Exploring the Landscape of Echinococcus granulosus Vaccine Research in Iran: Promising Antigens and Future Directions

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Abstract

Echinococcus granulosus is a parasitic cestode responsible for causing hydatid cyst disease in humans worldwide. This parasitic disease is a major threat to human and animal health. In addition to causing severe disease in individuals, it threatens public health and leads to significant economic losses in the livestock industry. Iran is endemic for this infection, highlighting the significance and prevalence of the disease in the country. Vaccination is considered one of the preventive strategies employed to control this disease. In recent decades, numerous studies have identified the protective antigens of *Echinococcus granulosus* and their role in immunizing various animal host species. The present study aimed to comprehensively identify and evaluate the most effective antigens that could serve as potential vaccine candidates against cystic echinococcosis. To achieve this goal, data were systematically extracted from eight databases, including *PubMed, ScienceDirect, Scopus, Google Scholar, Magiran, the Scientific Information Database (SID), IranMedex,* and *IranDoc*, covering the period from January 2000 to February 2025. Two researchers independently screened, data extraction, and quality assessment of the studies. Ultimately, 30 articles met the inclusion

criteria for this study. *EG95, P29,* and *protoscolex* were the most frequently utilized antigens in vaccine formulations designed against *Echinococcus granulosus*. Common adjuvants included *Freund's adjuvant, IL-12, Quil A,* and *alum*. Additionally, various antigen delivery methods, animal models, immune response assessments, and the extent of hydatid cyst reduction were examined in these studies. The results of this research suggest that multiepitope DNA vaccines containing *EG95* in combination with *P29, GST,* and *EgA31* yielded superior outcomes and induced stronger protective immune responses against cystic Echinococcosis.

Key words: *Echinococcus granulosus*, Vaccine, Antigens

1. Context

Cystic echinococcosis (CE), caused by the larval stages (metacestodes) of *Echinococcus granulosus sensu lato (E. granulosus s.l.)*, represents a significant zoonotic infection with a global distribution. This neglected parasitic disease imposes a significant burden on public health because of its high morbidity among human populations while simultaneously causing substantial economic losses in the livestock industry, making it a pressing concern in both medical and veterinary fields (1). The parasite's life cycle typically involves canids, particularly dogs, as definitive hosts and ungulates as intermediate hosts. Humans, who act as accidental and dead-end intermediates, become infected by ingesting parasite eggs found in the feces of definitive hosts, often through contaminated food or water. Once inside the body, these eggs develop into hydatid cysts, most commonly forming in the liver and lungs, which are the primary sites of disease manifestation (2). Several factors contribute to the risk of human infection with CE or hydatid disease (HD), including the presence of free-roaming dogs, feeding dogs with infected animal viscera, home slaughtering practices, dog ownership, rural lifestyles, and poor socioeconomic conditions (1). The incidence of CE varies widely, ranging from less than 1 to 200 cases per 100,000 people, yet its impact remains profound. The disease causes significant morbidity and disability among affected populations, with an estimated mortality rate of 2–4% (3-5). In various medical centers across Iran, between 1.18 and 3 cases of CE per 100,000 individuals have been identified during surgical interventions (6). On the other hand, serological studies estimate the prevalence of CE in humans to be approximately 5%, with 60% of positive cases occurring in rural areas and the remaining 40% reported in

urban regions (7). Therefore, in addition to its effects on human health, CE places considerable economic strain on the livestock industry due to losses associated with infected animals (4, 8, 9). The formation of hydatid cysts is the principal outcome of CE, although the process is typically slow and often persists for the duration of an individual's life (1). The clinical manifestations of the disease are largely determined by the extent of damage or dysfunction caused to the affected organs, leading to significant health complications and social impacts for those afflicted (10).

Efforts to control CE, including the use of anthelmintic drugs, enhanced hygiene in slaughterhouses, the treatment of dogs with praziquantel, and public health education programs, have been implemented globally. However, the disease continues to persist in many regions of the world (4). key obstacle is the prolonged under-dosing of antiparasitic medications, which can lead to the emergence of drug-resistant parasite strains. Additionally, the presence of drug residues in food products derived from treated animals has raised significant public health concerns, further complicating control measures (11). Given these limitations, vaccination strategies targeting both definitive and intermediate hosts have gained attention as more effective and sustainable methods for preventing CE. By reducing parasite transmission and overcoming some of the limitations of drug-based interventions, vaccines offer a viable pathway for long-term disease control (12). As a result, numerous studies have been carried out in Iran to develop vaccines for *E. granulosus*. These studies have focused on a variety of approaches, including the use of crude somatic or excretory/secretory (E/S) antigens extracted from the adult worm, crude extracts of protoscolices or the cyst capsule (13, 14), and in silico and in vitro (15-17). Additionally, some vaccine candidates have been tested in laboratory animals following the initial in vitro phase (18-20). Numerous studies in Iran have explored various *E. granulosus* antigens and investigated potential vaccine candidates. However, a comprehensive analysis of these antigens has yet to be conducted. Therefore, this systematic review seeks to consolidate the literature on this subject and highlight the most promising vaccine candidate antigens for future research.

