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REVIEW ARTICLE

Fasciolosis: Recent Update in Vaccines Development and Their Efficacy

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A B S T R A C T

Fasciolosis is caused by *F. hepatica* and *F. gigantica*. It is of economic and zoonotic importance. Several strategies for control of fasciolosis are being used; these include vaccination of animals that are at risk and control of the snails that carry the parasite. Several types of vaccines, such as recombinant cathepsin, mixed recombinant vaccine, fatty-acid-binding proteins, a cocktail of recombinant and fatty-acid-binding vaccines, nucleic acid-based vaccines and gene-silencing methods have been reported to show efficacy ranges of 32-75%, 52-79%, 8-36%, 43-68%, 74-100% and 90% respectively. These are currently undergoing experimental testing against fasciolosis. The study described in this paper was carried out to discover the comparative efficacy of these vaccines in the enhancement of the immune response in order to find the most effective method so that future research could focus on the development of that type of vaccine. Besides immunization, control of the intermediate host of the parasite (snail) is also an effective way to control fasciolosis. Snails are controlled through the use of physical, chemical and biological methods. The most effective of these is biological control using *Sphaerodema urinator* as a predator of snails.

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1. Introduction

Fasciolosis is an emerging zoonotically important disease in several (>70) countries (Mehmood *et al.,* 2017; Webb and Cabada, 2018). It is a food-borne disease that is caused by the parasitic trematodes *F. hepatica* and *F. gigantica* (El-Rahimy *et al.,* 2012). Infectious diseases cause serious threat to economy of livestock industry (Abbas *et al.,* 2021). Among infectious diseases, helminths pose high impact in terms of mortality, reduction in meat, milk and quality of hides. Fasciolosis leads losses worth an estimated cost of US$3 billion each year around the world to livestock industry (Piedrafita *et al.,* 2010; Mehmood *et al.,* 2017; McManus, 2020; Villa-Mancera *et al.,* 2021; Zafra *et al.,* 2021). It has been mentioned that Fasciolosis increases the mortality rate and decreases the production at domestic level and had serious effect on country economics (Dar *et al.,* 2005). It has been reported that about 180 million people are at risk of infection with food-borne zoonotic diseases and of them between 35-72 million are infected with fasciolosis (Cwiklinski *et al.,* 2016; Rehman *et al.,* 2016; Sabourin *et al.,* 2018). The rate of *Fasciola* infection depends upon the climate in which the potential infected lives, agricultural practices and the level of resistance that the parasites have developed against antitrematode drugs (Kelly *et al.,* 2016; Rehman *et al.,* 2016). Snails (*Lymnaea auricularia* var rufescens and *Lymnaea auricularia* var stricto) are the intermediate host of *Fasciola*. They are prevalent in tropical and temperate zones (Lalrinkima *et al.*, 2021). They reported that *Radix natalensis* (*Lymnaea natalensis*) also plays an important role in the transmission of *F. gigantica* (Dar *et al.*, 2005). It was reported that another species of snails like *Galba truncatula* (*L. truncatula*) is prevalent in Egypt and helps in transmission of parasite while other species like *Pseudosuccinea columella* (*L. columella*) and *Biomphalaria alexandrina* (Planorbidae) normally found habituating larvae of *Fasciola* sp. (Dar *et al.*, 2005). *F. hepatica* is prevalent in cold and temperate climate while *F. gigantica* is prevalent in tropical and subtropical countries whereas both species often mixed n subtropical areas (Zaman *et al.*, 2014). Unembryonated eggs passed in feces hatch in water into miracidia after embryonation. Miracidia penetrate intermediate host snails and molt into sporocysts, rediae and cercariae. Cercariae then come out of snails and encyst into metacercariae which are ingested by ruminant host. Metacercariae then excyst in duodenum and travel through liver to reach bile duct as shown in Fig. 1. Fasciolosis can be controlled by the application of antitrematode treatment to diseased animals and by restricting the size of the snail population. Variations in methods of antitrematode treatment in terms of dose rate according to efficacy and breed ultimately result in the development of drug resistance (Fairweather, 2011; Hanna *et al.*, 2015), which could lead to an alarming situation both now and in the future. Therefore, an effective vaccine must be produced in order to control fasciolosis infection (Dalton *et al.*, 2013; Dominguez *et al.*, 2018). Several recombinant, attenuated, subunit, cocktail, kunitz-type and nucleic-acid-based vaccines have been trialed in organisms to assess their enhancement of the hosts' immune responses to fasciolosis. The protection level achieved by these vaccines remains low (Toet *et al.*, 2014; Molina-Hernández *et al.*, 2015; Beesley *et al.*, 2018; Lalrinkima *et al.*, 2021). In this review article, we highlight and compare the several kinds of vaccines that have been studied to find out which offers the best method for the control of fasciolosis.

