The growing resistance: an overview of *Haemonchus contortus* and a proposed novel PCR method for its identification in small ruminants

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Background

For thousands of years, animals have been bred and raised with the intention of collecting products from them including milk, meat, and fiber. Civilizations across the globe developed methods of caring for large groups of animals so as to increase production. However, due to dense stocking rates, shared feed, and limited pasture, these animals are faced with a number of threats to their health. In the modern age of farming, the most dangerous threat to these animals is parasitic infection. This is due to the commonly used method of mass drug administration, in which entire herds are regularly treated with anthelmintic drugs. As a result of constant treatment over the past fifty years, parasitic resistance is now a worldwide issue, and broad spectrum anthelmintic drugs are losing any effectiveness (Kaplan 2004). *Haemonchus contortus*, also known as barber pole worm, has been named the most economically important parasite of sheep and goats (Laing et al. 2013).

Based on genetic data, the species *H. contortus* originates from an ancestral population in Africa, and spread corresponding with major human migrations and associated pastoral movement across the world (Salle et al. 2019). Sheep domestication first occurred in the Middle East around 10 thousand years ago (Larson et al. 2014). The gathering of large mammals, shared food sources and limited pasture all contributed to the propagation of parasites within these species. There was also a widespread increase in rainfall across central Africa about 10.5 thousand years ago, which would have supported *H. contortus* population expansion and dispersion (Campbell and Tishkoff 2010). Sheep were introduced to Eastern Africa and likely spread south through cultural diffusion. As sheep farming spread, *H. contortus* did as well. There is evidence that many parasite populations spread and mixed with one another through the transatlantic slave trade and colonization (Geggus 2001, Spangler et al. 2017). Today, *H. contortus* is found in both tropical and temperate regions across the world (Salle et al. 2019).

H. contortus is a gastrointestinal nematode of the family Trichostrongylidae. It feeds on blood within the abomasum after being ingested from the pasture. In the course of a day, an

adult barber pole worm can remove up to 30µl of the host's blood, and a single host can be infected with thousands of worms (Anderson et al. 1978, Dineen et al. 1965). The life cycle is about 20 days, the shortest of any gastrointestinal nematode, and each female produces between 5,000-15,000 eggs per day (Anderson et al. 1978). The adults feed and reproduce within the abomasum, or true stomach, of the host. The eggs produced by the female are passed in feces, and within the feces the eggs hatch. The larvae go through two phases of development (known as L1 and L2), which can take as little as 3-4 days if the environmental conditions are ideal. The L3 larvae then migrate up blades of grass through drops of moisture, and are ingested by a new host. From there, the L3 larvae enter the abomasum and develop into L4 larvae, the first blood feeding stage, then full adults (Zajac 2006).

The primary animal hosts of *H. contortus* are sheep and goats, with goats being considered the more susceptible of the two (Cotter 2019). However, *H. contortus* has been found to reproduce in several other species, including cattle, llamas and alpacas, and can also undergo limited development in some wildlife species. The usual clinical signs of infection in the host include weight loss and decelerated growth. More severe signs include anemia, hypoproteinemia, submandibular edema (or bottle jaw), weakness and collapse. The longer the infection is left untreated, the greater the risk of mortality. Unfortunately, the infection is difficult to diagnose early, because the initial symptoms are subtle and often go unnoticed, especially in larger herds on commercial farms (Hepworth et al. 2006).

Benzimidazole is one of the most common general anthelmintics used to treat *H. contortus* infection. Benzimidazoles have a high margin of safety, and therefore can be administered in double or triple doses to more effectively kill parasites without risking the host's life. Unfortunately, this has contributed to the development of resistance, as this has been the "go-to" treatment for many years (Hepworth et al. 2006). Benzimidazole functions by binding to tubulin monomers within the parasite, inhibiting cell division and leading to death (Mohonraj et al. 2017). It was also demonstrated that there is a direct interaction between another

anthelmintic drug, ivermectin, and tubulin, leading to paralysis and cell death (Ashraf et al. 2015). If these anthelmintics were to be used against a resistant population of *H. contortus*, there would be little to no effect. Instead, this would lead to selection of individuals with chemical resistance, further strengthening the parasite population. Therefore, it is very important to know whether or not an animal is infected with a chemically resistant population before administering anthelmintic treatment.

