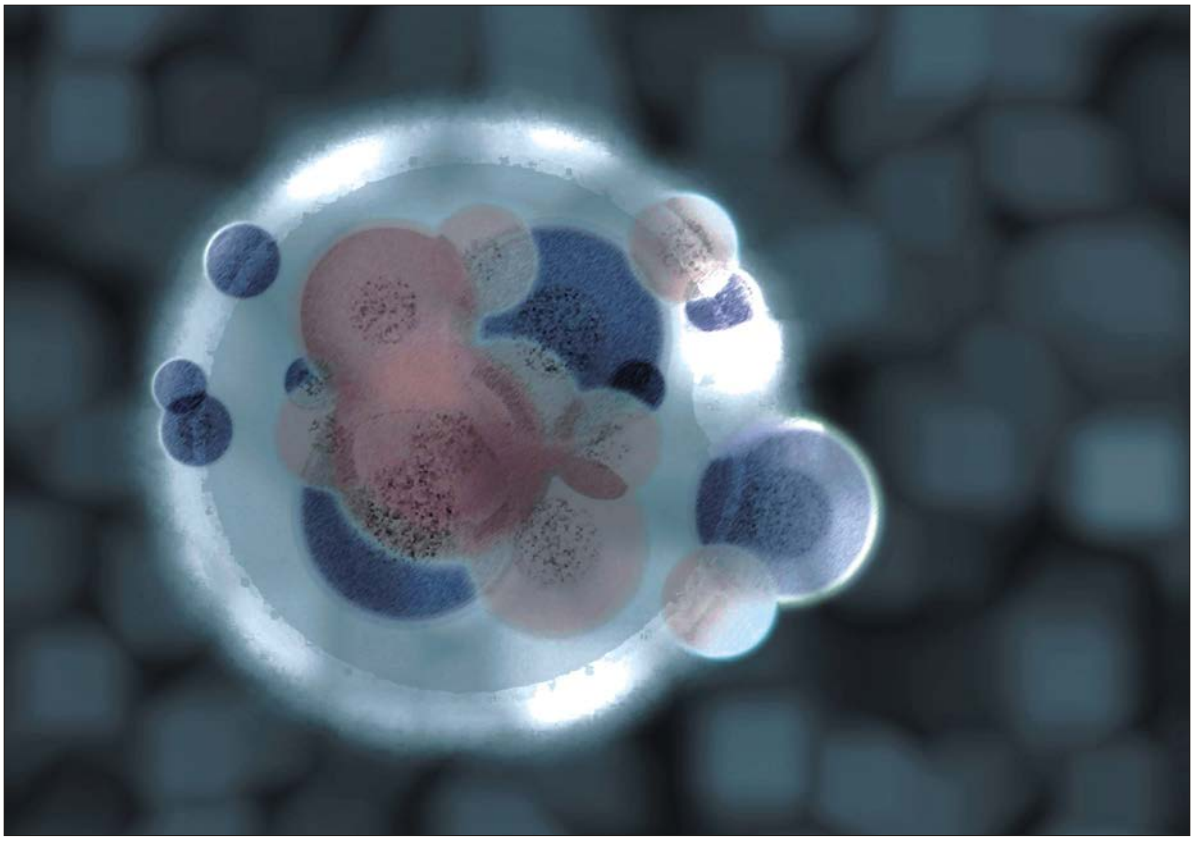

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Trends and Advances in Vaccines Against Protozoan Parasites of Veterinary Importance: A Review

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Abstract: By 2050 to feed the estimated human population of around 9 billion, there is requirement of 50% increase the food production, which can only be fulfilled by clean, healthy and sustainable food animal production. Livestock industry is facing considerable economic losses due to infectious diseases. So an effective control strategy is need of today to control these infectious diseases and contribute in augmentation of livestock production. Parasitic diseases have a major impact on livestock production, reproduction and hence economy. Protozoan parasites are major causes of human and animal disease causing extensive morbidity and mortality, particularly parasitic disease in tropical and sub-tropical climatic regions. Many protozoal parasitic diseases are zoonotic. Limiting the impact of parasitism in both man and livestock relies almost exclusively on the use of antiparasitic drugs. Development of resistance towards chemotherapeutic agents has forced the scientist to discover some alternative for control of parasitic diseases. Recent advances in immunology and biotechnology have sensitized the scientists or researchers to develop the newer and safer vaccines for control of parasitic diseases. This review is intended to provide state-of-art information to the reader with an overview on the trends, advances and perspectives in vaccines and vaccinology against important parasitic diseases of livestock and poultry viz., coccidiosis, anaplasmosis, giardiosis, babesiosis, *Neospora* infection, toxoplasmosis, theleriosis, sarcocyst infestation, leishmaniasis, trypanosomiasis and trichomoniasis, which altogether play crucial role in the prevention of protozoan parasitic diseases of animals.

