5-2012

Identification of Internal Parasites of Sheep and Goats

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IDENTIFICATION OF INTERNAL PARASITES OF SHEEP AND GOATS

by

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A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Animal and Veterinary Science)

The Honors College

University of Maine

May 2012

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Abstract

Abomasal worms are a major cause of small ruminant disease. Differentiation of the most pathogenic nematode, *H. contortus*, from the other common species can be difficult using standard diagnostic fecal floatation techniques because the ova are similar in size and morphology. Known pure culture *H. contortus* fecal samples from West Virginia University were used to develop morphologic assays using FITC-labeled lectin agglutination and immunocytochemistry to identify species of abomasal worms. These assays were applied to assess disease due to abomasal worms on selected small ruminant farms in Maine. The diagnostic tests were used to test the hypothesis that *H. contortus* is the most common internal parasite found on sheep and goat farms in Maine.

Acknowledgements

The author would like to acknowledge Dr. James Weber for his mentorship throughout the project. Completion of this research would not have been possible without his guidance and support. The author would also like to thank Anne Lichtenwalner for her aid in organization of the research and for her assistance in the presentation of scientific information.

The author would like to acknowledge Mimi Killinger for her assistance with the reading list and preparation for the defense. The author would also like to thank Robert Causey and David Markinkowski for their input on research design and for offering their time to serve on the thesis committee.

Finally, the author would like to thank Martin Stokes and Clare Thomas for their guidance in writing a scientific paper.

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Introduction

Gastrointestinal nematodes *Haemonchus contortus, Ostertagiai ostertagii, and Trichostrongylus axei* live in the abomasum and cause disease in sheep and goats. Parasitism results in economic losses to Maine producers, particularly the large organic component of the industry. Based on accessions to the Maine Animal Health Lab, it appears that nematode infections and associated production losses are prevalent in Maine. However, there are not currently any labs in the Northeastern U.S. that offer diagnostic testing to determine the species of nematode infecting an animal. Lack of species-specific information makes it difficult for veterinarians to advise clients on management of parasitic infections. Differentiation of the most pathogenic nematode, *H. contortus*, from the other common species can be difficult using standard diagnostic fecal floatation techniques because the ova are similar in size and morphology (Fig. 1) . Current diagnostic techniques for identification and differentiation between nematode species involve a time-consuming and expensive larval culture procedure.

Fig. 1. Nematode ova. Image by Amanda Chaney.

Haemonchus contortus, O. ostertagii, and T. axei are invertebrate nematodes that belong to the family Trichostrongylidae and live in the abomasum of sheep and goats.

Each species displays a similar life cycle in which adult female abomasal worms produce eggs that are passed with feces to contaminate pastures (Bowdridge, 2005). While on herbage, the eggs exsheath to form first stage larvae (L1). First stage larvae then shed their cuticle to form second stage larvae, which molt and develop into third stage larvae (L3). Third stage larvae are the infective juvenile worms that migrate up the blade of grass and are consumed by the host. Once in the abomasum, L3 molt to form fourth (L4) and then fifth (L5) stage larvae before they mature to adult stage parasites that are capable of reproduction and continuation of the life cycle (Fig. 2). If conditions are unfavorable, the L4 larvae can enter hyperbiosis, a period when the larvae arrest development in the lining of the abomasum. Triggers that arrest development are either hostile immunity or unfavorable environmental conditions such as dry or very cold periods of weather, when larvae deposited in the feces would not survive. When environmental conditions improve, development continues and the life cycle is completed. Triggers to continue development are not fully understood. It has been suggested that the hormonal changes that occur in the female around parturition cause a waning of resistance to parasites. Decreased immunity causes the larvae to emerge from arrest and the resulting parasite load is known as the spring rise (Kahn, 2005).
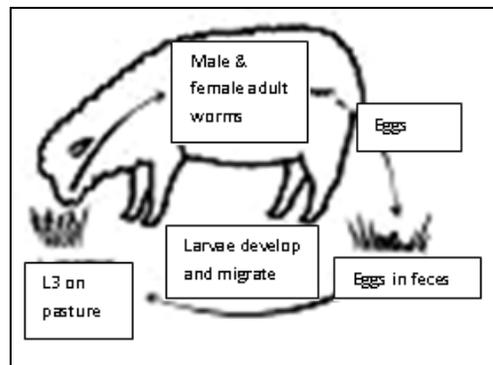

Fig. 2. The life cycle of a nematode. Image from http://www.basonevoice.org, modified by Amanda Chaney.

