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Review Article

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Vaccination against *Haemonchus contortus* in Small Ruminants: A Review

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A B S T R A C T

Haemonchus contortus is one of the most important nematode species responsible for huge economic losses to small-ruminant farming in India and world over. Control is primarily based on use of anthelmintics. Over reliance on and non-judicious use of anthelmintics has lead to development of anthelmintic resistance. This wide spread anthelmintic resistance has forced to look for alternate control measures. Vaccination can significantly protect livestock against and lower the economic losses incurred due to *H. contortus.* Different antigens have been tried with variable success. Use of recombinant antigens has not yielded desirable protection levels. Native antigens have given significant protection but has been considered non feasible. Recent discovery that the dose as low as 5 µg of native antigen can be protective has raised hopes of developing economically feasible vaccines. Barbervax, sub-unit vaccine derived from native gut integral glycoprotein is now commercially available.

Introduction

Diseases caused by parasitic nematodes pose a considerable medical and veterinary health problem. The diseases, they cause, are rarely fatal but instead are long term and debilitating. *Haemonchus contortus* is one of the most important nematode species responsible for economic loss in small-ruminant farming (Sykes, 1994). Reliance on and poorly managed use of anthelmintics has led to the continued development of drug resistance to each successive new anthelmintics class. The widespread appearance of *H. contortus* strains that are resistant to different classes of anthelmintic drugs (Wolstenholme *et al.*, 2004) and increasing concern about drug residues in the food chain and the environment has accelerated the need for studies on the development of alternative strategies to

prevent the economic impact of this parasite (Newton and Munn, 1999) such as vaccination, breeding 'worm-resistant' sheep lines (Bissett and Morris, 1996) and pasture control using nematophagous fungi (Larsen, 2000). Significant protection against livestock nematode infections has been achieved following vaccination. There are two types of antigens associated with nematode parasite: (1) soluble excretory and/or secretory (E/S) products; and (2) those fixed at external surfaces or within the parasite (the so-called somatic antigens). Some of the E/S products and exposed somatic antigens induce an immune response in the host during the course of infection and are designated natural antigens, while antigens that do not induce an immune response during infection are designated hidden antigens.

Attempts using recombinant antigens using different expression systems have largely been unsuccessful. This may suggest the importance of post-translational modifications, or that co-purifying components may be the true protective targets (Roberts *et al.,* 2013). Native proteins extracted from the adult parasite gut or from ES products are capable of inducing high levels of protection *i.e*. up to 90% reduction in faecal egg counts (FEC) and 75% reduction in worm burden (Newton and Munn, 1999). A number of promising candidate vaccine antigens have been identified (Smith, 1999). Significant among these are so-called 'hidden' antigens, such as H-gal-GP (Smith *et al.,* 1994), cysteine proteinases (Knox *et al.,* 1993), H11 (Smith *et al.,* 1997), in which H-gal-GP exhibits both aspartyl and metallo-proteinase activities (Longbottom *et al.,* 1997). Natural antigens include adult 15/24 kDa excretory/secretory antigens (Schallig *et al.,* 1994) and Hc-sL3 (Raleigh *et al.,* 1996). Trials with native antigen vaccines were encouraging but there are many practical problems associated with the use of vaccines based on native material. Most importantly, it is very difficult to obtain large quantities of worm material or native antigens from most helminths. An additional problem with native vaccines is the necessity to control for batch differences or to obtain a commercially stable formulation of native parasite material. However, with the discovery that the dose of native antigen required for protection was as low as 5 µg, it was then realized that a native antigen vaccine could be economically feasible provided methods could be found for collecting large quantities of adult worms cost-effectively (Bassetto *et al.,* 2014b). Also Barbervax, first commercially available sub-unit vaccine for *H. contortus* is derived from native gut integral glycoprotein (Smith *et al.,* 2014). This article reviews attempts done world over and India for development of vaccines against *H. contortus.*