Key words: Animal, cattle, livestock, parasite, protozoa, vaccine

INTRODUCTION

According to an estimate, by 2050 to feed the estimated human population of around 9 billion, there is requirement of 50% increase the food production. This requirement can only be fulfilled by clean, healthy and sustainable food animal production (Mahima *et al*., 2012a; Fitzpatrick, 2013). In modern days, the livestock industry is facing considerable economic losses due to infectious diseases of viral, bacterial, fungal, parasitic origin (Rogers and Randolph, 2006; Jones *et al*., 2008; Cascio *et al*., 2011; Verma *et al*., 2008, 2012a, b; Kumar *et al*., 2011; Dhama *et al*., 2013a, b). So an effective control strategy is need of today to control these infectious diseases and contribute in augmentation of livestock production (Innes *et al*., 2011; Dhama *et al*., 2013c, d). In past or till now, the limiting of impact of parasitism relies on use of chemotherapy like anthelminthic, antiprotozoal drugs etc (Vercruysse *et al*., 2004). Due to development of resistance towards these chemotherapeutic agents, scientists or researchers are now thinking towards the prevention of parasitic diseases through use of vaccines (Barriga, 1994; Vercruysse *et al*., 2004; Stern and Markel, 2005; Innes and Vermeulen, 2006; Paul-Pierre, 2009; Knox, 2010; Sharman *et al*., 2010; Liu *et al*., 2012; Dhama *et al*., 2008, 2013e). All these facts have forced the researchers to develop some alternatives for prevention and control measures like effective

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vaccination, immunomodulators and novel therapeutic regimens (Meeusen *et al*., 2007; Vercruysse *et al*., 2007; Manzano-Roman *et al*., 2012; Dhama *et al*., 2013d; Jacob *et al*., 2013; Mahima *et al*., 2012b). Effective ways to combat parasites are very limited due to their complex nature and complicated relationship with the hosts. Vaccine is a substance used to stimulate the production of antibodies and provide immunity against one or more diseases. It is prepared from causative agent of disease, its products, treated to act as antigen without causing disease (Stern and Markel, 2005). With the development of immunology, a continuous flow of vaccines to the market is going on, but among them the percentage of parasitic vaccine is very low (Vercruysse *et al*., 2004). The development of vaccines against the parasitic diseases for domestic animals is most fascinating, promising and challenging field (Innes and Vermeulen, 2006). Majority of the problems are due to their complex life cycle and difficulty in their *in vitro* culture (Cornelissen and Schetters, 1996).

Parasitic diseases have a major impact on livestock production worldwide with infection arising from a range of helminth, protozoan and ecto-parasites and out of these, many parasitic diseases are zoonotic (Innes and Vermeulen, 2006; Cornelissen and Schetters, 1996; Paul-Pierre, 2009; Dhama *et al*., 2013a, b, c, f; Reichel *et al*., 2013). This review is intended to provide state-of-art information to the reader with an overview of vaccines available against parasitic diseases of livestock viz., coccidiosis, anaplasmosis, giardiosis, babesiosis, *Neospora* infection, toxoplasmosis, theleriosis, sarcocyst infestation, leishmaniasis, trypanosomiasis and trichomoniasis, which are very useful for prevention and control of parasitic diseases in animals.

PROTOZOAN DISEASES

Protozoa are unicellular, eukaryotic, microscopic organisms, belongs to subkingdom protozoa, having a distinct nucleus as well as endoplasmic reticulum, golgi apparatus, mitochondria in the cytoplasm. About 65,000 species of protozoa have so far been named of which a great majority are free-living, while only 7000 protozoan species are parasitic both in vertebrate and invertebrate animals (Levine, 1985).

Metazoan and protozoan parasites are one of the major causes of disease causing extensive morbidity and mortality in animals (Knox, 2010). These parasites are major impediment to the introduction of high-productivity breeds in poorer or developing countries. Among these many of the parasites are zoonotic in nature that also increases their economic importance. At present, no vaccine for human protozoal disease is available; however, several veterinary vaccines are available in the market (Meeusen *et al*., 2007; Vercruysse *et al*., 2007). Among vaccines against protozoan diseases of livestock, many are based on live orgamisms, but recently there is progress in development and commercialization of killed subunit vaccines (Lightowlers, 1994; Sharman *et al*., 2010).

ANTIPROTOZOAN DRUGS- PROBLEMS IN THE NEAR FUTURE

Drugs against protozoan parasites are widely used to prevent the infection but protozoans are able to develop resistance against these drugs, which is a serious problem in human and veterinary (Vercruysse *et al*., 2007). Broiler industry is facing the crisis of antiprotozoan drug resistance against the common coccidiostats used in the farm (Stephan *et al*., 1997). This has lead to the shuttling of drugs used against *Eimeria* so as to keep resistance development under check. Resistances against antiprotozoan drugs are now reported in *Trypanasoma* (Geerts *et al*., 2001) and also in canine Babesiosis (Collett, 2000). Extreme use of antiprotozoan drugs has not only lead to the resistance against these drugs but also the residues in the animal products enter the food chain causing cross transfer of resistance (De Ruyck *et al*., 2000; Geary, 2002). Apart from these problems these drugs can also enter environment from the secretions and excretions of the animals, threatening the public further (Steel and Wardhaugh, 2002). Hence the current regime for the treatment several protozoan infections are not proper and the search for new antiprotozoan drugs has not yielded a fruitful result (Von Samson-Himmelstjerna and Blackhall, 2005). To solve these problems vaccination will be a good means to control the disease.

