Major Gastrointestinal Nematode Infections in Small Ruminants

Subjects: Parasitology

Contributor: P. G. Ashani S. Palkumbura , Thilini A. N. Mahakapuge , R. R. M. K. Kavindra Wijesundera , Viskam Wijewardana , Richard Thiga Kangethe , R. P. V. Jayanthe Rajapakse

Gastrointestinal parasitic nematode (GIN) infections are the cause of severe losses to farmers in countries where small ruminants such as sheep and goat are the mainstay of livestock holdings. There is a need to develop effective and easy-to-administer anti-parasite vaccines in areas where anthelmintic resistance is rapidly rising due to the inefficient use of drugs currently available.

helminth whole parasite purified parasite products vaccination

1. Introduction

Gastrointestinal parasitic nematode (GIN) infections are a major constraint in small ruminant production and cause severe economic losses to smallholder farmers. Economically important GIN parasites belong in the order Rhabditida, with a majority found in the family Trichostrongylidae [1][2]. The most economically significant GIN infections in this group are *Haemonchus* spp., *Teladorsagia* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Mecistocirrus* spp., *Nematodirus* spp., and *Cooperia* spp., along with *Bunostomum* spp. and *Oesophagostomum* spp. from the families Ancylostomatidae and Strongylidae respectively (the systematics of nematodes follows Hooda [3]). Most studies on GIN infections in small ruminants have focused on sheep, mainly because goats are more common in developing countries [4][5][6][7][8]. As a result, there is a lack of host-specific information on the species prevalence, geographical distribution, host immune responses, and appropriate prevention strategies of GIN infections in goats [9][10].

Haemonchus contortus is a highly pathogenic species in small ruminants in tropical and subtropical regions and can also be found in young cattle and some species of deer [11]. The abomasum is the most affected organ during infections due to the nematodes' haematophagous nature and the rapid development of worm burdens, making the parasites the most common nematodes that infect small ruminants globally [4][5][6][7][8][12][13]. Haemonchosis begins with the ingestion of third-stage larvae (L3) in pasture. L3 larvae migrate to the abomasum and penetrate glands that line the abomasum before moulting into fourth-stage larvae (L4). Complex immunological responses are elicited at this stage with various factors involved in developing resistance to subsequent infections by the host. These factors are associated with age, breed, and previous exposure to parasites [13]. Young animals are very sensitive to infection when compared to older animals, which acquire immunity after continuous or seasonal exposure [13][14]. In immunized animals, there is a rapid expulsion of parasites before L4 stage larvae are established [15]. *Teladorsagia circumcincta* is a major abomasal parasite species in temperate countries. Immunity

can be developed with repeated exposure to *T. circumcincta* and is related to the age of the animal, with weaned lambs highly susceptible to infection [16]. Most animals suppress the infection within 6 weeks after the development of IgE and IgA antibodies [16]. Mucosal mast cells have also been shown to play an important role in preventing larval colonization and the expulsion of adult parasites in association with parasite-specific antibodies [13][17][18]. *Ostertagia ostertagi* is a parasite predominantly found in cattle reared in temperate countries, with a life cycle similar to that of *Haemonchus* sp. The parasite is comparatively smaller, with third-, fourth-, and fifth-stage larvae inhabiting the abomasal gastric glands [19]. *Trichostrongylus* species are a significant problem in small ruminants due to the high fecundity of female parasites, the longevity of larvae in pasture, and a growing resistance to anthelminthic drugs [20]. Infection is initiated with the ingestion of L3 larvae in pasture, which subsequently invade the mucosa, where lesions develop as circular thickened areas several centimetres in diameter [21]. Affected animals are usually asymptomatic but may display diminished appetite and progressive weight loss, with young animals highly susceptible to the infection [22]. *T. axei* is the only parasite of the genus that can be found in the abomasum and has been recorded in cattle, sheep, goats, deer, pigs, and horses. *T. colubriformis* and *T. vitrinus* are primarily found in the intestines of small ruminants [2][23][24]. *Mecistocirrus digitatus* is a blood-feeding trichostrongyle with a pathology like that of *Haemonchus*, although *M. digitatus* adult parasites are much larger at approximately 40 mm and are typically found in tropical climates [25]. These larvae exhibit haematophagic behaviour and heavy infections will cause the common Trichostrongyloidea associated symptoms [20]. *Nematodirus* spp. are primarily intestinal parasites with *N. battus*, *N. fiticolis*, *N. spathiger*, and *N. helvatianus* affecting ruminants. *N. helvatianus* is the only species found in cattle, with the rest infecting small ruminants. These parasites are comparatively less pathogenic, but larval stages usually cause enteritis and intestinal necrosis. *Cooperia* spp. are important parasites in both cattle and small ruminants but are more often found in small ruminants. These are small intestinal worms, and the major species are *C. oncophora*, *C. punctata*, and *C. pectinata*. *Bunostomum trigonocephalum* is a hookworm that occasionally infects sheep and goats. *Bunostomum* infections can occur either via oral ingestion or direct skin penetration by the infective larvae [20]. *Oesophagostomum* spp. are primarily large intestinal nematodes of cattle and small ruminants. *Oesophagostomum columbianum* and *O. venulosum* occur in sheep and goats. Similar to the other GIN infections, transmission can occur via ingestion. Larvae penetrate the intestinal wall and become encysted, forming multifocal nodules throughout the gut, and can remain in the nodules for up to a year. The nodular lesions are used to identify *Oesophagostomum* infections during necropsy [2][20][23].

