ANTHELMINTIC RESISTANCE AND MANAGEMENT OF GASTROINTESTINAL PARASITES IN SMALL RUMINANTS: AN UPDATE

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INTRODUCTION

The problem of anthelmintic resistance in gastrointestinal nematodes (GIN) of small ruminants is world wide in distribution. In the past 20 years no new classes of anthelmintics have been developed for use in animals. Anthelmintic resistance has been documented to all classes of anthelmntics and multi-class resistance exists in many flocks. The challenge to veterinarians and producers is to utilize known and emerging technologies to control exposure to infection and reduce the use of anthelmintics to control GIN. The demonstration of anthelmintic resistance, the ecology of GIN, management to minimize pathological parasiste burdens and emerging technologies will be discussed.

Identification of Anthelmintic Resistance

1. **Fecal Egg Count Reduction Tests (**FECR)
   Fecal samples are collected on the day of treatment with the anthelmintic to be tested. Using a modified McMaster technique, an eggs per gram feces (EPG) is determined. Seven to 10 days later a second fecal sample is tested for EPG. The percentage in reduction in egg count is calculated. Authors vary in recommendations for cut off points in resistance. Egg counts should decrease by 80-95%. I usually look for at least 90% reduction for an anthelmintic to be considered effective.
   Larval Identification
   Ideally, if there is less than 90% egg count reductions, the eggs should be hatched and the larvae types. In the majority of cases these will be Hemonchus contortus.
2. **Egg Hatch Assays**
   Eggs from feces are incubated with concentrations of the anthelmintic to be tested and the eggs hatched. A dose-response curve is generated. (DrenchRite® test from Horizon Technology)
3. **Larval Development Tests** - Anthelmintic is incorporated into agar plates and eggs developed to third stage larvae and assess the effect on growth.
4. **Adult Development Tests** - Similar to larval development tests except using adults.
5. **DNA Probes** - Detection of genetic markers to identify resistant nematodes.

ECOLOGY OF GASTROINTESTINAL NEMATODES

Haemonchus contortus, Ostertagia (Telodorsagia) circumcincta, other Osteragia spp, Trichostrongylus axei, Nematodirus spp and Cooperia spp are all considered capable of causing clinical and subclinical disease in small ruminants. However, H. contortus is the major problem in North America, and in the southeast, in particular. Most of these nematodes have the same basic life cycle. Adult worms in the gastrointestinal tract mate and females lay eggs which pass out in the feces. The eggs undergo development to larvae which pass through two feeding stages in the fecal material to become the non-feeding, infective (L_3) stage. Eggs hatch within 1-5 days and larval development takes 7-10 days. The L_3 is the longest lived and most resistant stage of the parasite. It migrates to herbage and is ingested by a small ruminant. In the host, the L_3 goes through two more stages to become adults in 15-21 days. One H. contortus female can produce up to 10,000 eggs per day.

H. contortus eggs and larvae develop best when temperatures are above 50^{o}F (10^{o}C) and under high moisture conditions. Temperatures above 104^{o}F (40^{o}C) will kill eggs within 6 days and prevent hatching. Dry, hot summers and freezing temperatures during the winter will kill eggs. If larvae reach the third stage, they are more resistant to drying or freezing but populations will be decreased if conditions are prolonged. Most L_3 will survive 2-3 months but some can live as long as 12 months. There is some evidence that L_3 may get down into the soil and allow them to survive for longer periods.

IMMUNITY

Lambs and kids are the most susceptible to infection with L3. First exposure to infective larvae are from overwintered larvae or from new eggs passed by adult sheep. However, lambs are responsible for the majority of pasture contamination during the spring and summer. In general, worm burdens gradually increase in lambs and kids to peak in midsummer to early fall (July to September). Immunity in lambs gradually develops as lambs are exposed to parasites and is fully developed by 10-12 months of age. Immunity development in goats has not been well documented. Once immunity has developed, parasite burdens are restricted by sheep except during disease, malnutrition and stress.