In response to the rise of anthelmintic resistance in Australia among livestock, governmental organizations established integrated parasite management (IPM) programs. The goals of these programs were to broaden the impact of control measures, to reduce the frequency of chemical treatments to animals, and to prolong the effectiveness of available treatments. Researchers observed all aspects of host, parasite and environment in order to develop different types of control measures (Morley and Donald 1980, Barger 1997). For example, one such program was WormKill, a drenching regime which began in 1984. In animal husbandry, “drenching” is the method of administering drugs by force, usually by applying it into the animal’s throat. This program mainly focused on controlling *H. contortus* infections, as well as black scour worm (*Trichostrongylus* spp.) and small brown stomach worm (*Teladorsagia (Ostertagia) circumcincta*), and encouraged farmers to only drench animals with high parasite loads or during certain times of year (Love 2010). The WormKill program reduced the number of L3 worms on the pasture, which in turn lowered the amount of worm intake by animals. However, this and other IPM programs also led to selection for worms with chemical resistance, which greatly reduced the effectiveness of anthelmintic treatments in the following decade (Emery et al. 2016).

Therefore, a more targeted approach was developed. Researchers continue to investigate every aspect of the parasite’s life cycle in search of new ways to prevent infection. For example, there are studies which look for genetic responses to selective pressure, such as alleles associated with variation in virulence (including increased infectivity and reproductivity),

or the survival of L3 larvae in pasture (Emery et al. 2016). Other studies are focusing on the parasite's physical limitations, which could potentially be taken advantage of to decrease the rate of infection. A recent study by Almeida et al. (2020) has determined that *H. contortus* larvae do not survive as long on pasture during the summer as other months; the hot and dry conditions are detrimental to their survival. Generally, a contaminated pasture will continue to possess infective larvae for approximately six months to a year, depending on the weather and climate (Almeida et al. 2020). It has also been discovered that the general survival strategy of *H. contortus* has changed in the past few decades. About 30 years ago, the ingested larvae would remain in the L4 stage throughout the winter, then develop into reproducing adults in the spring. However, most populations today complete development as soon as they are ingested, and spend the winter as adults. It is likely that this adaptation increases species infectivity (Emery et al. 2016).

The "gold standard" for finding anthelmintic resistance in a parasite population is considered to be the Fecal Egg Count Reduction Test (FECRT), which derives a number of parasite eggs per gram of feces (Baltrusis et al. 2018, Coles et al. 2006). The fecal egg count provides an idea of general parasite load within individuals. It is used to test for anthelmintic resistance by comparing the egg count before and after treatment. However, the test is not very sensitive, and can vary greatly depending on different factors, including the type of forage consumed and how digestible it is. The test uses the number of parasite eggs present in the fecal sample as a proxy for the number of worms in the animal, which is a flawed assumption. *H. contortus* larvae in the L4 stage are already feeding on the host's blood, but are not yet producing any eggs (Shulaw 2011). A host could appear to have a low parasite load based on the FECRT results, but actually contain a heavy load of L4 *H. contortus* larvae. Additionally, different parasite species produce different amounts of eggs daily, so it is hard to truly judge the level of the infection from this test alone, and it is not recommended to make the decision to deworm an animal based solely on its results (Fernandez 2012). Finally, this test requires that

the infected animal is dosed with an anthelmintic in order to determine whether the parasitic population within it is resistant, thereby failing to avoid increasing the level of anthelmintic resistance within the population.