Significance of gastrointestinal nematodes

GINs are ubiquitous parasites of livestock species. Livestock is subject to severe economic losses from GIN infections, as a consequence of the serious diseases associated with infection (Stepek *et al.,* 2004). Diseases caused by GI nematodes in domestic ruminants represent one of the major impediments to livestock production and cause enormous economic losses in a wide range of agro-climatic zones (Waller, 1997). The diseases, they cause, are rarely fatal but instead are long term and debilitating. The major parasites of concern differ by the prevailing host animal species and climatic conditions in a particular geographic location and no farm animal species in general is free from GI parasitism. Among the GI nematodes of ruminants, *Haemonchus contortu*s is one of the most prevalent, highly pathogenic, possesses the highest biotic potential and economically important parasite. Its occurrence depends on the presence of biotopes suitable for the development and transmission of infective larvae to the

definitive host. *H. contortus* infection is a worldwide problem and prevails mostly in tropical and sub-tropical countries where temperature and humidity are comparatively higher than temperate regions (Kalita *et al.*, 1978). However, this parasite is also present in temperate regions with focal areas of similar climatic conditions (Waller *et al.*, 2006). In India, haemonchosis occurs as an acute disease of the small ruminants mainly sheep and goats as well as wild ruminants. Three species of *Haemonchus* have been reported in India *viz*., *H. contortus, H. longistipes* and *H. similis*. *H. contortus* occurs in the abomasum of sheep, goats, cattle and other ruminants. *H. longistipes* occurs in camels and *H. similis* has been reported from cattle and deer. Among these, *H. contortus* is of great economic importance (Soulsby, 1982).

Economic losses are incurred through morbidity and mortality and increased investment due to cost of preventive as well as curative treatments (Miller and Horohov, 2006). It is very difficult to assess the exact economic impact of this parasite in small ruminant farming due to the complicated nature of sub-clinical infection by multiple species of parasites. However, the economic losses in various countries due to helminthiasis including haemonchosis are high and therefore control and prevention need attention. As reviewed by Miller and Horohov (2006), GI parasitism has been a problem of moderate to high concern for US farmers. GI parasites in sheep alone cause annual losses ranging from US $ 42 million to US $ 222 million (Waller, 2006). Total losses in Australia due to all nematodes combined, of which *H. contortus* is a major contributor, was estimated to be US $500 million (Emery, 1991). One third of total sheep production, equivalent to $946 million was attributed to nematode infection in New Zealand (Vlassoff and McKenna, 1994). The estimated treatment cost alone for *H. contortus* per year in Kenya, South Africa and India was estimated at US $26, $46 and $103 million, respectively (Anon, 1999; McLeod, 2004; Waller and Chandrawathani, 2005).

The presence of *H. contortus* in the abomasum appears to interfere with the digestion and absorption of proteins, calcium and phosphorus (Sood, 1981). Increased susceptibility to *Haemonchus* infection due to deficiencies of vitamin A and calcium in goats has also been reported (Kumar and Deo, 1970).

Novel approaches for control of *Haemonchus contortus*

The aim of most parasite control strategies is not to eliminate the parasites totally in livestock, but to keep the population under a threshold, above which it would otherwise inflict harmful effects on the host population (Larsen, 2000). Development of alternative strategies for parasite control is essential for modern livestock farming due to widespread anthelminthic resistance (Burke and Miller, 2006) and increased consumer demand for "clean and green" animal products, free of residual chemicals and growth promoters (Waller, 2003). Novel approaches include breeding for "resistance to effects of infection" (Bisset and Morris, 1996) as well as breeding for "resistance to infection" (Kahn,2003); Use of nematophagous or parasite larvae trapping fungi species like *Duddingtonia flagrans* (Chandrawathani *et al.*, 2004) which results in reduced L3 pasture contamination; Use of Copper oxide wire particles (COWP) in boluses (Burke *et al.*, 2005); Use of dietary protein supplements (Bricarello *et al.*, 2005) and use of medicinal plants containing tannins (Iqbal *et al.*, 2007). Rotating more resistant mature animals with susceptible younger animals may also prove beneficial. However, this strategy may not be sufficient because of practical reasons (van Wyk *et al.*, 2006).

