bibliography lists were manually assessed for probably relevant references that were not noticed by a database search.

The keywords selected based on medical keyword terms included "*Echinococcus multilocularis*", "*E. multilocularis*", "Alveolar hydatid", "alveolar echinococccosis", "vaccination", "vaccine", "immunogenicity", "protective immunity", "antigen," and "immunization" alone or in combination with 'OR' and/or 'AND' operators. All references were manually checked to ensure no articles were overlooked. All citations were downloaded to EndNote. The study examined no gray literature or abstracts of articles presented at conferences. Articles were evaluated by two independent reviewers (M.H. and N.Kh.) to ensure eligibility. Additionally, discrepancies with the selected work have been reviewed by another author (D.A.).

2.2. Selection criteria and quality assessment

All the retrieved titles, abstracts, and full texts, if needed, were carefully screened, and the eligible papers were selected at least by two reviewers. Studies were considered eligible if they met the inclusion criteria. The main inclusion criterion was experimental studies investigating the characteristics of *E. multilocularis* antigens or assessing the protective effect of immunization against infection in different hosts. On the other hand, 1) studies lacking sufficient data, 2) articles in languages other than English, 3) those that did not use animal models to evaluate vaccines, and 4) irrelevant abstracts were excluded from the research procedure. The criteria by the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE; https://www.radboudumc.nl/en/research/departments/health-evidence/systematic-review-center-for-laborator-animal-experimentation) adapted from the Cochrane as a tool to assess the methodological quality were employed to assess the quality of the selected articles.

The 10-item SYRCLE statement is used for such biases as selection, detection, attrition, performance, and reporting. A higher SYRCLE score indicates better quality of biases in terms of attrition, detection, and reporting. Moreover, an increase in the SYRCLE score shows a higher quality of the articles.

2.3. Data extraction

The research team conducted the data extraction procedure, and any discrepancies were resolved through a consensus discussion. The extracted items from the eligible articles are as follows: Author/year, antigen delivery, adjuvant/carrier, antigen(s), animal model(s), challenge, and outcomes. Two experienced researchers performed a thorough search process, eliminated identical references, and evaluated relevant (eligible) articles. A data extraction form created in Microsoft Excel® was used to obtain the required data.

3. Result

3.1. Summary of the included studies

Out of 1611 articles identified, 540 were excluded from the screening process because they were duplicates. Subsequently, the titles and abstracts of the remaining 1071 publications were evaluated, leading to the exclusion of 944 investigations. Moreover, after reviewing the complete content of 127 articles, 86 items were excluded. The systematic review comprises 41 papers (6 crude antigen and 35 recombinant antigen), with no deadline for publication until March 1, 2023. Fig. 1 provides a comprehensive depiction of the systematic search process and article selection.

3.2. Crude or purified antigens or immunomodulators

In total, six articles investigated the immunization basis with crude or purified antigens against *E. multilocularis*. Protoscolex antigens were regarded as the most commonly used crude antigens. Table 1 tabulates the efficiency of crude antigen/purified vaccination against *E. multilocularis*.

3.3. Recombinant vaccines

The majority of the studies (n = 35) on AE vaccination used recombinant approaches. EM95 (n = 3), Antigen B (n = 3), EMY162, and TSPs genes (n = 4), recombinant Em14-3-3 alone (n =6) or in combination with other peptides or antigens (n = 5), and EmII/3 (n = 5) alone or in combination with BCG were some of the most common recombinant vaccines. Table 2 summarizes the approaches to immunization against AE using recombinant vaccines.

