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Research article

Haemonchus contortus: Review of recent molecular advances in anthelmintic resistance and vaccination

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Abstract

Haemonchus contortus is one of the world's major financial worms that attack ruminants. It is a blood-sucking parasite founded in the abomasum, particularly in cattle, sheep, and goats. Nematode infections may cause anemia, weight loss, or even death in animals that are severely affected. Current management practices against H. contortus largely depend on regular anthelmintic therapies in all countries with varying incidence across various regions.

H. contortus thus aims to form new action techniques in order to overcome resistance to anthelmintic agents. One option is a logical approach focused on a thorough knowledge of the molecular pathways in growth and reproduction cycles in the manufacture in modern anti-parasite drugs and vaccines. Key molecules may be defined as potential drug targets by a simple description of molecular, biochemical functions.

Besides, it is immediately essential to formulate immunological control of farm animal nematode infections. Important prevention has been accomplished after vaccination with native protein extracts, which shows that vaccination is possible. This paper explores the success of H. contortus science in the world. Particular fields of concern include epidemiological research, genetic analysis, and anthelmintic resistance identification using traditional and molecular techniques; morphological and chemical research of crucial molecules in mechanism expansion, parasitic organism-host interaction, and vaccine research. In the suggested form of these opinions, areas for potential exploration and alternatives for new or revised prevention strategies are described.

Keywords: Control, Vaccination, genetic, *Haemonchus contortus*, Ruminant.

Introduction

Nematodes have developed to take advantage of a wide variety of natural habitats. Although most stabilize a free lifestyle to reach maturity (Fig. 1), they depend on one or more hosts (1). A complex sequence of morphological features is taking place in several parasitic nematodes associated with migration from their hosts to create a mature infestation (2). Life cycles may include transitional hosts, vectors and, environmental-based stay when confronted with extreme and unpredictable circumstances, including freezing or dehydration (3). The risks, namely predation, atmosphere, and immune reactions, of a wide variety, both plant and animal hosts have been adapted (1–3). The evolution of parasites affects public health (which amounts to 10 million life-years adjusting to disabilities), or indirectly by major financial failures in plant and animal production and by investing in parasite control (4). Anthelmintic medications are used as a

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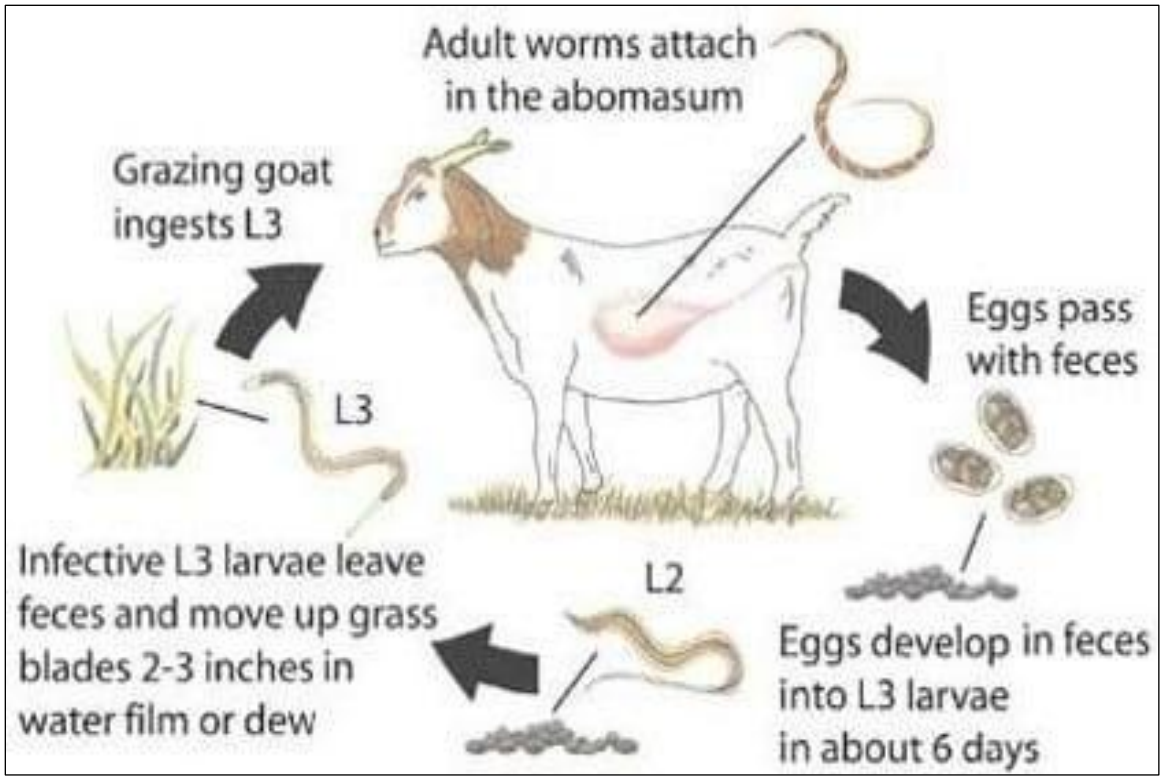
monitor, provided recurrently in livestock and through large-scale medical preventive initiatives (5). Although the effectiveness of these approaches was initially inevitable, the appearance or documenting inefficiency in human-infectious organisms of medicinal-resistant veterinary worms challenges continuous prevention efforts (4–7)



