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Review Article

Mammalian Parasitic Vaccine: A Consolidated Exposition

Abstract

Parasites are highly prevalent in livestock worldwide and infect over one fourth of the human population also. Parasites are successful in evading host immune responses, and vaccination can prove to be an effective way to control them. However, currently very few vaccines are available against parasitic infection. Two important limitations in the emergence of effective parasitic vaccines are incomplete understanding of the immunoregulatory pathways involved in immunity, and the lack of precise information regarding host-pathogen interactions. Precise identification of parasite genes and the role of their products in parasite biology may assist in the identification of useful antigens, which could then be produced in recombinant systems. Many recombinant parasitic antigens have been successfully used in livestock and new vaccines are under trail. Numerous vaccine antigens are defined to target a wide range of parasite species. Thus vaccines offer a green solution to control disease. Vaccines have multiple beneficial effects such as improvement of animal health and welfare by controlling animal infestations and infections; diminishing resistance to anthelmintics, acaricides and antibiotics; improving public health status by controlling food borne pathogens and zoonoses aspect related to animals; keeping animals and the environment free of chemical residues and maintaining biodiversity. This current review is an attempt to consolidate all commercial or under-trail vaccine for mammalian parasites.

Introduction

As an estimation done by FAO (Food and Agricultural Organization) and WHO (World Health Organization) the human population will reach around 9 billion by 2050, so in order to feed them along with agriculture, clean, healthy livestock population should be needed for alternate food resource because food requirement will increase up to 50% [1,2]. Moreover it was estimated that only with a 6% reduction in animal disease could provide food for an additional 250 million people [3]. Productivity of livestock is greatly hampered by various diseases (viral, bacteria, fungal and parasitic) out of which parasitic infection play a crucial role [2]. The word "parasite" was first used in 1539, derived from Greek language which means para-along site and sitos-food. Parasite are divided various groups viz. trematodes (flukes), cestode (flat worm), nematodes (round worms), arthropods and protozoan. Majority of parasites have a 2 host, one act as intermediate host while other act as definitive host. All parasites are responsible for causing diseases, some of which cause the most devastating and prevalent diseases in both humans and animals. In compared with exotic breeds of cattle, indigenous breeds have shown some resistance to these pathogens. But, the susceptibility of highly productive exotic breeds poses a major encumbrance to the development of the cattle industry and in the improvement of meat and milk production in developing countries [1,4].

As per WHO estimation, at present 3.5 billion people worldwide are affected by diseases and 450 million have diseases due to infecting parasites [5]. In Australia and New Zealand the annual losses caused by bovine neosporosis is about $ 100 million annually [1] while in Switzerland it is about 9.7 million Euros [6]. Whereas illness caused by water born outbreak of cryptosporidiosis causes a total loss of $ 96.3 million, out of which $ 31.7 million are lost in medical costs and $ 64.6 million are lost in productivity losses. The average total costs for persons with mild, moderate, and severe illness were $116, $475, and $7,808, respectively [7,8]. In order to come out from adverse effect of these parasites an urge of effective control is needed. Up till now the control strategy of parasites relies mainly on the use of chemotherapy like anthelminthes, antiprotozoal drugs and insecticides etc, as they are safe, cheap and effective against a broad spectrum of parasites [9,10]. But indiscriminate use of these drugs led to the emergence of drug resistance in many targeted parasites [11]. On the same time, issues of residues in the food chain and environment have arisen, which threaten their sustained use [12]. So scientist and researcher are now a days concentrating on development of alternate sustainable methods like vaccinations, novel therapeutic regimens and immnuo-modulations against these parasite [2]. The term "vaccine" was first coined by Edward Jenner in 1881; it was derived from *Variolae vaccinae* (smallpox of the cow). Vaccines are used to generate antibodies and boost immunity against a disease, and usually contain an agent which may be the microorganism, its product, toxins or one of its surface proteins, treated/modified to be used as an antigen without causing disease [13]. It can be prophylactic or therapeutic. Vaccination helps in the development of acquired immunity by inoculating non-pathogenic but immunogenic components of the pathogen, or closely related organisms. In animal science the vaccines comprise only approximately 23% of the global market for animal health products; the sector is growing consistently [14].

Discussion

The main types of vaccine used are (http://www.vaccines.gov/more_info/types/):

1. **Live attenuated vaccines:** These vaccines are produced

using the attenuated strains of microbe which has lost its pathogenicity but has antigenicity. Example: Paracox vaccine having eight precocious lines of *Eimeria* species. Livacox having precocious lines of only *Eimeria acervulina* and *E. maxima,* together with an egg-adapted line of *E. tenella* [15].

2. **Inactivated vaccines:** These vaccines has dead etiological agent of the disease done either by radiations/heat/chemical (formaldehyde/beta-propionlactone). Example: inactivated anti-*Philasterides dicentrarchi* vaccine [16].

3. **Subunit vaccines:** These vaccines have only the best antigen (epitope) part of microbe which can start best immune response. Subunit vaccines can contain anywhere from 1 to 20 or more antigens. These vaccines can be made in one of two ways:

- Microbe is first grown in the laboratory and then chemicals are use to break it apart and important antigens are collected.
- Using recombinant DNA technology the required antigen molecules from the microbe is manufacture. These vaccines are also called "recombinant subunit vaccines."

Example: Peptide-based subunit vaccines for malaria parasites [17], CoxAbic for Coccidia.