2. Data Acquisition

2.1. Database search strategy and study selection

This systematic review adhered to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (21). The search focused on studies related

to vaccination against *E. granulosus* published between 1st January, 2000, and up to February 2025. To ensure comprehensive coverage, searches were conducted across five English databases (PubMed, ScienceDirect, Scopus, Web of Science, and Google Scholar) and four Persian databases (Magiran, Scientific Information Database (SID), IranMedex, and IranDoc). Both English- and Persian-language publications were included in the search criteria. The keywords (MeSH terms) used included '*Echinococcus granulosus*,' '*E. granulosus*,' 'Echinococcosis,' 'hydatid cyst,' 'vaccine,' 'vaccination,' 'protective immunity,' 'in silico,' 'immunization,' and 'antigen,' applied alone or in combination via 'OR' and 'AND' operators. Conference abstracts and gray literature were not considered for inclusion in this review. All the references were thoroughly reviewed manually to ensure that no relevant articles were missed.

To ensure rigor, two independent reviewers (SK and AA) screened the titles and abstracts of the retrieved articles for relevance. Duplicate entries were identified and removed via EndNote® X9 software. Articles that passed the initial screening were then reviewed in full by the same pair of reviewers to confirm their eligibility. Any disagreements were resolved through consultation with a third reviewer (AD). Only studies that provided sufficient data on *E. granulosus* vaccine candidates were included in the final analysis.

2.2. Study criteria

This study included research that utilized at least one *E. granulosus* protein or gene in vaccine development. All the retrieved titles, abstracts, and, when available, full texts were meticulously reviewed by at least two independent reviewers to identify eligible articles. Conversely, studies were excluded if they 1) lacked sufficient data, 2) presented irrelevant abstracts, or 3) had inaccessible full texts.

2.3. Data collection process

The data extraction process for this study was conducted by a research team, with any discrepancies resolved through consensus discussions. The key information extracted from the eligible articles included the following: author/year, antigen(s), adjuvant or carrier, method of antigen delivery, animal model(s) used, type of challenge administered, and study outcomes. Additional data points such as molecular weight, allergenicity, instability index, types of antibodies, types of cytokines, and cyst parameters (size and number) were also documented where available. Two experienced researchers performed the database search, removed duplicate references, and carefully evaluated the eligibility of the retrieved articles. A

standardized data extraction form developed via Microsoft Excel® was used to ensure accuracy and consistency in collecting the required data.

3. Results

3.1. Overview and Summary of the Included Studies

Among the 322 articles identified, 98 were removed during the initial screening because of duplication or lack of relevance. The titles and abstracts of the remaining 224 articles were reviewed, leading to the exclusion of 174 studies. Following a full-text assessment of 50 articles, 20 were further excluded. Ultimately, 30 studies published between January 2000 and up to February 2025 were included in the systematic review. Figure 1 illustrates the process of article selection and systematic search.