Fifure 1: The life cycle of *Haemonchus contortus.* https://u.osu.edu/sheep/2019/07/30/ag-note-parasites-focus-haemonchus-contortus

Vaccines provide an enticing alternative control method for these parasites: however, while two approved vaccines are presently accessible for veterinary purposes, the vast parasite heterogeneity and immune procedural mechanisms are significantly hindered in the production of vaccine; transcriptomic plasticity after the vaccine attempt may be used to prevent infections (8). It is thus obvious that modern, efficient control methods are essential. Helminths can respond to control measures and therefore evade them because of genetic variation (9). A deeper understanding of the nature and dynamics of this diversity across its spectrum can provide better knowledge of the pathways by which they are modified and can recognize new goals that can be manipulated for influence (8,9).

The *H. contortus* (trichostrongylide) is a gastrointestinal worm of ruminants in tropical and temperate areas around the world, which has substantial effects on the economic and animal health of the sheep farm in a unique way (10). It also emerges as a prototype of a parasite nematode framework for functional and comparative genome sequencing, primarily due to its quick capability to collect resistance to medicines, its nearly equivalent tractability in controlled circumstances, the growth of enormous genomic inputs, and its special bond with other veterinary and medical nematodes of clad V (12,13).

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Resistance to anthelmintics

The fundamental strategy for the containment of parasitic nematodes like H. contortus is focused upon the utilization of anthelmintics, but an anthelmintic resistance has over-grown. Resistance to anthelmintic in several areas of the planet was identified and published (6). A concise-short study of the nature of anthelmintic resistance in several nations refers to the occurrence of the resistance in countries like the United States, Mexico, South Africa, Australia, New Zealand, European countries, and China. Albendazole, fenbendazole, and ivermectin were used to treat in dairy goat farm (400 Goats) with confirmed infection (all above 80%) (14). Albendazole and ivermectin (IM) showed low activity both, but fenbendazole displayed high effectiveness (6). This study showed a fecal egg count reduction test (FECRT) of 23,72% for the routine dose rate (5 mg /kg) of albendazole. In this field, ivermectin has not been successful, with a prescribed FECR of 52.29 percent (0.2 mg/kg) and a 2-fold FECR of 89 percent (0.4 mg/kg) (6,14). The drug resistance was tested using albendazole, levamisole, ivermectin, and a mixture of albendazole and ivermectin to check performance against the nematode. The findings were successful in the cases of levamisole, ivermectin, and the combination. The achievement of an FECR was above 95% and an FECR of albendazole of below 95% (6,14).