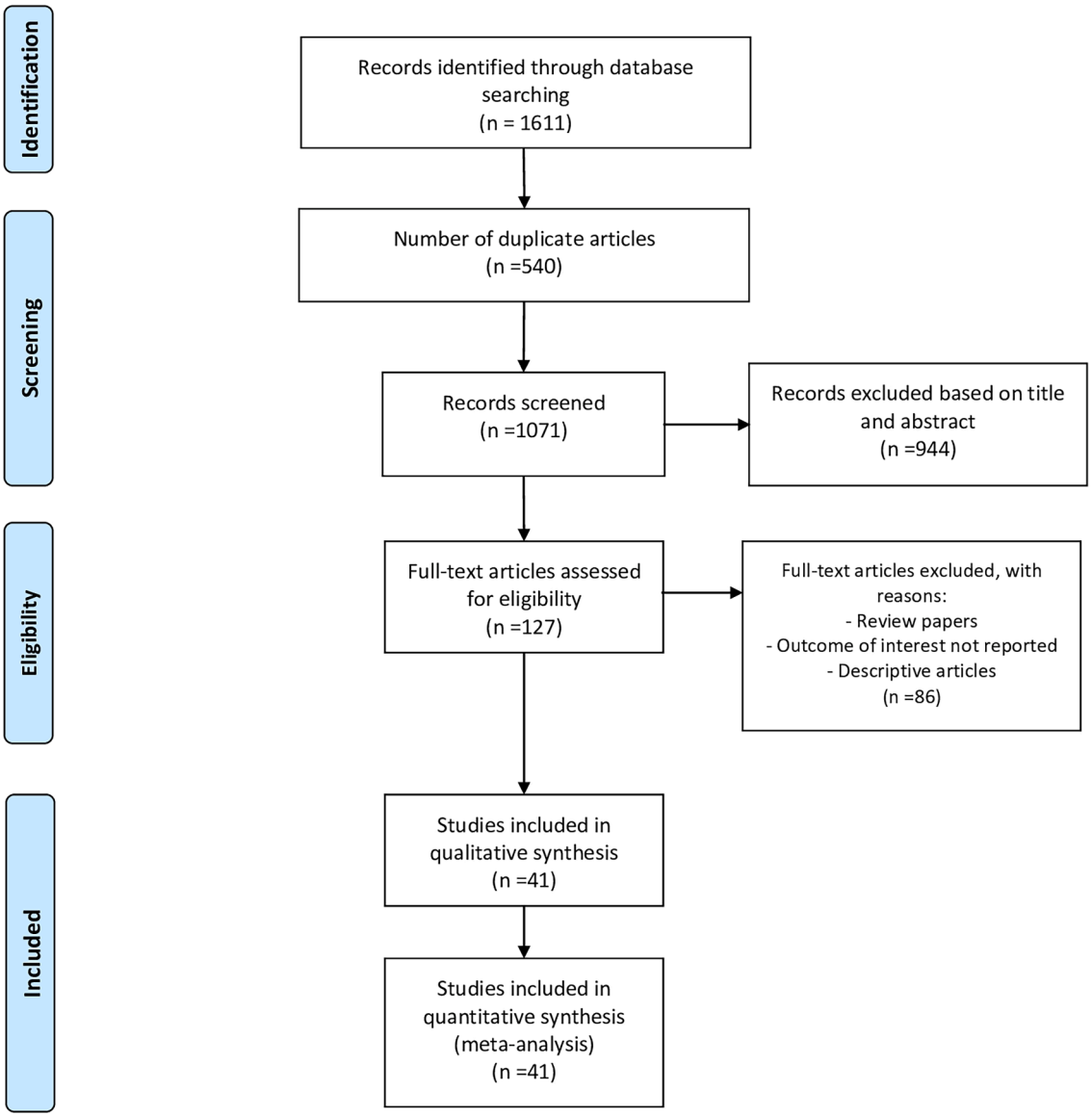

Fig. 1. PRISMA flow diagram describing included/excluded studies.

Fig. 1. PRISMA flowchart to describe the process of selected studies.

3.4. Adjuvants

The adjuvants that were included more than others were complete freund's adjuvant (CFA) (n = 15), incomplete freund's adjuvant (IFA) (n = 11), saponin (n = 3), CpG ODN (cytosine phosphoguanine oligodeoxynucleotides) (n = 3), and alum (n = 2). It should be mentioned that 13 articles employed no adjuvants in their vaccination protocols. Tables 1-2 summarize the other adjuvants.

3.5. Route and dose of vaccination

The vaccine candidates were administered through several methods, including intraperitoneal (i.p.; n = 10), subcutaneous (s.c.; n = 19), intranasal (i.n.; n = 9), intraoral/orally (i.o.; n = 4), and intramuscular (i.m.; n = 3). The optimal dosage for experimental recombinant protein vaccines was found to be 50 μg.

3.6. Animal models

Most of the studies (n = 37) used mice as their main animal model. The types of mice used were BALB/c (n = 26), C57BL/6 (n = 5), Cotton Rat (n = 3), AKR, DAB/2, C57B1/6J, C57B1/10, Athymic nude, and white outbred (one study per mouse). Other vertebrates regarded as animal models for immunization included dogs (n = 5), rabbits (n = 2), as well as Rhesus monkeys (n = 1) and pigs (n = 1).

3.7. Challenge

The i.p. or i.o. injection approach was extensively used to give parasites to animal models to determine if the vaccinated animals survived. The most common method of introducing the parasite into the animal models for testing was intraperitoneally (n = 17), with oral administration being the second most common (n = 7). No challenge was conducted in the eight experiments following immunization. Most

Table 1
Immunization with crude antigen or live vaccines against *Echinococcus multilocularis*

Antigen(s)	Adjuvant /Carrier	Antigen delivery	Animal model	Challenge	Result	Score	Authors/ year
E/S Antigen + Ribotan	-	60 μg (s.c.)	white outbred mice	*E. multilocularis* protoscoleces (i.p.)	The maximum protection was obtained in mice immunized with the combination of antigen and Ribotan (91.70 %).	9	(Rudneva et al., 2016)
SRF1	Cholera toxin (CTB) subunit B	500μg (i.n.)	Dog	5 × 105 *E. multilocularis* protoscoleces (i.o.)	Dogs vaccinated with SRF1 antigen showed an 87.6% reduction in worm counts compared to control dog. A weak serum antibody response was observed in dogs immunized with SRf1, but there was no correlation between antibody response and worm count.	10	(Kouguchi et al., 2013)
Purified alkaline phosphatase and crude extract antigen	alum	40 μg	BALB/c	*E. multilocularis* 2200±10 protoscoleces	The isotypic profile showed a prevalence of IgG1 and IgG3 in immunized infected mice compared to IgG2a and IgG2b. The cytokine profile was a mix of Th1/Th2 types in the infected and uninfected immunized mice. The comparison of the immune response showed an important immune response in mice immunized with purified alkaline phosphatase compared to mice immunized with the crude total antigen.	10	(Issaadi et al., 2006)
Em2	RIBI concanavalin A (Con A)	50 μg (s.c.)	AKR C57B1/6J C57B1/10	50 *E. multilocularis* metacestode (i.p.)	Resistance in C57B1/10 mice is associated with the ability of the host to synthesize antibodies to Em2 of the IgG3 and IgG1 isotype. In susceptible AKR and C57B1/6J mice, low levels of anti-Em2 antibodies of the IgG2a isotype were detected.	10	(Gottstein et al., 1994)
BCG-CW	-	150 μg (i.p.)	Cotton Rats	-	A single injection of BCG-CW, emulsified in Oil-Tween-saline, 2 weeks before the inoculation of the parasite completelyprotected against infection with *E. multilocularis*. Protection was correlated with an increase in the numbers of monocytes	8	(Reuben et al., 1979)
BCG	-	1-ml doses 10^1 to 10^7 CFU of BCG (i.p.)	Cotton Rats	-	Low prophylactic doses of BCG are effective in controlling experimental *E. multilocularis* infections without the concomitant formation of macroscopic granulomatous lesions.	8	(Reuben et al., 1978)