Most often, this test is supplemented by the animal's FAMACHA score (Fernandez 2012). The FAMACHA score takes advantage of the fact that increased parasitic infection in an animal leads to anemia, and the poor circulation is evident in the color of certain tissues, including the tissue of the inner eyelid. An animal is scored by comparing the color of its inner eyelid to a series of color photos on the scorecard (Fig. 1). A healthy animal would have red tissue, whereas an infected animal's inner eyelid would appear anywhere from pink to white, depending on the severity of the infection. The test has been found to be 92% accurate, and is therefore a good indicator of an animal's health (Pons 2005). This test is easy for farmers to conduct in the field on any animals they suspect might be infected (Mobini et al. 2019). Therefore, if an animal has a high egg count and a low FAMACHA score, then it is likely severely infected, and should be dosed.

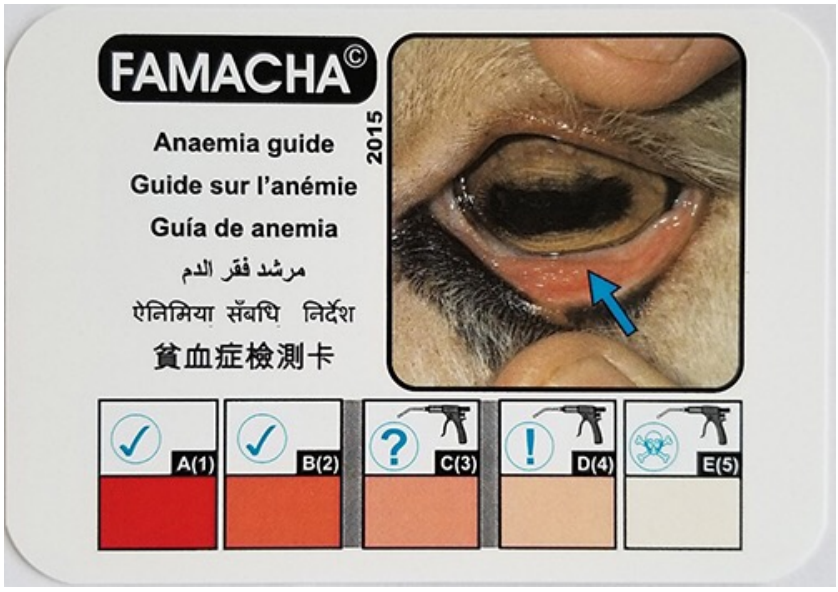

Figure 1: FAMACHA score card. Reprinted from Why and How To Do FAMACHA© Scoring, by Anne Zajac, Katherine Petersson, and Holly Burdett. Retrieved from https://web.uri.edu Copyright 2014 by University of Rhode Island.

While the FECRT and FAMACHA score have both helped lower the amount of mass dosing in sheep and goats, these tests are still insufficient. They provide no information on the species of parasite that the animal is infected with, nor any reliable information on whether or not it is a resistant population.

It is very important that *H. contortus* infection can be diagnosed quickly and reliably, so that treatment can be administered. Unfortunately, *H. contortus* eggs are morphologically very similar to the eggs of less damaging nematodes (Fig. 2), and so they cannot be easily identified using standard microscope methods. The only test that currently exists to diagnose *H. contortus* infection is the peanut-agglutinin (PNA) fluorescence test. The lectin PNA has been found to bind to sugars on the surface of *H. contortus* eggs, but not those of other nematodes (Abbas and Hildreth 2019). Therefore, researchers can tag the eggs derived from fecal samples via sugar centrifugation with fluorescent PNA, differentiating them from other nematodes and allowing them to be quantified (Jurasek et al. 2010). However, this test requires a long process of egg purification and does not provide any information regarding the resistance of the *H. contortus* population.

