CHAPTER 03

DEVELOPMENT AND ADVANCEMENT IN VACCINES AGAINST *HAEMONCHUS CONTORTUS*

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INTRODUCTION

Haemonchosis is the infection caused by *Haemonchus contortus* (barber's pole worm) which is one of the most pathogenic and socio-economically important parasites affecting cattle, goats and sheep of tropical and subtropical areas of the world (O'Connor et al. 2006). Haemonchosis is clinically characterized by decreased wool production, anasarca and chronic wasting resulting in poor carcass quality, anemia, hypoproteinemia, submandibular edema and death in severe cases. Small ruminants like sheep and goat share a major contribution to the agricultural economy of world in terms of their wool, skin, milk and meat production (Roos 2009; Roeber et al. 2013; Emery et al. 2016). Estimates of annual losses caused by haemonchosis to livestock account for 30 to 300 million dollars all over the world (Roeber et al. 2013; Emery et al. 2016). Primarily these parasitic infections are controlled by using anthelmintics but non judicious use of anthelmintics have led to the development of resistance against these drugs (Saddiqi et al. 2011). Resistance against different broad spectrum anthelmintics like morantel, levamisole, imidothiazoles-tetrahydropyrines, benzimidazoles, pyrantel, closantel, ivermectins, and macrocylic lactones has been reported across different parts of the world (Kaplan 2004; Kaplan and Vidyshankar 2012). So, the development of anti-parasitic vaccines and immumological approaches to control these infections is need of the hour. For the development of cost-effective methods to control *H. controtus* infections, we need a comprehensive knowledge of biology of *H. controtus* and immune responses involved in host-parasite interaction (Nisbet et al. 2016).

Life cycle of *H. controtus* starts with the sexual reproduction in the abomasum of the infected host resulting in the production of 5000-15000 eggs by a single female every day. Eggs are passed into feces and hatch to first larval stage (L1) and then moult to second larval stage (L2) within 4-6 days and start feeding on the bacteria present in the dung. When conditions are favorable like temperature ranging from 24 to 29 °C, L2 moults to L3 but does not shed its cuticle. L3 larvae are capable of moving up to the blades of grasses with in moisture drops. During grazing these are taken up by the host and reach abomasum where shedding of cuticle takes place and they burrow themselves into the abomasum. Within 48 hrs after burrowing they ex-sheath into L4 stage and ultimately into early (L5) and then ultimately into late adult worms. These adult worms start feeding blood. Infective L3 stage or early L4 stage instead of directly proceeding to next stage become dormant in the gastric glands of abomasum and are metabolically inactive. This phenomenon is called as developmental arrest or hypobiosis (Michel 1974). During unfavorable environmental conditions for the parasitic development proportion of hypobiotic worms is greater as the eggs shed in the feces have lesser chances of survival and development into the next stage (Waller at al. 2004). This mechanism depends on the environmental factor as well as immune responses of the host and genes involved in this phenomenon can be used as target antigen for developing new drugs and vaccines for control of *H. controtus* infections.

Importance

In last two decades various antigens have been identified from *H. controtus* and protective efficacy of these antigens as recombinant subunit and vector vaccines have also been evaluated (Knox et al. 2003; Tak et al. 2015; Wang et al. 2017). Potential efficacy of the use of gut proteases of *H. controtus* including an aminopeptidase H11 (Smith et al. 1997), cysteine protease with fibrinogenolytic properties (Boisvenue et al. 1992), H-gal-GP (gut membrane glycoprotein complex) (smith et al. 1994) which is a gut protein complex containing metalloendopeptidases (Redmond et al. 1997) and aspartyl protease activities as vaccine components have been evaluated previously. Among these, an integral membrane protein H11 having molecular weight of 10 kDa was found a potential candidate antigen with resulted in >90% reduction in fecal egg count and >75% reduction in abomasal worm load. A vaccine named Barbervax® has been recently licensed to be marketed in Australia which contain two native integral gut membrane protein H11 and H-gal-GP from *H. controtus* (Nisbet et al. 2016). During evaluation of safety and serological outcome of this vaccine significant antibody titers have been detected in the vaccinated animals (Vanhoy et al. 2018).