investigations used an infection dose ranging from 1000 to 2000 protoscoleces per animal.

3.8. Immune responses

The host immune responses were determined using specific antibodies and cytokine measurements. The main antibodies that were measured are as follows: IgG total (n = 14), IgG1 (n = 14), IgG2a (n = 14), IgG2b (n = 3), IgG3 (n = 4), IgA (n = 6), IgE (n = 5), and IgM (n = 4). Also given high marks were interferon gamma (IFN-γ) (16 people), interleukin 4 (IL-4) (15 people), IL-10 (n = 8), IL-12 (n = 3), IL-2 (n = 8), IL-6 (n = 2), IL-5 (n = 2), IL-17 (n = 3), and tumor necrosis factor alpha (TNF-α) (n = 6), TNF-β (n = 2), and TNF-γ (n = 2). In addition, lymphocyte and splenocyte proliferation assays were performed in 11 studies. Tables 1 and 2 contain the details of the immune responses for each study.

3.9. Protective effects

Vaccination can provide protection against *E. multilocularis* in both definitive and intermediate hosts and help decrease the spread of the parasite. Results of this review study showed that vaccinating definitive hosts can significantly decrease the number of worms and enhance both mucosal and systemic immunity. In intermediate hosts, vaccination results in a considerable reduction in cyst width and weight (Zhou et al., 2023; Kouguchi et al., 2013; Katoh et al., 2008). Recombinant vaccines made from 14-3-3 antigen mixed with saponin (97%) and gerbu (84.47%) gave the best protection against *E. multilocularis* infection (Table 2). In addition, even using crude or purified antigens, such as excretory/secretory antigens combined with ribotan, a significant level of protection (91.70%) was achieved (Table 1).

4. Discussion

AE, one of the most prevalent parasitic infections globally, resembles a tumor in its growth and invades the host's tissue. Treating *E. multilocularis* infections is still hard, and prevention is needed to stop cysts from fibrosis and infiltrating the liver. Generally, it is possible to control infectious diseases by protecting the final host against infection or by using vaccination to prevent disease in intermediate hosts. Vaccination against parasites becomes possible after intensive studies of protective immune responses that allow identifying the best antigens to elicit strong immune responses to protect against infection (Craig et al., 2007; Moghaddam et al., 2019). Vaccination is a successful technique for preventing AE because it inhibits the formation and metabolism of cysts and also induces a specific immune response (Wang et al., 2021). This study aimed to summarize the previously conducted research on the aforementioned topic in order to find the most effective vaccine candidates against AE that can be used in future research.

4.1. Immunization with crude or purified antigens or immunomodulators

According to our survey, a total number of six publications were done on the basis of immunization with crude antigen or purified antigens against *E. multilocularis*. In the study of Rudneva et al. which utilized the excretory-secretory antigen of protoscolex together with ribotan in outbred white mice, they achieved a significant level of protection (Rudneva et al., 2016). In another study carried out by Issaadi et al. on the mouse model, there was a considerable increase in IgG1 and IgG3 levels in subjects immunized with purified alkaline phosphatase and only IgM levels in those inoculated with crude antigens of *E. multilocularis*. Also, the levels of IL-6, IL-10, and IFN-γ were elevated in mice infected with pure alkaline phosphatase and crude antigen (Issaadi et al., 2006). Concerning the use of crude antigens, Koguchi et al. researched the use of SRf1, a glycoprotein component antigen, and

Table 2
Immunization with Recombinant vaccines against *Echinococcus multilocularis*