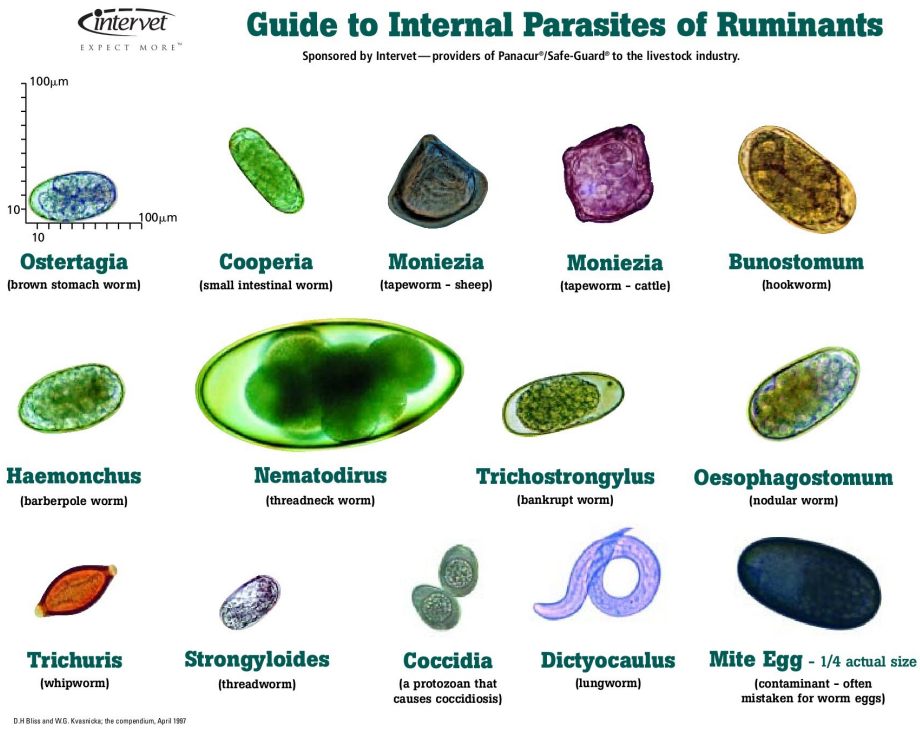

Figure 2: Common parasite eggs found in ruminant fecal samples. Reprinted from Guide to Internal Parasites of Ruminants, by Intervet. Retrieved from https://www.merck-animal-health-usa.com Copyright 2004 by Intervet Inc.

In the past few years, many researchers have undertaken investigations of the gene families within *H. contortus* and other prevalent parasitic species. These investigations have been mostly focused on identifying potential drug or vaccine targets. Population structure can be analyzed using genetic markers, so recent genetic analysis on *H. contortus* and similar species has focused on finding markers associated with developed chemical resistance (Emery et al. 2016). Both benzimidazole and ivermectin are significantly less effective than they were when they were first introduced because of evolved parasitic resistance. Resistance to benzimidazole (BZ) has been discovered to arise from point mutations within the parasite's β-tubulin gene, structural changes to the tubulin that inhibit the drug from binding to it. Three single nucleotide polymorphisms (SNPs) within the *H. contortus* gene have been identified in

previous research to be associated with BZ resistance. These single nucleotide polymorphisms are F167Y (TAC), E198A (GCA) and F200Y (TAC) within the gene (Shen et al. 2019).

I propose a new test with increased sensitivity and reliability that can provide more information about the infection itself. I also propose that the test will be more convenient to run than the PNA fluorescence test. This test will provide enough information about the level of infection and the resistance of the population for the farmer to develop a personalized treatment for the infected animal. This will not only increase the chances of the treatment's success, but it will also slow the rate of developing parasitic resistance.

Proposed Method

In the following section, I will outline the proposed qPCR assay I planned to develop with Dr. Tibbetts at Bard College, which would reliably diagnose sheep and goats with *H. contortus* infection from a stool sample.