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Efficacy evaluation of Barbervax® was carried out in two groups of periparturient ewes with two different nutritional supplements and results showed 80% reduction in fecal egg count and higher antibody titers of vaccinated ewes which suggested that *H. controtus* can be controlled by combined protective effect of vaccine and improved nutrition (Bassetto et al. 2018). But requirement of repeated vaccine doses to induce high level of antigen specific circulating antibodies was major drawback of this vaccine which has resulted in failure of application of vaccine at large scale and limited its access to international market. Recently use of soluble and recombinant protein vaccines to control *H. controtus* has unlocked a new insight into the field of host-parasite interaction (Wang et al. 2017). In current scenario vaccines can be suitable option to control parasitic infections but immunoregulatory properties and antigenic variation of parasites are major hurdles in the process of development of effective vaccines (Hewitson and Maizels 2014). Therefore, development of safe and effective vaccine for controlling *H. controtus* requires identification of molecular targets and utilization of advanced molecular techniques for structural as well as functional studies on potential candidate targets for vaccine development.

Immunity

Host's immune response to parasitic infection is complex and categorized into two stages; one being against the infective larval stage and other one against adult helminths. Immune reaction against helminths is mainly governed by level of eosinophilia and high levels of serum and mucosal immunoglobulins (IgA, IgE, IgG) which are associated with T-helper type 2 mediated response (Balic et al. 2006; Lacroux et al. 2006; Shakya et al. 2011). Resistance of host against *H. controtus* infection depends on many factors like specie, age, breed, nutrition and previous exposure to parasitic infection. Younger lambs are at a high risk of developing infection of *H. controtus* due to their poor immunity. Several events occur in the immunized host when it is exposed to parasite. These events include humoral and cell mediated responses characterized by recognition of antigens either somatic or secretory/ excretory by dendritic cells and presentation to T-cells as antigen presenting cells (APCs) (Meeusen et al. 2005). As a result, allergic inflammatory response is triggered which include mucosal eosinophils and mast cell, these cell release inflammatory mediators which check parasitic infection by decreasing egg production, preventing the establishment of larval and adult stage as well as paralyzing the worms (Jones et al. 1994; Emery 1996).

General immune response to helminths' infection consists of T-helper type 2 cells as a part of humoral immune response involved on the expression of cytokines at infection site and these cytokines are responsible for stimulation and activation of CD4+ T cells (Meeusen et al. 2005; Anthony et al. 2006). Helminths' infections are reported to induce strong Th-2 cell response characterized by production of high amount of interleukins (IL-4, IL-5, IL-9, IL-10, IL-13, IL-25 and IL-31) (Jackson et al. 2009; Maizels and Vazdanbasksh 2003; Wang et al. 2008) as well as high level of immunoglobulins (IgG1, IgG4, IgE) and stable mast cell and eosinophil responses. IL-4 and IL-13 were main regulators of humoral immunity stimulating B cells to produce IgE and regulating the production of major histocompatibility complex class II (Anthony et al. 2007). Generally, immunity against *H. controtus* is governed by eosinophils, mast cells, antibodies and inhibitory molecules (Bricarello et al. 2004; Balic et al. 2006). Development of various strategies to modulate or escape the immune response by helminths have also been reported such as infection of Ancylostoma caninum lead to upregulation of regulatory T cells which ultimately resulted in the suppression of Th-1 andTh-2 mediated responses (Ferreira et al. 2013). Similarly, study reported in-vitro suppression of maturation of dendritic cells by excretory/secretory products of Trichinella spiralis and both R- and S-from lipopolysaccharide induced upregulation of T regulator cells (Aranzamendi et al. 2012).

Exploitation of Genomic and Proteomic Profile of *H. controtus*

Exploitation of genomics and proteomics of *H. controtus* has resulted in identification of several novel vaccine components. According to genome-wide transcriptomic data of all stages of *H. controtus* , 23610 protein coding genes are responsible for reproduction, development, host-parasite relationship, immunity and disease (Schwarz et al. 2013). Proteomic analysis done by liquid chromatography tandem mass spectrometry (LC-MS/MS) and matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) has identified 107 identities from 102 spots (Yatsuda et al. 2003) which include zinc metalloproteases, aspartic and serine proteases, aminopeptidases (Karanu et al. 1993; Rhoads and Fettterer 1995; Shompole and Jasmer 2001) and excretory/secretory products of *H. controtus* (HcESPs) Hc-15, Hc-24, Hc-40 and apical gut proteins (Yatsuda et al. 2003; Schallig et al. 1997; Dicker et al. 2014). LC-MS/MS analysis at 3 days post infection also identified approximately 4400 unique proteins (Nagaraj et al. 2012). Twenty-two hundred (2200) protein identities were recognized during analysis of proteins differentially expressed between L3 and xL3 stages of *H. contortus* by 2D-DIGE (two-dimensional differential gel electrophoresis) and 124 of them expressed between L3 and xL3 (Wang et al. 2016). Previously 407 interacting proteins were identified with ranged from 13 kDa to 180 kDa by in-vivo analysis of HcESPs, 47 out of them were shared in among all developmental stages from L3 to adult (Gadahi et al. 2016). Analysis of whole protein extract from male and female *H. controtus* revealed 129 male specific, 123 female specific and 23 immunogenic proteins (Yan et al. 2010). Moreover, proteomic study of 2487 differential proteins from eggs, L3, L4 and adult male and female of *H. controtus* showed substantial protein profile transition among different stages (Wang et al. 2019). Purpose of reviewing genomic exploitation of *H. controtus* is to provide a major resource for scientific community extensive molecular, genomic, epidemiological studies and development of new and effective drugs or vaccines for the control of *H. contortus*.