Antigen(s)	Adjuvant /Carrier	Antigen delivery	Animal model	Challenge	Result	Score	Authors/year
Em CRT	CFA IFA	25μg (s.c.)	BALB/C	2000 Protoscoleces (i.p.)	Mice immunized with soluble rEmCRT formulated with Freund's adjuvant produced a 43.16% larval vesicle weight reduction against the challenge of *E. multilocularis* PSCs high titer of IgG, IgG1 and IgG2a antibody responses as well as high levels of Th1 cytokines (IFN-γ and IL-2) and Th2 cytokines (IL-4, IL-5 and IL-10.	10	(Chen et al., 2022)
Gp50 Eg95 B antigen	-	-	Dog Pig Dab/2 mice	5 × 105 *E. multilocularis* protoscoleces (i.o.)	Ag B antigen family dominated in early stage metacestodes, GP50antigen family dominated inactivated oncospheres andEg95 antigen are dominated in non-activated and activated oncospheres. Heat shock proteins and antigen II/3 which contain highly conserved domain in invertebrates and vertebrates are constantly expressed in the three stages. The reveal of various known antigens expression level during the parasite development stages, especially the stages of non-activated and activated oncospheres, will give fundamental information for choosing candidate genes used in early diagnosis.	9	(Huang et al., 2016)
EmAgB3	CFA	50μg 10μg (i.p.)	BALB/C	1000 protoscoleces (i.p.)	rEmAgB3 exhibited sensitivity of 90.9% and specificity of 98.5% by immunoblotting. The positive and negative predictive values were 89.9% and 98.6%, respectively. Immune responses to this EmAgB3 isoform were highly correlated with worm viability accompanied with AE progression.	10	(Ahn et al., 2015)
E95-1 E5-2	CFA IFA	0.5mg (s.c.)	Mice Rabbits	Protoscoleces	Our data suggest that B- and T-cell combined epitopes predicted from the Em95 antigen may be used for the construction of high-valence vaccines and as targets for prevention of echinococcosis.	10	(Wang et al., 2014)
EM-TSP3-FBP	CpG ODN	50μg (i.n.)	BALB/c	-	Both rEm-TSP3-FBP and rEm-TSP3+CpG evoked strong serum IgG and IgG1 responses, whereas only the latter induced a high level IgG2α production compared to that of rEm-TSP3 alone without any adjuvant. The results indicated that i.n. immunization with rEm-TSP3-FBP resulted in an increased IgG1/IgG2α ratio (a Th2 tendency), while rEm-TSP3+CpG caused a rapid Th1 response that later shifted to a Th2 response. Immunization with rEm-TSP3-FBP provoked significantly stronger IgA antibody responses in intestine, lung and spleen compared to those by rEm-TSP3+CpG.	9	(Dang et al., 2012a)
Hsp20	CFA IFA	10 μg	Dogs	5 × 105 protoscoleces	RT-PCR and Western blot analyses revealed that the putative hsp20 gene and its products were expressed in almost all stages of the parasite life cycle. Recombinant hsp20 showed specific reactivity to the sera from infected dogs, suggesting that this molecule may facilitate the development of a practical vaccine.	10	(Kouguchi et al., 2010)
TSPs	CFA IFA	Rabbit: 150μg (i.m.) BALB/C: 20 μg (s.c.)	BALB/C Cotton rats Rabbit	400 *E. multilocularis* eggs	The cyst lesion reduction rates induced by the seven tetraspanins in vaccinated and non-vaccinated mice were: 87.9%, 65.8%, 85.1%, 66.9%, 73.7%, 72.9% and 37.6%. Vaccination conferred protective rates to mice ranging from 0% (TSP5, 6, 7) to maximally 33% (TSP1, 3). The results indicated that recombinant tetraspanins have varying protective effects	10	(Dang et al., 2009)

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Table 2 (*continued*)

Antigen(s)	Adjuvant /Carrier	Antigen delivery	Animal model	Challenge	Result	Score	Authors/year
					against primary alveolar echinococcosis and could be used in vaccine development.		
EmY 162	-	-	dog	10^5 protoscoleces	EMY 162 can target both mucosal and systemic immunity in dogs because it is predicted to be a protein with a domain similar to type III fibronectin, while sera from infected dogs showed a strong IgG antibody response to recombinant EMY162.	8	(Katoh et al., 2008)
EMY162 EM95	CFA IFA	50 μg 20 μg (s.c.)	BALB/C	200 *E. multilocularis* eggs. (i.o.)	RT-PCR analysis revealed that the gene expression of emy162 was significantly higher than that of em95 at each life-cycle stage. Recombinant EMY162 antigen induced a significant level of host-protection (74.3%) in experimental infection with *E. multilocularis* eggs in mice.	10	(Kouguchi et al., 2007)
EmAgB	-	-	BALB/C Athymic nude mice	*E. multilocularis* metacestode (i.p.)	EmAgB transcripts were less abundant in nude mice during the early phase of infection (at one-month post-infection), and that EmAgB2 is simultaneously down-regulated when compared to the other three genes (AgB1, AgB3 and AgB4 genes). A negative relationship exists between the level of transcription and diversity of EmAgB genes. Moreover, no excess of non-synonymous substitutions was found among the distinct EmAgB alleles from a single host.	9	(Graichen et al., 2007)
14-3-3	-	10 μg	C57BL/6	Metacestode vesicles (i.p.)	These results provide a strong indication that the CD4(+) alphabeta (+) T-cell-mediated immune response contributes to the control of the parasite growth and to the regulation of production of the parasite 14-3-3 protein in metacestode tissues.	9	(Dai et al., 2004)
EM95	Saponin	20 μg (s.c.)	BALB/C	3000 *E. multilocularis* eggs (i.o.)	Immunization with the EM95 antigen induced statistically significant protection against infection with *E. multilocularis* in comparison with mice immunized with the control GST protein. This was evident when either saponin or oil was used as adjuvant. Immunization with EM95 plus saponin induced 82.9% protection.	10	(Gauci et al., 2002)
Salmonella typhimurium-delivered glyceraldehyde-3-phosphate dehydrogenase	STP (Squalen, Tween, Pleuronic)	(i.o.) or (i.p.)	BALB/C	*E. multilocularis* eggs (i.o.)	Recombinant glyceraldehyde-3-phosphate dehydrogenase of the *E. multilocularis* resulted in significant protection, reducing the number of developing metacestodes up to 79.8%. The sera of protected animals did not contain detectable amounts of antibody against glyceraldehyde-3-phosphate dehydrogenase of *E. multilocularis*.	10	(Müller-Schollenberger et al., 2001)
Em2(G11)	IL-12	-	C57BL/6	Metacestode vesicles (i.p.)	In vivo, the IgG response to major carbohydrate antigen Em2(G11) of *E. multilocularis* could take place independently of alphabeta+ CD4+ T cells and in the absence of CD40-CD40 ligand interactions; thus, the Em2(G11) antigen of the acellular LL represents a T-cell-independent antigen.	8	(Dai et al., 2001)
GILE recombinant protein (EMY162, LAP &GLUT1)	CFA IFA	50 **µg** (i.p.)	BALB/c	1000 protoscoleces (i.p.)	Mice immunized with GILE showed higher levels of serum antibodies compared to the PBS group. The results indicated that mice immunized with GILE secreted more IFN-γ and IL-4. Immunization with GILE also led to a significant decrease in the maximum diameter and weight of cysts and stimulated the production of $CD4^+$ and $CD8^+$ T Cell.	10	(Zhou et al., 2023)
rEM -LAP	CFA	10 μg (i.p.)	BALB/C	2000 protoscoleces (i.p.)	rEM-LAP could induce specific immunity response and produce high levels of IgG, IgG1, IgG2a, IgM, and IgA, and the serum levels of IFN-γ and IL-4 are significantly increased compared to the control groups,	10	(Zhou et al., 2022)