VACCINE AND ITS TYPES

Vaccines are dead or inactivated organisms or purified products derived from them. There are several types of vaccines in use (Table 1):

- Killed or inactivated
- Live attenuated
- Subunit
- DNA Vaccine
- Edible vaccine

Killed or inactivated: It is produced by killing the etiological agents of disease either by chemical (formaldehyde or beta-propiolactone)/heat/radiation and

Table 1: Types of protozoan vaccines

Vaccine type	Salient feature	Diseases/Pathogen	Advantages	Disadvantages/ Limitations	References
Killed or inactivated	Produced by killing the etiological agents of disease either by chemical (formaldehyde or beta-propiolactone) /heat/radiation	*Giardia, Neospora, Leishmania, Tritrichomonas Foetus*	Stable, constituents clearly defined, Unable to cause the infection	Need several dosages, Local reaction common, Short lasting immunity	Shkap *et al.* (2007)
Live attenuated	Prepared from attenuated strains that are almost or completely devoid of pathogenicity but having the immunogenicity	Coccidia, *Anaplasma, Babesia, Toxoplasma, Theileria, Sarcocystis*	Single dose is sufficient to produce long-lasting immunity, Strong immune response evoked, Local and systemic immunity produced	Requires refrigeration, Contraindicated in immunosupressed patient, Poor stability	Iunes *et al.* (2011)
Subunit vaccine	Contains only the essential antigens and not all the other molecules that make up of microbe	*Leishmania,* Canine babesiosis, Coccidiosis	Low adverse reactions Not infectious, so they can safely be given to immuno-suppressed animals	Do not stimulate immune system properly and requires boosters to have good immune response	Wallach *et al.* (1995) Wallach (1997) Ingolotti *et al.* (2010)
DNA vaccines	Small circular piece of bacterial DNA that has been genetically engineered to produce one or two specific protein	*Toxoplasma gondi, L. infantum, Anaplasma mariginale*	No risk of infection, Better stability, Cost effective, no risk	Risk of effective genes controlling cell growth, Chance of inducing mutation of reversion to virulence, Plasmid may get integrated into the cell and can lead to transfer of resistance gene	De Andrade *et al.* (2004) Dondji *et al.* (2005) Nielsen *et al.* (2006) Dhama *et al.* (2008) Kumaragurubaran and Kaliaperumal (2013)
Edible vaccine	Enters orally released in the intestinal tract elicits both humoral and mucosal immunity	Malarian parasite, *Eimeria tenella*	Eliminate the pain from injection, better immune response, free from contaminant microbes	Possibility of oral toleranceand side effects like allergy. Needs time and patience in expression	Sathish *et al.* (2011) Clemente and Corigliano (2012) Sathish *et al.* (2012) Dhama *et al.* (2013e)

such vaccines are more safe and stable than live vaccines. These are inactivated vaccines and easy to prepare. In this type of vaccine, the replicative function of etiological agent should be destroyed, while the outer coat of agent should be left intact. For its effectiveness, large amount of antigen is required in comparison to live vaccine. Excessive treatment can destroy immunogenicity whereas insufficient treatment can leave infectious agent capable of causing disease. The commercially available killed/subunit vaccines available against protozoan parasites are given in Table 2.

Advantages:

- Killed vaccines are stable
- Constituents clearly defined
- Unable to cause the infection

Disadvantages:

- Need several dosages
- Local reaction common
- Short lasting immunity (Shkap *et al.*, 2007)

Live attenuated: Live vaccines are prepared from attenuated strains that are almost or completely devoid of pathogenicity but having the immunogenicity therefore they are capable of inducing a protective immune response. They multiply in the host and provide continuous antigenic stimulation over a period of time. The commercially available live protozoal vaccine against animal diseases is given in Table 2.

Advantages:

- Single dose is sufficient to produce long-lasting immunity
- Strong immune response evoked
- Local and systemic immunity produced (Innes *et al.*, 2011)

Disadvantages:

- It required refrigeration
- Contraindicated in immunosupressed patient
- Poor stability

Subunit vaccine: Instead of entire microbe, subunit vaccines include only that best antigen which stimulates the immune system. It contains only the essential antigens and not all the other molecules that make up of microbe. It is of three types: (a) Natural tissue purified protein, (b) Recombinant protein antigens and (c) Chemical small peptide vaccines. Two subunit vaccines are available in market for canine babesiosis caused by *B. canis*. These vaccines contain soluble antigens from