History of Vaccine

In past various efforts have been made to develop an effective vaccine to control *H. controtus* . Several methods have been used for the development of vaccine which include DNA vaccines, recombinant subunit vaccines and protein vaccines which are under consideration and are in process for checking their safety and efficacy. Commercially development of vaccines against helminths started with the development of commercial vaccine against nematodes named Dictol in early

1950s. But soon research focus shifted towards the identification and development of ESPs and H-gal-GP based vaccines after the failure of previous vaccine against infective stage (Smith and Zarlenga 2006; Knox et al. 2003).

Types of Vaccines

Gut derived/ Hidden Antigens based Vaccines

Use of glycoprotein complex derived from gut of *H. contortus* as vaccine component resulted in >90% decrease in fecal egg count and approximately 70% decrease in fecal worm output in vaccinated animals (Smith and Smith 1996). This presents H-gal-GP as a strong immunogen and this a candidate for vaccine development. Glycan part of H-gal-GP was considered main immunogen (Knox et al. 2003). Other studies also support the immunogenic role of galectins as they decreased fecal egg count by 48% (Yanming et al. 2007), thus highlights the potential of use of galectin as antigens in vaccine against *H. contortus*. Another option is integral membrane glycoprotein complex H11 obtained from gut of blood feeding *H. contortus*. Five isoforms of H11 have been documented being H11-1, H11-2, H11-3, H11-4, and H11-5 (Roberts et al. 2013) but H11-1 is most immunogenic and its vaccination trails have reported 91% decrease in fecal egg count as well as 82% and 72% decrease in adult male and female worm burden respectively (Nisbet et al. 2016). Moreover, vaccination containing both H-gal-GP and H11 antigen have also be successful in reducing losses due to haemonchosis (Smith et al. 1999). But these combination vaccines have some demerits which include repeated dose requirement for developing good and long-lasting immunity (Ehsan et al. 2020). This is a major reason behind the failure application of these vaccines on large scale.

DNA based Vaccine

This method uses genetic engineering to produce a genetically engineered antigen and immune cells are directly exposed to antigen to produce diverse immune responses. DNA vaccines are considered superior that the conventional vaccine because of their ability to produce diverse immune responses. Development of an effective vaccine demands high level of specific antibodies against *H. contortus* in vaccinated animals. Study conducted by Zhao et al. (2012) showed that DNA fragments of caprine IL-2 and H11-1 served as good immunogens as they resulted in 57% decrease in fecal egg count and 47% decrease in abomasal worm burden in the vaccinated goats. Similarly in another study goat vaccinated with glutathione peroxidase and HC29 encoding gene resulted in 36% decrease in fecal egg count and abomasal worm burden with the production of appreciable level of HC29 specific antibodies IgG and IgA as well as intensification of CD4+ T cells (Sun et al. 2011). Moreover, extending studies over DNA vaccine also covered two more antigens from *H. contortus* named GAPDH and Dim-1 conjugate with pVAX1 recombinant plasmids which were administered to 10 months old goat. Vaccination with GAPDH resulted in 35% decrease in fecal egg count and 38% decreases in abomasal worm burden along with increased antigen specific antibodies as well as intensified CD4+ T cell population (Han et al. 2012) while vaccination with Dim-1 resulted in 46% decrease in fecal egg count and 51% decrease in abomasal worm burden (Yan et al. 2013).