Molecular identification of anthelmintics resistance

Resistance to Benzimidazole

Three specific β-tubulin SNPs were seen to be linked to H. contortus with resistance to benzimidazole (BZ), identified as F167Y (TTC to TAC), E198A (GAA to GCA) and F200Y (TTC to TAC) (15). A nucleotide sequence modification has resulted in alteration in the target protein and decreased BZ binding to the β-tubulin (16). In five H. contortus species, including two sheep and three goats species, a multiplex PCR for the β-tubulin consistent with BZ resistance has been established, which suggests that the resistant genetic makeup is not found in any observed community of the nematode (15–20). In the meantime, an analysis utilizing PCR-SSCP in the area of the F200Y community H. contortus of sheep showed a plurality of homozygous prone genotype (21). Also, one analysis employed PCR-RFLP system to detect F200Y in H. contortus sheep populations, which revealed that the homozygous prone genotype is the most common (21). In various regions, a resistance was earlier revealed in the detection of all three known SNPs in a β-tubulin gene with PCR-coupled sequencing, which has been commonly used for the management of worm load (21).

Molecular approaches may be utilized, but they could not test resistance at the quantitative rates, as a diagnostic aid in detecting BZ resistance in populations (22). In fact, in combination with this field, molecular testing and biological testing are to be used (23). To date, the molecular research of the identification of BZ resistance in field samples, including several species of nematodes, has not been tested explicitly for this reason (22). Moreover, in fecal samples that included nematode eggs (Fig. 2) directly, molecular trials were not used, which could save a great deal of time. For now and on, whether current approaches or modern molecular production are used (22,23).

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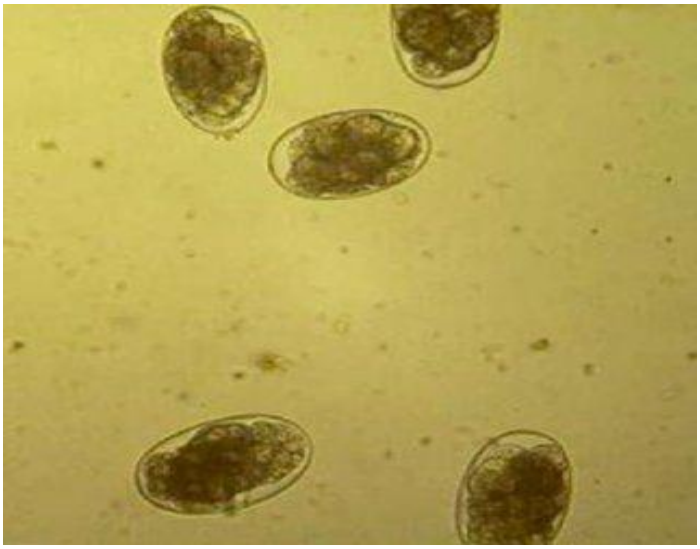

Fifure (2): The egg shape of Haemonchus contortus.
https://www.researchgate.net/publication/262932569_Efficacy_and_Safety_of_Albendazole_against_Haemonchus_Contortus_Infestation_in_Goats/figures?lo=1&utm_source=google&utm_medium=organic

Resistanse to Ivermectin

IM also relates to the leading anthelmintic groups and has been used disproportionately, contributing to widespread resistance (24). In addition to numerous genes that code for IM targets and efflux pumps, resistance to IM is considered to be very multi-gene based nature (25). SNPs around the entire genome were scanned using a 2b-RAD sequencing tool for further IM resistance-associated SNPs in goats in both sensitive and resistant nematodes (24–26). 2962 SNPs and 2667 SNPs in resistant isolates have been identified (27). Among resistant or sensitive species, identical and relatively smaller genetic variations were observed (28). However, 208 SNPs with a significant variation, 24 of which were SNPs (29). This process is likely to be the primary selection of IM, with seven of the nines genetic markers expected to code for those proteins, which may play a crucial role in the IM target or efflux pump and even in receptor complex factor proteins, including membrane or neurons for transcriptional regulating proteins (30). It was suspected that these genes are correlated with IM tolerance. The findings of this research revealed genes affecting the detection of IM and correlated with IM resistance in the genome of the nematode (31). Extensive SNP analysis utilizing the 2b-RAD sequencing method may be used to classify the worm (31).

