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**Review Article**

# Potential Vaccine Candidates against Nematodes of Veterinary Importance: An Overview

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Nematodes are highly pathogenic and economically important parasites in livestock globally. They strongly challenge the host immunity and in return can be ousted by host via prior anthelmintic treatment. However, this approach is sometimes not appropriate when resistance against anthelmintics develops by these parasites. The problem of parasitism can then be managed by alternative methods where vaccines are thought to play a crucial role. Efforts have been made in past to identify candidate vaccine antigens and are still going on for a wide range of nematode parasite species. The complexities arising due to host-parasite relationship have further challenged scientists to understand mechanisms of vaccine-induced immunity, causing more difficulties in producing effective vaccines though very few vaccines against nematodes have been successfully commercialised. This review highlights some antigens derived from nematode parasites used for the purpose of studying immunoprophylactic responses in livestock.

## Introduction

Phylum Nemathelminthes or perhaps Aeschelminthes commonly known as roundworms or nematodes, belong to the clade nematoida of the superphylum ecdysozoa in the kingdom Animalia forming the core of the helminth world. They are responsible for much economically devastating infection of the bovines, caprines, ovines, equines, porcines, canines and felines. To counter the deeds of such infestations, chemotherapeutics generally play by the rule book in eliminating a number of parasites (Jacob *et al.,* 2013). However, excessive usage of drugs has resulted in increased number of resistant parasitic strains highly

likely responsible for reduced efficacy of anthelmintics in eliminating them (Healy *et al.,* 2018). Furthermore, the rate at which this resistance problem is accelerating is at par than the rate of development of new anthelmintics (Healy *et al.,* 2018). We know disease control via chemotherapeutics/ chemoprophylaxis is an integral part of mitigation strategies (other being pasture management, breeding genetically resistant breeds, biological control, vaccination and nutrient supplementation) commonly termed as integrated parasite management. But relying solely on anthelmintics to curb parasitic menace would only land us with fewer to almost negligible available options in jeopardizing this problem. Hence, scientists

all over the world are engaged in process of finding promising potential candidates to dismantle the helminth infection in form of vaccines.

Developing vaccine against infections in animals has progressed through several scientific generations from live, attenuated, inactivated or killed, biochemical fractions, subunit, DNA vaccine and edible vaccines (Singh *et al.,* 2014) but very few amongst them have been exploited extensively on a commercial/market level with veterinary vaccines constituting about 23% of global market for animal health products (Meeusen *et al.,* 2007). However, these vaccines against veterinary diseases are mainly available to combat bacterial and viral diseases. The notion being that prokaryotic organisms have been extensively studied and exploited owing to their quite simple structural organisation in contrast to complexities thrown by eukaryotes (protozoa and metazoan). The low numbers of commercially available parasitic vaccines against helminths are perhaps due to the labyrinth posed by them in the form of their multicellular nature, various developmental phases, unusual adaptation inside vertebrate host, evading the host immune system by producing discrete antigenic molecules at each stage and chronicity in terms of infection (Hewitson and Maizels, 2014).

**Immune response against helminths**

Parasitic infestation is marked by a rise in IgE production, high eosinophil count, mast cell number and increased goblet cells activity. This response is mainly caused by type Th2 cells (except in few cases where Th1 comes into play) which influences cytokine and immunomodulatory cells production. It is universally proved that production of IgE is regulated by Interleukin-4, IL-5 regulating eosinophilia and IL-3, IL-4 and IL-10 regulating mastocytosis (Moreau and

Chauvin, 2009). Immune response against partial elimination of parasites has been evident with premune response (concomitant immunity) wherein establishment of further infection is prevented by the already on going infection with the same parasite.

**Vaccines against nematodes of veterinary importance**

Despite of brawling with difficulties and failures in getting desired immunogenic candidates against worms, the breakthrough in the vaccine development against pathogenic parasites was achieved in the previous century when scientists were successful in producing vaccines founding their way to the commercial/global market level.