2. Epidemiology of fasciolosis

The prevalence of fasciolosis is dependent on climatic conditions and whether the regions in which the animals live are tropical or temperate (Lalrinkima *et al.*, 2021). It has been reported that, in tropical and sub-tropical areas, fasciolosis is more prevalent in the rainy season in small ruminant as opposed to large ruminants (Bauri *et al.*, 2015; Swarnkar *et al.*, 2021). Similarly, it has been reported that the occurrence of infection by liver flukes (as the parasites are known) increases during and after the monsoon season but falls in the winter (Bauri *et al.*, 2015). The density of snails is correlated with the rate of fasciolosis infection after the monsoon when the raised water temperature and rainy weather lead to an increase in the number of snails and a direct increase in disease transmission (Silvane *et al.*, 2020). Fasciolosis is a zoonotically important disease; the World Health Organization (WHO) has reported that approximately 2.4 million people are infected (Ashdhir *et al.*, 2014). Diagnostic evidence of fluke in the bile duct and their ova in the stool have been reported (Brockwell *et al.*, 2014; Ramanan *et al.*, 2019).

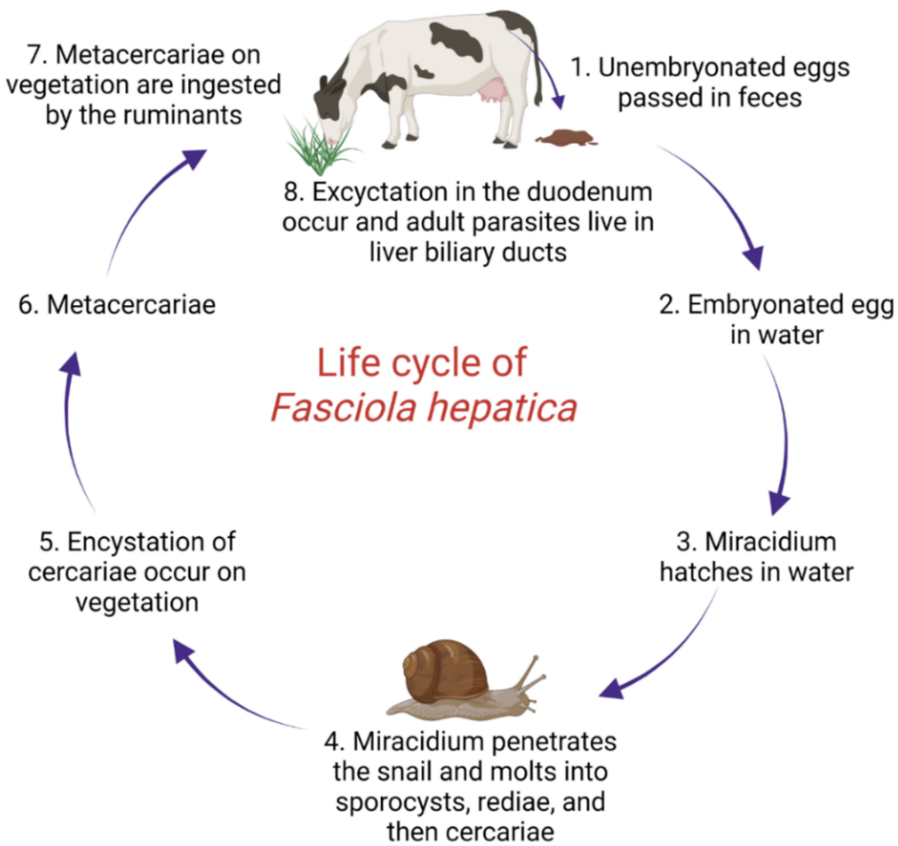

Fig. 1. Life of cycle of *Fasciola hepatica* in ruminants.