4. **Toxoid vaccines:** For those microbes which secrete toxins, or harmful chemicals, a toxoid vaccine might be the answer. The desired toxins are inactivated by treating them with formalin (a solution of formaldehyde and sterilized water). Such "detoxified" toxins, called toxoids; are safe for use in vaccines.

5. **Conjugate vaccines**: Many microbes has polysaccharides molecules on its outer coating as many harmful bacteria do, so scientists may try making a conjugate vaccine by using it. For conjugate vaccine targeted antigen (epitope) is linked so that infant's immune system can recognize to the polysaccharides. The helps the immature immune system react to polysaccharide coatings and defend against the disease-causing microbe.

6. **DNA vaccines:** These vaccines are also called third generation vaccine. This concept was introduced in 1990. Once the desired gene of microbe has been analyzed, direct intramuscular injection of plasmid DNA in myocytes was given for the induction of protein expression and immune system gets activated. DNA vaccines induce strong humoral and cellular immunity and have the potential to increase immunogenicity through modifications of the vector or incorporation of adjuvant-like cytokine genes. The cells of body take up the injected DNA and start secreting antigen, in other words the body's own cells become vaccine-making factories, creating the antigens necessary to stimulate the immune system. Examples: Vaccine against murine leishmaniasis are (Antigen-GP-63, dose- 2×100 μg IM, Parasite- *Leishmania major*; Antigen-LACK (*Leishmania major*-activated C kinase) dose- 2×30 μg IN Parasite- *L. amazonensis*), in *Phlebotomus papatasi* a salivary components i.e. SP15, was used as tested as DNA vaccines against *L. major*. For *Trypanosoma cruzi* (Antigen-TSA-1, type of antigen-TS family, dose- 2×100 μg IM) [18]. In *Schistosoma mansoni* large subunit of calpain (Sm-p80) and either mouse GM-CSF or IL-4 was used as DNA vaccine to determine their adjuvant effect in mice [19].

7. **Recombinant vector vaccines**: These are similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. "Vector" refers to the virus or bacterium which can be used as the carrier. Recombinant vector vaccines closely mimic a natural infection and therefore do a good job of stimulating the immune system. Example: Immunization with "Maxadilan" which is a potent vasodilator from sand-fly antigen (as a recombinant vaccine) protected mice against *L. major* infection [20].

Parasite vaccine production is rather very difficult as compare to other microorganism because of their large size, complex life cycle and difficulty in there *in vitro* culturing. So, precise work is to be done in this aspect.

Vaccine for cestodes

***Taenia* Vaccine:** In sheep Excretory-Secretory (ES) material from *Taenia ovis* oncospheres could induce sterile immunity when it is associated with antibodies binding to the 16, 18 and 45-kDa molecular weight antigens. Later on immunization of sheep with recombinant forms of these antigens (*T. ovis* antigens 45W, 16.17 and 18K) was also conducted successfully [21]. *T. ovis* 45W is a member of a family of genes comprising a minimum of 4; 45S differ from 45W at 11 of 985 nucleotides sequence of mRNA, animals vaccinated with other protein encoded by this variant gene were not protected against *T. ovis* infection. The 45W agent induces IgG_1 and IgG_2 antibodies; they provide a high degree of protection in animals [22]. The oil adjuvants, saponin and DEAE-dextran gave the highest antibody responses and greatest degree of protection against challenge infection with *T. ovis* eggs. For *T. solium* it was found that crude antigen preparations derived from oncospheres induce complete protection in pigs [22]. It was later noted that extracts of *T. crassiceps* cysticerci contain antigens which are also protective against *T. solium* infection in pigs. Homologues proteins of *T. saginata* (TSA-18 and TSA-9, similar to the TO 45-kDa antigen i.e. TO-45W) in cattle generated a good response [21]. Trials conducted in Mexico, Peru, Honduras, and Cameroon showed 99–100% protection against *T. solium* using TSOL 18 oncosphere antigen. Moreover this vaccine completely eliminated the transmission of *T. solium* by the pigs involved in the trial [23]. Other important agents are proteins such as TSOL 45 and TSOL 16. In case of *Taenia crassiceps* (mice) and *T. solium* (pigs) a vaccine candidate (designated S3Pvac) based on 3 synthetic peptides, KETc1, KETc12 and GK1 having 12, 8 and 18 amino acids, respectively were also shown to be effective [24,25]. It produces 90% protection in mice after successfully expressed in 19 different transgenic papaya clones [26].

Echinococcus: In case of *Echinococcus granulosus* oncosphere antigens provided high protection in sheep. In *E. granulosus* EG-95 and EM-95 from *E. multilocularis* provided protection in sheep and cattle up to 99%, moreover they are homologues to Taenia vaccine antigens [22]. Out of these EG-95 is the only field trial-tested vaccine

candidate against hydatidosis (Echinococcus infection). Now a days attempt has been made to express EG-95 in plants part along with a fibrillar antigen EG-A31. Alfa Alfa leaves are infected by modified *Agrobacterium tumefaciens* (updated name of *Rhizobium radiobacter*), which has a recombinant plasmid by electroporation (pBI–Eg95–EgA31). Its result showed significantly decreased (64.1%) in weight of hydatid cyst; moreover antigen specific IgG, IgG2b and IgE was also higher in BALB/c mice by oral immunization method [10]. When canine are vaccinated with adult-stage recombinant EgM proteins, it show great reduction in maturation to egg production as well as lowered the worm burdens very effectively [21]. Later on, trails on these cestode parasite (*T. ovis* and *E. granulosus*) was stopped because mainly these infection are detected at the time of slaughter and there is no loss to livestock owner, moreover the cost of manufacturing these vaccine agents was more than its return so funding industries took least interest [22].