2. Major GIN Infection Control Strategies against Nematode Infections

Control measures used against GIN infections are categorized as chemical, biological, and vaccination methods [26]. A fourth category that combines elements of these three is referred to as integrated parasite control. Chemical control is carried out using anthelmintic drugs and is the common method used to control GIN infections globally. Anthelmintics can be applied either as chemotherapy in infected ruminants or for chemoprophylaxis, a pre-emptive measure in susceptible animals against potential parasitic infections. However, the continued usage of the same anthelmintic drug has caused the rapid development of anthelmintic resistance by the nematode parasites, food

safety issues due to drug residues, and a limited availability of the effective drugs due to their high cost [26]. Other studies have administered drugs as topical applications to control nematode infections [27]. A study conducted in sheep with eprinomectin showed that it was effective against a host of nematodes, including *H. contortus*, *N. battus*, *N. spathiger*, *O. venulosum*, *T. circumcincta*, *D. filarial*, *C. curticei*, *T. axei*, *T. colubriformis*, and *S. papillosus* [28]. The use of eprinomectin orally in goats has been common due to their high susceptibility to GIN infections [29]. It has been argued that doses administered are suboptimal, with indications that this may lead to nematode resistance, which is more likely to emerge in goats when compared to other ruminants [30]. As an alternative, natural plant-based agents have been used as an alternative for controlling nematodes [31]. Herbal formulations of leaves from *Azadirachta indica* and *Nicotiana tabacum*, flowers from *Calotropis procera*, and seeds from *Tachyspermum ammi* have been shown to reduce the percentage of nematode eggs by 42%, 52%, and 70% respectively [32]. Tanniferous plants or tannins have also been used as an alternative approach for controlling the infection with varying degrees of success [33][34]. Other biological prevention measures include the introduction of the nematophagous fungi (*Duddingtonia* spp.) into pasture to reduce the pre-parasitic stages of GIN infections [26][33][34]. Integrated parasitic control measures utilise chemical, biological, and biotechnological control methods to reduce the extensive usage of chemicals and to achieve long-lasting protection in susceptible animals. A common example is the combination of both anthelmintic treatments and grazing management practices which together facilitate a more effective way of controlling parasites when compared to using them alone [26].