3. Vaccines against fasciolosis

In ruminants and humans, fasciolosis is caused by *F. hepatica* and *F. gigantica* (Dalton *et al.,* 2013). The infection has vast geographical distribution and affects the health of both farmers and livestock (Cooper *et al.,* 2012). Indiscriminate use of medication such as the anthelmintic triclabendazole, which is the drug of choice to treat this disease, has led to drug resistance in the parasites and the build-up of drug residues in meat and the environment (Piedrafita *et al.,* 2004; Fairweather *et al.*, 2011; Hanna *et al.*, 2015; Silvane *et al.*, 2020). This worrying situation highlights the urgency to producing an effective vaccine that can be used to control fasciolosis in animals including humans. Several studies have been conducted to enhance humoral or cell-mediated immunity to fasciolosis due to immunization with attenuated, recombinant, gene knockdown/silencing, or, nucleic-acid-based vaccines, or a cocktail of these (McManus *et al.,* 2006; Spithill *et al.,* 2012). The immunogenic efficacy of each method is summarized here to discover the most effective type of vaccine. Efficacy of mixed recombinant vaccine against fasciolosis infection is shown in Table 1.

3.1. Attenuated vaccines: The simplest way to produce a vaccine is through attenuation (irradiation and culturing) of a pathogen so that its pathogenic properties are eliminated. A whole pathogen contains several kinds of antigens which act as a source of an infection, so a vaccine that exhibits immune reaction to variety of antigens, is considered to provide better protection than other vaccines. However, the chances of the occurrence of allergic reactions to the vaccine in animals are much greater than when other vaccines (Golden *et al.,* 2010; Tadesse *et al.,* 2021). Use of irradiated metacercariae of *F. hepatica* can help us to control the number of flukes as well as development of infection, damage to liver and concentration of glutamate dehydrogenase and γ-Glutamyl transferase (Nansen, 1975). Metacercariae of *F. hepatica* attenuated with γ irradiation level of 3 Krad conferred significant resistance to calves through reduction in worm count and fecundity (Khan *et al.,* 2017).

3.2. Excretory/ secretory product vaccines: Excretory/ secretory (ES) products of *Fasciola* has immunogenic properties and can be used in immunodiagnosis and vaccine production. Polypeptide profiling of ES products of *F. gigantica* identified 24 polypeptide epitopes among which 12 epitopes were found to be immunogenic (El-Ridi *et al.,* 2007). ES product molecules exhibited considerable immunogenic response eliciting interleukin-12p40 mRNA response and production of good quantity of antibodies which bind to surface molecules of newly excysted juvenile worms. Cellular and humoral response induced by ES products result in moderate reduction of number of worms, however size of recovered worms is significantly reduced (Di Maggio *et al.,* 2019). Intermediate host species influence the immunogenic proteomic response of host. Immunogenic response to juveniles of *F. hepatica* derived from different snail species significantly differs (Kalita *et al.,* 2019) with difference in protein modification machinery, protease inhibitors, signal transduction, and cysteine-rich proteins.

3.2. Recombinant vaccine

3.2.1. Cathepsin proteins: The main *Fasciola* spp. protein classes that have been evaluated in vaccination trials are cathepsin B, cathepsin L, leucyl aminopeptidase, thioredoxin glutathione reductase, fatty-acid-binding protein-1 (FABP-1), saposin-like protein-2 and 14–3-3 protein epsilon) (Caffrey *et al.,* 2018). Several native and recombinant proteins have been identified as potential candidates for vaccine production against fasciolosis infection. Cathepsins (cathepsin L and cathepsin L mimotopes) are proteases. They are expressed abundantly in the liver fluke as they aid in tissue invasion by the parasite (Maggioli *et al.,* 2011; Cwiklinski *et al.,* 2019). Cathepsin L mimotopes (CL1 (DPWWLKQ), CL1 (SGTFLFS) and CL2 (PPIRNGK)) have been used in the control of fasciolosis infection in goats. These proteases have been used to decrease the liver-fluke burden (Molina-Hernández *et al.,* 2015). These researchers reported that the use of cathepsin L mimotopes caused a significant decrease (32.39-70.42%) in the egg burden laid by the liver fluke (Table 1). Moreover, it was found that goats treated with CL1 and CL2 mimotopes produced humoral and cellular immunity against the parasite (Th1/Th2), and the titers of antibodies (immunoglobulin G (IgG)1 and IgG2) increased four weeks after vaccination (Molina-Hernández *et al.,* 2015; Shaukat *et al.,* 2019). It has been reported that haemoglobin, cathepsin (L1 and L2) and leucine aminopeptidase of *F. hepatica* have been used collectively to control fasciolosis infection. After the use of this mixed vaccine (cathepsin L1 and L2 with haemoglobin), the researchers found a significant decrease in the liver fluke burden up to 53.7% (Dar *et al.,* 2005). Similarly, another study reported a 98% anti-embryonated effect on eggs after the use of CL2 and haemoglobin of *F. hepatica* (Haçarız *et al.,* 2012). Similar findings reported that the production of a mixed multiepitope vaccine (cathepsins L and B, leucine aminopeptidase, saposin-like protein-2, thioredoxin glutathione reductase and FABP) showed a good immunogenic effect against fasciolosis infection (Caffrey *et al.*, 2018). Efficacy of recombinant cathepsin (cathepsin L and cathepsin L mimotopes) proteins vaccine is shown in Table 1.