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Table 2 (*continued*)

Antigen(s)	Adjuvant /Carrier	Antigen delivery	Animal model	Challenge	Result	Score	Authors/year
					indicating that treatment with rEM-LAP leads to a Th1 and Th2 mixed-type immune response.		
LTB-ETBM	CFA IFA CpG	0.5 mg/mL	BALB/c	1000 protoscoleces (i.p.)	The LTB-ETBM significantly inhibited the formation of cysts in mice challenged with 1000 E.m. protoscoleces. Decreased the growth of protoscoleces and the formation of cysts. LTB-ETBM may be efficacious for activating the immune system and for use as a prophylactic or therapeutic agent against E. m. infection.	10	(Li et al., 2022)
TSP3	CFA	50 μg (i.p.)	BALB/C	-	The B-cell epitopes and T-cell epitope subtypes Th1, Th2, and Th17 were identified as having good immunogenicity.	9	(Pang et al., 2022)
Em14-3-3	CFA IFA	50 μg	BALB/C	-	Two dominant antigen epitopes of B cells, two Th1 dominant antigen epitopes, and one Th2 dominant antigen epitope were validated.	9	(Wei et al., 2021)
rEm-LAP	CFA IFA	50 μg (i.p.)	BALB/C	2000 protoscoleces (i.p.)	rEm-LAP could induce a Th1 and Th2 mixed-type immunological response and produce high levels of IgG, IgG1, IgG2a, IgM, and IgA. Furthermore, serum IFN-γ and IL-4 secretion were increased compared with the control groups.	10	(Wang et al., 2021)
Emy162	CFA	50μg (i.p.)	BALB/C	-	The effect of Th2 and B predominant antigen peptide on the stimulation of lymphocytes in the Emy162 immune group and the PBS immune group had obvious differences, and it was higher in the Emy162 immune group.	9	(Pang et al., 2020)
LTB-EMY162	CFA IFA	50 μg (s.c.)	BALB/C	1000 protoscoleces (i.p.)	LTB-EMY162 induced high-titer specific IgG against EMY162 and *E. multilocularis* protoscoleces protein in BALB/c mice and promoted sensitized T lymphocyte cell proliferation, and LTB-EMY162 stimulated Th cell to secrete IL-4 and IFN-γ and induced a Th1/Th2 mixed type immunological response.	10	(Li et al., 2018)
Em14-3-3	Quil A Alum Murray Dipeptide	(s.c.)	Rhesus monkeys	-	Vaccination of rhesus macaques with Em 14-3-3 recombinant antigen induces parasite-specific binding antibodies, and alum is the strongest adjuvant in terms of antibody level and longevity.	9	(Lampe et al., 2017)
rEmp29	Saponin	20μg (i.p.)	BALB/C	*E. multilocularis* 50 metacestode (i.p.)	The mean parasite weight in mice vaccinated with rEmP29 was reduced by 75% and 59%, respectively, compared to control mice treated with NaCl or saponin. Mice treated with rEmP29 immunotherapy showed a mean parasite load that was reduced by 53% and 49% compared to control mice treated with NaCl and saponin, respectively.	10	(Boubaker et al., 2015)
rEm-TSP1 rEm-TSP3	CpG ODN CFA IFA	50μg (i.n.) (s.c.)	BALB/c	1000 protoscoleces (i.o.)	Both the immunization routes evoked strong serum IgG, IgG1 and IgG2α responses; i.n. immunization induced significantly higher IgA responses in nasal cavity and intestine compared with s.c. immunization. Both immunization routes induced extremely strong liver IgA antibody responses. S.c. immunization resulted in a reduction in the IgG1/IgG2α ratio (Th1 tendency), whereas i.n. immunization caused a shift from Th1 to Th2.	10	(Dang et al., 2012b)
VF Em2(G11) Rec-14-3-3	LPS	1 μg 2 μg 1 μg 100 ng	C57BL/6	*E. multilocularis* metacestode (i.p.)	While LPS and rec-14-3-3-antigen were able to induce CD80, CD86 and (to a lower extent) MHC class II surface expression, Em2(G11) and, strikingly, also VF-antigen failed to do so. LPS and rec-14-3-3 yielded elevated IL-12, TNF-α and IL-10 expression levels, while Em2(G11) and VF-antigen didn't.	10	(Margos et al., 2011)