While the life cycles of *H. contortus, O.ostertagii, and T. axei* are very similar, there are physiologically important differences in the life cycle which leads to differences in their pathogenicity and economic impact. Third stage *H. contortus* larvae are ingested and develop into fourth stage larvae (L4) in the lumen of the abomasum within forty-eight hours. Fourth stage larvae have a small buccal cavity with several teeth (Fig. 3) that are used to pierce the mucosal cavity and feed on blood. The final molt occurs within three days and the adult worms lay eggs and continue to feed on blood. Blood feeding causes severe anemia that results in decreased pigmentation of the mucus membrane (Fig. 4). *Haemonchus contortus* infection also causes edema due to hypoprotonemia that is commonly seen as bottle jaw (Fig. 5) (Schmidt and Roberts, 1981). Emaciation is also a result of *H. contortus* infection; in the chronic phase of the disease, edema of the abomasum can cause an increase in pH, which leads to gastric dysfunction and further weight loss (Merial Australia, 2011). The severity of infection determines the level of production loss and may result in death.

Fig. 3. Head region of *H. contortus,* showing teeth located in buccal cavity. Image from www.sheepandgoat.com

Fig. 4. Mucus membrane of anemic sheep. Image from

www.sheepandgoat.com

Fig. 5. Bottle jaw in sheep. Image from www.sheepandgoat.com

Third stage *Ostertagia* larvae inhabit abomasal (gastric) glands, where they molt

to develop into L4 (Fig. 6). Fourth stage larvae cause hyperplasia of cells of the gastric

gland, resulting in nodules. After the final molt to reach the adult stage, the worms

emerge from the gastric gland and cause cytolysis. As part of the natural bodily

response, the destroyed parietal cells are replaced with undifferentiated cells that do not

produce the gastric juices normally secreted by the gland. Lack of gastric juices leads to

an increase of abomasal pH, impairing metabolism of protein and energy. The results are

loss of body condition, hypoprotonemia, decreased milk production in ewes, and scours. Severe diarrhea may cause death (Kahn, 2005).

Fig. 6. *Ostertagia* L4 in gastric gland of abomasum**.**

http://cal.vet.upenn.edu/projects/merial/nematodes/images/el4F.JPG

Infective L3 *Trichostrongylus axei* are ingested and molt in the abomasum. The fourth stage larvae and the adult worms erode the villi of the abomasum. Since villi contain capillaries and lymph vessels, erosion causes bleeding into the lumen of the abomasum. This bleeding causes dark diarrhea, decreased appetite, loss of body condition, and slight anemia (Merial Australia, 2011).

Productive loss due to gastrointestinal nematode infections is an ongoing global problem. In an attempt to identify the source of productive losses, several studies have been completed that focus on species identification. The modified McMaster technique is used to determine prevalence of parasitic infection. Individual or pooled fecal samples are subjected to a floatation solution and a portion of this slurry is transferred to a McMaster Chamber. Nematode eggs can be visualized in the McMaster chamber and a quantitative egg count can be completed (Fisheries and Food Ministry of Agriculture, 1971). Information gained from the McMaster technique identifies whether or not parasite infection exists, and estimates the extent of infection based on the number of

eggs counted. Eggs of *Haemonchus contortus, Ostertagia, and Trichostrongylus* are morphologically similar (Fig. 2), so it is not possible to identify the infective species based on microscopic evaluation of Nematode eggs (Jurasek et al., 2010). In addition, new research completed in the Kaplan lab at the University of Georgia has found that L3 morphology may not be a consistent and accurate method to differentiate between *Ostertagia* and *Trichostrongylys*.

Since species identification cannot be determined from evaluation of Nematode eggs, researchers in the 1970's developed a technique in which they hatched the fecal eggs and identified the species based on morphological differences of L3 larvae. Eggs were incubated until L3 developmental stage was achieved. The L3 were then stained with Grams Iodine and visualized under a microscope; species were identified based on shape, internal structures of the head region, and tail morphology (Fisheries and Food Ministry of Agriculture, 1971). Third stage larval isolation and identification is currently the standard method used for identification and differentiation between *Haemonchus, Ostertagia, and Trichostrongylus.* However, there are several downfalls to this identification technique. The person completing the procedure must be trained to identify morphological differences in larvae, and the incubation and identification is a time-consuming and expensive process (Jurasek et al., 2010).