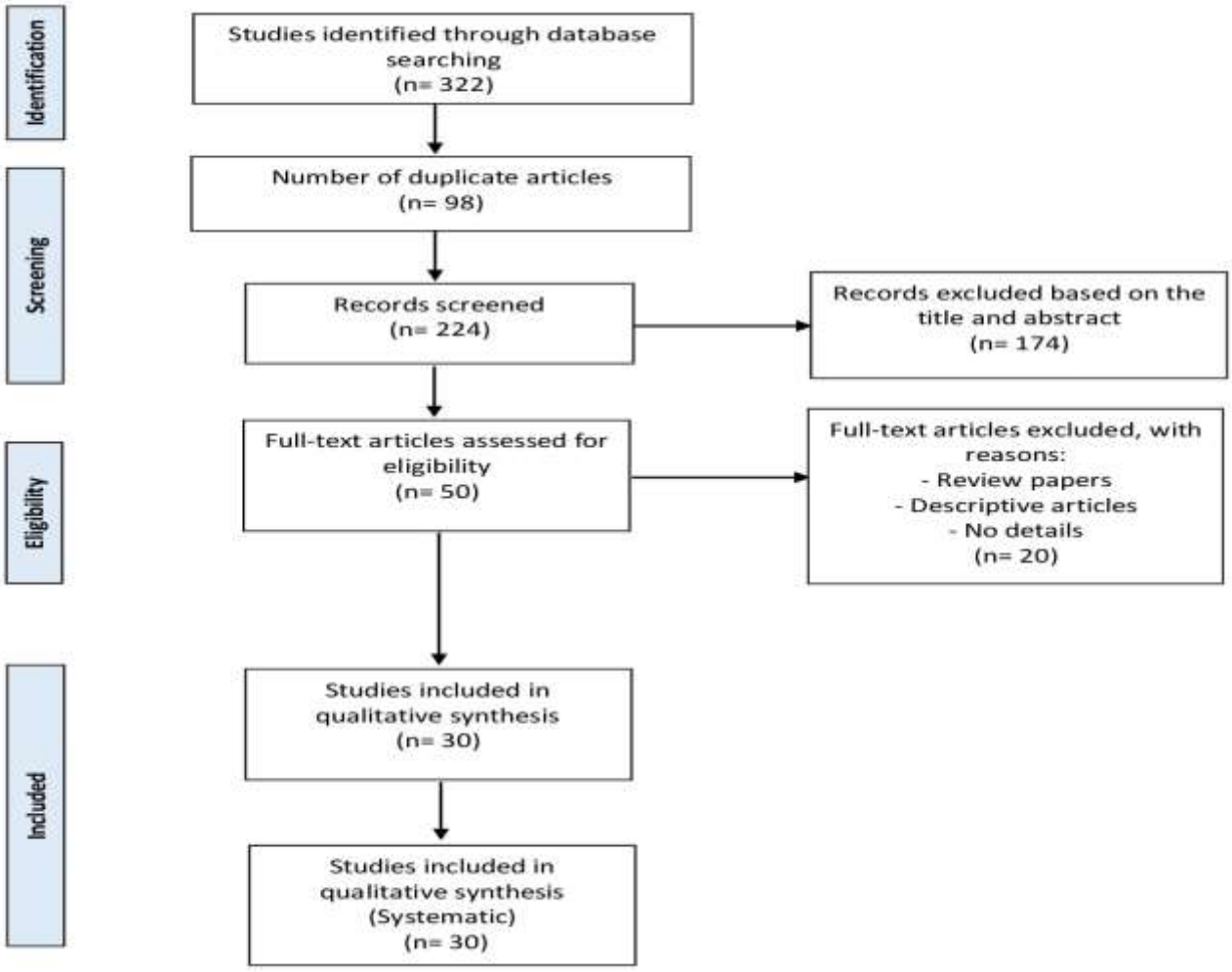

Figure 1: PRISMA flow diagram to describe the study selection process.

3.2. Crude antigens or live vaccines

A total of 10 studies focused on immunization via crude antigens or live vaccines against *Echinococcus granulosus*. Among the crude antigens, protoscolex antigens were the most commonly used, appearing in five studies. This was followed by hydatid fluid and whole-body protein, each of which was used in four studies. Oncosphere and E/S antigens were included in two studies each, crude antigen and immune dominant antigen were also included in two studies, and eggs were ultimately investigated in one study. Notably, one study used a combination of oncosphere and protoscolex antigens for immunization. Since some studies have evaluated multiple antigens for immunization, the total number of antigen applications exceeds the 10 studies conducted. The effectiveness of these crude antigens and live vaccines against *E. granulosus* is summarized in Table 1.

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

AntAaigen	Adjuvant	Antigen delivery (Route)	Animal model	Challenge (Route)	Results	Authors (Year)
Eggs Oncosphere	FCA	40 mg of eggs (I.M) 40 mg of oncosphere (I.M)	Buffalo calf	500 eggs (Oral)	•Significant reduction in the number of cysts formed in the vaccinated group compared to the control group.	Navidpour et al (2003)
Hydatid fluid Protoscolices protein Whole body protein	FCA, FIA	Mice: 100 µg hydatid fluid (I.M) Mice: 100 µg protoscolices protein (I.M) Lamb: 1 mg whole body protein (I.M)	BALB/c Lamb	Mice: 2000 protoscolices (I.P) Lamb: 2000 protoscolices + 10 gravid proglotid (I.P)	•Protective immunity in mice with protoscolex protein was 72.1% and hydatid fluid 82.6% •Protective immunity in lamb with whole body homogenate of *E. granulosus* was 90.9%	Hashemitabar et al (2005)
Whole body protein	FCA, FIA	1 mg (S.C)	Lamb	2000 protoscolices + 10 gravid proglotid (I.P)	•The control group had over 10 hydatid cysts in each lamb, while only one vaccinated lamb had two and one cysts in the liver and lung. •Protective immunity was approximately 90%	Hashemitabar et al (2005)
Whole body protein	FCA, FIA	100 µg (S.C)	BALB/c	2000 protoscolices (I.P)	•The control group had over 50 viable hydatid cysts in each mouse, while none of the vaccinated mice had viable cysts. •Protective immunity was 100%.	Hashemitabar et al (2006)
Oncosphere Protoscolices	Aluminium Phosp	2000 oncosphere (S.C)	Sheep	2000 eggs (Oral)	•Only one or two fertile cysts were observed in the vaccinated group, while all cysts in the control group were fertile.	Soleimani et al (2007)