Table 2: Progress in protozoan vaccines and their commercial availability

Pathogen	Example/Commercially availability	Type	Salient features/Remarks	Reference
Coccidia	Coccivac®-B, Coccivac®-D, Coccivac®-T, Nobilis® COXATM, Eimeriavax -4 m, Immucox, Paracox®-8, Livacox, CoxAbic	Live, Sporulated oocyst vaccine	Provide protection against different coccidian parasites viz., *Eimeria acervulina*, *E. maxima*, *E. necatrix*, *E. hagani*, *E. praecox*, *E. tenella*, *E. brunetti*, *E. mivati*, *E. dispersa*, *E. meleagrimitis*, *E. adenoides* and *E. gallopavonis* etc.	Chapman (1994), Johnson and Reid (1970) and Mathis and McDougald (1989) Vermeulen *et al.* (2001), Gore *et al.* (1983), Lee (2006) and Shirley *et al.* (2005), Shirley and Bedrnik (1997), Sharman *et al.* (2010)
Anaplasma	Anaplaz	Killed vaccine	Protect the animal from development of clinical disease	Brock *et al.* (1964)
	Anavac	Modified live vaccine	Animals become infected with the vaccine strain of *Anaplasma* and are "immune carriers"	Ristic (1960)
Giardia	Giardiavax	Killed culture trophozoite vaccine	Prevent the disease and shedding caused by *Giardia lamblia* in dogs	Olson *et al.* (2001)
Babesia	Pirodog/Nobivac® Piro	Soluble Parasite Antigen (SPA) of supernatants of *in vitro* cultures	Provides immunity upto six months	Schetters (2005)
Neospora	Bovilis® Neoguard	Killed tachyzoite with spur adjuvant	Reduces abortion in cattle by 50% Gives protection upto 1 year	Meeusen *et al.* (2007)
	Live tachyzoite vaccine	Live vaccine	Vaccine protects the losses due to death of foetus	Williams *et al.* (2007)
Toxoplasma	S48 strain (Toxovax)	Tachyzoite that loss ability to form bradyzoite or oocyst	Prevent from spreading of oocyst in the body, Placenta as well as meat contamination for 18 months	O'Connell *et al.* (1988)
	T263	Live vaccine containing bradizoites of mutant *T. gondii*	Reduce/prevent oocyst shedding in cats	Verma and Khanna (2012)
Theileria	Rakshavac-T	Cell culture attenuated vaccine	Provide protection against *Theileria* infection	Brown *et al.* (2006)
Leishmania	Leishmune	Purified fraction (gp63 protein) of *L. donovani* adjuvanted in saponin	Block the transmission of *Leishmania* parasites	Dantas-Torres (2006)
	Leish111f	Recombinant vaccine	Vaccine efficacy is very high reaching upto 99.6%	Coler *et al.* (2007)
Sarcocystis	EPM vaccine	*In vitro* cultured merozoites	Gives protection against a neurological disease in horses caused by infection with *Sarcocystis neurona*	Marsh *et al.* (2004)
	S. neurona SAG1 protein vaccine	Subnnit vaccine	Better protection	Ellison and Witonsky (2009)
Trypanosoma	Beta-tubulin	Recombinant vaccine	Beta-tubulin gene of *Trypanosoma evansi* (STIB 806) cloned and expressed in *Escherichia coli*	Li *et al.* (2007)
	TSA	DNA vaccine		Silva *et al.* (2009)
	MAPp15	Recombinant vaccine	100% protection from lethal challenge of a heterologous strain of *Trypanosoma brucei*	Rasooly and Balaban (2004)
Tritrichomonas Foetus	Trichguard	Killed vaccine	Protect cattle from infection by *Tritrichomonas foetus*	Baltzell *et al.* (2013)

the pathogen of canine babesiosis. NobivacPiro® is a newly marketed vaccine which contains soluble protein antigen of two pathogens namely *B. canis* and *Babesia rossi* that broadens the immunity. ABIC Veterinary Products, Israel markets a novel killed subunit vaccine for poultry coccidiosis (Wallach, 1997). The peculiarity of this vaccine is that it targets macrogametocyte stages of coccidia which results in formation of oocyst unlike live vaccines which target merozoite stages. Laying hens are immunized by this vaccine rather than chicks, transferring immunoglobulins to the yolk which is the added advantage of this vaccine (Wallach *et al.*, 1995). A subunit vaccine comprising of antigens such as Gam82 and others purified from the Eimeria gametocytes has been reported to be safer and effective in preventing coccidiosis in birds (Vermeulen, 1998; Wallach *et al.*, 2008; Jang *et al.*, 2010).

Advantage:

- Low adverse reactions
- Not infectious, so they can safely be given to immuno-suppressed animals

Disadvantage:

- As native structure of antigens is difficult to retain, so antibodies produced after immunization may not recognize the same protein on the pathogen surface
- Do not stimulate immune system properly and requires boosters to have good immune response (Ingolotti *et al*., 2010)

DNA vaccines: These vaccines are the third generation vaccines, in which DNA responsible for particular protein is directly injected into host to produce the desired immune response. At present this technology is proving helpful in control of protozoan infections (Dumonteil, 2007; Liu *et al*., 2006). DNA vaccines are developed for a handful of protozoan infections of animals and their studies on laboratory animals showed good results that these vaccines can be adopted for animals (Da'dara and Harn, 2005; Dhama *et al*., 2008; Carvalho *et al*., 2010; Kumaragurubaran and Kaliaperumal, 2013).

DNA vaccine for *Toxoplasma gondi* targeting the bradyzoite stage antigens BAG1 and MAG1 reduced cyst burden in mice when challenged with infection (Nielsen *et al*., 2006). Recombinant plasmid using *T. gondii* surface antigen 1 (SAG1) and 14-3-3 protein has been found to induce protective immunity in BALB/c mice (Meng *et al*., 2012). Recently, a DNA vaccine expressing eukaryotic translation initiation factor (eIF4A) protein of *T. gondii* has been reported to be promising in inducing protective immunity against acute toxoplasmosis in mice (Chen *et al*., 2013). Development of DNA vaccines has been tried against protozoan parasites, Leishmania and *Trypanosoma cruzi* also which affects human being. Leishmaniasis, a major public health zoonoses, is a complex disease caused by at least 18 species of genus Leishmania transmitted by hematophagous sandflies while *T. cruzi* is the causative agent of Chagas disease. Cross-protection studies showed that infection by one species of Leishmania may or may not protect from subsequent infection by another species of same genera (Xu and Liew, 1994, 1995). In dogs, therapeutic DNA vaccine has also been developed against *Trypanosoma cruzi* infection (Quijano-Hernandez *et al*., 2008). Another DNA vaccine targeting the *Leishmania infantum* acidic ribosomal protein P0 (LiPO), *Leishmania major* DNA vaccine encoding PSA-2 antigen, Cysteine Proteinase (CP) a and b DNA vaccines also had good immune response studies in mice (Dondji *et al*., 2005). Another DNA vaccine encoding *L. donovani* nucleoside hydrolase NH36 is therapeutically active against murine visceral leishmaniasis produced by *L. chagasi* (Handman *et al*., 2000; Aguilar-Be *et al*., 2005; Gamboa-Leon *et al*., 2006). Nucleoside hydrolase DNA vaccine (Leishmune®'s) has been found effective for immunotherapy of canine visceral leishmaniasis (Borja-Cabrera *et al*., 2012). DNA vaccine for *Anaplasma mariginale* targeting the major surface protein namely MSP1b yielded good antibody response and fair protection was noticed in challenge studies in cattle (De Andrade *et al*., 2004). Development of DNA vaccine targeting babesiosis disease in canines has been attempted with protein p50 gene coding the *Babesia canis,* which generated protective immunity, while the p36/LACK antigen gene and *L. major* GP63 antigen encoding gene inducing Th1 immune response has been utilized for Leishmaniosis (Walker *et al*., 1998; Fukumoto *et al*., 2007). DNA vaccines have also been developed against coccidiosis in birds, employing the 3-1E and EtMIC2 genes (Min *et al*., 2001; Ding *et al*., 2005).

