



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2017; 5(6): 1016-1021

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Received: 13-09-2017

Accepted: 15-10-2017

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Vaccines against protozoan parasites of veterinary importance: A review

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Abstract

Protozoan infections in animals are responsible for significant losses of production and many protozoan parasites are of zoonotic importance. Among various parasitic infections, several protozoan parasites cause severe morbidity and mortality in animals and thus affect the economy of livestock in tropical and sub-tropical regions including India. The most common way to combat protozoan diseases is based on using anti-protozoal drugs. However, increasing evidence of anti-protozoan resistance and drug residues in food producing animals has emerged as the major concern for protozoan control programmes in various parts of the world. The most efficient and cost effective way to control protozoan infections is to vaccinate animals to control such infections. Although initial cost of vaccination is high, the long lasting immunity resulting from vaccination of animals offer a cheaper and effective alternative to control such infections. Based on this objective, this review underscores up to date information to the reader to overview on the trends, advances and future prospects of vaccine development against important protozoan disease of livestock and poultry viz, coccidiosis, babesiosis, theileriosis, anaplasmosis to name but a few.

Keywords: Protozoan parasites; vaccines; control

1. Introduction

It is anticipated that by 2050 to feed the estimated human population of around 9 billion 50% increase of food production is required which can only be fulfilled by clean, healthy and sustainable food animal production [1, 2]. In modern animal farming, the livestock industry is facing considerable economic losses due to infectious diseases of viral, bacterial, fungal or parasitic origin [3-6]. So an effective control strategy is need of today to combat these infectious diseases and for increase of livestock production [7-9]. Till date, the limiting of impact of parasitism relies on use of chemotherapy like anthelmintic, antiprotozoal or ectoparasitic drugs. Parasitic diseases have a major impact on livestock production worldwide with infection arising from a range of helminth, protozoan and ecto-parasites and many parasitic diseases are of zoonotic importance [10-14]. Due to development of resistance towards these chemotherapeutic agents and drug residue problems in food animals, scientists or researchers are now thinking towards the prevention of parasitic diseases through use of vaccines [15-24]. Effective ways of preparing vaccine to combat parasites are very limited due to the complex nature of the parasites and complicated relationship with the hosts. It is prepared from causative agent of disease or its products and treated to act as antigen without causing disease.

1.1 Protozoan Diseases

Protozoa are unicellular, eukaryotic, microscopic organisms belonging to subkingdom protozoa. They are characterized by having a distinct nucleus as well as endoplasmic reticulum, Golgi apparatus and mitochondria in the cytoplasm. About 65000 species of protozoa have so far been identified majority of which are free-living, while only 7000 protozoan species are parasitic both in vertebrate and invertebrate animals [25]. Protozoan parasites are considered one of the major constraints causing extensive morbidity and mortality in animals [16]. Among these many of the protozoan 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 marketed with varying efficacy [21]. Among vaccines available against protozoan diseases of livestock, many are based on live organisms, but recently there is progress in development and commercialization of killed subunit vaccines [21, 26].

1.2 Antiprotozoan Drugs- Problems In the near Future

Several anti-protozoan drugs are used against protozoan parasites to prevent the infection but protozoans are able to develop resistance against these drugs, which is a serious problem in human and animals. Broiler industry is facing the crisis of antiprotozoan drug resistance against the commonly used coccidiostats in the farm [27]. Resistance against antiprotozoan drugs is now reported even to *Trypanosoma* spp [28] and also in canine babesiosis [29]. Extensive use of antiprotozoan drugs has not only resulted to the resistance against these drugs but also the residues in the animal products to enter the food chain causing cross transfer of resistance [30, 31]. Apart from these, drugs can also enter environment from the secretions and excretions of the animals and thus threatening the public further [32]. To solve these problems vaccination will be an alternative option for controlling protozoan diseases.

1.3 Vaccines available against some of the protozoan parasites

1.4 Vaccines against Coccidia

1.5 Coccivac B

This live, attenuated vaccine produced from isolates that were initially developed in the late 1940s, before the current anticoccidial products were introduced into the market. This vaccine is prepared from anticoccidial-sensitive strains of *E acervulina*, *E mivati*, *E maxima* and *E tenella* and 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 [33]. In experimental study on birds, this vaccine has shown variable degrees of protection with the five different strains of *E. tenella* [34].

1.6 Coccivac D

It is a live, sporulated oocyst vaccine containing different spp of *Eimeria*. In order to produce complete immunity, the original dose of coccidial oocysts must complete at least four life cycles in the flock [35].

1.7 Coccivac T

It is a live anti-coccidial vaccine containing sporulated oocysts of *E dispersa*, *E meleagrititis*, *E adenoids* and *E gallopavonis*. It 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 shown highly sensitive to all anticoccidials [36].