3.2.2. Fatty-acid-binding protein: Parasite extract or recombinant antigens can be used to control fasciolosis infection (Haçarız *et al.,* 2012). *Fasciola* cannot synthesize long-chain fatty acids or steroids, which are usually produced by the beta-oxidation pathway (López-Abán *et al.*, 2008). Yet FABPs are very important for the survival of liver fluke, and therefore the parasite depends on the host for FABP supply (López-Abán *et al.,* 2007). In studies, the supply of native and recombinant FABPs in sheep and cattle led to control of fasciolosis up to 55% (Nambi *et al.,* 2005; López-Abán *et al.,* 2007). Recombinant FgFABP was used with Freund's adjuvant; vaccinated animals showed both humoral and cell-mediated immunity with a reduced (35.8%) chance of fasciolosis infection in buffalo (Varghese *et al.,* 2010; Lalrinkima *et al.*, 2021). Another study also reported that the use of FgFABP by cell-penetrating peptides (R-19, R-22 and R-25) obtained from infectious bursal disease virus triggered an immune response (Th1 and Th2), which was evidenced by increased levels of immunoglobulins (IgG1, IgG2α and IgG2β) and cytokines (IFN, TNF, IL-2, IL-4 and IL-5) (Bozas *et al.*, 1995). Efficacy of Fatty-acid-binding protein type of vaccine is shown in Table 1.

Table 1: Efficacy of recombinant cathepsin proteins, Mixed recombinant vaccine, Fatty-acid-binding protein (FABP) and Cocktail vaccine