Advantages:

- Safe as do not contain any form of pathogen
- No risk of infection
- Administration is easy by either I/M or I/D routes
- Manufacture of multivalent vaccines by combining multiple DNA
- Stability of the vaccine for storage and shipping especially in tropical zones
- As compared to live attenuated or recombinant vaccines DNA vaccines are more stable
- Cost effective, hence ideally affordable
- Produces both humoral and cellular immune response
- Unlike live attenuated vaccine there is no risk of reversion to virulence

Disadvantages:

- Risk of effective genes controlling cell growth
- Possibility of including antibody production against DNA
- Chance of inducing mutation
- Complexity in vaccine formulation
- Plasmid may get integrated into the cell and can lead to transfer of resistance gene (Kumaragurubaran and Kaliaperumal, 2013)

Edible vaccine: Recent advances in sciences has made it possible that vaccine can be administered orally as edible vaccines hence eliminating the problem of pain which is associated with the needle pricks during vaccination (Dhama *et al*., 2013e). Though the concept of producing

edible vaccine started in 1990's but it had rapid strides and all sorts of plants are being exploited in the recent years so as to get good expression of the required gene of interest so that good immunity may be elicited against a particular organism (Curtiss and Cardineau, 1990). Edible vaccine which enters orally released in the intestinal tract elicits both humoral and mucosal immunity. More work has been carried out the malarian parasite which of importance in human aspect (Clemente and Corigliano, 2012). Tomato has been used as an expression plant so that it can be easily consumed by human (Chowdhury and Bagasra, 2007). In livestock sector edible vaccines has been developed against coccidiosis in poultry which is an important disease in poultry industry, caused by *Eimeria tenella*. Microneme protein EtMIC2 of this deadly pathogen was expressed in tobacco leaves (Sathish *et al*., 2011). Feeding trails of this tobacco leaves with the microneme protein resulted in good antibody synthesis and there was low oocyst shed down in the droppings. The combination of EtMIC2 and EtMIC1 yielded good antibody response along with increase in weight gain (Sathish *et al*., 2012).

Advantages:

- Plant cell wall act as a barrier protecting the antigen of interest to reach the target place safely (Streatfield, 2006; Hayden *et al*., 2012)
- Plants contain some phytochemicals which act with co-ordination with the antigen so that it provides good immune response even in the absence of adjuvants (Pasquevich *et al*., 2011)
- Secondary plant metabolites like lectins, saponins, alkaloids, phenolic compounds and flavonoids act as an immune stimulant (Kostrzak *et al*., 2009)
- Edible vaccines are free from bacteria, fungi and viruses because plant pathogens are not deleterious to animals
- Needle prick is eliminated and hence there is no pain and no damage to muscles
- As the administration of oral vaccine is much easier than injection, it can be applied for mass vaccine and time can be saved
- Production can be done in large scale with available plants locally and can be stored for longer period (Dhama *et al*., 2013e)

Disadvantages:

- Negative impact on public because of the use of edible plants for production of vaccine
- Possibility of inducing oral tolerance and side effects like allergy
- To produce highly expressive plants it needs time and patience (Jacob *et al*., 2013)

VACCINES AVAILABLE AGAINST SOME OF THE PROTOZOAN PARASITES

Vaccines against coccidia

Coccivac®-B: It is a live, sporulated oocyst vaccine produced from isolates that were collected in the late 1940's, before the current anticoccidial products were introduced. This vaccine is prepared from anticoccidial-sensitive strains of *Eimeria acervulina*, *E. mivati*, *E. maxima* and *E. tenella*, unlike present-day field oocysts, these isolates had never been subjected to selection pressure by anticoccidials resulting in resistance. Coccivac-B vaccine is a valuable tool to restore the performance of existing anticoccidials (Chapman, 1994). In experimental study on birds, this vaccine produced variable degrees of protection with the five different strains of *E. tenella* (Awad *et al*., 2013).

Coccivac®-D: It is a live, sporulated oocyst vaccine containing different species of *Eimeria* viz., *Eimeria acervulina*, *E. maxima*, *E. necatrix*, *E. hagani*, *E. praecox*, *E. tenella*, *E. brunetti* and *E. mivati*. To induce complete immunity, the original dose of coccidial oocysts must complete at least four life cycles in the flock (Johnson and Reid, 1970).