Haemonchus contortus and diapause-presented genetic markers

Haemonchus contortus may evolve diapause while on an infective-free-living L3s (iL3s), or beginning of the L4 (Fig. 3) of the nematode stage in order to safeguard it against a harsh environment (32). Knowing this transformation could reveal major molecules as new drug attractions and provide new insights into protection and management (33). The L3 (iL3s)-based nematodes have been proven to have higher common phenotypic, behavioral, and biological characteristics with the long-standing phase of free-living Caenorhabditis elegans (34). The "dauer hypothesis" considers that the iL3 is comparable to that of the dauer stage of C. elegans in terms of developmental and

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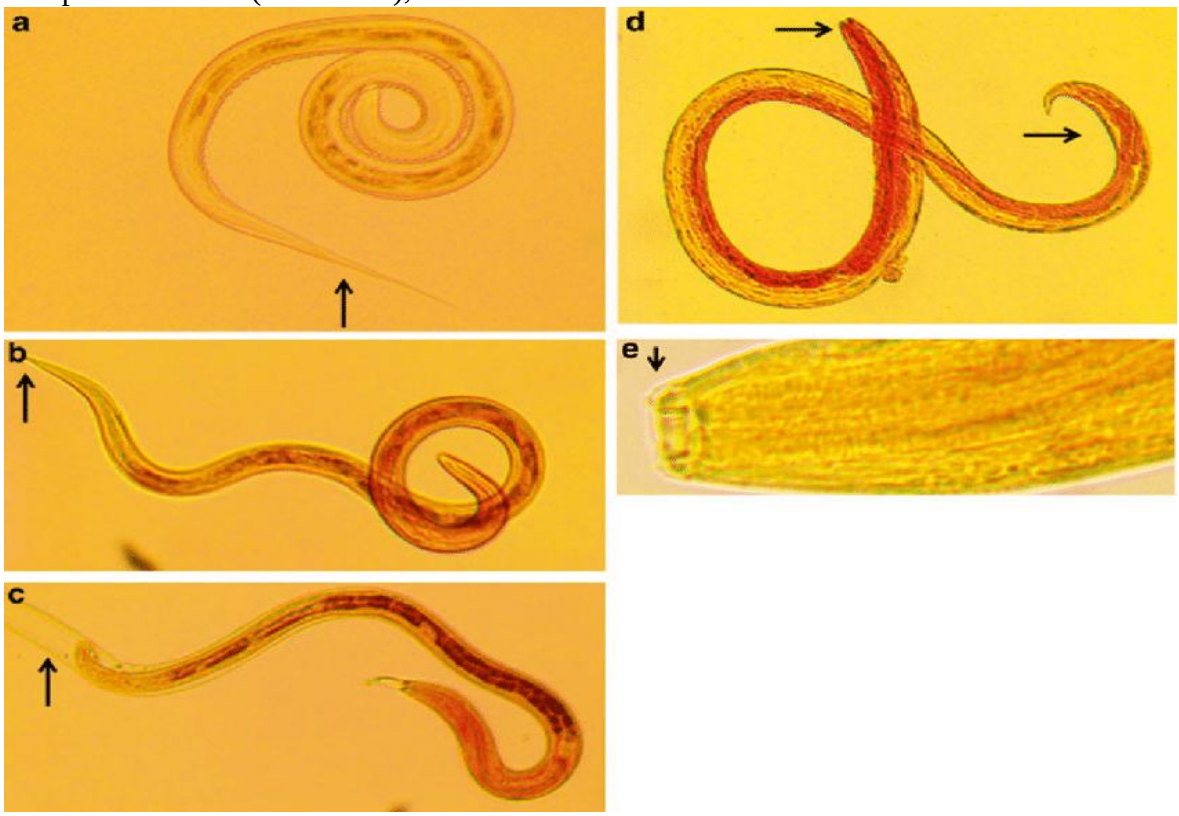
functional applications and controlled by a relatively similar method (35). The insulin-like component regulates the long-term development of C. elegans preserved in many parasitic species (35). The insulin-like signaling pathway genes are fork head transcription factor (Hc-daf-16), insulin receptor (Hc-daf-2), phosphoinositide 3-kinases (PI3Ks) component catalytic subunit (Hc-age-1), regulatory subunit (Hc-aap-1), and phosphoinositide dependent protein kinase-1 gene (Hc-pdk-1) (32–35).