Oncosphere + Protoscolices	hate Gel	500 µg protoscolices (S.C) 2000 oncosphere + 500 µg protoscolices (S.C)				
Protoscolices Hydatid fluid	FCA, FIA	1 mg protoscolices (S.C) 1 mg hydatid fluid (S.C)	Lamb	2000 protoscolices + 10 gravid proglotid (I.P)	•Protective immunity in lambs immunized with protoscolex was 54.5% and hydatid fluid 75.75%. •The average size of hydatid cysts in hydatid fluid was 3.1 mm, protoscolex 3.6 mm and control group 9.5 mm.	Hashemitabar et al (2007)
Hydatid fluid Protoscolices protein Whole body protein	FCA, FIA	2 mg hydatid fluid (S.C) 2 mg Protoscolices (S.C) 2 mg whole body protein (S.C)	Lamb	2000 protoscolices + 10 gravid proglotid (I.P) and Oral	•A significant association between antibody production in the vaccinated groups and the control (P<0.05). •Lambs immunized with whole body of *E. granulosus* showed the highest antibody production.	Hashemitabar et al (2009)
excretory/secretory antigens Crude antigen Immunodominant antigen	FCA, FIA	1 mg E/S antigens (S.C) 1 mg crude antigens (S.C) 1 mg immunodominant antigens (S.C)	BALB/c	not applicable (N/A)ted	•The highest level of antibody produced in mice immunized with crude antigens. •Crude antigen immunization induced the highest levels of IFN-γ, IL12 and IL-4, followed by E/S antigens.	Rahimi et al (2011)
Protoscolices Hydatid fluid	FCA, FIA	50 mg protoscolices (I.M) 50 mg hydatid fluid (I.M)	Dog	8000 protoscolices Oral	•The severity of the infection was determined by counting the total worms, with 197 worms recovered in hydatid fluid group , 207 in protoscolex, and 382 in the control group.	Youssefi et al (2011)
E/S antigens Crude antigen Immunodominant antigen	FCA, FIA	100 µg E/S antigens (S.C) 100 µg crude antigens (S.C) 100 µg immunodominant antigens (S.C)	BALB/c	2000 protoscolices (I.P)	•crude antigens are suitable candidates for the vaccination. •Protective immunity with E/S antigens was 90%, crude antigens 98.7% and immunodominant antigens 76.2%.	Rahimi et al (2017)

3.3. Recombinant protein vaccines

In the studies conducted in Iran, recombinant proteins were the least explored vaccine approach, with only five studies focusing on them. Of these, EG95 was the most frequently used antigen, featured in two studies, whereas P29, EgA31, and Antigen B were each examined in one study. Further details regarding the recombinant protein-based vaccination are provided in Table 2.

Table 2. Immunization with recombinant protein vaccines against *Echinococcus granulosus*

Antigen	Adjuvant	Antigen delivery (Route)	Animal model	Challenge (Route)	Results	Authors (Year)
Subunit of antigen B	FCA, FIA	1 mg (I.D)	Rabbit	not applicable (N/A)	•A higher level of antibody in immunized rabbits compared to the control group.	Abdi et al (2012)
EgA31	FCA & FIA	50 µg (S.C)	BALB/c, C57Bl/6	500 protoscolices (I.P)	•High level of IFN-γ in immunized C57Bl/6 mice. •No significant levels of IL-10 and IL-4 in immunized C57Bl/6 mice and controls •A significant reduction (~60%) with EgA31- FCA & FIA in cyst load in immunized Balb/C mice	Esmaelizad et al (2013)
Eg95	FCA & FIA & Alum	20 µg (S.C)	BALB/c	not applicable (N/A)	•Humoral and cellular immune responses to rEG95 with a mixed Th1/Th2 pattern and a stronger inclination toward Th1. •Higher level of IgG2a in mice vaccinated with the FCA/FIA adjuvant compared to the Alum adjuvant. •Higher levels of TNF-α, IFN-γ, IL-12, IL-10, and IL-4 in the vaccinated group compared to the control group.	Pirestani et al (2014)
Eg95	Quil A	20 µg (S.C) and Oral	BALB/c	not applicable (N/A)	•Live L. lactis enhanced sIgA level, whereas heat-killed L. lactis induced a significant increase in IgG levels. •A significant increase in IFN-γ in the groups receiving live and heat-killed recombinant L. lactis. •No significant difference in IL-4 and IL-10 between the vaccinated and control groups.	Ebrahimzadeh et al (2021)
P29	-	100 µg (I.M)	BALB/c	2000 protoscolices (I.P)	•The level of protection with P29: 93%. •The size of the cysts formed in the vaccine group: 1.1 mm. •Control group: 5.28 mm.	Gharibi et al (2021)

3.4. DNA vaccines

Among the included studies, 15 experimental DNA vaccines were examined. The antigens used either alone or in combination were as follows: the most frequently used DNA vaccines were EG95 (n = 4), GST (n = 3), P29 (n = 3), EgA31 (n = 2), Eg14-3-3 (n = 2), and enolase (n = 2). Other vaccines included EgTrp (n = 1), EgG1Y162 (n = 1), Myophilin (n = 1), Antigen B (n = 1), Cyclophilin (n = 1), Calreticulin (n = 1), and Adenylate Kinase 1 (n = 1). Table 3 presents the immunization approaches against CE using DNA vaccines.

Among the DNA vaccine studies conducted, 11 focused on bioinformatics features, protein structures, and physicochemical properties. Of these, 10 were purely in vitro studies, whereas only one study, in addition to the bioinformatics analysis, also included an in vivo component, evaluating the vaccine's efficacy in laboratory animals. The bioinformatics characteristics and properties of these studies are presented in Table 4.

3.5. Adjuvants

Among the 30 studies, 20 were in vivo studies, with 19 utilizing adjuvants, whereas only one study did not employ adjuvants. The remaining 10 studies focused on in vitro experiments and evaluated bioinformatics indicators and physicochemical properties. Among the adjuvants used, FCA was the most common, appearing in 15 studies, followed by FIA in 13 studies. Other adjuvants included aluminum sulfate gel in one study, IL-12 in two studies, aluminum hydroxide in one study, and Quil A in one study.