Protein based Vaccines

Over last two decades several studies have been conducted to identify various antigen from *H. contortus* for efficient vaccine development which can provide high level of specific and long-lasting immunity. During blood feeding stages various proteins called as ESPs are released into the environment by *H. controtus* (Rathore et al. 2006; Marcilla et al. 2012). Survival of helminths within their hosts depends on their ability to modify host's immune responses (Maizels and Yazdanbaksh 2003). Ability of parasite to modulate its host's immune responses improves its survival within host. Nematodes contain two type of antigens one being soluble (ES products) while other one being somatic antigens that are present either on the surface or present within the parasite. ES antigen that induces immune response in host are known as natural antigens while those that do not do so are called as hidden antigens (Munn 1997). An antigen that is a good candidate for vaccine must be presented to antibodies and cells involved in regulating the immunity of the host. HcESPs contain antigen candidates for the development of vaccine that can provide up to 90% protection in sheep against *H. contortus* infections (Yatsuda et al. 2003). Previously study conducted by Schallig and Leeuwen in 1997 reported 99.9% decrease in fecal egg count and 97.6% decrease in abomasal worm burden of animals vaccinated using two adult somatic extracts enriched in ES-15kDa and ES24-kDa. Similarly, a reduction of >90 % in fecal egg count and 72 to 80% decrease in abomasal worm burden has been reported in animals vaccinated using gut membrane antigens of *H. controtus* (Jasmer and McGuire 1991; Andrews et al. 1997). All of these studies hint the potential of using ES antigen in the development of protective vaccine.

Binding Proteins as Vaccine Agents

When a parasite infects an individual, it releases large number of molecules into the host which are responsible for immune reactions within the host (Cox et al. 1990). Excretory/secretory (ES) products contain many proteins which act as immunogens and can be used as antigens in vaccine.

Challenges to the Development of Effective Vaccine

Various vaccine trails have been conducted in which vaccine against *H. contortus* produced by several strategies have been test for their safety and efficacy. Although advancement in the development of vaccine against *H. controtus* is obvious but at the same time none of the vaccine is completely capable of to eliminate haemonchosis completely or they nullify its transmission. These vaccines are only capable of reducing fecal egg count and abomasal worm burdens partially to varying extent. So, still a gap is present that can be filled and further and more comprehensive studies are needed for the development of an effective vaccine against *H. controtus* .

Diversity of *H. controtus*

Various studies have been conducted in past regarding the genetic diversity of *H. controtus* but a more comprehensive overview of genetic diversity is needed for the development of effective vaccines or drugs and knowledge about

epidemiology, molecular genetics and drug resistance of *H. controtus* . Study conducted by Charlesworth in 2009 on the population genetics of *H. controtus* revealed that genetic diversity is dependent on many factors that include population size, gene flow, geographical restrictions and life history. Therefore, extensive genetic diversity among a population is suggestive of a larger population size or increased rate of mutation in *H. controtus* (Gilleard and Redman 2016). Similarly, a high degree of genetic variation has also been reported among laboratory strains of *H. contortus* from different countries (Redman et al. 2008). On the other hand, various genetically different isolates have also been detected in sheep and goats from same geographical locality. Hunt et al. in 2008 infected ten sheep with five different laboratory isolates of *H. controtus* from different regions of Australia and reported increased fecal egg count and abomasal adult worm burden resulted from difference in establishment rate. Difference in the pathogenicity among genetically different isolates of *H. controtus* collected from different geographical areas of United States has also been reported (Gilleard and Redman 2016). Poeschel and Todd (1972) performed an experimental study to check pathogenicity of 18 isolates of *H. controtus* and reported that three of these isolates were more less while two were more pathogenic than the control. Furthermore, reporting of the fact that the antigens associated the development of protective immunity are also not conserved in different species and different isolates of a specie (Maizels and Kurniawan 2002) has further added to the challenges faced in development of vaccine against *H. controtus* . Keeping in view the facts about genetic diversity and difference in immune response to different parasitic stage has highlighted the need of development of a vaccine that can be effective to be used in young animals against L3 stage of *H. controtus* in pre-seasonal as well as eliminates the necessity of administration of booster shot in post-seasonal period. This task can probably be performed by developing a vaccine that contain the antigens from different stages of *H. controtus* such as it L3 surface antigen and H-gal-GP from L4 and abomasal parasites can be used in combination (Nisbet et al. 2016). Although it is a difficult task to be performed because it requires the collection of different parasitic stages from naturally infected individual and complete of their antigenic profile which needs financial resources (Willadsen 2008). But development of such an effective vaccine will revolutionize the control of *H. controtus* as well as will open a way for the development of vaccines against other parasitic diseases as well.