Coccivac®-T: It is a live coccidiosis vaccine containing sporulated oocysts of *Eimeria dispersa*, *E. meleagrimitis*, *E. adenoides* and *E. gallopavonis* that is administered to day old turkey poults via spray cabinet. The strains of *Eimeria* in Coccivac-T were isolated prior to common use of modern ionophore and chemical anticoccidial products and are highly sensitive to all in-feed anticoccidials (Mathis and McDougald, 1989).

Nobilis® COXATM: This vaccine is a live vaccine having a special property of being active even in presence of ionophore compounds. The vaccine contains strains of *E. acervulina, E. tenella and E. maxima* which are tolerant to ionophore compound. The benefit of this vaccine is that apart from production of immunity the vaccine allows the use of ionophore compounds during the 3-4 weeks of age when the immunity is poorly developed (Vermeulen *et al*., 2001).

Eimeriavax -4 m: This vaccine contains viable oocysts of *Eimeria acervulina* Strain RA, *E. maxima* Strain MCK_{+10},

E. necatrix Strain mednec$_{3+8}$ *and E. tenella* Strain Rt $_{3+15}$ suspended in phosphate buffered saline (PBS). Each dose comprises a minimum of *E. acervulina* 50 oocysts, *E. maxima* 100 oocysts, *E. necatrix* 100 oocysts and *E. tenella* 150 oocysts, with a minimum predicted titre of 1.6×10^4 oocysts mL^{-1} at the end of the shelf-life (Gore *et al*., 1983). The advantages of *Eimeria vax* 4 m are:

- Simple, single dose, eye-drop or oral application
- Safe in chickens from day-old
- Immunity induced within 10 days against the four major species of *Eimeria* (*E. acervulina*, *E. maxima*, *E. necatrix* and *E. tenella*)
- Effective in broiler breeders, broilers, free range and layer flocks
- Supplied ready to use
- Anticoccidial medications are not required
- Provides productivity improvements (McDonald and Shirley, 2009)

Immucox: Oral coccidiosis vaccine of live oocysts of *Eimeria* spp. designed to help healthy Chicken Broilers and Roasters to develop immunity to coccidiosis. This is a one-time vaccination that delivers protective immunity through the productive life of the bird. It is a vaccine approved for water or gel delivery in the hatchery (Lee, 2006).

Paracox®-8: It is a live attenuated oral vaccine consisting of translucent, suspension of oocysts derived from eight precocious lines of coccidian, used for the active immunization of chickens against different *Eimeria* spp. viz., *Eimeria acervulina*, *E. tenella*, *E. brunetti*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. maxima* (Shirley *et al*., 2005). The dose of vaccine is 0.1 mL per chicken, which can be administered orally either by spray on feed or in drinking water or by hatchery spray.

Livacox: It is live attenuated coccidiosis vaccines for domestic poultry (*Gallus domesticus*). Its range consists of LIVACOX® T for broilers and LIVACOX® Q for breeder and layer pullets (Shirley and Bedrnik, 1997).

CoxAbic: It is first commercially available subunit vaccine for poultry and contains purified antigens isolated from the macrogametocyte (sexual stage) of *Eimeria maxima* (Belli *et al*., 2004). Vaccination using gametocyte antigen through the breast muscle will guide to production of antibody response (Sharman *et al*., 2010).

Vaccines for anaplasma

Anaplaz: It is the first anaplasmosis vaccine manufactured for cattle in the United States by Fort Dodge. More recently, Mallinkrodt (later Schering-Plough) marketed a vaccine called Plazvax®. Both of these vaccines protect against Anaplasmosis by similar mechanisms. The vaccines contain killed *Anaplasma marginale*, harvested from infected cattle (Brock *et al*., 1964). The vaccines do not prevent the animal against infection by the *Anaplasma* organism, but protect the animal from development of clinical disease. They are "immune carriers". That is to say, they are "immune" to becoming sick from the agent but are carriers of the agent. Dose rate of 1 mL by sec $cycle^{-1}$ route and repeated after 3-4 week and revaccinate annually by single dose, 1 mL.

Anavac: It is a modified live vaccine, which is safe and effective when given to young cattle. They become infected with the vaccine strain of *Anaplasma* and are "immune carriers". Dose rate of 2 mL i/v and is given at 6-12 month of age. Booster doses are recommended every 1-2 years depending on herd history (Ristic, 1960).

Vaccines against giardia

GiardiaVax: It is a killed culture trophozoite vaccine for dogs and prevent the disease and shedding of *Giardia lamblia*. The vaccine is derived from *G. duodenalis* isolated from sheep. Dose of vaccine is 1ml by subcutaneous route. First dose at 8 weeks of age and second after 2-4 weeks and then repeated annually. Dogs which had failed to be cured of giardiasis following chemotherapeutic measures were treated with a *Giardia* vaccine (2-3 injections). After immunization, the clinical signs diminish within 16-42 days, therefore it is a good method for treating giardiasis in dogs.

Vaccines against babesia: It is live attenuated vaccine, developed by *in-vitro* culture (Levy and Ristic, 1980). Cattle were vaccinated with exo-antigen from culture (MASP) elicit humoral and cell mediated immunity (Alexis *et al*., 1993).