Periparturient egg rise in small ruminants is a well known phenomenon. However, the exact mechanism is not entirely understood. The stress of lambing and lactation allows the immunity of ewes to be overcome until approximately 4 weeks after lambing. Adult worms that are established during this period allow exposure of susceptible lambs to infective larvae. Nutritional supplementation with protein during this period can abolish the periparturient egg rise.

MANAGEMENT

Pasture Management

The goal of pasture management is to provide safe pastures for grazing. A safe pasture is one that has had no sheep or goats grazed on it for 6 months during cool/cold weather or 3 months during hot, dry weather. Weaning sheep and goats at 2 months of age, and rotating them through pastures ahead of the adults will minimize the exposure to large numbers of infective larvae. Pastures should at least be rotated following any administration of anthelmintics to the animals. It has been advocated to keep dewormed animals in a holding pen for 24 hours following deworming and then move them to a safe pasture. Small ruminants can graze pastures after cattle and this is considered to be a safe pasture (assuming cattle are under adequate parasite control themselves).

Pastures should be subdivided into smaller lots to allow rotation of pastures. Pastures that have a heavy thatch or extensive overgrowth provide a good environment for larval survival. UV light is effective in killing larvae. Keeping pastures clipped will assist in weed control as well as parasite control. Short duration grazing carries pasture rotation to a level that maximizes forage production and harvesting by controlled animal grazing. It is management intensive but can be effective in controlling parasite burdens.

Pastures which have become heavily contaminated due to mismanagement can be tilled and reseeded. This is an opportunity for pasture improvement and shorten the time that an area needs to remain ungrazed to become a safe pasture. In the future, pastures may be reseeded or overseeded with forages containing condensed tannins to take advantage of their anthelmintic effects. Taking a cutting of hay from a pasture is another method of giving a pasture time to reduce infective larvae. However, there is one report of GIN and tapeworms developing in "worm free" lambs after feeding hay from heavily infected pastures.

Depending on the available facilities, it may be necessary to dry lot the flock and feed hay and grain from elevated feeders if pastures have become dangerously contaminated with resistant nematodes.

Weather Conditions

Anthelmintic administration should be coordinated with the weather. Many producers religiously deworm their flocks according to a set schedule. During hot, dry weather there will be little or no exposure to infective larvae. As soon as there is significant rainfall (0.5-1.0 inches) larvae exposure goes up exponentially as previously inactive larvae become active and new larvae are hatched. The producer's should be trained to plan deworming within three weeks of significant rain after a dry spell. Similar strategies can be used during cool weather. Once ambient temperatures drop below 50^{o}F, the flock can be dewormed and no further treatments are necessary until temperatures become favorable to larval development and activity.

Treatment Strategies

Recent work in South Africa has developed a patented FAMCHA^{R} plan. Study in this country has been led by Dr. Ray Kaplan, University of Georgia. Instructions and producer kits may be obtained from the webpage http://www.scsrpc.org/FAMACHA/famacha.shtml. Only veterinarians may order the kits and must first sign an agreement to distribute the kits after training the producer how to use them. This plan utilizes a chart of mucus membrane color to determine whether an individual should be dewormed. The initial research in sheep exposed to heavy contamination with H. contortus only dewormed those with PCV below 15% to prevent death. The majority of the flock (69%) required no treatment and 21% required only one treatment. The use and expense of anthelmintics was greatly reduced.

This approach revolutionizes traditional deworming strategies. The goal is to maintain a susceptible population of nematodes in the environment that controls the population of resistant nematodes. Only animals developing pathological parasite burdens are treated. Indiscriminant selection for resistant parasites by blitz treatment is avoided.

Condensed Tannins (CT)

CT are secondary plant metabolites which are defense mechanisms against insects and herbivores. There are hydrolysable and condensed tannins but the CT are more common. CT can be detrimental to monogastrics and at high levels will decrease DMI in ruminants. CT containing forages increased weight gains, wool growth and milk production while decreasing the effects of GIN in red deer and sheep. The direct parasitic effects include decreased fecal egg counts and decreased L_3 viability. The indirect effect of CT is by binding to dietary protein, which allows it to bypass rumen and thus increases protein availability in the small intestine. The most commonly investigated forages are Lotus pedunculatus (big or large trefoil), Lotus corniculatus (birdsfoot trefoil), Hedysarum coronarium (sulla) and Onobrychus vicifolia (sainfoin). Quebracho is a cold soluble extract from Schinopsis sp. tree bark. It can be used as a drench or incorporated into pelleted feeds. Further research is needed to determine appropriate dosages.