Vaccine for trematode

Fasciola hepatica: In this case many agent has been tried; proteases such as the leucine aminopeptidase (LAP) which are involved in parasite blood digestion, reduces worm loads in rabbits by >75%. Other important agents targeted against *Fasciola hepatica* and *F. gigantica* in sheep and cattle are GST (glutathione-S-transferase), trematode hemoglobin, cathepsin proteases (CP) L1, L2 and FABP (fatty acid binding protein), among all these agents CP play a key role in migration, immune evasion and feeding through host tissue material [27]. In cattle it produce high levels (>70%) of protection. Recombinant DNA constructed by encoding *F. hepatica* GST had a high humoral response to the mice. In lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*) a 981 nucleotide cDNA fragment encoding the catalytic domain of the CP of *F. hepatica* was incorporated and this also induces an effective immune response in mice [10]. Moreover a schistosome protein Sm14 provides cross-protection between the two trematode parasites [27].

Schistosoma Vaccine: High levels of immunity (up to 90%) were developed in mice and primates against multiple exposures of irradiated cercariae of schistosome [21]. Some somatic agents which were used are cytosolic structural proteins (paramyosin) and glycolytic enzymes (aldolase) but they fail to generate high levels of immunity [21]. The most important vaccine target of the schistosome is the tegument. For *Schistosoma mansoni* - TSP-2 (Tetraspanin: found in outer tegument) has been used for development for human vaccine antigen in sub-Saharan Africa and Brazil, later on recombinant TSP-2 reduces adult worm burdens and liver eggs by >50 and >60% respectively [28]. In Europe and Africa for *S. haematobium* a recombinant 28 kDa Glutathione S-transferase (GST) was also used. Another candidate i.e. Sh28-GST (Bilhvax) appears to be immunogenic and well-tolerated in healthy conditions [28]. Moreover Sm14 which is a fatty acid binding protein has been use against both human schistosomiasis and fascioliasis in cattle and was effective. Immunization with the cercarial surface protein SmTOR reduced worms by up to 64%. Immunization with the membrane-associated large subunit of calpain Sm-p80 resulted in up to 70% worm reductions in mice and >50% worm reductions in baboons [21]. Other agents which can provide protection are Sm29, SmCD59-like and Sm200 [21]. In case of *S. japonicum* a 23 kDa membrane protein (Sj23) plays an important role in producing immunity and this antigen exists in all stages of the parasite [29]. Three doses of 3 plasmids encoding *S. japonicum* antigens, Sj62, Sj28 and Sj14 induced high levels of IFN-γ and partial protection from challenge infection when administered in mice [30]. These plasmids also produce antigen-specific IgG in mice moreover this gene was transferred into *M. sativa* through Agrobacterium. In pigs for *S. japonicum* an antigen i.e. SjCTPI (triose-phosphate isomerase) was used and 60% of vaccinated animals demonstrated antigen-specific antibodies against the parasite. Moreover significant reduction in hepatic worm burden (48.3%) and size of liver egg granulomas have also been noted [31].

Vaccine for nematodes

Hookworm Vaccine: For hook worm in 1964, infective stage of larvae i.e. L_3 of *Ancylostoma caninum* was attenuated using 40,000 roentgens of X-ray and was used as vaccination agent by Miller et al. [32]. In 1970 for canine after US licensing, its commercial industrial manufacture was started as a first hookworm vaccine consisting of gamma-irradiated infective *A. caninum* L_3 larvae [33]. But in year 1975 this vaccine was discontinued due to some drawbacks (vaccinated dogs were found to have eggs in faeces, cost of production and maintaining laboratory-canine model was high, deficiency of *in vitro* test to determine the efficacy of immune response and short shelf-life). Major target for vaccination against gastrointestinal nematode infection are the human hookworms i.e. *Ancylostoma duodenale* and *Necator americanus*. In human now a days one group i.e. Human Hookworm Vaccine Initiative (HHVI) is working for vaccine development. For control of *N. americanus* a 21 kDa protein i.e. Na-ASP-2 has been used as a vaccination agent [34]. In 2012 *Necator americanus*-glutathione S-transferase 1 (Na-GST-1) was also tried in Brazil [35]. To provide protection against *A. caninum* infection several antigens have been tried like VAL and ASP-2 [36]. ASP-2 increases the level of IgE and evokes allergic adverse effects. These agents lead to reduced egg production, as well as a lower degree of blood loss and anemia in infected patients. In case of canine the antigens targeted are Ac16 and As14 [37].

Haemonchus: In *Haemonchus contortus* infection younger animals remain highly susceptible, but in adults natural immunity develops after its repeat exposure. Earlier vaccination using irradiated larval was done but it acted poorly in lambs [38]. Up till now for *H. contortus* main vaccine targets are: A) **H_{11}:** It is a microvillar integral membrane glycoprotein complex obtained from detergent extracts of *H. contortus* adult worms and generates 70-90% reduction in parasite loads [39]. Later on recombinant rH_{11} using a baculovirus-derived insect cell homogenate was also tried but it induced disappointingly low level of protection (30%). B) **H-Gal-GP:** H-gal-GP (Haemonchus galactose-containing glycoprotein) is also obtained from detergent extracts of adult *H. contortus,* followed by peanut agglutinin affinity chromatography, which binds to Gal b1,3 GalNAc disaccharide motifs, it resulted in >70% reduction in adult worm counts [40,41]. C) **TSBP (Thiol-Sepharose binding protein):** It was isolated using a method designed to purify cysteine proteases associated with *H. contortus* gut extracts [42]. First extracts of adult *H. contortus* are depleted from Hc-gal-GP by lectin binding, then subjected to thiol-