Recent studies have been conducted that aim to overcome the shortfalls of larval identification. Researchers have identified three flourescein isothiocyanate (FITC)-labeled lectins that bind to genus-specific carbohydrates on the surface of *Haemonchus, Ostertagia,* or *Trichostrongylus* eggs. Osage orange seed agglutinin binds to the eggs of *H. contortus, O. circumcincta,* and *T. colubriformis*. Jack bean agglutinin binds to the

eggs of *H. contortus* and *O. circumcinus*. Most importantly, Peanut agglutinin binds selectively to *H. contortus.* Since the lectins have a fluorescent tag which can be visualized under a fluorescent microscope, selective binding of Peanut agglutinin (PNA) provides a rapid, simple, and less expensive identification technique for *H. contortus* (Palmer and McCombe, 1996). Other studies have been completed to improve certain aspects of the identification method. For example, Jurasek et al.(2010) conducted a study that made adjustments to the PNA-FITC procedure to further reduce the time required for identification of *H. contortus.*

Another recent study used an alternate technique for identifying anemic individuals that may be infected with clinically relevant levels of *H. contortus*. Kaplan et al. (2004) used the FAMACHA score system developed in South Africa to clinically identify anemic sheep. In this system, the color of the ocular conjunctiva of sheep and goats is compared with the images on the FAMACHA scoring card, which ranks ocular conjunctiva color on a scale of one to five, with one being a red color of a healthy animal and five being an almost white color of a severely anemic animal. Kaplan et al. (2004) tested the reliability of the FAMACHA system by comparing FAMACHA evaluation of animals with packed cell volume (PCV) and fecal egg counts (FEC) of the same animals. The study concluded that there was a correlation between eye score, PCV, and FEC. Anemic sheep display decreased PCV, increased FEC, and increased eye score. Since *H. contortus* is the main parasite which causes severe anemia in sheep and goats, the FAMACHA system can be used to identify animals infected with this nematode. Identification of infected animals without extensive lab testing reduces costs while still

providing evidence of *H. contortus* infection; this information is useful in developing treatment plans to reduce parasite prevalence.

Although recent research has developed assays for species-specific identification of abomasal worms, these techniques are not available in labs in Maine. Gastrointestinal nematode infections negatively impact the Maine sheep and goat industry. The objective of this project was to streamline a diagnostic procedure for species-specific identification that could be used by the University of Maine diagnostic lab to provide Maine famers with an affordable service for parasite identification. This would benefit Maine sheep and goat producers by allowing them to reduce parasitic disease on their farms, thus increasing production.

Materials and Methods

Sheep fecal samples were obtained from Dr. Scott Bowdridge from the research farm at West Virginia University (WVU) and from several commercial sheep and goat farms in Maine. The WVU fecal samples were known positive for *H. contortus.* Each of the following techniques was applied to each sample.

Total fecal egg count

The Modified McMasters technique was used to obtain a total fecal egg count. To complete this technique, 2 grams of feces were weighed out and placed it in a 50 ml centrifuge tube. A small amount of saturated sodium chloride solution (~15 ml) was added and the feces were allowed to soak for one minute. The saturated sodium chloride solution works to dissolve the feces and allow the ova to go into solution. A scoopula was used to break up the fecal pellets, and the fecal solution was run through a mesh

strainer to further break up the feces if needed.   Once the feces were well broken up, more sodium chloride was added to bring the total volume to 30 ml. The McMasters chamber was prepared by wetting it then gently tapping it on paper towel to remove excess water. The centrifuge tube containing the fecal slurry was rocked back and forth 10 times and a pipette was used to draw up enough suspension to fill one chamber of the McMasters slide. The rocking and pipetting procedure was repeated to fill the other side of the chamber (Fig. 7). The McMasters chamber was allowed to stand for 1-2 minutes to allow any eggs to float to the top. A microscope was used on 100X power to count the nematode eggs within 60 minutes to prevent drying or crystal formation of sample in the chamber. The number of eggs per gram of feces was calculated by multiplying the total egg count (for both chambers) by a factor of 50 (Kaplan et al., 2011).
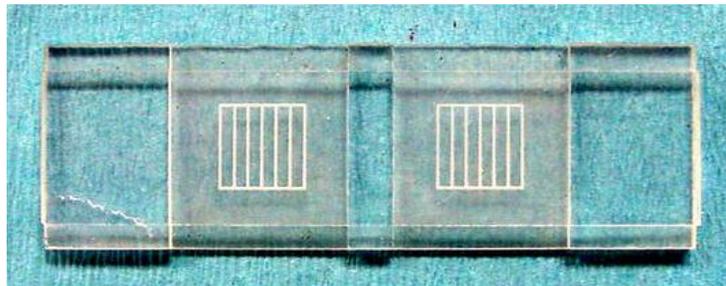
Fig. 7. Modified McMasters Chamber. Image from http://loudoun.nvcc.edu