2.1. Vaccination as a Parasite Control Method

Vaccination could be considered the safest and most cost-efficient way to control GIN infections, as it offers a natural and chemical-free method that does not contaminate grazing pasture and is an effective prophylactic method when compared to anthelmintics, which can lead to parasite resistance with repeated use [26][35][36]. Vaccination using the irradiated bovine lung worm *D. vivaparus* has been successful when compared to previous attempts that used antigen preparations from adult worms, thus indicating the importance of hidden and conformational epitopes that can only be presented by living but non-infectious parasites produced via irradiation [37]. With the commercial success of irradiated *D. vivaparus* as a vaccine, other irradiated GIN trials have been attempted, including gamma-irradiated *B. trigonocephalum*, *Oe. Columbianum*, *T. colubriformis*, and *H. contortus* in sheep; *A. caninum* in dogs; and *Trichinella spiralis* in pigs. Nematodes are large extracellular parasites with complex genomes that are required for escaping various immune responses for successful establishment in the host [38]. An effective vaccine would need to stimulate many different host immune pathways to sufficiently survive subsequent infection. Using whole irradiated L3 larvae that have lost their ability to establish an infection but are still alive and therefore mimicking a natural infection, ensures that the host is able to induce all the variable, but pertinent immune response is required to prevent infection [39]. It is therefore not surprising that most of the experimental vaccines do not confer sterilising immunity but rather reduce parasite shedding to a minimum which, when combined with other integrated control measures, would effectively stop all infection in a herd; e.g., irradiated *Trichostrongylus*, with an efficacy of almost 80%, is sufficient to prevent disease [40]. In order to reduce shedding, three parameters need to be fulfilled. First, the larvae need to be less infective with a compromised ability to establish as adults. In addition, the number of worms shed needs to be lower compared to natural infections due to compromised pathogenicity after irradiation, and lastly, reduced fecundity amongst adult female worms that results

in decreased egg numbers must occur [41]. When *A. caninum* L3 larvae irradiated at 400 Gy were used as a vaccine, approximately 75% of the irradiated larvae failed at gut establishment after dying in the lungs, with larvae in vaccinated dogs a great deal less motile and thus unable to evade the vaccinated host immune response when compared to naïve dogs [42][43][44][45][46]. The presence of dead larvae in the lungs of vaccinated dogs stimulates a strong immune response, and although they continue to shed parasite eggs when challenged, the numbers are much lower when compared to a natural infection, and they do not display any of the symptoms seen during natural infection [45]. This was however not replicated in 2-month-old lambs, with poor responses upon challenge [47][48]. Further experiments with lower radiation doses were also explored [49]. Revived studies using *H. contortus* parasites irradiated at the lower dose of 200 Gy was effective when used to immunise 4-month-old goats [39]. This would suggest that previous experiments that did not test the viability of irradiated parasites before inoculation failed to provide younger animals with crucial conformational antigens that are necessary for inducing protection. Using doses that kill instead of producing live but non-infective parasites has negative consequences, especially when immunising younger animals [39]. It would therefore be advisable to analyse the viability of irradiated parasites before immunisation when producing commercial batches. A study comparing murine *Trichinellosis* vaccination with parasites irradiated at 300 Gy to treatment using *Punica granatum* confirmed how effective using irradiated parasites can be in preventing infection [50]. Vaccinated mice exhibited a significant reduction in muscle larvae at 72.5% when compared to a treated group at 56.3%. A combination of vaccination with treatment as an adjuvant was postulated to give higher levels of protection [50]. A threshold of more than 5000 parasites is required to generate immunity, with success seen at 2 doses of 20,000 parasites [51]. A study using gerbils as a model host for ruminant GIN infections revealed strong mucosal antibody responses following vaccination and challenge [40].

The ruminant host immune system can also be stimulated by ES parasite antigens/proteins that can be utilised as successful vaccine candidates compared to using whole parasite vaccines. In some cases, such as *O. ostertagi*, the recombinant version of the purified protein did not elicit the expected response and performed subpar when compared to the purified native protein, indicating the complexities involved in developing parasite vaccines [52]. Due to their complex structure and pathogen–host interactions, GIN vaccines require various considerations. This includes understanding the exact level of protective immunity that should be induced by the vaccination, selection of the most suitable antigen able to elicit an effective level of immunity, and selection of the most appropriate vaccine formulation, such as adjuvants, to obtain optimal success in a combination of the antigen and mode of delivery [26][53]. The development of immunity to the appropriate vaccine antigen will however not perform equally in all ruminant recipients, as this will further depend on various host factors. These include age, breed, genetics, individual animal variation, the plane of nutrition, health status, level of management, climate, and the complexity of the parasite vaccinated against [26][53][54][55]. Native parasite antigens as vaccine candidates and commercially viable subunit vaccines for ruminant GIN infections have recently also been discussed in recent publications [56][57][58].