Parasite	Vaccination agent	Reference
Trichinella spiralis	Excretory–secretory, Ts87 and gp43 recombinated in Salmonella, TspE1 (31kDs)	[82-84]
Strongyloides ratti	HSP60 in alum	[85]
Strongyloides stercoralis	Ss-IR	[86]
Brugia malayi	Irradiated infective mosquito-borne L3 larvae, ALT-1 and ALT-2	[87]
Litomosoides sigmodontis	Plasmids encoding *L. sigmodontis* antigens (including ALT)	[88]
Brugia pahangi	Excretory–secretory	[89]
Dictyocaulus viviparus	Radiation-attenuated larval vaccines (Dictol, Huskvac), rAChE, Excretory–secretory	[90]
Dictyocaulus filari	X-irradiated larvae vaccine (Difill)	[91]
Trichostrongylus colubriformis	CarLA (heat-stable Glycolipid: carbohydrate larval antigen),	[92]
T. circumcincta	Tci-MIF; apyrase, Tci-APY-1; TGFb homolog, Tci-TGH-2, cathepsin F, Tci-CF-1; Astacin metalloprotease, Tci-MEP-1; a 20 kDa protein of unknown function; VAL/ASP protein, Tci-ASP-1, Tci-SAA-1	[93]
Strongylus vulgaris	Irradiated larval vaccines	[94]
Ascaris suum	Recombinant protein vaccines As 14, As 16,As 24	[95,96]
Toxocara canis	Excretory–Secretory	[97]
Onchocerca volvulus	Irradiated L3 larvae, tropomyosin, CPI-2, FAR1, ALTand RAL2(cocktail of subunit vaccine and recombinant antigens)	[98]
O. ostertagi	Oo-gal-GP, ES-thiol' OPA	[21]
C. sinensis	FABP (fatty acid binding protein)	[31]
Teladorsagia circumcincta	Excretory–Secretory	[99]
Cooperia spp	Native and recombinant proteins	[99]
Nematodirus spp	Native and recombinant proteins	[99]

sepharose affinity chromatography, to purify proteins with free cysteine residues, including (but not limited to) cysteine proteases. This showed 43-52% protection against challenge infection of Haemonchus. TSBP does not react with antisera to H_{11} or H-gal-GP but contains a different range of antigens, including a major glutamate dehydrogenase and minor cathepsin B-like cysteine proteases (hmcp-1, 4 and 6) which are the actual protective targets [21].

Against *H. contortus* infection the immunogenic properties of recombinant Cu/Zn superoxide dismutases, P46, P52, and P100 have also been assessed.

Dictyocaulus: Against *Dictyocaulus viviparous* a commercial vaccine (containing X-irradiated infective larvae of lung worm) is available for cattle in Europe under trade name "Dictol". The vaccine consisting of 2 doses each 'containing 1000 irradiated larvae given at one month interval has been used with outstanding success. Calves are immunized at 3-7 weeks of age. The vaccination program of calves dairy should be completed before they go to grass in spring or early summer. In endemic areas, immunity is maintained by continuous exposure to infection. In India, X-irradiated larvae vaccine was developed against *Dictyocaulus filarial* infection in sheep and goat with similar success and marketed as "Difil" [43].

Vaccine for protozoa

Malaria Vaccine: Malaria is cause by various *Plasmodium* species and transmitted by various species of mosquitoes**.** The extracellular sporozoites and intracellular liver stages produce no clinical symptoms, so they are also regarded as an ideal target for vaccine intervention [44]. In 1960 for immunization against malaria, trials on mice were conducted by using irradiated sporozoites [45]. The live sporozoites attenuated by irradiation (IrrSpz) provided complete protection against sporozoite challenge in primate and mouse model [44]. In 2005 genetically attenuated parasites (GAP) produced sterile, protective immunity comparable to IrrSpz immunization, where protective immune responses are also critically dependent on $CD8^+$ T-cells. In Colombia peptide base vaccine SPf66 was developed for primates but its trails in Asia and Africa failed [46]. Until now most effective vaccine tested is a hybrid protein molecule (pre erythrocytic) i.e. RTS,S also called as Mosquirix (RTS,S recombinant vaccine is based on the major *Plasmodium* sporozoite surface antigen; circumsporozoite protein-CSP) with adjuvant AS01 (having liposomes). This vaccine produce high level of antibodies, in its 1st trial 51% reduction of clinical cases has been reported from Kenya [47], 55% reduction cases occur when its trial were conducted in sub-Saharan region of Africa at 11 different sites in 2011. It is also observed that before use of this agent in vaccination programs, 34.3% of infants were positive with low titers for anti-circumsporozoite antibodies. After vaccination, 99.7% were positive at high titers (209 EU/ml) for anti-circumsporozoite antibodies. Its entire trial will be over in 2015 [5]. For anti-malarial vaccination in children of sub-Saharan African region, the European Medicines Agency (EMA)'s decision paves the way for a policy recommendation by the WHO [48]. Now a days many target gene are been use as vaccination agents to eliminate malaria such as UIS 3, UIS 4, P 32, P52, SAP1, SLARP, FabB/ F, PDH E3, PALM, LISP etc [28] moreover, parasitic antigens are also expressed in plants (*Arabidopis thaliana* seeds, Tobacco, *Brassica napus*, and Lettuce) such as MSP4/5 (Merozoite surface protein), MSP119, AMA1 (Apical membrane antigen), MSP1, CSP (Circumsporozoite protein), P230 (Gametocyte antigen), P25 (Surface antigen) etc [10]. Furthermore now a days various antigen has been identified that will help in targeting the liver stage of malaria [49].