Selective treatment of individual animals instead of treating all animals is another economic strategy for control of *H. contortus*. The FAMACHA system, which involves comparison of conjunctival mucous membrane color with an eye color chart to determine the severity of anemia, is used to decide whether an animal needs treatment (Kaplan *et al.*, 2004). This method has facilitated quick identification of *H. contortus* infected sheep and goats without the aid of any laboratory procedures and delivers the treatment only to those who require it (van Wyk *et al.*, 2006). This system has enabled farmers to limit the expense of anthelmintics and at the same time reduce undue exposure of the worm to anthelmintics which will slow the evolution of resistance but it is labour intensive and needs huge funding.

Immunoprophylaxis, an alternate way of control

Successful vaccination against nematodes may be the most effective and long term strategy for prevention and control. Hence a substantial amount of effort has been put into research and development of vaccines against helminth parasites including *H. contortus*. Ideally, vaccines should have a high efficacy and be commercially viable for their proposed use in the livestock sector. Useful levels of protection can be defined as "reducing parasitism below that which causes a significant production loss" (Klei, 1997). It is unlikely that anti-parasite vaccines will attain the almost 100% efficacy associated with new anthelmintics and bacterial/viral vaccines (Emery, 1996) but computer modelling of sheep–trichostrongylid interactions (Barnes and Dobson, 1995) predicts that adequate control can be achieved with vaccine efficacies of about 80%. The early approaches in the development of vaccine were to attenuate the L3 through irradiation. Vaccination with irradiated L3 was reported to confer a very high level of protection (Smith and Christie, 1979). The lack of commercially available vaccines may be attributed to the lack of complete understanding of the protective immune responses to the helminth parasites and the inability to produce recombinant antigens equivalent to the natural antigens.

Targets for immunoprophylaxis in haemonchosis

Although sheep can be successfully immunized with irradiated third-stage GI nematode larvae (Jarrett *et al.*, 1959), it has not been feasible to use these as commercial vaccines due to failure to protect vulnerable young lambs (Smith and Christie, 1979). There are two types of antigens associated with a nematode parasite: (1) Natural or Conventional antigens and (2) Hidden, concealed /cryptic or covert antigens, while natural antigens evoke an immune response in the host during the course of infection, the hidden antigens do not induce an immune response during natural infection.

Natural or conventional antigens

Natural antigens are recognized during the infection and include excretory/secretory (ES), surface somatic antigens and can be effective against both blood and non-blood feeding nematodes (Newton and Meeusen, 2003). An advantage of the so-called natural antigens is the possibility of natural boosting whilst being continuously infected on pasture. ES products have been shown to be likely targets for immune and biochemical control of infections (Ring *et al.*, 1993). The ES products of adult *Haemonchus contortus* comprise of at least 15 polypeptides with molecular weights ranging from 10 to > 100 kDa. Schallig *et al.*, (1997) identified adult 15 and 24 kDa as immunogenic polypeptides in ES products of *H. contortus*. Gomez-Munoz *et al.*, 1996 identified and purified 26 kDa somatic antigen but Cornelissen, 1996 concluded that 26-kDa

somatic and the 24-kDa ES antigens of *Haemonchus contortus* one and the same differing in glycosylation.

Hidden, concealed /cryptic or covert antigens

A hidden antigen is defined as one which is not recognised by the host following infection and do not induce an immune response during infection (Emery *et al.,* 1991). Hosts are usually not exposed to proteins on the gut membrane of non-invasive metazoan parasites such as ticks and gastro-intestinal nematodes. If, however, these species are also blood feeders, the surface of the parasite intestine is exposed to host immunoglobulin and potentially to antibodies directed against it.

Different Hidden antigens used as vaccine candidates against *H. contortus* include

Contortin

The microvillar surface is coated with a layer of electron dense amorphous material (glycocalyx). In *H. contortus* there are helical filaments, composed of contortin associated with this layer, which fill the spaces between the microvilli. Contortin is not attached to the plasma membrane and some lies free in the lumen of the intestine and is also present in large amounts in the pharynx although this may not be the case *in vivo* (Munn, 1997). Geldhof *et al.,* (2008) found that contortin comprises two major proteins, Hc-PCP1 and Hc-PCP2, with homology to prolyl-carboxypeptidases. Addition of contortin to a fibrinogen solution significantly inhibited blood coagulation in a dose-dependent manner.