1.8 Nobilis COX ATM

This vaccine is a live vaccine having a unique property of being active even in the presence of ionophore compounds. The vaccine contains strains of *E acervulina*, *E tenella* and *E maxima* which are tolerant to ionophore compound. One of the major advantages of this vaccine is that apart from production of immunity the vaccine allows the use of ionophore compounds during 3-4 weeks of age when the immunity is poorly developed [37].

1.9. Eimeriavax 4m

This vaccine consists of viable oocysts of *Eimeria acervulina* strain RA, *E maxima* Strain MCK+10, *E. necatrix* Strain mednec3+8 and *E tenella* Strain Rt3+15 suspended in PBS. It promises productivity improvements [38].

1.10 Immucox

This orally prepared vaccine of live oocysts of *Eimeria* spp is designed to help healthy Chicken Broilers and Roasters to develop immunity to coccidiosis. This is a one time vaccination that delivers protective immunity throughout the productive life of the bird. This vaccine is approved for water and gel delivery in the hatchery [39].

1.11 Paracox-8

It is a live attenuated oral vaccine consisting of translucent, suspension of oocysts derived from 8 precocious lines of coccidian and is used for the active immunization of chickens against different *Eimeria* spp [40].

1.12 Livacox

It is live attenuated coccidiosis vaccines for domestic poultry. It consists of LIVACOX T for broilers and LIVACOX Q for breeder and layer pullets [41].

1.13 Cox Abic

It is the first commercially available subunit vaccine for poultry and contains purified antigens isolated from macrogametocyte of *Eimeria maxima*. Vaccination using gametocyte antigen through the breast muscle results production of antibody response [42].

1.14 Vaccine candidates for Coccidia

1.15 MZ5-7

It is a DNA vaccine co-expressing *Eimeria tenella* MZ5-7 and chicken IL-17 gene. MZ5-7 gene encodes surface protein MZP5-7 of 2nd generation merozoite [43].

1.16 TA4

A DNA vaccine of *E. tenella* which is co-expressed with IL-12 [44].

1.17 EtMIC2

Recombinant antigen, in-ovo administration co-vaccination with IL-8, IL-16 and TGF-4 (Transforming growth factor) [45].

1.18 GX3262

It is a recombinant antigen from sporulated oocyst of *E. tenella* which gives partial protection [46].

1.19 EmTFP250

It is derived from asexual stage *E. maxima* (a part of microneme complex) [47].

1.20 p250

It is a recombined merozoite antigen of *E. acervulina*. It give 50% protection [48].

1.21 Vaccines for *Anaplasma*

1.22 Anaplaz

It is the first commercially available vaccine against anaplasmosis manufactured for cattle in the US by Fort Dodge. More recently, Mallinkrodt marketed a vaccine called Plazvax. Both o vaccines give significant protection against Anaplasmosis. The dose rate is 1mL p/sec cycle route and repeated after 3-4 week and then boosting annually [49].

1.23 Vaccines for *Giardia*

1.24 Giardiaavax

It is a killed culture derived trophozoite vaccine prepared for dogs and it is found effective to prevent the disease and

shedding of *G. lamblia*. The vaccine is derived from *G duodenalis* isolated from sheep. Dose rate is 1mL subcutaneously. The 1st dose is given at 8 weeks of age and 2nd dose after 2-4 weeks and then repeat annually [50].

1.25 Vaccines against *Babesia*

1.26 Pirodog/Nobivac Piro

It is a soluble parasite antigen prepared from supernatants of *in vitro* culture. It gives 80% protection and the immunity lasts for about 6 months. The 1st dose is given when the animal is 6 months old and booster dose is 3-6 weeks after the initial vaccination and repeated every 6 months by I/M route [51].

Different vaccine candidate of *Babesia* in different species are shown in Table No. 1.

1.27 Vaccines against *Neospora*

1.28 Bovilis Neoguard

It is a killed tachyzoite of *Neospora caninum* with spur adjuvant which reduces abortion in cattle by more than 50% [21]. But the drawback of the vaccine is that it may increase the early embryonic death, if used in pregnancy. It is administered in 2 doses of 5ml at one month apart. The 1st dose is given between day 75 and 90 of gestation then booster in 3-4 weeks with 2 annual boosters 3-5 weeks apart by sub-cut route [21].

1.29 Vaccines against *Toxoplasma*

1.30 Live attenuated vaccines

1.31 T263

It is a bradizoite of live mutant *T gondii* that does not form an oocyst. The administration of T263 leads to reduction/prevention of oocyst shedding in cats [52].

1.32 S48 strain (Toxovax)

It is produced by repeated passage in mice (x3000) resulting losses in ability to form bradyzoite or oocyst. It reduces abortion and neonatal mortality by 75% [53].

1.33 TS-4

It is a temperature sensitive RH strain. It protects from cyst formation and congenital toxoplasmosis [54].

Recombinant vaccines and DNA vaccines against *Toxoplasma* are shown in Table No. 2 and Table No. 3 respectively.