Leishmania Vaccine: The disease caused by Leishmania spp has its zoonotic importance. In case of human mainly if a subject recovers from leishmaniosis it become resistant for further infection. Moreover it is also observed that vaccine against *Leishmania* provide protection against more than one species [8,50]. Various forms of vaccine have been tried such as: **A) Live Leishmania vaccine:** It is used in Israel, Russia, Iran and Uzbekistan but not yet licensed. In this promastigotes

of *L. major* were cultured and used. Despite of adverse effect such as immune suppression, lesion etc [51] in Uzbekistan mixture of live virulent and killed parasite has been used. **B) Whole/fractions of killed vaccine:** In early 1940 whole-killed promastigotes were also tested as vaccines against CL and VL (cutaneous leishmaniosis and visceral leishmaniosis), in Brazil [52]. For canine, leishmaniosis parasite lysate vaccines fractionation led to the development of a glycoprotein enriched mixture termed as "FML antigen" and it provided 92% protection. In 1970 killed vaccine having five isolates of Leishmania of four different species was developed by Genaro and co-worker [53]. In Venezuela autoclaved *Leishmania mexicana* was used by Convit and his coworker [54]. For old world leishmaniosis, autoclaved *L. major* + BCG (Bacille Calmette Guerin) have been extensively studied and it depicted 18-78% reduction in case of CL. In mice and rabbits a subunit vaccine utilizing the fucose mannose ligand antigen has been shown to be a potent immunogen, moreover for sero-testing in human and canine kala-azar it act as a sensitive, predictive and specific antigen [55]. **C) DNA, recombinant proteins vaccines and combinations:** To stimulate lifelong protection, genetically altered Leishmania parasites are used because they lack cystein proteases or dyhydrofolate reductase enzyme [56]. In case of canine VL saponin formulation of fucose mannose ligand was found to be safe and is licensed as Leishmune (76-80% protection) veterinary vaccine [57]. The antibodies (Abs) produce by this vaccine do not allow the development of promastigote in fly. Moreover in dogs LiESAp-MDP produces long-lasting protection [58]. Now a days for the effective control of leishmaniosis some of the important agents for vaccines include: kinetoplastid membrane protein-11, amastigote specific protein A2, sterol 24-c-methyltranferase, K26/HASPB, Leishmania-activated C kinase, PSA (parasite surface antigen), LACK (Leishmania activated C kinase), gp63 (surface expressed glycoprotein leishmaniolysin reconstituted in liposomes), Leish-111f (Leishmania derived recombinant polyprotein), cysteine proteinase B, KMP11, nucleoside hydrolase, open reading frame F and tryparedoxin peroxidase [13,59]. Out of these Leish-111f product (99.6% protection) is the first defined vaccine against leishmaniosis to be use in to primate clinical trials [60]. This contains *L. major* stress inducible protein-1, *L. major* homolog of eukaryotic thiole-specific antioxidant, *L. braziliensis* elongation and initiation factor, in formulation with MPL-SE, and the results denoted that it provide protection in mouse models for CL and VL, but failed to prevent natural *L. infantum* infection. For *L. major*, liposomal soluble antigen incorporated with phosphorothioate CpG ODN (PS CpG) or phosphodiaster CpG ODN (PO CpG) has also been tested for CL. **D) Live-attenuated Leishmania vaccines:** In case of mice use of dihydrofolate reductase thymidylate synthase (dhfr-ts) parasites led to the effective protection. Currently use of *L. donovani* centrin null mutants (LdCEN-/-) in mice showed reduced parasitic burden in the spleen [61]. Biochemically and radio attenuated parasite have also provided high protection in hamsters and rodents without any adjuvant [62].

Recently an intranasal vaccine for Leishmania amazonensis antigens (LaAg) to provide protective immune responses against *Leishmania (infantum) chagasi* by using the CAF01 association has also been tried. A significant reduction in their parasite burden in both the spleen and liver, along with an increase in specific production of IFNγ and nitrite, and a decrease in IL4 production was observed in LaAg/CAF01 vaccinated mice. Furthermore there was increased lymphoproliferative immune response after parasite antigen recall [63]. In recent times a polyproteins vaccine when administered in association with an adjuvant, provide protection against VL. This vaccine has two *Leishmania infantum* hypothetical proteins present in the amastigote stage, LiHyp1 and LiHyp6, were combined with a promastigote protein, IgE-dependent histamine-releasing factor (HRF) [64,65].

Amebiasis Vaccine: Entamoeba spp mainly causes diarrhea (watery or contains blood and mucus) and vomiting. In case of mammalian cells the binding of trophozoites of *Entamoeba histolytica* is mediated by a protein (serine rich). The galactose and N-acetyl-D-galactosamine-specific lectin on the surface of the amoeba is a potent immune-dominant molecule that is highly conserved and has an essential role in the stimulation of immune responses. The structure of the lectin has been defined, and the heavy subunit with its cysteine rich region has been demonstrated in animal models (mice) to have some efficacy as a possible vaccine agent for prevention of amoebic infection [66]. Moreover the N-Acetyl-D-galactosamine-inhabitable *E. histolytica* lectin (GAL/GALNAC) also mediates the adherence of trophozoites. This antigen had shown protective effect on 66% of the animals against the amebiasis. Other candidates under investigation in case of developing vaccine for amebiasis are oral/intranasal administration of the galactose and N-acetyl-D-galactosamine lectin, cysteine proteinases, the serine rich *E. histolytica* protein, lipophosphoglycan, amebapores and 29-kDa protein (peroxiredoxin) [5]. Some workers are using the vaccination agent in plants (lectin) against *E. histolytica* (LecA) by Plastid transformation [10]. When DNA plasmids encoding either *E. histolytica* cysteine protease 112 or adhesion 112 were co-administered to hamsters, they provided protection against liver abscess formation [30].