Aminopeptidase H11 glycoprotein

Protein H11, the most effective hidden antigen is found on the microvillar surface of the intestinal cells of fourth larval stage (L_4) and adult parasites. This protein was purified from contortin using concanavalin A lectin-affinity chromatography combined with, on some occasions, Mono Q anion-exchange chromatography. H11 is the shorthand designation for H110D that runs on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as a doublet with a mean value of 110 kDa. H11 is expressed only in the parasitic stages and only on intestinal microvilli. It has microsomal (membrane) aminopeptidase M and microsomal aminopeptidase A activities attributable to distinct isoforms (Smith *et al.,* 1997).

Haemonchus galactose-containing glycoprotein complex

The H-gal-GP complex was first described by Smith and coworkers in 1994. Several different techniques, including lectin screening of worm sections and radiolabelling have been used to target integral membrane glycoprotein on the luminal surface of *H. contortus* intestinal cells. These proteins were then purified from detergent extracts of whole worms. It is a 1000 kDa glycoprotein complex that can be extracted from the brush border of the intestinal cells of *H. contortus*. This fraction is glycosylated and selectively binds to lectins with a preference for N-acetylgalactosamine (Smith *et al.,* 1994, 1999). Biochemical analyses of H-gal-GP have indicated that it contains aspartyl, metallo- and cysteine proteinases (Smith *et al.,* 1999). The major component of the H-gal-GP complex is a family of four zinc metalloendopeptidases, designated MEPs 1–4. MEP3 appears to be the most abundant member of this metalloendopeptidase family. H-gal-GP also contains a family of galectins with a molecular mass of about 35 kDa. Galectins are soluble lectins which specifically bind β-galactoside sugars and have diverse roles in cell adhesion, immune function and apoptosis.

p52 and p46

Antigens obtained from extracts of adult *H. contortus* by affinity chromatography (Jacalin lectin, in combination with ion exchange and gel filtration chromatography) using a carbohydrate-specific monoclonal antibody yielded a mixture of two gut surface proteins of 52 and 46 kDa, with trace amounts of a 100 kDa protein. Although selected on the basis of having a carbohydrate epitope in common, there is no evidence that this epitope contributed to the protective effect.

The p46 molecule had been described earlier as a 45 kDa glycoprotein. N-terminal sequence analysis of p46 revealed significant homology with that predicted from a cDNA fragment which encoded the 45 kDa protein (Sharp *et al.*, 1992). Furthermore, the N-terminal data from p52 was almost identical to the predicted product of a second, closely related cDNA fragment (Sharp *et al.*, 1992), now known to be part of a recently isolated full length gene, designated Hc40 (Rehman and Jasmer, 1998).

Hc40 would be predicted to encode a protein of 51 kDa, in near agreement with the estimated mass of p52.The sequence of *Hc40* suggests that the N and C terminal halves of p52 are closely related, with many motifs repeated in each half (Rehman and Jasmer, 1998). The p52 component has a glycerophosphatidyl inositide anchor and 47% sequence identity with p46 (Jasmer *et al.*, 1996).

GA1 and P1 antigen

Monoclonal antibodies were used to identify and purify a group of proteins of molecular weight 46, 52 and 100 kDa. N-terminal protein sequence analyses of the three proteins, termed $p46^{GA1}$, $p52^{GA1}$ and $p100^{GA1}$, and cDNA library immunoscreening showed that all three were encoded by the same GA1gene and are initially expressed as a polyprotein ($p100^{GA1}$, Jasmer *et al.*, 1996).

The GA1 gene product showed closest homology with bacterial Tolb proteins that are associated with the bacterial membrane and periplasm and may be involved in transport. Smith *et al.*, (1993) identified a group of three peptides (P1) which were separated from H11 by ion-exchange chromatography. The constituent peptides (45, 49 and 53 kDa) showed some similarities to the GA1 proteins. The proteins, designated p45, p49 and p53, form a complex (p150) which is a ubiquitous constituent of the microvillar membrane of the intestinal cells. p53 has a membrane anchor and associates non-covalently with a disulphide bridged dimer of p45 and p49.