Genetic Diversity of Host

Genetic diversity of host basically affects the immune response to parasitic antigens. Effect of host genetics on the development of innate as well as acquired immunity against nematode infection in a herd has been studied previously (Smith and Zarlenga 2006). Generally, it was considered that vaccination can ensure 100 percent immunity in individual infected naturally in past in comparison to those involved in host parasite relationship for longer periods (Lightowlers et al. 2003). Studies reported variation of fecal egg count of grazing animals with the genetic diversity of host, furthermore demonstrated that a major cause of parasitic transmission was a small proportion of highly vulnerable animals within the population (Barnes et al. 1995). Similarly, vaccination of that small proportion of population that is highly vulnerable can be used to decrease pathogenicity and transmission of parasites in a herd (Smith and Zarlenga 2006). Parasitic infections can be more challenging in genetically less diverse populations. There has been an increase in the evidences supporting the hypothesis which suggests that genetic diversity host is also reduced as result of environmental changes, pollution, global warming and decrease in the geographical range of host specie (Ekroth et al. 2019). At the same time increase in the parasite dominancy has resulted in increased chances of co-infection of different parasites among genetically homogenous populations (Whiteman et al. 2006). Pathogenicity in a population might also depend on timing and intensity of parasitic infection (Ekroth et al. 2019). A potential association also exists between lower genetic diversity of host and new emerging infections. So, conserving genetic diversity of host and vaccination of most vulnerable individuals within populations might be a suitable strategy to control emerging parasitic diseases.

Complexity Due the Different Developmental Stages of Parasite

Like many other parasites life cycle of *H. contortus* also consists of various developmental stages that differ in their antigenic profile (Gadahi et al. 2016). Similarly, immune responses of host against each stage also differ greatly which makes vaccine development against *H. controtus* more challenging. Vaccine containing antigen from one stage fails to protect against infection by other stages of parasite. Combination of genetic diversity and multistage complexity of parasite raises questions on the idea that a vaccine containing antigen from a single stage of parasite can provide long lasting immunity. So, a vaccine that contain suitable antigens from all developmental stages of *H. controtus* can be suitable options for providing long lasting immunity against all developmental stages of the parasite. Moreover, another challenge in vaccine development is the ability of parasite to modulate immune responses of the host in such a way that causes a delay or inability of host to resist parasitic infection.

Composition of Effective Vaccine

In the process of vaccine development against *H. controtus* one thing that needs to be emphasized is the quality and durability of immunity produced by vaccine. In this context adjuvants are considered an important component of vaccine in regard of production of vaccine that has better stability, safety, lower requirement in terms of volume and frequency of vaccination as well as capable of producing high and rapid immunity by increased differentiation of B lymphocytes (Chauhan et al. 2017; Reed et al. 2013). Selection of adjuvant from those available nowadays depends on immunogenicity of antigen adjuvant complex as well as lesser side effects associated with the vaccine administration. Various adjuvants have been used and their outcomes have been recorded in host models (Stutzer et al. 2018). But these has some disadvantages such as use of saponins results in tissue damage at the site of administration that leads to induction of improper immune response (Chauhan et al. 2017). Recently a new technology named as microencapsulation of antigens has been developed as an alternative to conventional adjuvants used in vaccines that has proved to be more reliable, effective

and promising vaccine delivery system (Himly et al. 2017). When considering adjuvants as most effective vaccine delivery system studies have shown that vaccination of lambs containing native or recombinant type antigens of *H. contortus* named as Hc23 and rHc23 respectively and Al(OH)3 as adjuvant resulted a decrease of 70 to 80 %in fecal egg count and abomasal worm burden of vaccinated animals (Fawzi et al. 2014; Fawzi et al. 2015). Another study conducted on the efficacy of vaccine containing rHc23 antigen and Al(OH)3 as adjuvant as showed similar results (González-Sánchez et al. 2018).

Future Perspectives

Extensive studies have been conducted all over the world about the biology of *H. contortus* which include molecular genetics for the search of antigen candidates from various its developmental stages that can be used for the development of an effective and protective vaccine against haemonchosis as well as various efficacy trials have also been conducted to test efficacy and safety of these vaccines. In spite all of these efforts lack of an effective vaccine against all stage of *H. controtus* still persists. Researchers have explored most of the complexities associated with host-parasitic relationship, life cycle, development of resistance and antigenic diversity of *H. controtus* . Similarly, valuable and more specific as well as sensitive diagnostic techniques have been developed which would help minimizing losses caused by haemonchosis through an early diagnosis and treatment. Furthermore, studies on the molecular genetics, proteomics as well as transcriptomics have enabled us to use most advanced gene editing technologies like CRISPR-Cas technology for the development of an effective vaccine development tool. By the use of these modern technologies, we are determined to develop and effective and safe vaccine against *H. controtus* in near future.

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