Trypanosome: Earlier the beta tubulin gene of *Trypanosoma evansi* (STIB 806) after cloning in *E. coli* [2] was used as vaccine agent, later on recombinant beta tubulin was also expressed in *E coli*. For *T. brucei* DNA vaccine (TSA protein) provide protection of 60% cases. A recombinant agent MAPp15 (microtubule protein) provided complete protection against haemoparasitic infection. For protection against *T. cruzi* intramuscular DNA vaccine containing the TcPA45 gene (39kDa) was used and it was observed that there is 85% decrease in parasitaemia levels after challenge with infective forms of the parasite. When its recombinant form i.e. rTcPA45 protein was used as intra peritoneal injection there was decrease up to 95% parasitaemia level in mice after a lethal dose of *T. cruzi*. Both protocols were able to trigger specific B cells and high levels of antibodies anti-rTcPA45 were also detected in sera [67]. Moreover an enzyme "cyclophilin" was identified and trails were conducted on its recombinant form in *E. coli*.

Trichomoniasis: *Tritrichomonas foetus* mainly causes abortion in cattle. A killed whole-cell protozoan vaccine (Trichguard) provides protection when given @ 1-2 ml [2]. In vaccination trail it was observed that during and after the 90-day breeding period heifers immunized showed faster rise in systemic antibodies level as well

as better pregnancy rates. In conclusion, if this vaccine was given before breeding and early in the breeding season by both SQ and intravaginal route, it can yield superior protection for heifers exposed to bulls infection [68]. In case of bulls vaccination with whole cell antigen showed that IgG antibodies specific for protective antigens of *T. foetus* in the preputial secretions and serum [69].

Coccidia: In late 1940 a live sporulated oocyst vaccine (Coccovac-B) was produced. Its strains are *E. tenella, E. acervulina, E. maxima* and *E. mivati*. Another vaccine based on live sporulated oocyst (Coccivac-D) having 8 different species of *Eimeria viz., E. maxima, E. burnetti, E. acervulina, E. mivati, E. necatrix, E. hagani, E. tenella* and *E. praecox* has been developed in 1970. Coccivac-T having live sporulated oocysts of *E. gallopavonis, E. adenoids, E. meleagrimitis* and *E. dispersa* was also used for vaccination [5]. Despite the presence of ionophore compound a live vaccine (COXATM) remain fully active. It has 3 main strains i.e. *E. tenella, E. acervulina* and *E. maxima*. Another vaccine (*Eimeria vax* 4 m) having *E. tenella* (150 oocysts) strain Rt_{3+15}, *E. maxima* (100 oocysts) strain MCK_{+10}, *E. acervulina* (50 oocysts) strain RA, and *E. necatrix* (100 oocysts) strain $mednic_{3+8}$ in PBS (phosphate buffer saline) are been used with a titer of 1.6×10^4 oocysts ml^{-1}. It is safe in day old chick. Others commercially used vaccines are: CoxAbic (subunit vaccine form macrogametocyte of *E. maxima*), Immucox (Oral vaccine-developed in Canada by Vetech Laboratories), Livacox T/Q (live attenuated vaccine), Paracox-8 (*E. tenella, E. maxima, E. acervulina, E. mitis, E. burnetti, E. necatrix* and *E. praecox*). In U.S another vaccine named as Advent was recently developed by Viridus Animal Health. It has more viable oocysts (truly sporulated oocysts that can cause immunity) than other vaccines. As Coccivac, immucox, advent are not are not "attenuated" so they can actually cause some lesions and occurrence of coccidiosis in birds. On other hand, vaccines such as Paracox, and Livacox used in Europe are attenuated. They are altered because the coccidia used in the vaccine are designed to mature quickly and have a short (precocious) life cycle and low fertility. They are not pathogenic-disease causing and are less costly to produce than the non-attenuated vaccines. These vaccines are marketed in other countries but not currently in the U.S [70].

Recently a microneme protein, EtMIC2 of *E. tenella* was incorporated by using Agrobacterium in tobacco leaves [5]. Feeding of this transgenic plant resulted in the higher weight gain, reduction in oocyst output and high antibody production. Later on EtMIC1 was also used in poultry and its efficacy was compared with EtMIC2, it was observed that serum antibody response and weight gain was better in former one [10].

Anaplasma: In United States first trial to develop vaccine against anaplasma was conducted, it contains killed *Anaplasma marginale* and marketed as "Plazvax", moreover "Anaplaz" (non living lyophilized preparation with adjuvant) was also commercialized later. In 1989, irradiated *A. marginale* was used in deers and sheep. Later on vaccine having this stock (Anavac) has been developed by 58 passages at university of Illinois. These vaccines only protect animal form the development of clinical disease but have no effect on infection by anaplasma. Other agents used for vaccination are MSP1b (major surface protein), it produced significant antibody response and partial protection (two out of six immunized animals were protected) when challenged with cryo-preserved parasites [31]. Moreover infection with *A. centrale,* an organism originally isolated in South Africa provides partial crossimmunity against *A. marginale* challenge. In *A. central* two surface proteins (36 and 105 kDa) induce a protective immune response in calves to homologous and heterologous challenge.