Cysteine proteinases and glutamate dehydrogenase

Cysteine proteases are present in almost all life forms such as plants, mammals, parasites and virus and this fact make them one of the most important and interesting enzyme groups. Parasite cysteine proteases are functionally diverse and are involved in immunoevasion, enzyme activation, tissue and cellular invasion, excystment, hatching and moulting.

The genes encoding these enzymes constitute approximately 2% of the genomic content. The *H. contortus* GDH gene encodes a protein of 538 amino acids with a predicted molecular mass of 60 kDa. *H. contortus* gut membrane extracts exhibit intense cysteine proteinase activity (Knox *et al.*, 1993). The expression of the GDH and cysteine protease encoding genes coincides with the onset of blood-feeding and immunolocalisation studies showed that the former was expressed in the cytoplasm of the intestinal cells (Skuce *et al.*, 1999a) while the latter was expressed on the microvillar surface (Skuce *et al.*, 1999b).

Table.1 Vaccination by recombinant antigens

Antigen	Animal specifications	Protection	References
r15/24	Lambs Adults	55% FECR 49% FECR	Vervelde *et al.*, 2002
rHcp26/23	All age groups	No significant protection	García-Coiradas *et al.*, 2010
rH11	6 m old lambs	No significant protection	Roberts *et al.*, 2013
H-gal-GP	Blackface × Leicester male lambs, aged 9 months	No significant protection	Cachat *et al.*, 2010
Cysteine Proteinase	Sheep	29-38%	Redmond and Knox, 2004
rHc23	Lambs (6-7 m)	>80% FEC 85% WB reduction	Fawzi *et al.*, 2015
rHc23	Lambs	>80% FEC >70% WB reduction	GonzaÂlez-SaÂnchez *et al.*, 2018

FECR= Faecal Egg Count Reduction, WB= Worm Burden

Table.2 Vaccination with native natural antigens

Antigen/dose	Adjuvant	Animal specifications	Efficacy	Reference
15 and 24 kDa 50-100µg	DDA (Dimethyl dioctadecyl ammonium bromide)	Lambs	72.9%FEC reduction 82.2% WB reduction	Schallig *et al.*, 1997
15 and 24 kDa 50-100µg	DDA	Sheep	82% WB reduction in 9 m old 77% in 6m old 0% in 3m old	Kooyman *et al.*,2000
p26/23	DDA	Lambs	>60% FEC >61.6% WB reduction	Domınguez-Torano *et al.*, 2000
Cysteine protease	Aluminum hydroxide	Lambs	77%FEC reduction and 47% WB reduction	De Vries *et al.*, 2009
Hc-*sL3* *20* µg	Aluminum hydroxide	Five month old lambs	61% FEC reduction 69% WB reduction	Jacobs *et al.*, 1999

Table.3 Vaccination with native hidden/gut antigens

Antigen	Animal specifications	Efficacy	Reference
Contortin	Lambs	75% reduction in worm burden	Munn *et al.*, 1987
p52 and p46	14-month-old goats	34% FEC reduction and 53% WB reduction	Jasmer *et al.*, 1993
Cysteine proteinase ▪ **S3 TSBP** ▪ **S3 TSBP** ▪ **S3 TSBP** ▪ **Purified cysteine protease** ▪ **S3 TSBP** ▪ **Purified cysteine protease**	 Sheep Goats Lambs Lambs Lambs	 95% FECR,50% WBR 77% FECRand 47%WBR 89% FECRand 68%WBR Comparable results Significant protection Significant protection	 Knox *et al.*, 1995 Knox *et al.*,1999 Ruiz *et al.*, 2004 Knox *et al.*, 2005 Redmond *et al.*, 2004 Molina *et al.*, 2015