Larval Identification

The eggs in the fecal sample were hatched and L3 larvae at 7-10 days post-hatch were identified to determine the relative amounts of the three parasites. To hatch the eggs, the feces were incubated. Two grams of feces were weighed out and placed into a 6in x 1in x 6 in Tupperware container with a cover.   A small amount of water was added to moisten the feces, and the feces were broken up with a spatula. Peat moss was mixed

in to form a thin layer covering the bottom of the container. Water was sprinkled on top

until the mixture became moist. The covered container was incubated at 29°C for 7 days.

The Baermann larval collection technique (Fig. 8) was used to isolate the L3

larva from the feces. Motile L3 larvae were collected from the cultured fecal mix by

suspending 2 g of feces in a Kimwipe paper towel. The edges of the Kimwipe were

folded together, and the package was placed in a glass funnel filled w/warm water (the

funnel's outlet was plugged to retain water). Larvae were collected from the bottom of

the funnel after 4-12 hours.
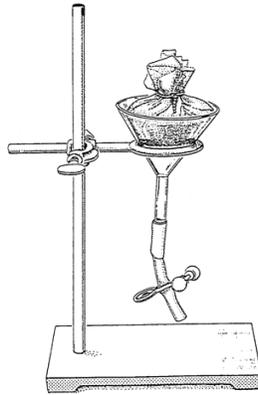
Fig. 8. Baermann larval collection technique. Image from
www.sheepandgoat.com

Grams iodine was added in a 1:1 ratio to the slide to stain free living nematodes

and to enhance visualization the internal structure of the L3 larvae. The sample was

viewed under the microscope to identify L3 of *H. contortus, O. ostertagii, and T. axei.*

Larval counts were used to determine the relative amount of each species causing parasite

infection.

Isolation of eggs

Nematode eggs were isolated from the fecal sample to be used in the fluorescent-labeled lectin agglutination and immunocytochemistry assays. To isolate the eggs, the Modified Wisconsin Sugar Floatation Method was used. Three grams of feces were weighed out and placed in a beaker. Next, 10 ml of Sheather's solution was added to the feces and the sample was mixed well to form a slurry. The liquid portion of the slurry was transferred to a 15mL centrifuge tube using a funnel in between a strainer and the centrifuge tube. A tongue depressor was used to press the fluid out of remaining solid fecal matter. The tube was then centrifuged at 280 xg for 4 minutes. Sheather's solution was added to fill the tube until a meniscus formed just over the top of the tube. A cover slip was placed onto the meniscus and the experiment was allowed to sit for a minimum of five minutes to allow the strongyle eggs to float to the top and collect on the cover slip. To remove the eggs, the cover slip was rinsed with a small amount of PBS into a 1.5mL Eppendorf tube. The tube was then filled to 1.5mL with PBS and spun in the microcentrifuge for 2 minutes at 6000 xg to rinse the eggs. The supernate was removed, and the rinsing process was repeated. The supernate was removed and the ova in the pellet were isolated for the next experiment (Jurasek et al., 2010).

A new step was added to clean the ova. The ova were placed in a sucrose gradient (3 layers of sucrose at increasing densities). Ova migrated to the interface between the top and middle layers.

Fluorescent-labeled lectin agglutination test

Once the eggs were isolated as described in the previous experiment, they were incubated with FITC-labeled peanut agglutinin (PNA) to identify *H. contortus* versus non-Haemonchus ova. The peanut agglutinin lectin used was Archis hypogaea—FITC conjugate.