Giardia: In canines, a killed cultured trophozoites vaccine (Giardiavax) is been used against *Giardia lambia*. It mainly has a crude preparation of disrupted, axenically cultured *G. duodenaalis* isolates derived from sheep [31].

Toxoplasma: Earlier in pig partial protection from the development of *Toxoplasma gondii* tissue cysts was developed by using crude fraction of *T. gondii* rhoptry proteins incorporated into an ISCOM (immune stimulating complexes) adjuvant. Recently in pig intradermal inoculation of *T. gondii* GRA-1-GRA-7 DNA cocktail, developed a strong humeral immune response [1]. S48 strain (Toxovax), is a live vaccine and it inhibit development of *T. gondii* in both cat and sheep [69]. INF-gamma target bradyzoites or oocyst and clear parasite within 14 days after infection. This was originally isolated from an aborted ovine foetus in New Zealand and was passaged over 3000 times in laboratory mice initially to provide a source of antigen for diagnostic purposes. This live vaccine Toxovax (live organisms of an attenuated strain of *Toxoplasma gondii*-incomplete Strain 48) is currently the only commercial vaccine for toxoplasmosis worldwide. A bradizoite of live mutant *T. gondii* (T263) also help in providing protection. MIC6, MIC8 (micronema protein 6 and 8) has also been used as vaccine agents. In some country a recombinant PDI (rTgPDI) was also used for vaccination. Now a day the potential proteins are also available for vaccine purpose. On the whole in *T. gondii* there are about 1,360 specialized protein families [71]. Some proteins such as Surface antigen glycoproteins (SAGs) are important for host cell attachment and host immune evasion, and *T. gondii* possesses 182 SAG-related sequences. Out of which the main are SAG1 and SAG2, which are the most abundant proteins in tachyzoites, they can be used as a vaccine agents. Some worker are using the vaccination agent in plants (Tobacco leaves) also such as SAG1 (surface antigen), GRA [40 kDa] (dense granule protein) by Agro-infiltration method [10]. Other important protein is AMA1 (Apical membrane antigen 1) this helps in host cell penetration; because of this property, AMA1 is also considered to be a potential vaccine candidate. Rhoptry proteins are also been targeted out of which ROP2, ROP3, ROP4, ROP7, and ROP8 are of veterinary importance [72,73]. Some other useful antigen targeted for vaccination purpose are: TS-4 (temperature sensitive mutant) 2×10^4, TLA (*Lactobacillus casei* as adjuvant), rROP2/4, Zj111/pSAG1-MIC3, KO-strain, pVAXROP16, pVAXROP18, RON4, AdSAG3, AdSAG2, AdSAG1, pME18100/HSP70 etc [74]. In *T. gondii* infection the cytokines secreted by the immune response include up regulated factors (IFNγ, IL-2, TNFα, IL-1, IL-7, IL-12, IL-15) and down regulated factors (IL-4, IL-6, IL-10) [75].

Theileria: Attenuated schizont culture *Theileria parva* infected lymphoblastic cell line $@10^8$ attenuated cells was used for vaccination by sub-cut route in cattle. Cock tail of strains [*T. parva* (Muguga), *T. parva* (Nugong) and *T. Lawrence*] in GUTS was also used in many African countries. In cattle for *T. parva* infection agent named p67

(67kDa MW) was used earlier in Kenya. Later on its recombinant form was also tested with recombinant *vaccina* virus and *Salmonella typhimurium* but failed to give good result. For *T. annulata* agent used was SPAG-1 [1]. In *T. annulata* infection *in vitro* cell culture attenuated vaccines (Rakshavac-T) provide protection of about 95-100%. It should be avoided in pregnancy. In *T. parva* infection mixture of sporozoite and schizont provide good immunity response. The major merozoite surface antigen of *T. annulata* (Tams-1) has also been expressed in recombinant form and used in small scale immunization trials. Later on it was observed that animal recovered from infection has special cytotoxic T-cells which destroy the lymphocyte infected with *T. parva*. Schizoints in lymphocyte has two proteins which stimulate this response [5]. Genes responsible for this was identified and its recombinant form were used as vaccination agent. Tissue cultured attenuated schizoint vaccine for *T. annulata* was also developed in which parasite multiplication is confined to 1×10^9. In National Dairy Development Board (1989), Anand, India a vaccine (Raksha Vac-T) was developed using *T. annulata* ODE strain by 150 passages @5×10^6. It can be given in calf more than 2 year of age. In Punjab agricultural university (PAU) Ludhiana, India, a vaccine was developed which can be given to 7 day old calf by using Hissar isolate, by 100-150 passage @1×10^6. The seed culture should be tested for some viral infection (infectious bovine rhinotrachitis, blue tongue virus, bovine viral diarrhea, bovine leukemia virus, bovine cyncytial virus, bovine immunodificency virus, bovine parainfluenza virus type –II and rinderpest virus).