Table.4 Vaccination with native H11

Antigen/dose/adjuvant	Animal specifications	Efficacy	Reference
H11 50 µg FCA/FIA	Young Dorset lambs	78% FECR 83% WB reduction	Tavernor *et al.*, 1992
H11 fraction 0.35µg FCA/FIA	Young merino lambs	70.7% FECR 70% WB reduction	Munn *et al.*, 1993
H11 140µg **H11 200µg**	4 month old lambs	94.6% FEC reduction 86.5% and 93.5% male and female WB reduction respectively	Smith *et al.*, 1994
H11 from resistant strains150µg FCA/FIA	Lambs	99% FECR 90% WB reduction	Newton *et al.*, 1995
H11 (4 isolates)150µg FCA/FIA	Lambs	82–96% FECR 55.9–93.8%WB reduction	Newton *et al.*, 1995
H11 50 µg FCA/FIA.	Pregnant ewes	91% FECR 86% WB reduction	Andrews *et al.*, 1995
H11 40 µg Vax saponin	Lambs	99.9% FECR 93.6% WB reduction	Roberts *et al.*,., 2013

Table.5 Vaccination with native H-gal-GP

Antigen/dose/adjuvant	Animal specifications	Efficacy	Reference
H-gal-GP 200 µg in FCA	Suffolk x Dorset 3-5 m old lams	89% FECR 69.5% WB reduction	Smith and Smith., 1996
H-gal-GP 100µg FCA/FIA	5-6 m old lambs	56.5–69.7 % FECR 40–53.5%% WB reduction	Smith *et al.*, 1999
H-gal-GP 100 µg Quil A	Lambs	93% FECR 60–64%WB reduction	Smith *et al.*, 2001
H-gal-GP 100 µg Quil A	Lambs	88.5% FECR 72.3% WB reduction	Cachat *et al.*, 2010

Table.6 Field trials in sheep using H11 and H-gal-GP

Location	Animal specification	Antigen, dose and adjuvant	Efficacy	Reference
Lousiana, USA	Suffolk ewes>2y	100 µg H11+100 µg H-gal-GP in 5 µg Quil A	>65%FECR	Kabagambe *et al.*, 2000
South Africa	12-18 m old Dorper sheep	100 µg H11+100 µg H-gal-GP in 5 µg Quil A	>82% FECR	Smith *et al.*, 2001
Australia	Grazing merino lambs	100 µg H11+100 µg H-gal-GP in 5 µg Quil A	>85% FECR High levels of IgG1 and IgG2	LeJambre *et al.*, 2008
Brazil	Periparturient ewes	5 or 50 µg H11 and 1mg of saponin as adjuvant	78% FECR	Bassetto *et al.*, 2014

Vaccination by recombinant antigens

Many largely unpublished attempts have been made in several laboratories to produce protective recombinant versions of the protective antigens, but without success. Different published attempts of vaccination using recombinant antigens are summarized in Table 1.

Reasons for failure of recombinant vaccines

Despite the success of native proteins in inducing protective immunity, recombinant forms expressed in bacteria, yeast or insect cell expression systems are far less effective (Newton and Meeusen, 2003; Vercauteren *et al.*, 2004). Possible explanations include

incorrect folding, lack of glycosylation of bacterially-expressed proteins, inappropriate glycosylation of yeast or insect-expressed proteins, induction of lower avidity antibodies or, alternatively, that the dominant proteins identified in protective native fractions are not solely responsible for protection (Newton and Meeusen, 2003). Roberts *et al.,* (2013) suggested that this failure might be due to differences in the glycosylation and/or conformation between the native and recombinant proteins. These authors were able to show that *H. contortus* H11 expressed in *Caenorhabditis elegans* was enzymatically active, and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry identified di- and tri-fucosylated structures that were similar to those on native H11. Some glycan structural differences were observed. Serum antibodies raised against native H11 bind to *C. elegans* recombinant H11, and most of the antibodies to recombinant H11 or native H11 are directed to glycan moieties. Despite these similarities, no reductions in worm burdens or FECs were observed following the immunization of sheep with *C. elegans*-expressed recombinant H11 protein. These findings suggest that the di- and tri-fucosylated N-glycans expressed on recombinant H11 do not contribute to the protective effect of H11, and that additional components that are present in native H11-enriched extract are likely required to enhance the antibody response that is necessary for protection (Roberts *et al.,* 2013). Like H11, the native H-gal-GP complex loses most of its protective activity if it is dissociated, which indicates that conformational epitopes are important and explains why bacterially expressed insoluble recombinants do not work (Smith *et al.,* 2003a, b). Also the failure may be due to failure to identify the protective component of the complex, or the requirement for the antigen(s) to be presented in a complex form (Newton and Meeusen, 2003). For other antigens, such as ES15 and ES24, failure is most likely due to redundancy of function, due to the very high number of members of these gene families (Newton and Meeusen, 2003). García-Coiradas *et al.,* (2010) expressed p26/23 and found lack of protection was apparently due to lack of glycosylation in recombinant protein.