Nematophagous fungi

Nematophagous fungi are microfungi which utilize nematode larvae as their main source of nutrients. The fungi are ingested by ruminants, pass through the digestive tract and colonize fecal material. Three predaceous fungi have been identified but only one is suitable for including in ruminant diets. Duddingtonia flagrans has thick-walled spores that can be fed to ruminants and passes safely through to the feces. The spores must be fed daily to maintain the reduction in L3 numbers.

Vaccine Development

Several natural antigens have been used to develop protection through vaccination but none have been mass produced. Vaccines have been developed to the "hidden" antigens and "natural" antigens. Hidden antigens are those which do not cause a detectable immune response in the host with natural infections and are thought to be internal antigens of the nematode. H11 is the best known and is from the intestinal mucosa of H. contortus. Protective immunity was achieved using H11 purified from the parasite. Recombinant H11 vaccines have not provided the same protection as the natural H11 and have not been commercialization.

The interaction of immunity and hypersensitivity to parasites is complex. Many of the undesirable effects of parasitism (scouring, and reduced production of muscle, wool and milk) may be due to an exaggerated hypersensitivity reaction. This may have a genetic predisposition. Some research has looked at modifying the immune reaction using cytokines.

Nutrition

The strongest link between nutrition and parasitism has been illustrated between protein intake and resistance to GIN infection. The most dramatic has been the abolishment of the periparturient egg rise in lambing ewes by providing protein at 130% of requirements. Immunity is closely related to protein repletion. GIN increase the demands for amino acids by the sheep. Conversely, one of the first responses of the animal is to decrease feed intake. Some hypothesize that anorexia may be immunostimulatory or that the host is becoming selective in their diets. Lambs will voluntarily select a higher protein diet when infected with GIN compared to uninfected lambs.

Supplementation with phosphorus has been shown to prevent worm establishment. Cobalt deficiency has also been associated with reduced immunity to GIN. Adequate copper levels are necessary for development of immunity to GIN. Recent work suggest treatment of lambs with copper oxide wires orally reduces Haemonchus contortus burdens. However, copper toxicity would be a concern with these treatments. Surprisingly, addition of molybdenum at 6-10 mg Mo/day decreased worm burdens in lambs that was not attributable to the expected copper deficiency. Molybdenum may have a role in increasing jejunal mast cells and blood eosinophil numbers.

Genetics

Discussions of genetic selection of sheep include the terms resistance and resilience. Resistance is the ability of the host to prevent or limit establishment of GIN infection. Resilience is the ability to maintain a reasonable level of production when subjected to parasitic challenge. Resistance is as heritable as most production traits (h^2=0.35) and is based on fecal egg counts. Resistant animals may not be desirable as there is a negative correlation with production traits and a positive correlation with the incidence of dag and breech soiling. Resistant animals utilize a larger proportion of their resources to developing their immune responses. On the other hand, high producing animals funnel the majority of their resources to growth and less to immunity, thus becoming more susceptible to parasitism. Under conditions of nutritional adequacy, resilience should be improved and may be more realistic that selecting for resistance. However, resilience is more difficult to identify and select for. The heritability of resilience is lower (h^2=0.1-0.19). Resilience was based on drench on demand programs that deworm based on loss of body condition. Resilience has been documented in rams. Resilience and resistance were found not to correlate well. A commercial program, WormFEC™ performs fecal egg counts and calculates breeding values for rams based on several traits including presence of serum antibody to GIN. The best way for producers to incorporate these traits is to purchase replacement rams that have been suitably evaluated.

Genetics of the GIN must also be considered. New gene silencing technology uses double stranded RNA (dsRNA) to turn off specific genes in the target organism. A free living nematode has been used as model to identify the genes of interest (embryo development, fecundity and larval development). Biological anthelmintics are also being investigated. These are phages that are carried into the parasite and either block normal protein replication or provide new binding sites for anthelmintics.

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