Fifure(3): The Larva shape of Haemonchus contortus
https://www.researchgate.net/publication/266152011_Evaluation_of_the_Role_of_Galectins_in_Parasite_Immunity/figures?lo=1

They are intestinal genes which regulate the growth of the nematodes (36). It was found that C. elegans daf-2 mutant can be rescued by Hc-daf-2, which means that similar functions of Hc-daf-2 to Ce-daf-2. PI3K kinase, Hc-age-1, and Hc-aap-1 are the downstream of Hc-DAF-2. The C. elegans age-1-mutant can not be rescued by the Hc-age-1 like Ce-age-1 (36). RNAseq analysis shows that these four genes have the maximum amount of transcription expression in iL3, which displayed that these genes could play a significant role in controlling iL3 against an unpleasant surrounding environment (36). In summary, the results from these experiments indicate that insulin-like signals are functionally conserved between the two worms. In contrast, a bioinformatic analysis further supports the mapped insulin-like signal route of transcriptomic and genetic information for this parasite (36).

Besides, researchers are actively working on other signals such as a TGF beta signal pathway that is important for the normal growth of C. elegans, and their function in H. contortus diapause in iL3 must be understood (37). Besides being in a position to protect themselves from drying, H. contortus iL3s have also established genes that participate in this biological cycle (38). Two versions of the major gene transcript were called Hc-ubq

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and Hc-gst based on their homologous ubiquitin in C. elegans and glutathione-S-transferase in H. contortus, respectively (37,38). The silencing in L3 of H. contortus of Hc-ubq or Hc-gst by RNAi decreased the rate of survival, leading to its resistance (37). The Hc38 silencing, first observed with the blot of Northern and strongly expressed in the intestinal microvilli by in situ position, was decreased by 50 and 48.6 percent, respectively by taking iL3 from H. contortus into dsRNA, which were utilized to invade sheep (37,38).

The L4 has short body, low metabolic rate, and crystal rod-like in the intestinal canal to safeguard them in the cold niches (39). Gene expression includes Hc-daf-22 (3-ketoacyl-CoA thiolase), Hc-maoc-1 (enoyl-CoA hydratase), and Hc-hsp-70 (heat-shock protein 70). Hc-daf-22 and Hc-maoc-1 have similarities in C. elegans, Ce-daf-22 and Ce-maoc-1, respectively, and may act in the growth of the nematode and its peroxisomal β-oxidation (40). The Hc-hsp-70 overexpression manages the reduction of hsp-1 in C. elegans, indicating similar activity to that of the Ce-hsp-1 that helps of parasitic survival and invasion. Hc-fau, a human fau and C. elegans Ce-rps30 analogs (ribosomal protein S30 coder), with a conserved domain of the S30 (expressed in the nucleus) and a diverged ubiquitin-like (UBiL) protein domain (cytoplasm-located expression) (41). They act in the egg-laying process and the life-span range of the C. elegans, indicating potential activities in L4 diapause based regulation in H. contortus (42).