Vaccination with native natural antigens:

Attempts using different native natural antigens have been done. Varying degrees of FECR and WB reduction have been achieved as shown in Table 2.

Vaccination with native hidden/gut antigens

Attempts using different native hidden antigens have been done with variable success. Different studies have been done as shown in Table 3, 4 and 5. Besides, H11 and H-gal-GP, most studied native hidden antigens, other hidden antigens used as vaccine candidates have been summarized in Table 3 below:

Field trials with sheep

Owing to the success in experimental trials, field trials were performed in different countries (USA, South Africa Australia and Brazil) to determine whether vaccination with 100µg native H11 and 100 µg native H-gal-GP in 5µg Quil A adjuvant could provide similar protection against natural *H. contortus* infections in grazing sheep (Kabagambe *et al.,* 2000; Smith *et al.,* 2001; LeJambre *et al.,* 2008, Bassetto *et al.,* 2014). Different field trials conducted in sheep have been summarized in Table 6 below:

Although the trials described above with native antigen vaccines were encouraging, it was considered that only recombinant vaccines could be commercially viable.

However, with the discovery that the dose of native antigen required for protection was low (5 µg), it was then realized that a native antigen vaccine could be economically feasible provided methods could be found for collecting large quantities of adult worms cost-effectively.

Recently, Smith (2014) developed a vaccine for the Barber's Pole worm *H. contortus* called 'Barbervax' using purified native gut antigen of the adult worms in Moredun Research Institute, Edinburgh, UK. Barbervax was the first commercially available sub-unit vaccine for *H. contortus*. The active constituent in the vaccine was *H. contortus* integral membrane glycoprotein antigen and used at a dose of 1 ml by subcutaneous injection into the neck of sheep and lambs from 3 weeks of age. The availability of vaccine was 250 doses in 250 ml pack specification as 5.0 µg/ml and 1 mg/ml saponin (Quil-A) as adjuvant. The dosage includes one injection from 3 weeks of age and three injections 3 to 6 weeks apart were needed to induce protection. For the remainder of the Barber's Pole worm risk period, immunity can be maintained by boosters given at 6 week intervals for up to 6 months. This vaccine was used for reduction of pasture larval contamination and disease caused by *H. contortus* in lambs. No harmful effects were expected in small ruminants. Local tissue reactions in the form of swelling at the injection site may occur and last for up to 17 days. Animals may show a moderate rise of temperature for up to 3 days. The commercial scale 'Barbervax' manufacturers are Animal Health Laboratories located in Albany, Western Australia.

Research work on Immunoprophylaxis against *H. contortus* in India

Joshi and Singh (1999) identified two low molecular weight immunoprotective antigens, 15 kDa and 22 kDa from the extract of *H. contortus* by gel filtration conA-sepharose and affinity chromatography on antibody-sepharose. These proteins were also identified in the ES products of adult parasites. Joshi and Singh (2000) purified acetylcholinesterase (AChE) from adult *H. contortus* ES products as 144 kDa antigen. Enzyme specific antibodies against this antigen were showed by *H. contortus* infected animals in western blot. These antibodies along with antibodies directed against other molecules may be responsible for the phenomenon of self-cure observed in hypersentitive sheep infected with *H. contortus*. This raises the possibility of using AChE alone or in combination with other antigens as a possible protective immunogen. Suchitra and Joshi (2005) characterized *H. contortus* calreticulin (CalR) in the ES products of adult worms. The calcium binding protein showed reactivity approximately at 62 kDa in western blotting. They suggested that CalR has role in preventing blood clotting by binding to Ca^{2+} and clotting factors thus enabling parasite to feed on the host blood and also modulate the host immune response by binding to complement C1q thereby facilitating parasite's survival within the host. Rathore *et al.,* (2006) identified a 66 kDa *H. contortus* excretory/ secretory antigen that inhibited host monocytes function *in vitro*. Antibodies against this protein caused loss of adult worm motility *in vitro* resulting in the death of the parasite. The fact that the protein is recognized by the host together with *in vitro* killing of adult parasites by antibodies makes this protein a promising candidate for vaccination trial. Garg *et al.,* (2007) studied the protective effect of a low molecular weight protein antigen (LMWPAg) and crude somatic antigen (CSA) in Barbari goats. The LMWPAg of 16 and 20 kDa were partially purified by gel chromatography. Maximum of 70.79% FEC reduction was seen in the experiment. Prasad *et al.,* (2007) found that