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Table 2 (*continued*)

Antigen(s)	Adjuvant /Carrier	Antigen delivery	Animal model	Challenge	Result	Score	Authors/year
VF Rec14-3-3	Gerbu	10 μg (s.c.)	C57BL/6	*E. multilocularis* clone KF5as (i.p.)	Results Mice vaccinated with rEg14-3-3 and challenged with protoscoleces revealed significant protective immunity of 84.47%. Immunized mice generated specific high levels of IgG and the prevailing isotypes of IgG were IgG1 and IgG2a. Splenocytes from mice immunized with rEg14-3-3 showed a significant proliferation response. The secretion of IFN-y and IL-2 increased significantly in the vaccinated mice whereas there was no significant difference in IL-4 and IL-10 levels between vaccinated and control mice.	10	(Margos and Gottstein, 2010)
rBb-Emll/3-Em14-3-3	-	(s.c.) (i.m.) (i.n.) (i.o.)	BALB/C	50 protoscoleces (i.p.)	The level of IFN-γ, IL-12 and TNF-α were greatly higher than that of PBS control group, and the level of IL-10 was lower. The level of IFN-γ, IL-12, TNF-α and IL-10 in each group with EmAg and ConA or LPS stimulation was greatly higher than that in the group without stimulation.	9	(Yang and Li, 2008)
rBCG-Em14-3-3	-	(s.c.) (i.n.)	BALB/C	Protoscoleces	Significant increase of IFN-γ and TNF-α were found in the immunized mice. But the IL-4 was decreased after the vaccinations. The levels of TNF-α were higher in the intranasal vaccinated mice than in the subcutaneous vaccinated mice.	9	(Li et al., 2008)
Mix rBCG-EmII/3 and BCG-Em14-3-3	-	(s.c.) (i.n.)	BALB/C	Protoscoleces	In the groups of immunization, the rate of reduced alveolar echinococcus weight was 45.29%~76.47%, the level of IgG, IgG2a, IgG2b and IgE. Intranasal vaccination with mix recombinant BCG-EmII/3 and BCG-Em14-3-3 vaccine may be a good route.	8	(Jiang, 2007)
rBCG-Em II/3	-	(s.c.) (i.m.) (i.n.) (i.o.)	BALB/C	50 protoscoleces (i.p.)	• Apoptotic rate of splenocytes in all groups with or without ConA stimulation was obviously lower than that of PBS group.	9	(Yang, 2007)
Mix rBCG-EmII/3 and BCG-Em14-3-3	-	(s.c.) (i.n.)	BALB/C	Protoscoleces	The levels of IFN-γ and TNF-α increased obviously but the level of IL-4 decreased. The level of TNF-α in subcutaneous group was higher than that in intranasal group.	9	(Li et al., 2007)
14-3-3 protein (rE14t)	Saponin	20 μg (s.c.)	BALB/c	2000 *E. multilocularis* eggs (i.o.)	Major differences became apparent between secondarily and primarily infected animals: whereas no protection against secondary infection was achieved by vaccination, vaccinated animals were protected by 97% against challenge primary infection with *E. multilocularis* eggs. The parasite 14-3-3molecule appears crucially involved in the early stage of the host–parasite interplay and exhibits potential to be used as target molecule for the development of protective tools against AE.	10	(Siles-Lucas et al., 2003)
rBCG-EmII/3 and BCG-Em14-3-3	-	(s.c.) (i.n.)	BALB/C	Protoscoleces	• In the immunized groups, the rate of reduced alveolar echinococcus weight was 45.29%-76.47%; the levels of IgG, IgG2a, IgG2b and IgE increased apparently while IgG1 and IgG3 decreased remarkably. • CD4+ subset and the ratio of CD4+/ CD8+ increased obviously, but CD8+ subset had no change.	9	(LI et al., 2003)
S. typhimurium +pVM II/ 3-10	-	(s.c.) (i.o.)	C57BL/6J Dog	-	• The vaccine infected C57BL/6J mice and dogs stimulated antibody production and lymphocyte activation against S. typhimurium and *E. multilocularis* antigens. • Two immunized dogs showed a robust humoral immune reaction, but no lymphocyte growth was observed.	8	(Gottstein et al., 1990)

cholera toxin B subunit (CTB) as an adjuvant to accelerate the development of vaccines for *E. multilocularis* in a dog model. They noted a significant decrease in the parasite population following immunization in dogs (Kouguchi et al., 2013). Another study used the Em2 antigen as a crude antigen in different mice. The study discovered that resistance in C57B1/10 mice is associated with the host's ability to generate antibodies against Em2 of the IgG3 and IgG1 isotypes. AKR and C57B1/6J mice that were vulnerable have low levels of IgG2a isotype anti-Em2 antibodies (Gottstein et al., 1994).