*Dictyocaulus*

*Dictyocaulus viviparous* and *D. filarial* are two notorious pulmonary nematodes affecting large and small ruminants respectively, causing parasitic bronchitis. The disease produced by them is often referred to as 'husk' or 'hoose'. The pioneer work on *Dictyocaulus* was done by W.F.H. Jarrett who was able to induce immunity in calves against *D. viviparus* with gamma globulins (Jarrett *et al.,* 1955) as well with whole worm extract mixed with Freund's adjuvant (Jarrett *et al.,* 1960). In 1959, vaccine was launched by the name of 'Dictol' by Allen and Hanburys Ltd, U.K. but now continues to be marketed as 'Bovilis Huskvac'. It contains irradiated L3-larvae (truncated life cycle) usually given twice at a monthly interval with each dose having 1000 larvae and administered orally to young calves of above 8 weeks of age. The vaccine is reported to give a year long protection against the parasite. On the contrary, a vaccine against *D. filaria* was developed at the Nuclear Research Laboratory of Indian Veterinary Research Institute in

1970-71 (Sharma *et al.,* 1988). After successfully testing the vaccine at laboratory and field conditions, it was subjected to a large scale production at an irradiated laboratory in Srinagar (J&K) in 1973. The vaccine was launched by the name 'Difil' in the Indian market.

The vaccines contain gamma rays attenuated third stage larvae. The attenuation is done at 40 kDr or 50 kDr. The lambs of 6-8 weeks of age are vaccinated orally with the immunity lasting for a year. Usually 2 doses of 1000 and 2000 infected larvae are administered orally at an interval of 4 weeks.

## *Haemonchus contortus*

When it comes to being public enemy number one, *H. contortus* grabs the topmost position. A voracious blood sucking nematode parasitizing the gastrointestinal tract of small ruminants causing high morbidity and thus hitting hard the economy of the livestock industry worldwide. So far a number of potential candidate antigens have been evaluated down the memory lane, the earliest being contortin (Munn, 1977; Munn *et al.,* 1987) which was documented to provide up to 70% protection against *H. contortus* challenge (Munn *et al.,* 1987). Thereafter extensive studies have been done to find some promising vaccine candidates, out of which following have been stated below:

Gut-derived antigens: H11: a 110 kDa integral membrane glycoprotein complex found in five isoforms (H11, H11-1, H11-2, H11-4 and H11-5) (Newton and Munn, 1999; Roberts *et al.,* 2013)
H-gal-GP: a 981 ± 10 kDa galactose containing glycoprotein complex (Smith *et al.,* 1994)
Thiol Sepharose-Binding Protein (TSBP): contains cysteine proteases and glutamate dehydrogenase as its principal constituents

(Knox *et al.,* 2005)
GA1: contains a set of three glycosylated proteins of 42, 52 and 100 kDa (Jasmer *et al.,* 1993)
Surface antigen: Hc-sL3, a 70-90 kDa third stage larva surface antigen, showing an efficacy of 50-70% reduction in worm burden (Piedrafita *et al.,* 2013).
Excretory-Secretory antigens (Smith *et al.,* 2009)
Recombinant forms of H-gal-GP components: Aspartyl proteases, Zinc metalloproteases, cystatin (Nisbet *et al.,* 2016)
Recombinant forms of H-11 components (Roberts *et al.,* 2013)
Others: 15 and 24 kDa ES proteins, galectins, Hc23, enolase, cysteine proteases (Nisbet *et al.,* 2016)

Barbervax: first commercially available vaccine against *Haemonchus* developed by Moredun Research Institute, Scotland (U.K.) containing native integral gut membrane protein enriched for H-gal-GP and H11. A single subcutaneous shot of 1ml contains 5ug native antigen + 1mg saponin adjuvant /dose. Usually 5 shots are given with each one at an interval of six weeks (Nisbet *et al.,* 2016).

## *Ostertagia ostertagi*

*O. ostertagi* harbours the gastric mucosa of the ruminants and is one of the parasites responsible for causing gastritis in cattle. The parasite proliferates with ease at a lower temperature thus indicating its importance in temperate regions of the world. A lot of vaccine candidates (antigens) have been explored for the purpose of immunoprophylactic studies mostly categorising into two broad antigenic types viz. native and recombinant antigens. Third and fourth larval stages and adults have been used to induce immune responses. Native antigens derived from third and fourth larval stages include whole ES, somatic ES

fractions, ConA lectin purified material (only L4) whereas those derived from adult worm include ConA gut proteins, P-ConA gut proteins, H-Gal-GP, ES-thiol, Cysteine proteases, polyprotein allergen, globin etc. whereas recombinant derived antigens include polyprotein allergen (L3), heat shock protein (adult), SCP/TAPS protein (L4 and adult) and aspartyl protease inhibitor (L3, L4 and adult) (Rinaldi and Geldhof, 2011).