Babesiosis: After the discovery that these organisms can be attenuated by sequential passage in splenectomised calves the commercial live attenuated vaccine became available. In earlier days by passaging (20-30 times) 10^7 parasites a vaccine was prepared but later on it showed many short coming. Its shelf life was only 5-7 days at 5ºC. In Venezuela *Babesia bigemina* cultured derived soluble exo-antigen vaccine with saponin as an adjuvant was developed; it has stability of 2 year at 4ºC. For *B. bovis* the agents targeted by chromatography for vaccination were 11C5, 12D3 and 21B4. For canine babesiosis two subunit vaccines have been developed [11]. They consist of soluble parasitic antigens (SPA) that are released into the culture supernatant by *in vitro*-cultured parasites, combined with adjuvant. The first vaccine released was "Pirodog" (France), for *B. canis* cultures, whereas recently released "NobivacPiro" contains SPA from *B. canis* and *Babesia rossi* in an attempt to broaden the strain-specific immunity. In cattle an *in vitro* cultured live attenuated vaccine can produce good immunity mainly for *Babesia bovis*. For culturing Micro Aerophilus stationary phase (MASP) technique is used [14].

Neosporosis: For neosporosis there are no live vaccines commercially available to provide protection. Later on attenuated vaccine was also used and it was observed that NC-Nowra strain was more virulent than NC-Liverpool strain in mice [1]. In cattle a tachyzoite base vaccine [Neoguard (A killed vaccine having Neospora tachyzoites with an adjuvant -Havlogen)] has been used to prevent abortion caused by *Neospora caninum*. 50% reduction in abortion was seen but it should be avoided in pregnancy. In some country live tachyzoites maintained on vero cell line in certain specific condition (37ºC, 5 % CO_2 and RPMI 1640 medium with 2 % horse serum and penicillin-streptomycin) was also used as vaccine [14].

Sarcocystosis: Chemically treated *in vitro* culture merozoites mixed with adjuvants (EPM vaccine) is used as vaccine for sarcocystidae family members. In equines it provides protection against neurological sign (equine protozoal myeloencephalitis) due to infection from *S. neurona*. A vaccine having rSnSAG1 (recombinant *S. neurona* surface antigen gene 1) can also protect horses [22].

Cryptosporidiosis: It is an emerging topic due to its zoonotic aspect. Animals vaccinated with killed *C. parvum* oocysts showed reduced oocyst levels and diarrhea in controlled condition, but this agent did not prove to be efficacious when tested under field conditions. Recombinant *C. parvum,* C7 protein containing the 101C terminal of the P23 antigen was used to immune Holstein cows in their late gestation, and there colostrums showed a significant reduction in oocyst shedding and also provided protection against diarrhea. When recombinant *C. parvum* oocyst surface protein rCP15/60 was used for vaccination, it helps to prevent cryptosporidiosis in livestock [1,5].

Vaccine for arthropods

Among arthropods most important species are the ticks, they transmits various diseases. In India alone the cost of Tick and Tick Born Diseases in animals has been estimated in the tune of US$ 498.7 million [67]. Several agents have been tested in vaccination programs which are derived from ticks. Earlier whole body homogenate of salivary gland was used as vaccine agent. For *Bhoophilus microplus* a subunit vaccine was made i.e. Tick GUARD in Australia. It has recombinant tick gut concealed antigen BM86 (located in mid-gut of the tick). In 1996 this vaccine was re-registered as TickGARD plus (*E. coli* expressed BM86+BM91). "Gavac" was another version of tickguard and was developed in Cuba; it was having recombinant BM86 in *Pichia pastoris* and provided protection upto 85%. Now a days various endosymbiont was also identified and they are used as vaccination agents because ticks depend only upon hosts blood and that may not provide all the nutrient so endosymbionts are necessary for them [60]. Sterility was observed in healthy tsetse flies fed with tetracycline (2500 µg/ml) due to damage to the mycetome bacterial endosymbionts [76]. In *Haemaphysalis longicornis* a 29 kDa salivary gland-associated protein was identified and its recombinant protein produced in *E. coli* showed reduction in adult female engorgement weight and 40% and 56% mortality of larvae and nymphs post-engorgement. In *R. appendiculatus* a 15 kDa protein (64P), its recombinant versions (64 TRPs) resulted in reduction of the nymphal and adult infestation rates by 48 and 70%, respectively [77]. DNA vaccination in Merino crossbred sheep against *B. microplus* using Bm86 full length gene was also tried [78]. Moreover, covaccination with Bm86 and GM-CSF plasmids gave statistically significant reduction in the fertility of ticks. Development of combine vaccine against *T. parva–R. appendiculatus* and *T. annulata–H. a. anatolicum* systems has also been tried. In Indian Veterinary Research Institute (IVRI), India the recombinant Tams 1 antigen of *T. annulata* (Parbhani strain) and the Bm86 homologue antigen of *H. a. anatolicum* (Izatnagar isolate), rHAA86, were produced in *E. coli* and *Pichia pastoris*. Against *T. parva,* infection novel subunit vaccine has been recently evaluated, additionally; the homologue of Bm86 has

been discovered in *R. appendiculatus* (Ra86). Some genes have been identified that are present across phylogenetically distant species in *I. scapularis* and were designated as 4D8 (also identified as subolesin), 4F8 and 4E6 [79,80]. Subolesin protective antigen was demonstrated to extend to other tick species [81]. In case of *A. variegnatum* and *A. americanum* whole nymph and gut extract was used respectively. For *Lucilia cuprina* an agent has been isolated from its peritropic membrane (peritrophins 95, 48 and 44) and used for vaccination. In *Hypodermis bovis* hypodermin A from first instar provided the greatest level of protection. In *Anophelese quadrimaculata*, whole female homogenate provided protection in rabbits.

Other Parasite and their antigenic agent targeted (Table 1).

Conclusion

Concise information provided in this review regarding the progress in the field of vaccine development for various parasitic diseases of livestock and humans may be a guideline for veterinarian clinician and academician.

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