Parasite	Host	Immunization with	Adjuvant	Type of Response	Vaccine	Decrease Worn Load (%) or Efficacy (%)	Reference(s)
F. hepatica	Cattle	rCL	------	IgG1 & IgG2	Recombinant Cathepsin Proteins	37	(Garcia-Campos *et al.*, 2019)
		rCL	------	IgG1 & IgG2		48	(Tadesse *et al.*, 2012)
	Goat	rCL	------	IgG & IgG1		38	(Buffoni *et al.*, 2012) (Pérez-Écija *et al.*, 2010) (Dominguez *et al.*, 2018) (Villa-Mancera *et al.*, 2021)
		rCL	------	No response		39	
		FhPrx	------	------		33	
		CL1 (DPWWLKQ)	------	Th1/Th2 (IgG1 & IgG2)		55	
		CL1 (SGTFLFS)	------	Th1/Th2 (IgG1 & IgG2)		70	
		CL2 (PPIRNGK)	------	------		32	
	Sheep	FhCL2	------	------		34	(Dominguez *et al.*, 2018)
	Rat	recombinant FhCL3-2	------	Th1/Th2 (IgG1 & IgG2a)		53	(Wesołowska *et al.*, 2018)
F. hepatica adult excretion/ secretion	Sheep	CL1 + CL2 + LAP	FCA/FIA	------	Mixed recombinant vaccine	79	(Dominguez *et al.*, 2018)
		CL1 + CL2	FCA/FIA	------		60	
	Cattle	CL1 + Hb	FCA/FIA	------		51	
		CL2 + Hb	FCA/FIA	------		72	
F. gigantica	Mice	recombinant proFgCatL1H	------	Th1/Th2 (IgG1 & IgG2a)	Recombinant Cathepsin Proteins	66	(Sansri *et al.*, 2015) (Changklungmoa *et al.*, 2016) (Kueakhai *et al.*, 2021)
		recombinant mature FgCatL1G	------			58	
		FgCatL1H and FgCatB3	------			75	
F. hepatica or *F. gigantica*	Mice	FABP	DNA + mannosylated-polyethylenimine	Elevation of Th1 cytokines	Fatty-acid-binding protein		(Lalrinkima *et al.*, 2021)
		FABP	DNA + cell-penetrating peptide	Elevation of Th1/Th2 cytokines		------	
		FABP	DNA + polyethylenimine	No protection		------	
	Buffalo	Recombinant FABP and glutathione S-transferase	Montanide 70 M-VG	------		35.8	
		Recombinant leucine aminopeptidases and peroxiredoxin	Montanide 70 M-VG	------		8.4	
		rFABP	Freund's adjuvant	------		35.8	
	Cattle and sheep	Native and rFABP	Freund's adjuvant	Humeral and cell mediated immunity	Fatty-acid-binding protein	35.8	(Brockwell *et al.*, 2014)
		recombinant FgFABPs	Freund's adjuvant	Humeral and cell mediated immunity			(Ramanan *et al.*, 2019)
	Sheep (Group A)	Recombinant-CL1 + recombinant-HDM + recombinant-LAP + recombinant-Prx	Montanide	------	Cocktail vaccine	42	(Zafra *et al.*, 2021)
	Sheep (Group B)	recombinant-CL1 + recombinant-HDM + recombinant-LAP + recombinant-Prx	Alhydrogel	------		58	
	Sheep (Group C)	Positive control	-----	------		67	
F. hepatica	Sheep (Group1)	Irradiated metacercariae of *F. hepatica* with Irradiation dose of 30 grays	Gelatin Capsule	Humoral response	Attenuated Vaccine	18.6	(Trelis *et al.*, 2022)
	Sheep (Group2)	Irradiated metacercariae of *F. hepatica* with Irradiation dose of 60 grays	Gelatin Capsule	Humoral response	Attenuated Vaccine	13.4	
	Sheep (Group3)	Irradiated metacercariae of *F. hepatica* with Irradiation dose of 120 γ rays	Gelatin Capsule	Humoral response	Attenuated Vaccine	11.2	
	Sheep (Group4)	Irradiated metacercariae of *F. hepatica* with Irradiation dose of 240 γ rays	Gelatin Capsule	Humoral response	Attenuated Vaccine	7.7	
	Infected control	------	------	------	------	22.5	

rCL1 = *F. hepatica* recombinant cathepsin; rHDM = *F. hepatica* recombinant helminths defence molecule; rLAP = *F. hepatica* recombinant leucine aminopeptidase; rPrx = *F. hepatica* recombinant peroxiredoxin.

3.2.3. *F. hepatica* Kunitz-type molecule: *F. hepatica* Kunitz-type molecules (FhKTM) are used as antigens (Silvane *et al.*, 2020). FhKTM is a single polypeptide of 58 amino acids. It is present in large quantity in gut, parenchymal tissue and tegument of adult *F. hepatica* (Mulcahy *et al.*, 1998). Apart from this abundance, this molecule is potent inhibitor of proteases and best suit as vaccine candidate (Silvane *et al.*, 2020). They are formulated by liquid crystal nanostructure with the assembly of a 6-O-ascorbyl palmitate ester (Coa-ASC16) and a synthetic oligodeoxynucleotide that contains unmethylated cytosine-guanine motifs (CpG-ODN) for vaccine production against *F. hepatica* (Silvane *et al.*, 2020). The molecules enhance the humoral immune response against *F. hepatica* (Cervi *et al.*, 2004; Toet *et al.*, 2014; Fracasso *et al.*, 2017). Studies have reported that the introduction of FhKTM/CpG-ODN/Coa-ASC16 increases the production of interleukin (IL)-17A and interferon-gamma (IFNγ), which are effective in the control of the fasciolosis infection (Lin *et al.*, 2009; Falcón *et al.*, 2012). It has also been reported that vaccination with FhKTM/CpG-ODN/Coa-ASC16 prevents liver damage and improves the survival of the host (Silvane *et al.*, 2020). Similarly, other studies have reported that IFNγ and IL-17A work synergistically by initiating the production of nitric oxide in macrophages to provide protection against *Fasciola* infection (Kumar *et al.*, 2012; Nascimento *et al.*, 2015; Gao *et al.*, 2016).