3.6. Animal models

Among the 20 animal studies conducted, the primary animal model used was the mouse model (12 studies), which included 11 BALB/c studies and one C57Bl/6 study. Other vertebrates, including lambs (4 studies), sheep (2 studies), dogs (2 studies), buffalo calves (1 study), and rabbits (1 study), were also used for immunization studies. This discrepancy arises because two studies used two animal species.

3.7. Vaccination route and dosage

The vaccine candidates were administered through different delivery routes. The subcutaneous route was the most commonly used route and was applied in 13 studies. Intramuscular administration was reported in 6 studies, whereas intradermal and oral administrations were less frequently employed, with each being used in 1 study. The vaccine dosage varies depending on the animal species. Specifically, the doses used ranged from 20 µg in mice to

500 µg in dogs, 1000 µg in sheep, and 40 mg in calves. However, the most commonly used dosage across studies was 50 µg.

Table 3. Immunization with DNA vaccines against *Echinococcus granulosus*

Antigen	Adjuvant	Antigen delivery (Route)	Animal model	Challenge (Route)	Results	Authors (Year)
GST, Eg95, EgA31, Trp, P14-3-3	FCA, FIA	50 µg (S.C)	BALB/c	500 protoscolices (I.P)	•Higher production of IFN-γ in vaccinated mice compared to the control group (>1300 pg/mL). •No statistical difference in IL-10 and IL-4 production between the vaccinated and control groups. •Protective immunity was 99.6%.	Esmaelizad et al (2013)
Subunit of antigen B	IL-12	50 µg (I.M)	BALB/c	not applicable (N/A)	•Mice that received the vaccine along with IL-12 as an adjuvant produced the highest levels of IFN-γ and IgG2a. •The group that received pcDNA alone produced more IL-4 compared to other groups, such as the subunit antigen B and IL-12.	Azizi et al (2016)
P29	FCA	10 µg (S.C)	BALB/c	not applicable (N/A)	•The designed peptide induced an increase in IFN-γ in immunized mice compared to the control and adjuvant groups. •no significant changes were observed in the levels of IL-4 and IL-10 in mice.	Jafari et al (2016)
Eg95, Eg14-3-3, Enolase	FCA, FIA	Dog: 500 µg (S.C) Sheep: 1000 µg (S.C)	Dog Sheep	Dog: 105000 protoscolices (Oral) Sheep: 2000 eggs (Oral)	•IL-4, IgG, and IgE showed a significant increase in the vaccine plus adjuvant groups compared to the control group. •Vaccinated dogs were fully protected against challenge with live E. granulosus PSC. •In sheep, the vaccine demonstrated relative efficacy (~85%) following a challenge infection with parasite eggs. •The average cyst size was 2.21 mm in vaccinated sheep and 7.44 mm in control sheep.	Pourseif et al (2021)
Eg95, P29, GST	IL-12	100 µg (I.M)	BALB/c	2000 protoscolices (I.P)	•Protective immunity was 97.6%. •The average cyst size was 0.3 cm in vaccinated mice and 1.3 cm in control mice. •IFN-γ and IgG2a levels were significantly higher in vaccinated groups, indicating a strong Th1 response. •The minimal or absent hydatid cyst formation in vaccinated BALB/c mice	Khazaei et al (2025)

highlights the vaccine's strong immunogenic potential.

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203 **Table 4.** In Silico Studies on *Echinococcus granulosus* Vaccine Design and Bioinformatics
204 Characteristics

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Antigen	Adjuvant	Antigen delivery (Route)	Animal model	Challenge (Route)	Results	Authors (Year)
Eggs Oncosphere	FCA	40 mg of eggs (I.M) 40 mg of oncosphere (I.M)	Buffalo calf	500 eggs (Oral)	•Significant reduction in the number of cysts formed in the vaccinated group compared to the control group.	Navidpour et al (2003)
Hydatid fluid Protoscolices protein Whole body protein	FCA, FIA	Mice: 100 µg hydatid fluid (I.M) Mice: 100 µg protoscolices protein (I.M) Lamb: 1 mg whole body protein (I.M)	BALB/c Lamb	Mice: 2000 protoscolices (I.P) Lamb: 2000 protoscolices + 10 gravid proglotid (I.P)	•Protective immunity in mice with protoscolex protein was 72.1% and hydatid fluid 82.6% •Protective immunity in lamb with whole body homogenate of *E. granulosus* was 90.9%	Hashemitabar et al (2005)
Whole body protein	FCA, FIA	1 mg (S.C)	Lamb	2000 protoscolices + 10 gravid proglotid (I.P)	•The control group had over 10 hydatid cysts in each lamb, while only one vaccinated lamb had two and one cysts in the liver and lung. •Protective immunity was approximately 90%	Hashemitabar et al (2005)
Whole body protein	FCA, FIA	100 µg (S.C)	BALB/c	2000 protoscolices (I.P)	•The control group had over 50 viable hydatid cysts in each mouse, while none of the vaccinated mice had viable cysts. •Protective immunity was 100%.	Hashemitabar et al (2006)
Oncosphere Protoscolices Oncosphere + Protoscolices	Aluminium Phosphate Gel	2000 oncosphere (S.C) 500 µg protoscolices (S.C) 2000 oncosphere + 500 µg protoscolices (S.C)	Sheep	2000 eggs (Oral)	•Only one or two fertile cysts were observed in the vaccinated group, while all cysts in the control group were fertile.	Soleimani et al (2007)