Pirodog/Nobivac® Piro: It is a Soluble Parasite Antigen (SPA) of supernatants of *in vitro* cultures (*B. canis* and *B. rossi*) gives 80% protection and immunity last for about 6 months (Schetters, 2005). It is given at 6 months of age and booster vaccination is required 3 to 6 weeks after the initial vaccination and thereafter revaccination every 6 months by intramuscular route. Both the vaccine produces some local reaction at the site of injection but this reaction is more in case of Nobivac Piro vaccine (Freyburger *et al*., 2011).

Vaccine against neospora: An ideal vaccine have to provide protection against both infection and the clinical

signs, so there is requirement of vaccine that can induce a non-foetopathic cell mediated immune response (Goodswen *et al*., 2013). Recently, a *Neospora caninum* killed tachyzoite based vaccine has been reported to be efficacious in preventing abortion in dairy cattle (Williams *et al*., 2007; Weston *et al*., 2012). Proteins, which have important role in adhesion/invasion or other parasite-host-cell interaction processes can provide protection against Neospora infection and can be targets for the development of an effective vaccine against this important protozoan parasite (Hemphill *et al*., 2013).

Bovilis® Neoguard: It is prepared by killed tachyzoite of Neospora caninum with spur adjuvant which reduces abortion in cattle by more than 50% (Meeusen *et al*., 2007; Weston *et al*., 2012) but may increase the early embryonic death, if used in pregnancy (Weston *et al*., 2012). The vaccination induce antibody at a high level which give protection upto 1 year, so booster vaccination after 1 year is required. It is administered in two doses of 5 ml at one month apart, the first dose given between day 75 and 90 of gestation, booster in 3-4 weeks with 2 annual boosters 3-4 weeks apart by subcutaneous route.

Live tachyzoite vaccine: Tachyzoites were maintained under conditions in a continuous passage of vero cell-lines at 37°C and 5% CO_2 in air and in RPMI 1640 medium supplemented with 2% horse serum and penicillin-streptomycin (100 IU mL^{-1} 100 g mL^{-1}). This vaccine protects the losses due to death of foetus (Williams *et al*., 2007; Hecker *et al*., 2012; Goodswen *et al*., 2013).

Vaccines against toxoplasma: Effective vaccines against *Toxoplasma gondii*, a protozoan parasite infecting both animals and humans, are very helpful in preventing and controlling toxoplasmosis (Innes *et al*., 2009; Liu *et al*., 2012). Invasion factors of *T. gondii* viz., microneme protein 6 and 8 (MIC6, MIC 8) have also been proposed to be a useful vaccine contender for toxoplasmosis (Peng *et al*., 2009; Liu *et al*., 2010). Some experimental trials have been going on the protective efficacy of recombinant *T. gondii* PDI (rTgPDI) as a vaccine candidate for combating toxoplasmosis (Wang *et al*., 2013).

S48 strain (Toxovax): It is a live vaccine containing originally isolated tachyzoite from aborted placenta and maintain in laboratory by repeated passage in mice. It was initially developed for sheep but in cats it inhibits sexual development of *T. gondii* (Verma and Khanna, 2012). Loss ability to form bradyzoites or oocysts and eliminated within 14 days by host immune response (INF-gamma). The S48 strain when ingested by cat after voided by cat does not cause production of oocyst. It prevent from spreading in the body; placenta as well as meat contamination for 18 months. It is given 4 weeks before mating by intramuscular injection on the neck (O'Connell *et al*., 1988). It should be given @ 2 mL intramuscularly. Basic vaccination should be given as single dose at least 3 weeks prior to mating. Re-vaccination after 2 years with a single dose atleast 3 weeks prior to mating is recommended.

T263: It is a bradizoite of live mutant *T. gondii* that does not formed an oocyst. The administration of T263 yield leads to reduction/prevention of oocyst shedding in cats (Verma and Khanna, 2012).

Vaccine against *Theileria*: The process of development of *Theileria annulata* cultures involves cultivation of the organism for a prolonged time (Pipano and Tsur, 1966). The organisms are collected from the infected animal either by Lymph node biopsy or whole blood. The *in vitro* culture yields agamogenic strain of the organism. This cell culture attenuated vaccine (Rakshavac-T) has an efficacy approaching 95-100% (Brown *et al*., 2006). The site for inoculation is mid-neck region@ 3 mL. Calves of 2 months age and above only should be vaccinated. This vaccine should not be used in advanced stage of pregnancy. Attenuated schizont vaccine are effective in prevention of theileriosis in cattle (Barriga, 1994; Pipano and Shkap, 2000) however more safer and effective vaccines need to be developed utilizing advances in molecular tools and techniques for control of theileriosis in bovines and ovines (Yin *et al*., 2008). Vaccines against Bovine theleriosis caused by Theileria parva should be mixture of several antigens derived from both sporozoite and schizont stages, leading to strong immunity (Morzaria *et al*., 2000).

Vaccine against *Leishmania*: Different types of vaccines for prevention of leishmaniasis have been developed (Brodskyn *et al*., 2003; Coler *et al*., 2007; Nagill and Kaur, 2011).

Leishmanization: It is inoculation of exudates from the active lesion of an infected person to non-infected susceptible person to give an immunization. It has been use from older times but the use is now limited.

Killed vaccine: The killed promastigote are use for immunization with or without BCG. *Leishmania braziliensis* promastigote killed by autoclaving give good results.