Vaccination

Increasing consumption of anthelmintics for the care and mitigation of human and animal parasitic nematode diseases has created significant problems with anthelmintic and pharmaceutical resistance around the world (43). The vaccine is an effective method to manage parasitic nematodes, like H. contortus (44). Remarkable improvement in the identification of several possible antigens from H. contortus over the past two decades has been done in order to enhance influential immune thresholds in the immune system (43,44). Here, recombinant subunit vaccine and DNA vaccination have been synthesized the molecular properties and defensive potency of the key-antigens (44).

Recombinant gene subunit-based vaccines

In terms of the production of consumer vaccines, special attention has been given to gene recombination, an essential biological innovation (14). Partial safeguards with H. contortus recombinant antigens has been granted for immunization (45). A 110kDa central membrane glycoprotein complex was the strongest described nominee for the H. contortus antigen named H11 (14,45). The immunogen (> 90% decrease in fecal egg counts (FECs), > 75% lowering of the weight on a worm) (45). Consequently, some scientists anticipate the recombinant vaccine for potential large-scale development to be produced to promote the realistic usage of the hosts for immune defense (14). Escherichia coli had H11-1 and H11-2 variant expression, which had slightly higher aminopeptidase activity than H11-1 with enzyme activity (14,45). It has been inoculated twice with H11-1, H11-2 and H11-1 and H11-2 mixed with phosphate buffered saline. Partial protection has been afforded through immunization with H11-1 and H11-2 blends (29% reduction in FECs and 18% lowering of worm load) (14,45). The three (H11-1, -2, and -3) fragments of H11 gene have also been inserted in the vector for yeast expression and the lithium chloride

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process used to convert recombinant Pichia Pastoris X-33. RT-PCR identified transcriptions and SDS-PAGE, and Western blot verified glycosylation of the proteins. Even so, the recombinant molecule containing three isoforms of H11 did not show protection (14,45).

As a medium, Celegans were also attempted to convey H11 to boost safety apart from E. coli and P. pastoris. (46) In this experiment, a flanking area of 1517bp 5″ and part of the first H11 exon. Celegans are subcloned into the upstream region of the pPD95,77 vector, the contortus and homologous genes of the C. elegans were respectively sub-clones (47). Microinjected into the C. elegans gonads, respectively. The findings revealed numerous trends of transcriptional expression powered by free-living and blood-sucking nematodes from their promoters, demonstrating the heterogeneous supply of C. elegans (46). The trans-HPS recombinant has been developed (a 1710 bp isoform gene fragment of the H11 gene) (46). Vaccination from transgenic worms with crude Trans-HPS led to a decrease in the FEC's by 38% and a decrease in worm weight by 25% (46,47). However, E. coli presented that in the immunization studies sheep were not secured by a gene fragment from 670 bp to 1710 bp isoform H11. In addition to possible antigen H11, the recombinant galectin Hco-gal-m / f (obtained respectively from male and female nematodes) is expressed from H. contortus in E. coli Vaccinations with 200 µg protein reduced 48-46 percent fecal egg output, with Freund's adjuvant. Vaccination with a mixture of Hco-gal-m / f recombinant proteins has a function to play in the defense of goats (46,47).

DNA-based vaccines

DNA vaccination is a bioengineered DNA defense strategy that allows cells to actively produce antigens and vaccinated animals to develop safe immune responses to diseases (48). In contrast with traditional vaccines, DNA vaccines have several benefits, including a broader variety of specific immune responses (48). This is a new approach to managing infectious parasites. For the illustration, direct injection of antigen-specific immune-based plasmid codes for exogenous antigenic release (48). Several observations of partial safety in goats after DNA vaccination were reported and identified. Immunization of goats between 8 and 10 months of age, with HC29, DNA encoder (48). Different antibodies and partially immune defense (36% declines in FECs and worm burdens), comparison to goats who obtained just PBS, have been caused by the GPX (contortus glutathione peroxidase) (48). Vaccination with DNA Vaccines, comprising three fragments, encoding sections for H11-1 and caprine interleukin 2 (IL- 2), led to a significant reduction of the fecal output, the abomasal worm and lymphocytes by 57 and 47 percent of specific serum immunoglobulins G (IgG), IgA, CD4 + T lymphocytes and CD8 + T lymphocytes (48).