### *Trichostrongylus colubriformis*

Just like *O. ostertagi*, *T. colubriformis* is of more importance in temperate regions affecting the economic profit of sheep industry. Vaccinating animals against this parasite has not been successful in the past. Harrison *et al.,* (2003) identified a potential candidate of 35 kDa molecule termed as *T. colubriformis* 35-kDa carbohydrate larval antigen (Tc35CarLA). However, Maass *et al.,* (2009) reported that this nematode can successfully evade host immune system by undergoing antigenic variation within Tc35CarLA.

### *Ascaris suum*

*A. suum* is a cosmopolitan roundworm of swine affecting neonates and adult pigs. Protecting animals from *A. suum* has been achieved through chemotherapeutic intervention, however, to avoid the situation of resistance against drugs other options are being exploited. The infection is transmitted primarily by the consumption of embryonated eggs (L2 inside the eggshell) so efforts have been made in the past to vaccinate pigs by orally infecting with ultraviolet irradiated attenuated embryonated (containing L2) eggs (Urban *et al.,* 1988). Antigens derived from pseudocoelomic fluids, cuticles, excretory-secretory fractions have been studied to impart protection against ascariasis (Zhan *et al.,* 2014). Effects of recombinant antigenic

fractions like 14-kDa antigen and (As14)16-kDa antigen (As16) of *A. suum* L3 were studied in mice model where a 60% reduction in larval count was achieved (Tsuji *et al.,* 2001; 2003).

Other molecules discovered includes As24 showing 58% reduction in lung larval burden with elevated levels of IgG, IFN-g and IL-10 (Islam *et al.,* 2005), As37, another larval surface eliciting 69% reduction in liver and lungs larval count (He *et al.,* 2012), As-Enol-1 found in adult ES products and As-GST-1 (Zhan *et al.,* 2014).

### What debars/challenges the commercial success of vaccines?

Despite working rigorously for years in laboratories, conducting field trials on small scale and then large scale with good results many other factors become responsible for commercial viability of the vaccine. Some of them include:

Safety: The vaccine should be safe to use in animals and should not pose any harm to the handler. Special care should be taken while handling live vaccines. It should be made sure that it doesn't revert to virulence. Sutkowsky and Gruber (2006) in a study stressed that vaccine should be marketed only if it is safe to use in trial animals at their lowest and highest passages using the most sensitive target animal species.

Quality and efficacy: The product made should be free from undesirable microbes and in field conditions also it should confer protection almost up to same level what it has claimed during study trials.

Stability and shelf life: One of the most important hurdle in commercialization of vaccines is lowering of potency of the product during the course of time. The temperature

range at which the product should be kept for maximum stability and efficient working should be carefully and logically determined.

Onset and duration of immunity: Proper documentation supporting the onset and duration of immunity following single vaccination supported by experimental data including specific time for booster vaccination.

Routes of administration: For each routes of administration, safety and efficacy data must be made available and clearly stated on the leaflet.

Compatibility: Safety data supporting the claim whether a vaccine can be physically mixed with another product as stated on the leaflet.

Conclusion of the study is as follows

A bitter truth to admit is that parasitic population will persist until the end of the universe. Scientists have tried to eliminate parasites using chemotherapy and chemoprophylaxis only to end up with the problem of anthelmintic resistance just because some of the parasites were smart enough to handle the drugs on their own by means of mutation in its genome. Besides chemical approach, biological control has also been used but with limited feasibility in field. Therefore, control of economically important parasites can be achieved best through immunoprophylactic approach by developing suitable vaccines. With the advancement of molecular techniques, it may be expected that vaccines will provide another alternative to combat parasitic diseases in near future. Whenever commercial vaccines are made available, they can be a part of integrated parasite management (IPM). By alternating chemotherapeutics and vaccination it will definitely prove to be an effective and easy approach in controlling anthelmintics resistance.

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