Based on our results, in relation to immunization with crude antigen or live vaccines against the *E. multilocularis*, it seems that protection percentage of this method is 87.6 to 91.7 and the best candidate is the *E. multilocularis* protoscoleces. Recent research has shown that vaccines made from crude antigens are not able to provide full immunity, stimulate both humoral and cellular immunity, or generate enough antibodies for a prolonged period. Additionally, vaccines with crude antigens have a short duration of effectiveness and pose a risk of transmitting parasites to vulnerable animals. Consequently, these vaccines are not commonly utilized in current research. (Kebede, 2016; Miguez et al., 1996).

4.2. Immunization with recombinant vaccines against E. multilocularis

Our findings revealed that the included studies had 35 experimental recombinant vaccines. DNA vaccines and protein recombinant vaccines are both types of recombinant vaccines that utilize genetic engineering techniques to generate an immune response, but they differ in the way they deliver the antigen to the immune system.

DNA vaccines involve the direct injection of a small, circular piece of DNA (plasmid) containing genes that encode specific antigens from a pathogen. Once inside the body, the host cells take up the DNA and use their own cellular machinery to produce the encoded antigen. The antigen is then presented on the surface of the host cells, triggering an immune response and the production of antibodies and T cells against the antigen. DNA vaccines have the potential to induce both humoral and cellular immune responses (Li et al., 2012; Wedrychowicz, 2015).

Single or combinational formulations of EM95, EMY162, 14-3-3, TSP and antigen B genes were among the frequently used DNA vaccines against *E. multilocularis*. Protective and successful vaccinations have antigens that elicit a strong immune response. For this purpose, adjuvants are compounds that, when combined with vaccine antigens, induce an effective and more potent immune response to the vaccine than the vaccine alone does (Corradin and Giudice, 2005; Kenney RT, 2004). Recombinant vaccine studies have used CFA, IFA, saponin, STP (Squalen, Tween, and Pleuronic), CPG ODN, and IL-12 as adjuvants for *E. multilocularis*. The study by Chen et al. used soluble rEmCRT formulated with Freund's adjuvant (FA) in BALB/C mice and showed high levels of IgG, IgG1, and IgG2a antibody responses, high levels of IFN-γ, as well as IL-2, IL-4, IL-5, and IL-10 (Chen et al., 2022). Another study using the EM95-EMY162 antigens with CFA and IFA adjuvant in the BALB/c model demonstrated that at each life-cycle stage, EMY162 expression was remarkably higher than that of EM95. Furthermore, it has been reported that the EMY162 antigen is expressed in all worm stages and induces remarkable host protection in an experimental infection with *E. multilocularis* eggs in mice (Kouguchi et al., 2007). In addition, it has been proven that EMY162 induces a strong IgG antibody response in dogs (Katoh et al., 2008). Gauci et al. (2002) reported high-level protection against *E. multilocularis* infection employing the EM95 antigen with saponin adjuvant in the BALB/c model (Gauci et al., 2002). Moreover, Ahn et al. (2015), in a study of using EmAgB3 antigen in the BALB/c model, showed that the i.p. injections of this antigen were highly correlated with worm viability and accompanied by AE progression (Ahn et al., 2015). In another study, Dang et al. (2009) utilized the TSP antigen with CFA and IFA adjuvants in BALB/C, cotton rats, and rabbit models. They observed that TSP has different protective effects against primary AE and could be used in the development of a vaccine (Dang et al., 2009). Also, C57BL/6 immunized with 14-3-3 antigen indicated a strong indication that the CD4+ immune response contributes to parasite growth control (Dai et al., 2004).

The research showed that the DNA vaccine design used antigens such as EmCRT, Gp50, Eg95, B antigen, EM-TSP3-FBP, HSP20, EM95, EMY162, TSPs, 14-3-3 antigen, and glyceraldehyde-3-phosphate dehydrogenase from *Salmonella typhimurium*. Most of these antigens are correlated with a high titer of IgG antibody responses and high levels of Th1 and Th2 cytokines, as well as high protection against *E. multilocularis*, and among them, EM95 and EMY162 induce a stronger IgG antibody response than a Th1 response (Table 2). Fewer *E. multilocularis* cysts were found in the livers and lungs of mice to test how well the EMY162 and EM95 vaccines worked. The use of DNA vaccines against *E. multilocularis* in animal models has shown promising results in inducing protective immunity and reducing parasite burden. While further research is needed to evaluate their efficacy and safety.

Recombinant protein vaccines are made by inserting genes that encode specific antigens from a pathogen into a different organism, such as a bacterium, yeast, or virus, which then produces the antigen. The antigen produced by the recombinant organism is purified and used as a vaccine (Lemaire et al., 2012; Nascimento and Leite, 2012). As shown in Table 2, most studies on AE vaccination have involved recombinant protein approaches. Recombinant EmII/3 and Em14-3-3 alone or in combination with other peptides/antigens were the proteins of choice for such immunizations. Accordingly, antigens specific for recombinant protein vaccines against *E. multilocularis* were as follows: GILE recombinant protein (EMY162, LAP, and GLUT1), rEM-LAP, LTB-ETBM, TSP3, Em14-3-3, rEm-LAP, Emy162, LTB-EMY162, rEmp29, SRF1, rEmTSP1, rEmTSP3, Rec 14-3-3, rBb-Emll/3-Em14-3-3, rBCG-Em14-3-3, Mix rBCG-EmII/3 and BCG-Em14-3-3, rBCG-EmII/3, and *Salmonella typhimurium* +pVM II/3-10. Em14-3-3-based protein vaccines yielded the highest protection rate against *E. multilocularis* infection. Its various formulations included 14-3-3-Saponin (97%) and 14-3-3-Gerbu (84.47%) (Margos and Gottstein, 2010; Siles-Lucas et al., 2003).