Leishmune: It blocks the transmission of canine visceral leishmaniasis (Nogueira *et al*., 2005). It has gp63 protein of *L. donovani* that is adjuvanted in saponin (Parra *et al*., 2007). This vaccine has 76-80% efficacy. It is transmission blocking because:

- The number of *Leishmania* protozoa in the skin of a dog is reduced in number. Thus the Phlebotomine flies does not readily get the the protozoa during blood feeding
- The antibody produced does not allow the development of an infective promastigote inside the fly vector

Leish111f: One of the most promising finding is the recombinant protein called Leish 111f along with an adjuvant MPL-SE (Monophosphoryl Lipid-stable emulsion) shown by Coler *et al*. (2007). It is a recombinant protein of:

LeIF: *L. braziliensis* initiation and elongation factor
TSA: Thiol-specific antioxidant
LmSTI1: *L. major* stress inducible protein

The Leish 111f is having an added advantage like the vaccine efficacy is very high reaching upto 99.6%. Another is that it gives cross protection i.e., *Leishmania donovani*, which can be also used as a therapy especially trial in man. In mucocuteneos Leishmaniosis caused by *L. briziliensis* and other cuteneous Leishmaniosis. But the treatment schedule takes a very long time.

Vaccines for *Sarcocystis*:

EPM vaccine: It consists of *in vitro* cultured merozoites, obtained from the spinal cord of horse, which are chemically inactivated and mixed with suitable adjuvants (Marsh *et al*., 2004). It gives protection against a neurological disease in horses caused by infection with *Sarcocystis neurona*, equine protozoal myeloencephalitis. It is given@ 1 mL intramuscularly and booster vaccination 3 to 6 weeks after the first dose, then revaccination annually.

***S. neurona* SAG1 protein vaccine:** It is subunit vaccine prepared from major Surface Antigen Gene 1 (SAG1), which is conserved among members of Sarcocystidae (Elsheikha and Mansfield, 2004). Horses were vaccinated on days 0 and 21 with 1ml adjuvanted rSnSAG1 (50 μg) or 1 mL adjuvant alone by intramuscular injection in the left side of the neck (Ellison and Witonsky, 2009). Booster vaccination is required after 3 to 6 weeks of the first dose and then revaccination annually.

Vaccine against *Trypanosoma*:

Beta-tubulin: The beta-tubulin gene of *Trypanosoma evansi* (STIB 806) was cloned and expressed in *Escherichia coli* (Li *et al*., 2007). Beta-tubulin is important for cellular structure and physical functions. Recombinant beta-tubulin was expressed as inclusion bodies in *E. coli*.

TSA (*T. brucei* DNA vaccine encoding TSA Protein): The DNA vaccination process was able to protect 60% of mice submitted to a challenge assay with the infective form of *T. brucei brucei* parasites (Silva *et al*., 2009).

MAPp15 (Microtubule associated protein): In an experimental study, vaccination of mice with p15 (native or recombinant) provides complete protection from *Trypanosoma brucei* suggesting it as an effective vaccine (Rasooly and Balaban, 2004).

Vaccine against *Tritrichomonas Foetus*

Trichguard: It is killed protozoan vaccine for cattle for prevention of infection by *Tritrichomonas foetus*. It is given to cattle @ 1-2 mL subcutaneously, booster vaccination 2-4 weeks after first dose and then revaccination annually. The last injection should proceed the breeding season by 4 weeks.

COBWEB FOR VACCINE DEVELOPMENT AGAINST PROTOZOAN INFECTION

There are lot many factors which causes the pitfall for development of a successful protozoan vaccine which is of the supreme importance to keep infection at the gate way. The important factors are pathogen associated, vaccine market associated or funding (Vercruysse *et al*., 2007). Among all the factors pathogen associated problems needs to be dealt in a more scientific and clever way as some protozoa are clever enough to escape the immune system. One of the most important protozoa of this type is the *Trypanasoma* spp. which causes a serious problem in case of animals as well as humans (Vanhamme *et al*., 2001; La Greca and Magez, 2011). One of the noteworthy features of this protozoa is that it can change its antigenic surface protein in such a rapid manner so that the immune system of host cannot trace it (Pays *et al*., 2004). Many protozoa also have a complex life cycle during their survival in the host. Two stages of life cycle namely sexual and asexual exist in the life pattern of many protozoa and hence selecting a vaccine candidate becomes a difficult task. Certain situation occurs when a single host cell have protozoa at different stages of development giving a feel to the host immune system that there a number of protozoa in a single cell (Vercruysse *et al*., 2007).

CONCLUSION

Drugs remain central to alleviating clinical disease and for larger scale disease control programmes. However, available drugs have often been in use for decades and drug resistance in the target parasites is now prevalent and, particularly in the case of livestock, threatening sustainable control. Control is threatened by the widespread appearance of drug-resistant parasites in animals. Development of drug resistance could limit the use of anti-parasitic drugs which will remain important for a long time. Drug residues found in the food animal, development of drug resistance and lack of development of new drug are all major problems or reasons which diverts the scientist to make efforts on vaccine development related research. Many vaccines are included in the control of parasitic diseases programs, as these approaches may lead to a substantial reduction in the use of chemical drugs for prevention and control of parasitic diseases. The need of hour is to apply the immunization results of murine/lab animal model in veterinary science and for humankind. Vaccination may also aim to improve public health, so there is a need of cheap and effective vaccine to be developed and reach with success in the market. Development of vaccine against protozoan parasite is cumbersome process because of their complex life cycle, many developmental stages and sub clinical form of disease. Efforts for making economically feasible and effective protozoan vaccine, strategies to discover new antigen to act as novel vaccine candidates are still in continuation.

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