1.34 Vaccines against *Leishmania*

1.35 Leish111f

One of the most promising finding is the recombinant protein called Leish 111f along with an adjuvant MPL-SE. It is recombinant protein of LeIF: *L braziliensis* initiation and elongation factor, TSA: Thiol-specific antioxidant and

LmST11; *L. major* stress inducible protein [55].

1.36 Vaccines against *Sarcocystis*

1.37 EPM vaccine

It consists of *in vitro* cultured merozoites which are obtained from the spinal cord of horse. The merozoites are chemically inactivated and mixed with suitable adjuvants [56]. It gives protection against a neurological disease in horses caused by infection with *S neurona*, the causative agent of equine protozoal myeloencephalitis. It is administered by IM at a dose rate of 1ml and booster vaccination after the 1st dose, then annually [56].

1.38 Vaccines for *Trypanosoma*

1.39 Beta-tubulin

The beta-tubulin gene of *T evansi* was cloned and expressed in *E coli* [57]. Beta-tubulin is important for cellular structure and physical functions. Recombinant beta-tubulin was expressed as inclusion bodies in *E coli* [57].

1.40 MAP p15

A subunit vaccine which gives 100% protection in mice from homologous challenge with *T. brucei* [58].

1.41 Trans-sialidase (TSA)

A DNA vaccine that protects 60% against *T. brucei*.

1.42 Vaccine against *Theileria*

1.43. Rhakshavac T

Rakshavac-T contains live schizonts grown in lymphoblast cell culture, attenuated by prolonged in-vitro passage. This cell culture derived vaccine has an efficacy up to 95–100% [59].

1.44. SPAG1

It is a surface antigen of sporozoite stage of *T. annulata*.

1.45. TAMS1

A merozoite surface antigen of *T. annulata*.

1.46. p67

Sporozoite surface antigen of *T. parva*

1.47. Cobweb for vaccine development against protozoan infection

There are lot of factors which cause the pitfall for development of a successful protozoan vaccine which is of the supreme importance to keep infection at the gateway. The important factors are pathogen associated vaccine market associated or funding. One of the most important protozoa of this type is the *Trypanosoma* spp which cause a serious problem in case of animals as well as humans [60].

Table 1: Different vaccine candidate of *Babesia* spp

Vaccine Candidates	Species	Type
GST-12D3	<i>B. bovis</i> , <i>B. bigemina</i> , <i>B. canis</i> , <i>B. divergens</i>	Recombinant vaccine
GST-11C5	<i>B. bovis</i> , <i>B. ovis</i> , <i>B. canis</i> , <i>B. divergens</i>	Recombinant vaccine
GST-T21B4	<i>B. bovis</i> , <i>B. bigemina</i> , <i>B. canis</i> , <i>B. equi</i> .	Recombinant vaccine
Bd37	<i>B. divergens</i>	Exo-antigen of culture - supernatant
SBP1	<i>B. bovis</i>	Recombinant vaccine
MSA-1 & MSA-2	<i>B. bovis</i>	Recombinant vaccine
Ribosomal Phosphoprotein PO	<i>B. divergens</i> and <i>B. bovis</i>	Recombinant vaccine

Table 2: Recombinant vaccines against *Toxoplasma* spp

Recombinant Vaccine Candidates	Level of Protection	Adjuvants
SAG1	90% survival (intra-nasally)	Quil A
	85% survival	Cholera Toxin
MIC1 & MIC 4	80% survival and 68 % brain cyst development	Quil A
GRA2 and GRA6	GRA2 – reduction of brain cyst	MPL
	GRA6 – no reduction of brain cyst	
GRA4 and ROP2	Protection against brain cyst	Alum
GRA 1 and SAG 1	89% brain cyst development reduction	FCA
GRA7, MIC2, MIC3 & SAG1	79% brain cyst development reduction	GARBU Adj.

Table 3: DNA vaccines against *Toxoplasma* spp

DNA Vaccine	Protection
SAG1	100 % protection against lethal challenge
GRA7 and ROP2	79 % protection from acute toxoplasmosis
GRA1 and GRA7	89 % protection from acute toxoplasmosis
GRA 4	Protects against brain cyst development
SAG1 & ROP2	Prolonged protection
MIC2, MIC3, MIC4, M2AP, AMAI	84% cyst reduction
BAG1 and MAG1	62% reduction of brain cyst development.

2. Conclusion

The development of drug resistance, drug residue in food chain and lack of development of new drugs are the major constraints for effective control of protozoan infections of veterinary importance. The need of hour is to immunize animals by means of vaccination. Although, the progress in the development of effective commercial vaccine against many important protozoan infection is slow, new vaccines are coming to the market and the number of effective recombinant vaccines is increasing. Vaccine technology continues to advance rapidly, especially through the use of modern molecular techniques and through our increased understanding of immune mechanisms and way to optimize immune responses to achieve maximal protection.

3. Acknowledgements

The authors are thankful to the Dean, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, India for providing necessary support.

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