Protoscolices Hydatid fluid	FCA, FIA	1 mg protoscolices (S.C) 1 mg hydatid fluid (S.C)	Lamb	2000 protoscolices + 10 gravid proglotid (I.P)	•Protective immunity in lambs immunized with protoscolex was 54.5% and hydatid fluid 75.75%. •The average size of hydatid cysts in hydatid fluid was 3.1 mm, protoscolex 3.6 mm and control group 9.5 mm.	Hashemitabar et al (2007)
Hydatid fluid Protoscolices protein Whole body protein	FCA, FIA	2 mg hydatid fluid (S.C) 2 mg Protoscolices (S.C) 2 mg whole body protein (S.C)	Lamb	2000 protoscolices + 10 gravid proglotid (I.P) and Oral	•A significant association between antibody production in the vaccinated groups and the control ($P<0.05$). •Lambs immunized with whole body of *E. granulosus* showed the highest antibody production.	Hashemitabar et al (2009)
excretory/secretory antigens Crude antigen Immunodominant antigen	FCA, FIA	1 mg E/S antigens (S.C) 1 mg crude antigens (S.C) 1 mg immunodominant antigens (S.C)	BALB/c	not applicable (N/A)	•The highest level of antibody produced in mice immunized with crude antigens. •Crude antigen immunization induced the highest levels of IFN-γ, IL12 and IL-4, followed by E/S antigens.	Rahimi et al (2011)
Protoscolices Hydatid fluid	FCA, FIA	50 mg protoscolices (I.M) 50 mg hydatid fluid (I.M)	Dog	8000 protoscolices Oral	•The severity of the infection was determined by counting the total worms, with 197 worms recovered in hydatid fluid group , 207 in protoscolex, and 382 in the control group.	Youssefi et al (2011)
E/S antigens Crude antigen Immunodominant antigen	FCA, FIA	100 µg E/S antigens (S.C) 100 µg crude antigens (S.C) 100 µg immunodominant antigens (S.C)	BALB/c	2000 protoscolices (I.P)	•crude antigens are suitable candidates for the vaccination. •Protective immunity with E/S antigens was 90%, crude antigens 98.7% and immunodominant antigens 76.2%.	Rahimi et al (2017)
Eggs Oncosphere	FCA	40 mg of eggs (I.M) 40 mg of oncosphere (I.M)	BALB/c	2000 protoscolices (I.P)	•Significant reduction in the number of cysts formed in the vaccinated group compared to the control group.	Navidpour et al (2003)

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207 FCA: Freund complete adjuvant, FIA: Freund incomplete adjuvant, I.M: Intramuscular, S.C: subcutaneous, I.P:
208 Intraperitoneal, E/S: Excretory/Secretory

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3.8. Challenge

To evaluate cyst formation and determine the level of vaccine-induced protection, intraperitoneal inoculation was the most frequently used challenge route and was applied in 9 studies. The oral route was employed in 4 studies, while one study utilized a combination of both intraperitoneal and oral administration. However, in 16 studies, no challenge was performed. For the challenge experiments, eggs, gravid proglottids, and protoscolices were used across different studies. Among these, protoscoleces were the most commonly utilized, with a standard inoculum of 2,000 protoscoleces. Additionally, the timeframe from challenge to necropsy and internal organ examination ranged from 3--16 months. However, the most frequently reported incubation period was approximately 7--8 months.

3.9. Immune responses

Host immune responses were assessed by measuring specific antibodies and cytokine levels. The key antibodies assessed included IgG total (n = 8), IgG1 (n = 4), IgG2a (n = 4), IgG2b (n = 1), IgG3 (n = 2), sIgA (n = 1), and IgE (n = 1). Additionally, cytokine analysis focused on IFN-γ (n = 8), IL-12 (n = 2), IL-4 (n = 9), IL-10 (n = 6), and TNF-α (n = 1). A detailed overview of the immune responses observed in each study is provided in the Tables 1 to 3.

3.10. Protective immunity

Vaccination has the potential to provide protection against *E. granulosus* in both definitive and intermediate hosts, thereby contributing to a reduction in parasite transmission. Findings from one study indicated that immunization of definitive hosts significantly decreased worm burden while enhancing both mucosal and systemic immunity as well as in intermediate hosts, and vaccination resulted in a substantial reduction in cyst diameter and weight (20). The use of whole-body protein antigen conferred complete (100%) protection, while crude antigen administration in another study achieved a protective efficacy of 98.7%. Additionally, a study conducted by the authors of this review demonstrated that a multi-epitope DNA vaccine incorporating the Eg95, GST, and P29 genes provided 97.6% protection.