3.3. Cocktail vaccine: For successful migration and development in the host, liver flukes require the expression of multiple genes including FgFABP and Fg-glutathione S-transferase through the use of mountainside 70M-VG adjuvant or a delivery agent (Spithill *et al.*, 2012). The use of a cocktail vaccine that incorporates both of these genes triggers the IgG1 and IgG2 antibody responses and hyper-eosinophilia, which has been shown to provide protection upto 35% in animals (Kofta *et al.*, 2000). A diverse combination of recombinant vaccines includes *F. hepatica* recombinant cathepsin L1 (rCL1), *F. hepatica* recombinant peroxiredoxin (rPrx), *F. hepatica* recombinant helminths defense molecule (rHDM) and *F. hepatica* recombinant leucine aminopeptidases (rLAP) in two different (Montanide and Alhydrogel) adjuvants. The results shown in Table 1 indicate that a certain level of protection has been achieved against *F. hepatica* with the help of rCL1, rHDM, rPrx, rLAP and Montanide ISA 61 VG (Zafra *et al.*, 2021). Efficacy of Cocktail vaccine is shown in Table 1.

3.4. Nucleic-acid vaccines: Nucleic-acid vaccines against fasciolosis infection offer an advanced vaccine production technique that has eliminated the difficulties associated with recombinant vaccines (Carmona *et al.*, 1993). It has been found that cysteine proteases are crucial in host-parasite interactions (Carmona *et al.*, 1993) as they play a major role in migration, protection of the parasite from the host immune system and the feeding of *Fasciola* (Smitha *et al.*, 2010). One study has reported that the use of naked FgFABP DNA with nosylated-polyethylenimine-FgFABP shows significant production of Th1 cytokines (IFN-γ and tumor necrosis factor) in mice and these protect the host against the parasitic infection as shown in Fig. 2 (Kofta *et al.*, 2000). Cysteine proteases with complementary DNA vaccination have been found to provide 100% protection against liver fluke infection (Kaplan, 2001) in male mice and 74% protection in females (Carmona *et al.*, 1993; Kofta *et al.*, 2001). Briefly, 50µg of complementary DNA cysteine proteases in 250µl of saline was mixed with 0.05% bupivacaine (Carmona *et al.*, 1993), and this vaccine blocked the parasite at an early stage of life; evidence came from the eosinophil range and rate of liver damage in mice (Anderson *et al.*, 1997; Waine *et al.*, 1997).

3.5. Vector control: Control of intermediate hosts that support any parasitic infection is the best choice to eradicate diseases caused by the parasite. There are physical, chemical and biological processes that can be used to control hosts. Physical control methods include reducing the snail population through environmental management (elimination of water bodies, hu-man settlement, effective drainage, and rotation of aquatic and xeromorphic crops) (Leighton *et al.*, 2000; Li *et al.*, 2016; Bajwa *et al.*, 2022).

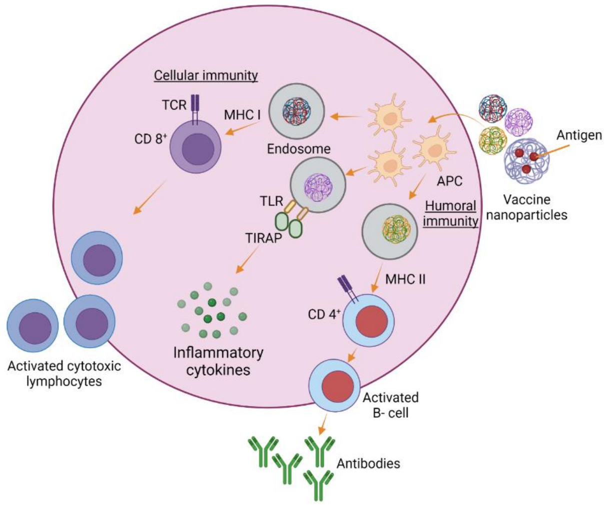

Fig. 2: Mechanism of nanoparticle vaccine. APC = Antigen presenting cells (Dendritic cells), TCR = T cell receptor, TIRAP = Toll-interleukin-1 receptor domain containing adaptor protein, MHC = Major histocompatibility complex, TLR = Toll-like receptor.