60 and 120 kDa polypeptides were immunodominant in somatic antigen of adult *H. contortus* which may be utilized further for immunodiagnosis/ immunoprophylactic purposes. Allaie *et al.*, (2010) studied the immunodominant polypeptides 60, 120 and 170 kDa from L3 larval stage of *H. contortus* and polypeptides of 60, 120 and 170 kDa were found to be immunodominant in western blotting with prepatent sera from experimentally infected sheep. They suggested that these polypeptides may be enzymes including proteases, which may further be exploited for immunoprophylaxis against haemonchosis in young animals. Arunkumar *et al.*, (2012a) reported that thiol-sepharose affinity chromatography purified E/S antigen of *H. contortus* produced more antibody levels compared to crude extract antigen and unimmunized control animals. The crude E/S antigen showed five reactive bands at 24, 29, 46, 66 and 93 kDa and thiol-purified antigen showed a single reactive band at 66 kDa on western blot analysis. Immunization trials in sheep with 500 µg of antigen revealed higher serum antibody levels in ELISA with purified antigen as the absorbance values were significantly higher up to 20 weeks post immunization in group-I (purified antigen) than group-II (crude antigen) compared to unimmunized control animals. The EPG values were lower in group-I (200 to 400) than group II (2200 to 5100) and the percentage reduction in mean FEC was 88.5 %. Similarly, the mean abomasal worm counts was lower in group-I (808.33 ± 78.29) than group-II (3280 ± 147.19) and the percentage reduction in mean abomasal worm count was 75.40 % (Arunkumar, 2012; Arunkumar *et al.*, 2012b). Suchita (2012) purified the native cysteine proteinase of adult male and female *H. contortus* worms using 60-75 % alcoholic precipitation. Hyper immune serum raised for this cysteine proteinase identified immunodominant polypeptides of 31, 43, 60 kDa size in purified cysteine proteinase fractions from both male and female *H. contortus* in western blotting. Ishfaq (2016) evaluated protective efficacy of immunoaffinity purified immunodominant polypeptides of adult somatic antigen of *H. contortus* using $Al(OH)_3$ in Muzzafarnagri breed of sheep and observed reduction in worm burden of 82.41% in immunised group as compared to unimmunised control group and 79.37% as compared to adjuvant control group while the FECR was 70.32% and 67.79% reduction in FEC as compared to unimmunised and adjuvant control group, respectively.

Vaccination offers best alternative control measure against *H. contortus*. Recombinant proteins used as vaccine candidates have not induced significant protective immunity. Of late other expression systems have been used like yeast, insect cell expression and *Caenorhabditis elegans* (a free living nematode) have been used but to no avail. This shortcoming hinders use of recombinant proteins as vaccine candidates. However, with the discovery that the dose of native antigen required for protection was low (5 µg), it was then realized that a native antigen vaccine could be economically feasible provided methods could be found for collecting large quantities of adult worms cost-effectively. Barbervax, first commercially available sub-unit vaccine for *H. contortus* is also derived from native H11 antigen.Also in the future; it is unlikely that nematode control in small ruminants will rely on single approach. The sustainability of control will be largely conditioned by the ability to achieve an integrated parasite management strategy by combining several solutions, corresponding to the different principles (Waller, 2006).

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