4. Conclusion

E. granulosus is a significant cyclophyllidean cestode responsible for cystic hydatidosis, a zoonotic disease that imposes a considerable global burden on both public health and the livestock industry each year. The parasite's life cycle is maintained through definitive hosts,

particularly dogs, which become infected by consuming the cyst-containing viscera of intermediate hosts, predominantly sheep (22). The mature proglottid harbors numerous eggs capable of contaminating extensive areas. In regions where echinococcosis is endemic, the sheep-dog strain of *E. granulosus* (G1 genotype) plays a predominant role in transmitting the parasite to humans (23, 24). In Iran, the G1 genotype has been reported as the most prevalent strain, followed by G6 and G3 (25). Iran is classified as a hyperendemic region because of the prevalence of traditional livestock farming practices and the frequent exposure of dogs to slaughterhouse waste (26, 27). The annual economic impact of CE in the country has been estimated at approximately USD 232.3 million (6, 28). Given these considerations, implementing preventive measures and vaccination programs has been acknowledged as a fundamental strategy for minimizing this risk. Nevertheless, the planning and execution of such programs entail a significant financial burden for many countries dealing with this challenge. Furthermore, pharmacological interventions not only lack prophylactic efficacy but also may lead to undesirable side effects in both humans and animals (29, 30).

E. granulosus can infect multiple host species and possesses immune evasion mechanisms, both of which present significant challenges in the development of an effective vaccine against this complex parasite. Consequently, selecting an optimal vaccination strategy remains a critical challenge. An ideal vaccine should be effective in both definitive (canine) and intermediate (ovine) hosts. Given that dogs have a smaller population than sheep do and that infected dogs can contaminate the environment by shedding thousands of eggs daily, canids serve as the primary source of parasite transmission worldwide (9, 31). Moreover, combating such complex pathogens necessitates the development of innovative vaccine platforms. The design of an optimal vaccine should incorporate advanced immunoinformatics analyses while considering key immunological and biochemical parameters to increase its efficacy and applicability (32, 33).

This systematic review was conducted to evaluate immunization studies against *E. granulosus* infection and to provide a summary of relevant research to identify the most promising vaccine candidates for future investigations in hydatid cyst control.

4.1. Vaccination using crude antigens or live vaccines against *E. granulosus*

On the basis of our review, a total of 10 studies have been conducted in Iran on immunization against *E. granulosus* using either crude antigens or live vaccines. In this context, a study conducted by Navidpour and colleagues in 2003 utilized *E. granulosus* eggs as antigens in

calves, resulting in a reduction in cyst formation and providing 84.5% protection (34). Furthermore, a study conducted by Hashemitabar et al. demonstrated that the greatest protective immunity was achieved in BALB/c mice using hydatid fluid and in sheep via whole-body homogenate of *E. granulosus* (14). In another study by Hashemitabar et al. on a lamb model, the highest antibody levels were induced by utilizing the whole body of *E. granulosus*. Additionally, hydatid fluid is associated with a significant level of protective antibody production (35). Regarding the use of crude antigens, a study conducted by Soleimani et al. on sheep demonstrated a high level of protection (82%) when a combination of oncosphere and protoscolex was used as the antigen (36).

A study conducted by Youssefi et al. evaluated the immunogenicity of hydatid cyst fluid and protospecies proteins under 30 kDa in dogs experimentally infected with *E. granulosus*. The results indicated that, compared with control dogs, dogs immunized with these proteins presented a reduced worm burden, with the lowest number of adult worms (197) observed in the group receiving hydatid cyst fluid proteins (37). According to our results from studies on immunization with crude antigens or live vaccines against *E. granulosus*, the protection rate in this approach varies from 50.2% to 100%, with whole-body protein being the best vaccine candidate. The wide range of immunization rates, inability to stimulate both cellular and humoral immunity simultaneously, and insufficient and unstable production of specific antibodies have made this approach less favorable. Consequently, it is not widely used today. Moreover, these vaccine models have additional drawbacks, including a shorter lifespan, lower stability, and the potential risk of parasite transmission to other susceptible animals. Furthermore, their use poses significant concerns in individuals with immunodeficiency, chronic health conditions, or a history of organ transplantation, making them less favorable for widespread application (38-40).

4.2. Vaccination using recombinant protein vaccines against *E. granulosus*

Recombinant vaccines activate the immune response against pathogens by utilizing one or more specific antigens, particularly when they are administered alongside adjuvants or non-pathogenic carriers such as bacteria, viruses, or plasmids. These vaccines offer several advantages over those based on purified macromolecules, including a reduced risk of contamination with unwanted components and the prevention of toxoid reversion to toxic forms, as observed in diphtheria and tetanus toxoid vaccines. Furthermore, this technology

addresses the challenge of producing sufficient quantities of purified antigenic components (41, 42).

According to our research, recombinant vaccines have had the lowest application in hydatid cyst vaccination in Iran, with only five studies conducted in this field. Among them, the recombinant EG95 protein has been the most widely used protein for immunization. The antigens utilized in recombinant vaccines against *E. granulosus* include EG95, EgA31 (multi-epitope), a subunit of antigen B, and P29. The lowest protection rate was observed with EgA31 (60%), whereas the highest protection rate was achieved with P29 (93%).

EG95 is recognized as one of the most effective recombinant proteins for stimulating the immune system. Studies have demonstrated its remarkable ability to induce immune responses and protect animals against *E. granulosus* infection, with protection rates ranging from 96% to 98% (43). The EG95 antigen can generate a robust immune response against *E. granulosus*, providing significant immunity to the vaccinated host. Research has identified EG95 as a multigene family comprising seven isoforms (EG95-1 to EG95-7) (44). Allelic polymorphisms in the EG95 gene may influence the efficacy of EG95-based vaccines. Therefore, understanding the genetic diversity of EG95 in different *E. granulosus* isolates is crucial for optimizing vaccine effectiveness (45).