Chemical control involves the use of either natural or synthetic chemicals (molluscicides). The application of these chemicals remains an efficient method for the control of snails (Xia *et al.*, 2014). Synthetic chemicals such as N-tritylmorpholine, copper sulfate, niclosamide, sodium pentachlorophenol, bromoacetamide and nicotinamide were used to control snails in Africa, Asia, China and South America from the 1950s to 1970s (King *et al.*, 2015; Liu *et al.*, 2021; Hussain *et al.*, 2021). Among these synthetic molluscicides, only niclosamide is recommended by the WHO; however, it is expensive and toxic to aquatic animals. Therefore, a 50% wettable powder of niclosamide ethanolamine salt (WPN) is used in China, where it is the only synthetic compound available to eradicate snails and is widely used (Carmona *et al.*, 1993; Kofta et al 2001; Akram *et al.*, 2019). To overcome these challenges, a novel molluscicide, a quinoid-2′, 5-dichloro4′-nitrosalicylanilide salt, has been developed that has the same molluscicidal effects as WPN but is cheaper and significantly less toxic to fish (Kofta *et al.,* 2000). Another new molluscicide (niclosamide suspension concentrate) is more effective, more stable and less toxic than WPN (King *et al.,* 2015). Due to high cost, toxicity and resistance and environmental contamination caused by chemical molluscicides, natural molluscicides produced from several kinds of environment friendly plant extracts are also used (Lu *et al.,* 2018). Biological control involves the use of environmentally acceptable living organisms to kill the target/harmful organism. Examples of these organisms include prawns, water bugs and *Sphaerodema urinator*. They share the habitat of freshwater snails and control their numbers (Leighton *et al.,* 2000; Lu *et al.,* 2018). Biological control is mutually beneficial to nature, including humans (Xia *et al.,* 2014).

4. Future perspective

The development of a vaccine against fasciolosis infection poses a major challenge to the scientific community due to the various life stages of the parasite that must be controlled in the host. As yet there is insufficient knowledge of immunogenic proteins to produce vaccines against the parasite. Further studies are required regarding the changes that occur in the structure of the reproductive organs of liver flukes after vaccination. The cock-tail vaccine is a good choice but the interaction between the antigen in the multivalent vaccine and fasciolosis is not yet properly understood. According to this review of various studies, the maximum protection rate was found after vaccination with cysteine protease cDNA, which was reported to provide 100% protection in male mice and 74% in females. However, further trials in cattle are required. An important challenge in vaccine production against *F. hepatica* is the Th2-type of immunosuppressive response, which may hamper efforts to eliminate the parasite. Moreover, proteins in the parasite gut, parenchymal tissue and the tegument of juvenile and adult parasites act as protease inhibitors, which protect the parasite from the destruction of the tissue that it must penetrate.

Conclusions: Genus *Fasciola* comprises two-host (snail and mammals) trematode parasites. It is of zoonotic importance. Resistance to the drugs that have been used to control parasitic infection has become a major challenge. To combat this problem, researchers are focused on the development of vaccines to eradicate fasciolosis. Most parasites undergo several life stages in a host, which reduces the efficacy of vaccines. Researchers have developed several kinds of vaccines in laboratories that show variable efficacy. Fasciolosis is hepatobiliary disease in which major damage is caused by migrating juveniles of liver fluke and indirectly by host immune response to ES products of *Fasciola* resulting in host's tissue damage. In most cases, efficacy of a vaccine is judged by reduction in number of worms reaching bile duct examined in postmortem examination of experimental animals. However at this stage, liver damage cannot be easily graded. Hence, importance should be given to parameters quantifying liver damage at early stage i.e., damage caused during migration of newly excysted juveniles. Most effective vaccine would be one directed against newly excysted juveniles and their ES products with aim of preventing penetration of liver capsule by juveniles. Among all types of vaccines currently under investigation, the review found that RNA silencing and nucleic-acid vaccines significantly reduced infection rates in vaccinated animals, by 89.6 and 74-100% respectively. Additionally, control of the intermediate (snail) host has also proved to be an effective way to eradicate fasciolosis in endemic areas.

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