2.2. Mucosal Immune Responses in Vaccine Development

The major effector mechanisms, especially in live vaccines, are the production of antibodies, induction of strong $CD8^+$ T cell responses, and $CD4^+$ T cell responses against the infection. However, in most situations, inactivated or

killed vaccines do not produce a sufficient quantity of antibody titres in mucosal surfaces [59]. Exposure of GINs to mucosal surfaces often facilitates the development of mucosal immunity in both the innate and adaptive arms of the immune system [60]. However, almost all vaccines developed to date have been applied to the host through non-mucosal parenteral routes, directly to the cells of the immune system in tissue or blood where the major effector mechanism of mucosal immunity is not secretory IgA, but $CD4^+$ and $CD8^+$ T lymphocyte-mediated immunity. More than 80% of the entire mucosal lymphoid population is comprised of T lymphocytes, while $CD4^+$ Th2-type responses are more effective in inducing mucosal immunity in sheep. In addition, eosinophil production, mast cells primed with specific IgE production, increase in the smooth muscle contractility, increase in the epithelial cell turnover rate, and mucus secretion are other effector mechanisms involved in parasite expulsion [60][61][62]. For gastrointestinal tract diseases, particularly parasitic infections, the oral route will be the preferred route of vaccination, as this would mimic how GIN infections are acquired and this would generate an effective immune response on mucosal surfaces. Intranasal vaccines are used to induce a rapid interferon response basically due to the higher concentration of NALT in the respiratory lymphoid system. The absence of interference with maternal antibodies will be an added advantage of intranasal vaccines. Induction of NALT also has the effect of inducing immunity in other mucosal surfaces because of the common mucosal immune response theory [63][64]. Oral vaccines are exposed to NALT as a major portal entry, where they induce a rapid immune response. Immunization of GALT is largely dependent on the reaching time of the vaccine to the lymphoid tissue such as Peyer's patches. If oral vaccines were administered within the first 24 h of birth, there would be a high risk of neutralizing the vaccine by the maternal antibodies of the colostrum [65]. Several experimental vaccines have attempted mucosal application. In a study conducted with *H. contortus*, mucosal antigen extracted from the parasite was inoculated into the abomasum and rectum via intra-mucosal injections; significant lymphocyte proliferation in the abomasal mucosae was detected following vaccination [66]. In another study, 3-month-old lambs were immunized via the intranasal route with a recombinant part of the catalytic region of the serine/threonine phosphatase 2A (PP2Ar). The immunized lambs showed a strong immune response with reduced faecal egg count against *Haemonchus contortus* and *Teladorsagia circumcincta* parasites, establishing the possibility of using the intranasal route to induce immunity against GIN infections [67]. Other additional advantages of using mucosal vaccines include a long-lasting immunological memory, the development of herd immunity due to secondary contact immunization, and easy administration [68].

2.3. Parasite Immune Modulation of the Host Mucosal Microenvironment

The gastrointestinal tract in ruminants is home to a variety of commensal microorganisms that play a large role in host nutrition, homeostasis, and the development of the host's immune system [69]. The effect of GIN infections in the gut can be quantified in the increase or decrease of alpha and beta diversity in the host gut that signifies the regular mean microbial diversity and the ratio between the normal and nematode-infected microbial species diversity, respectively [69][70]. It has been shown in several GIN infections that infecting nematodes are able to modulate the population of the host microbiome to their advantage. In a trial vaccination study using *T. circumcincta* in lambs immunised with a cocktail of recombinant antigens, 16s rRNA sequencing of faecal samples from both immunised and control groups was carried out [70][71]. Results showed an overrepresentation of *Prevotella* spp. in vaccinated and adjuvant groups compared to untreated groups post-challenge [70]. Members of

the genus *Prevotella* are associated with peptide degradation, and their increase is hypothesised to compensate for low protein levels during *T. circumcincta* infections [69]. It has been proposed that communication between infecting nematodes and gut microflora is facilitated by helminth derived extracellular vesicles (EVs) that contain immunomodulatory factors, such as transforming growth factor β (TGF-β) and peroxiredoxins, that induce responses to infection and regulate anthelmintic type-2 responses [72]. EVs have also been implicated in parasite migration during changing larval stages and for nutrition, and their presence in the gut can affect a number of beneficial commensals such as probiotics [73]. A deeper understanding of how parasite mucosal vaccines are affected by changes to the microbiome could help develop new vaccine antigens and increase the efficiency of vaccine delivery in the host.

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