A single-protein vaccine might not be enough to protect against infection with *E. multilocularis* because different stages of *E. multilocularis* development (egg, protoscolex, and adult tapeworm) produce different types and amounts of protein. The rational design of multi-epitope vaccines is crucial for vaccine efficacy (Gottstein et al., 2017). Using bioinformatics software to find and predict the most important antigenic epitopes of *Echinococcus* antigens and proteins has gotten a lot of attention as part of the development of multi-epitope vaccines (Lightowlers and Heath, 2004).

According to numerous earlier studies, the advantages of the multi-epitope vaccine include a stronger immune protection effect, safety, and stability. It also has a better protective effect compared to the subunit vaccine (Guo et al., 2014). The increased IFN- and IL-4 secretion in mice given the vaccine, according to Zhou et al. (2023), indicated that the multi-epitope vaccine GILE (EmEMY162, EmLAP, and EmGLUT1) was crucial for the development and survival of *E. multilocularis* in the host. After the GILE vaccination, the cysts' biggest size and weight went down, and the production of CD4+ and CD8+ T cells went up (Zhou et al., 2023). Moreover, Zhou reported that the rEM-LAP antigen with CFA adjuvant could inhibit invasion and cyst growth. Moreover, it induces a specific immune response in BALB/c mice infected with E. multilocularis. Also, treatment with rEM-LAP reduces weight, number, fibrosis, and invasion; it also results in high levels of IgG, IgG1, IgG2a, IgM, IgA, IFN-γ, and IL-4 (Zhou et al., 2022). LTB-EMY162 helped treat and stop *E. multilocularis*, but it didn't completely protect (Li et al., 2018). On the other hand, the levels of IgG, IgG1, IgG2a, IFN-γ, and IL-4 went up a lot with the LTB-ETBM (EMY162 and TSP3) multi-epitope divalent vaccine. This could inhibit cyst formation effectively and minimize the number of vesicles significantly, which has a positive effect on *E.* multilocularis prevention and control (Li et al., 2022). Different levels of 14-3-3 proteins are found in different stages of echinococcosis, such as the metacestode, the germinal layer, and the

extracellular vesicles. This shows how important they are and has been studied recently (Pourseif et al., 2018). Lampe et al. gave rhesus monkeys long-lasting immunity by immunizing them with the recombinant antigen 14-3-3 protein of *E. multilocularis* along with an alum adjuvant (Lampe et al., 2017). The Em14-3-3 vaccine could induce a high level of protective immunization and a high level of IgG, IFN-γ, and IL-4 (Wei et al., 2021). Li et al. have vaccinated BALB/c mice with recombinant BCG-Em14-3-3 and observed an increase in IFN-γ and TNF-α, but the IL-4 level decreased after the vaccinations. rBCG-Em14-3-3 vaccines induced a Th1 response in mice to fight against the challenge of E. multilocularis protoscoleces (Li et al., 2008). Moreover, immunization of BALB/c mice with the mix recombinant BCG-EmII/3 and BCG-Em14-3-3 vaccines of *E. multilocularis* increased the levels of IgG, IgG2a, and IgG2b, as well as the levels of IFN-γ and TNF-α (Jiang, 2007; LI et al., 2003; Li et al., 2008).

Research has shown that protective efficacy against *E. multilocularis* infection is closely linked to both cellular and humoral immunity. T cells, especially CD4+ T cells, are involved in controlling the growth and development of *E. multilocularis* larvae in the host. These cells can produce cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), which are important for activating macrophages and other immune cells to eliminate the parasite. Humoral immunity, mediated by antibodies produced by B cells, also contributes to protection against *E. multilocularis*. Antibodies can bind to parasite antigens, neutralize their activity, and facilitate their clearance by other immune cells (Zhou et al., 2023; Wang et al., 2021).

Vaccination against *E. multilocularis* aims to induce both cellular and humoral immune responses to provide protection against *E. multilocularis*. Experimental studies have shown that vaccination with recombinant protein or DNA vaccines can stimulate T cell responses and antibody production, leading to reduced parasite burden and increased survival in animal models. Understanding the specific immune responses that confer protection can guide the design of more efficacious vaccines (Lampe et al., 2017; Li et al., 2018). While there is ongoing research into the development of recombinant vaccines against *E. multilocularis*, it is important to note that no commercially available vaccine is currently approved for use in humans or animals. However, the exploration of recombinant vaccines represents a promising avenue for potentially preventing AE in the future.

5. Conclusions

In summary, this study indicated that recombinant vaccines containing EMY162, EM95, and EmII/3-Em14-3-3 antigens and crude or purified antigens containing ribotan-formulated excretory/secretory antigens exhibited the most favorable outcomes and elicited protective immune responses against to *E. multilocularis*. This systematic review has provided a comprehensive analysis of the current status and future prospects of *E. multilocularis* vaccine candidates, highlighting emerging technologies and research directions that hold promise for advancing this field. This includes the potential for multi-stage or multi-antigen vaccines, as well as the integration of immunoinformatic and systems biology approaches to optimize vaccine design.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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