The assay would be developed to target areas in the parasite genome which have evolved to be resistant to chemical anthelmintics. We decided that resistance to benzimidazole (BZ) would be the best anthelmintic to focus on, because this anthelmintic is widely used in countries across the world, and therefore it can be assumed that BZ resistance is widespread (Zhang et al. 2016). Multiple SNPs associated with BZ resistance have been found within the isotype-1 β-tubulin gene, and so we decided to focus on the SNPs found within this gene, and designed primers that will surround and amplify this sequence in *H. contortus*, but not the sequence of the most closely related species (determined through a BLAST search to be from the species *Cooperia oncophora*). We found eight BLAST sequences of *H. contortus*, which we used to design our primers (Table 1). None of these sequences had the F167Y SNP present, one had the F200Y SNP, and three had the E198A SNP. None of the sequences had multiple SNPs. We decided to focus on the two SNPs present, and designed our primers to surround

and amplify the sequence TTGGTAGAAGACACCGATGAAACGTTCTG (locations of the SNPs are highlighted).

Table 1: Primer design

Primers	**Sequence**
Forward	5’-CTACCCTTTCCGTCCATCAA-3'
Reverse	5’-ACAGAGCTTCGTTGTCAATA-3’

The qPCR products were to be analyzed using a high resolution melt curve analysis. Since evolved resistance confers differences in the amplified genetic sequence, resistant and non-resistant parasitic DNA will have different melt curves at the end of the PCR run. Real-time PCR with a SYBR Green melt curve analysis is extremely informative and has been used to differentiate between closely related organisms within a sample, including identifying different strains of a pathogen (James and Varga 2006). Therefore, one should be able to use the melt curves produced from a qPCR run of the parasitic DNA to determine which resistance type of *H. contortus* exists in the sample.

We would conduct preliminary tests of these primers using a known DNA sample of *H. contortus* and SYBR Green fluorescent dye. SYBR Green has been found to be more reliable, sensitive, and low cost than specific probe based assays such as TaqMan (James and Varga 2005). SYBR Green is an intercalating dye, and will bind to any double-stranded DNA in the mix. Throughout the qPCR, the concentration of amplicons increases, causing the amount of dsDNA—and fluorescing SYBR Green—to subsequently increase. So, the amount of *H. contortus* DNA within the sample can be quantified by how the amount of fluorescence changes throughout the qPCR.

The samples would then be mixed into a range of dilutions to test the sensitivity of the assay, and a gradient PCR would be run to determine the ideal annealing temperature. Once

the annealing temperature has been set, a confirmatory test will be conducted by running positive and negative controls through the qPCR assay, alongside water as a no-template control, and comparing the results to the standard PNA fluorescence test. The negative controls would include DNA samples of other parasite species that are commonly found in mixed infections of small ruminants, such as *Teladorsagia circumcincta* or *Trichostrongylus colubriformis*. It is very likely that these species will be present in fecal samples alongside *H. contortus*. While we considered including *Haemonchus placei* because of its genetic similarity to *H. contortus*, we decided it was not a necessary negative control for the following reason: *H. placei* is a parasite of cattle, and while it can be transferred to small ruminants in pasture, it is quickly outcompeted by *H. contortus* when both are present, and goes extinct in the absence of cattle (Dos Santos et al. 2020). Therefore, even though a DNA sample of this species is very similar and may result in a false positive, the presence of *H. placei* in large numbers within a small ruminant is very unlikely, and is more likely to be *H. contortus*.

The next stage of development would be field trials. The DNA samples used in these trials would be derived from fecal samples provided by a local veterinarian, who can ensure that the samples are known positive and negative *H. contortus* infections. A number of sheep or goat fecal pellets would be blended into a homogenous mixture, then a standard amount of the blend would be taken and run through a DNeasy blood and tissue kit to isolate the template DNA. The template DNA from each sample will then be subjected to both the developed assay and the confirmatory PNA fluorescence test. The researcher conducting the study should be blind to the identity of each sample, and diagnose each sample based solely on the test results. The results of the assay can then be compared to the PNA test to see which is more sensitive and reliable.

Discussion

I predict that the developed assay will be significantly more sensitive and informative than the PNA fluorescence test. It will not only be able to detect *H. contortus* within a sample at

much lower concentrations than the fluorescence test, but it will also describe the resistance-type of the population within the infected animal. Using these results, one can decide whether or not to dose an animal with anthelmintics, and can decide if they should use benzimidazole or a different treatment, based on the amount of resistance within the population.