In H. contortus, Glyceraldehyde-3-phosphate dehydrogenase, as a protection against experimental infections of H. contortus in goats was evaluated with DNA vaccine (49). The analysis indicates that the vaccinated adds essential qualification peripheral and local mucosal immune responses and supported the development of lymphocytes of CD4+T and B but supplied only limited protection in comparison with control groups (35% decrease in FECs, and 38% decrease in the worm weight) (49). DNA vaccine may be much more secure (46% decrease in FECs and 51% decrease in worm burden) against the resulting disease in the goats through vaccination of disorganized muscle family member (Dim-1) (49).

The processes recognition of the immune control in order to identify useful vaccine antigens. Several experiments

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were performed during a parasite invasion to examine the function of immune suppression by studying the interplay of parasite and host cell molecules (50). To achieve so, venipuncture has isolated the target peripheral blood mononuclear cells (PBMCs) of goat blood. PBMC is the mixture of activated cell subpopulations, primarily lymphocytes, monocytes and dendritic cells (T cells, B cells and NK cells) (50). The process for communicating host cells with several essential parasite molecules is crucial for any subpopulation. For instance. The Hco-gal-m / f (rHco-gal-m / f) recombinant Contortus Galectin peptides are grown using PBMC of caprines and investigated in the PBMC the impact of rHco-gal-m / fon the induction of apoptosis (50). The pathways underlying immunomodulation caused by rHco-gal-m / f were also studied together in a proteomic and transcriptomic way (50). PBMC. The results indicate that rHco-gal-m/f can bind to the surface of the PBMC and serve as an inflammatory reaction to ease H. contortus immune escape (50).

Based on the analysis, two H-co and F binding partners, Transmembrane Protein 147 (TMEM147), and Transmembrane Protein 63A (TMEM63A) were also discovered through further yeast two-hybrid testing (51). The communication between galectin and TMEM147 mainly mediates cell division, death of cells, and the transcription of cytokine in the PBMC (51). Along with TMEM63A, this membranous protein participates in the control of galectin and the development of the PBMC in the phagocytosis and nitric oxides. Even so, in the migratory galectin control and IFN-β transcript of goat PBMC, TMEM63A performs a significant role than TMEM147 (51). These research results provide a unique view for the clarification by nematodes and parasite-host interaction of the processes involved in immune avoidance (51).

As a vaccine nominee, H. contortus excretory and secretory items include different proteins that may activate or suppress the host's immune system and are implicated in the pathophysiology of worms (52). Work suggested that the PBMC in vitro is suppressed by H. contortus excretory and secretory items. The items were used to inhibit IL-4, IFN-α supply, boost cytokine suppressive IL-10, strengthen inflammatory modulator IL-17, and repress the manufacturing of the nitric oxide (52). Protein engagement with the PBMC in vivo using liquid chromatography/tandem mass spectrometry from these materials in various developmental stages showed a total of 407 particles were detected interacting with the PBMC, 14-3-3 protein in all parasites as PBMC-interacting proteins (52). IL-4 output declined, and the PBMC in vitro proliferation repressed by the 14-3-3 isoform 2 (rHcftt-2) (52).

In addition , an important immunogenic component was also observed in 24 kDa H. contortus excretory / secretary proteins (53). The immune interactions between HcES-24 and Goat PBMC recombinant protein have shown an improvement in IL 4, IL-10, IL-17, and cell migration. Nonetheless, PBMC multiplication and NO development were greatly inhibited by the relationship (53). The results showed that the rHcES-24 had significant regulatory impacts on the PBMC (53).

Conclusion

The use of molecular-based approaches is critical in identifying the resistance type and its mechanism for the best utilization of therapeutic drugs against *Haemonchus contortus*.

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