4.3. Vaccination using DNA vaccines against *E. granulosus*

DNA vaccines offer numerous advantages, including enhanced stability and prolonged immune response, stimulation of both humoral and cellular immunity, simpler production processes with lower costs, the potential to target multiple antigens, safety due to the absence of live organisms, and the ability to generate antigens in their native conformation (46).

Moreover, DNA-based vaccines exhibit strong immunogenicity and induce immune responses distinct from those of natural infections (47). However, challenges remain in the development of DNA vaccines for parasitic diseases, such as enhancing immunogenicity, selecting optimal antigens, and identifying effective delivery strategies. Nevertheless, recent advancements in genetic engineering, novel adjuvants, and innovative delivery methods have raised hopes for the successful development of effective DNA vaccines against parasitic infections (48).

According to the findings of our study, among the studies conducted on *E. granulosus* vaccination in Iran, 15 focused on DNA vaccines. Some of these studies employed in silico approaches for vaccine design, analyzing physicochemical properties, structural modeling, and

predicting immunogenic B- and T-cell epitopes. In addition to computational analyses, the immunogenicity of the vaccines was further assessed through in vivo experiments. Single or combination formulations of EG95, EgA31, and P29 are among the most commonly used DNA vaccines. Effective and protective vaccines typically comprise antigens with the highest capacity to stimulate the immune system when combined with potent adjuvants. In general, adjuvants are designed as integral components of vaccines to increase the magnitude and durability of the immune response, thereby improving vaccine efficacy. In DNA vaccine studies against *E. granulosus*, adjuvants such as FCA, FIA, and IL-12 have been utilized. In a study by Esmaeilzad et al., immunization of mice with a multi-epitope vaccine incorporating EgGST, Eg95, EgA31, EgTrp, and P14-3-3 antigens resulted in 99.6% protection (19). Similarly, in a study conducted by Khazaei et al., a multi-epitope vaccine based on the Eg95, P29, and GST genes conferred 97.6% protection in mice (49). This vaccination also significantly increased the levels of IFN-γ, total IgG antibodies, and the IgG1 and IgG2a subclasses in the immunized group (50). Furthermore, Pourseif et al. evaluated Eg95, Eg14-3-3, and enolase antigens in both dogs and sheep. The results revealed a significant increase in the IL-4, IgG, and IgE levels in the vaccinated groups receiving adjuvants compared with those in the control group. This preliminary field study further indicated that dogs immunized with the vaccine were completely protected against challenge with live protoscolices of *E. granulosus* (20). In a study conducted by Azizi et al., the use of antigen B (HydI) with and without the IL-12 adjuvant yielded the following results: (i) in the pcHydI+IL12 group, a significant increase in lymphocyte proliferation and IFN-ɣ, IL-4, and IgG2a levels was observed; and (ii) in the pcHydI group, an increase in IgG1 levels was noted. Many of these antigens effectively stimulate humoral immune responses and are recognized as DNA vaccines (Table 3). Among them, antigen B not only strongly induces humoral immune responses but also adequately stimulates cellular immunity, particularly in the activation of lymphocytes. Furthermore, it has been established that antigen B alone can effectively elicit both cellular and humoral immune responses; however, the Th2 response is more prominently triggered than the Th1 response. This could be attributed to the specific structure of this antigen, which contains epitopes for both T and B cells. To modulate this response and direct it toward Th1 cells, the use of a strong pcMIL12 adjuvant has been proposed as a potential solution (18). Overall, immunization against *E. granulosus* has shown promising potential as a preventive strategy, although its effectiveness largely depends on the type of vaccine used. In this context, DNA vaccines containing antigen B and recombinant vaccines based on EG95 have demonstrated the highest success in eliciting a strong protective immune response. Furthermore, the inclusion

of effective adjuvants or suitable delivery systems has significantly enhanced vaccine efficacy. Although no vaccine has yet been developed that provides complete immunity for both definitive and intermediate hosts, ongoing advancements in immunology and bioinformatics offer a promising outlook for achieving this goal. However, the challenges associated with vaccine development extend beyond antigen selection, as factors such as production costs, risk assessment, and large-scale feasibility must also be carefully considered. Ultimately, an effective vaccine against *E. granulosus* could not only reduce the disease burden in endemic regions but also contribute to public health improvement and economic sustainability by disrupting the transmission cycle between humans and livestock.

Ethical approval

As a synthesis of existing literature, ethical approval was not required.

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Authors' contributions

Study concept and design: S.K. and A.D.

Acquisition of data: S.K.

Analysis and interpretation of data: A.D. and S.K.

Drafting of the manuscript: A.D. and S.K.

Critical revision of the manuscript for important intellectual content: A.D.

Statistical analysis: A.D. and S.K.

Administrative, technical, and material support: A.D.

Conflict of interest

The authors have declared no conflicts of interest.

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Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Figure legends

Figure 1: PRISMA flow diagram to describe the study selection process.

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