The chemical classes of widely-used commercial anthelmintics are benzimidazoles, macrocyclic lactones and imidazothiazoles (Puspitasari et al. 2016). The latter two are conceivably the only other options available to farmers once benzimidazole has lost its effectiveness. Unfortunately, resistance to ivermectin, a type of macrocyclic lactone, is also becoming a growing issue among sheep and goat farmers in many countries (Le Jambre 1993). The most common alternative farmers turn to is giving the infected animal a combination of doses from different chemical classes. However, even this eventually becomes ineffective as *H. contortus* develops a cross-resistance (Puspitasari et al. 2016). There needs to be further research into the development of chemical resistance in *H. contortus*. Once we have a better understanding of what confers resistance to ivermectin, we can begin to develop qPCR assays that can target these areas of the species genome.

The sampling methods used for this assay should provide a consistent and accurate representation of the presence of *H. contortus* within the individual. Therefore, the sampling methods should be closely evaluated when establishing the protocol for this assay. For example, a number of fecal pellets will be taken from the main fecal pile to be used in this assay. These pellets should be taken from different parts of the main pile to represent the entire pile (based on the assumption that the organization of the pile is similar to the organization within the GI tract). To test the probability of a false negative diagnosis, multiple groups of fecal pellets can be taken from one pile, and their results can be compared. It is possible that increasing the number of pellets taken per sample can minimize the possibility of a false negative. Another example is the protocol for taking extracts from the homogenized fecal sample. Multiple samples should be taken from one blend and run through the assay to test the variation of the output. A

large amount of variation would mean either the amount being used is too small, or the blending procedure is faulty.

Unfortunately, the proposed procedure currently relies on the assumption that each resistant and non-resistant type of *H. contortus* DNA will be clearly identifiable in the melt curve analysis. It is possible that when there are various types in one sample, the output will be more difficult to analyze. A possible future experiment could more closely analyze the melt curves of mixtures of *H. contortus* resistance types. Mixtures of *H. contortus* DNA could be made, each containing different proportions of resistant and non-resistant types, and could then all be run through the assay and compared. Once there is a better understanding of how the melt curves are affected by mixed populations, the assay can then be more widely used to diagnose infected individuals.

Conclusion

H. contortus is considered the most economically important parasite in recent history (Laing et al. 2013). This species can cause a great deal of damage to a herd of small ruminants if the infected individuals are not diagnosed and treated quickly. Additionally, as a result of the parasite's extreme adaptability and the common method of mass drug administration over the past few decades, anthelmintic resistance has become a major issue for farmers trying to treat infected animals. Therefore, a simple and fast method of diagnosis must be developed to allow the infection to be confirmed before a treatment drug is administered to the animal. This method must also provide information about the parasitic population's resistance to chemical anthelmintics, so that a different treatment may be used if necessary, thereby avoiding selection for resistant individuals. Then, rather than mass drug administration, targeted treatments will be used to combat infection. The qPCR assay proposed in this paper is a potential solution.

Additionally, new treatments can be developed. One promising new method of treatment is the use of copper oxide wire particles, which, when administered, acts like an anthelmintic

within the animal. Beyond anthelmintics, there are preventative methods and treatments that *H. contortus* could not develop resistance to, and are therefore more sustainable options. For example, recent studies have begun to analyze a species of nematode-trapping fungi which is non-toxic to livestock. This fungi could reduce pasture contamination by feeding on developing larvae in feces. Another alternative to anthelmintics is the use of secondary plant compounds, such as condensed tannins, to reduce the number of nematodes within the abomasum. These suggested treatments require further study before they can be used, but could be potentially huge advancements in the field (Burke and Miller 2020).

References

Abbas, I., Hildreth, M. (2019). Egg autofluorescence and options for detecting peanut agglutinin binding for the identification of *Haemonchus contortus* eggs in fecal samples. Vet. Parasitol., 267, 69-74.

Almeida, F. A., Albuquerque, A. C. A., Bassetto, C. C., Starling, R. Z. C., Lins, J. G. G., Amarante, A. F. T. (2020). Long spelling periods are required for pasture to become free of contamination by infective larvae of *Haemonchus contortus* in a humid subtropical climate of São Paulo state, Brazil. Vet. Parasitol., 279, 109060.

Anderson, N., Dash, K. M., Donald, A. D., Southcott, W. H., Waller, P. J. (1978). The epidemiology and control of gastro-intestinal parasites of sheep in Australia. Division of Animal Health, CSIRO, 23-51.

Ashraf, S., Beech, R. N., Hancock, M. A., Prichard, R. K. (2015). Ivermectin binds to *Haemonchus contortus* tubulins and promotes stability of microtubules. International Journal for Parasitology, 647-654.

Baltrusis, P., Halvarsson, P., Höglund, J. (2018). Exploring benzimidazole resistance in *Haemonchus contortus* by next generation sequencing and droplet digital PCR. International Journal for Parasitology, 411-419.

Barger, I. A. (1997). Control by management. Vet. Parasitol., 72, 493-506.

Burke, J. M., Miller, J. E. (2020). Sustainable Approaches to Parasite Control in Ruminant Livestock. Vet Clin North Am Food Anim Pract., 36(1), 89-107.

Campbell, M. C., & Tishkoff, S. A. (2010). The evolution of human genetic and phenotypic variation in Africa. Current biology : CB, 20(4), R166–R173.

Coles G.C., Jackson F., Pomroy W.E., Prichard R.K., von Samson-Himmelstjerna G., Silvestre A., Taylor M.A., Vercruysse J. (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol., 136, 167–185.

Cotter, J. (updated 2019) Barber's pole worm in sheep. Agriculture and Food.

Dineen, J. K., Donald, A. D., Wagland, B. M., Offner, J. (1965). The dynamics of the host-parasite relationship III. The response of sheep to primary infection with *Haemonchus contortus*. Parasitology, 55, 515-525.

Dos Santos, M. C., Amarante, M. R. V., Amarante, A. F. T. (2020). Is there competition between *Haemonchus contortus* and *Haemonchus placei* in a pasture grazed by only sheep? Vet. Parasitol., 279, 109054.

Emery, D. L., Hunt, P. W., Le Jambre, L. F. (2016). *Haemonchus contortus*: the then and now, and where to from here? International Journal for Parasitology, 46(12), 755-769.

Fernandez, D. (2012). Fecal Egg Counting for Sheep and Goat Producers. University of Arkansas at Pine Bluff.

Geggus, D. (2001). The French Slave Trade: an overview. William Mary Q., 58(1), 119-38.

Hepworth, K., Neary, M., Hutchens, T. (2006). Managing Internal Parasitism in Sheep and Goats. Purdue University.

Intervet (2004). Guide to Internal Parasites of Ruminants. Intervet Inc., Retrieved from https://www.merck-animal-health-usa.com.

James, D., Varga, A., 2005. Nucleotide sequence analysis of Plum pox virus isolate W3174: evidence of a new strain. Virus Res. 110, 143–150.

James, D., Varga, A., 2006. Real-time RT-PCR and SYBR Green I melting curve analysis for the identification of Plum pox virus strains C, EA, and W: Effect of amplicon size, melt rate, and dye translocation. Journal of Virological Methods 132, 146–153.

Jurasek, M. E., Bishop-Stewart, J. K., Storey, B. E., Kaplan, R. M., Kent, M. L. (2010). Modification and further evaluation of a fluorescein-labeled peanut agglutinin test for identification of *Haemonchus contortus* eggs. Veterinary Parasitology, 209-213.

Kaplan, R. M. (2004). Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol., 20(10), 477-81.

Laing, R., Kikuchi, T., Martinelli, A., Tsai, I. J., Beech, R. N., Redman, E., Holroyd, N., Bartley, D. J., Beasley, H., et al. (2013). The genome and transcriptome of *Haemonchus contortus*, a key model parasite for drug and vaccine discovery. Genome Biology, 14, R88.

Larson, G., Piperno, D. R., Allaby, R. G., Purugganan, M. D., Andersson, L., Arroyo-Kalin, M., Barton, L., Climer Vigueira, C., Denham, T., Dobney, K., Doust, A. N., Gepts, P., Gilbert, M. T., Gremillion, K. J., Lucas, L., Lukens, L., Marshall, F. B., Olsen, K. M., Pires, J. C., Richerson, P. J., de Casas, R. R., Sanjur, O. I., Thomas, M. G., Fuller, D. Q. (2014). Current perspectives and the future of domestication studies. Proceedings of the National Academy of Sciences of the United States of America, 111(17), 6139–6146.

Le Jambre, L. F. (1993). Ivermectin-resistant *Haemonchus contortus* in Australia. Australian Veterinary Journal., 70(9), 357.

Love, S. (2010). WormKill - the basics. PRIMEFACT 1079.

Mobini, S. Y. (2019). Evaluation of emerging anthelmintics and non-chemical combination methods for targeted selective treatment of gastrointestinal nematodes in small ruminants. USDA REEIS.

Mohonraj, K., Subhadra, S., Kalyanasundaram, A., Ilangopathy, M., Raman, M. (2017). Genotyping of benzimidazole resistant and susceptible isolates of *Haemonchus contortus* from sheep by allele specific PCR. Journal of Parasitic Diseases, 41(1), 282–288.

Morley, F. H. W., Donald, A. D. (1980). Farm management and systems of helminth control. Vet. Parasitol., 6, 105-134.

Pons, L. (2005). Eyeing a Test for Barber Pole Worm. USDA Agricultural Research Service.

Puspitasari, S., Farajallah, A., Sulistiawati, E., Muladno. (2016). Effectiveness of Ivermectin and Albendazole against *Haemonchus contortus* in Sheep in West Java, Indonesia. Tropical life sciences research, 27(1), 135–144.

Sallé, G., Doyle, S. R., Cortet, J., Cabaret, J., Berriman, M., Holroyd, N., & Cotton, J. A. (2019). The global diversity of *Haemonchus contortus* is shaped by human intervention and climate. Nature communications, 10(1), 4811.

Shen, D., Peng, Z., Hu, M., Zhang, Z., Hou, Z., Liu, Z. (2019). A detection of benzimidazole resistance-associated SNPs in the isotype-1 β-tubulin gene in *Haemonchus contortus* from wild blue sheep (*Pseudois nayaur*) sympatric with sheep in Helan Mountains, China. BMC Veterinary Research, 15, 89.

Shulaw, W. P. (2011). What Do Fecal Worm Egg Counts Tell Us? Ohio State University.

Spangler, G. L., Rosen, B. D., Ilori, M. B., Hanotte, O., Kim, E. S., Sonstegard, T. S., Burke, J. M., Morgan, J., Notter, D. R., & Van Tassell, C. P. (2017). Whole genome structural analysis of Caribbean hair sheep reveals quantitative link to West African ancestry. PloS one, 12(6), e0179021.

Zajac, A. M. (2006). Gastrointestinal nematodes of small ruminants: life cycle, anthelmintics, and diagnosis. Vet. Clin. North Am. Food Anim. Pract., 22(3), 529-541.

Zajac, A. M., Petersson, K., and Burdett, H. (2014). Why and How To Do FAMACHA© Scoring. University of Rhode Island, Retrieved from https://web.uri.edu.

Zhang, Z., Gasser, R. B., Yang, X., Yin, F., Zhao, G., Bao, M., Pan, B., Huang, W., Wang, C., Zou, F., Zhou, Y., Zhao, J., Fang, R., & Hu, M. (2016). Two benzimidazole resistance-associated SNPs in the isotype-1 β-tubulin gene predominate in *Haemonchus contortus* populations from

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