Once the eggs were obtained from the *Isolation of eggs* step, enough PBS was added to the tube to bring the total volume to 1mL. Half of the samples were treated with galactose that bound to the PNA, preventing binding of PNA to the egg surface. This acted as the negative control. To create the galactose mixture, galactose was mixed to 10X concentration by combining 0.54g galactose to 200mL of PBS. One hundred mL of the galactose solution was added to appropriate ova samples and allowed to incubate for 1 hour.

Next, the ova samples, both those treated with galactose and those not treated with galactose, were incubated with the PNA-FITC. Thirty uL of PNA-FITC was added to the samples without the galactose treatment, and 35uL was added to the samples with the galactose treatment. Samples were incubated for 1 hour on a rocker and in the dark. Then, the samples were washed two times in PBS by adding PBS to the sample to bring the total volume to 1.5 ml, centrifuging at 6000 x g for 10 seconds, and then removing the supernatant. The pellet containing the eggs was re-suspended in a small amount of PBS buffer. Twenty uL of each sample were pipetted from the tube and onto individually labeled microscope slides. A coverslip was placed on top, and the slides were examined with a fluorescence microscope using FITC filters (Jurasek et al., 2010). The presence

of PNA-ova binding was confirmed by bright green fluorescence of the egg when viewed under UV light (Fig. 4).

HRP-labeled lectin agglutination test

In addition to the experiments described above, we also attempted to develop an assay in which the *Haemonchus contortus*-specific PNA lectin was linked to horseradish peroxidase (HRP) and reacted with a substrate, tetramethylbenzidine (TMB), to produce a color change that indicated the presence of the species to which the lectin specifically bound. The HRP is a plant-derived enzyme that catalyzes a color change when reacted with a specific substrate, TMB. The resultant is a blue-colored product that becomes insoluble and precipitates in the vicinity of the HRP enzyme. A microscope was then used to determine presence or absence of color change around the parasite to determine which species were prevalent in the fecal sample.

To complete this procedure, a Corning centrifuge tube was first prepared by washing the filter with PBS, and then incubating the filter with PBS-Tween (1 part Tween-20 per 2000 parts of PBS) for 1 hour. The purpose of this was to coat non-specific sites on the filter. After the hour incubation, the Corning tube was centrifuged for 10 seconds at 6000 x g to remove the fluid. Next, 500 ova in water were added, and the tube was centrifuged for 10 seconds to remove the fluid. The ova were rinsed with 1mL PBS and spun down to remove the fluid. Two different concentrations of PNA-HRP was then added, either a 100x or a 400x, and the sample was allowed to incubate for 1 hour at room temperature. After the incubation, the sample was centrifuged for 10 seconds to remove the fluid before undergoing several washes. The washes consisted of 2 washes

with PBS-Tween and 1 wash with PBS. To wash, 1mL of solution was added to the sample, and the sample was centrifuged to remove the fluid. Once the washes were completed, 0.5mL of the substrate, TMB, added. At time zero, and every five minutes after, a drop of sample was viewed under the microscope. Once a blue shell formed around the ova, the reaction was stopped by spinning down the tube, then rinsing once with PBS-Tween and once with PBS. The ova were then resuspended in ~200uL of PBS to obtain a concentrated sample for microscopic evaluation. Before transferring to a slide, the ova were displaced from the membrane by gently pipetting up and down several times before removing all fluid from the Corning centrifuge tube into a fresh tube.

Results and Discussion

Total Fecal Egg Count

The Modified McMasters technique was applied to a fecal sample obtained from Old Oak Farm in Maxfield, Maine. Greta, an Ewe, had 1500 strongyles/g and ~100 coccidia/g. Stella, an Ewe, had 400 strontyles/g and 1250 coccidia/g (Table 1). The main purpose of this experiment was to ensure that the technique could be replicated.

	Strongyles/g	Coccidia/g
Greta (ewe)	1500	100
Stella (ewe)	400	1250

Table. 1. Results of total fecal egg count for Old Oak Farm in Maxfield, Maine.

Larval Identification

Feces from Old Oak Farm in Maxfield, Maine were placed in larval culture to obtain L3 larvae for larval identification. For the first trial, 10g of feces were combined with 10g of peat moss. When the sample was viewed under a microscope 7 days later, there were many free-living nematodes. An explanation could be that the peat moss contained many free-living nematodes that contaminated the sample.

For the second trial, the peat moss was first autoclaved to destroy any microbes before being mixed with the feces. This resulted in a clean L3 sample, which was beneficial for larval identification purposes.

The L3 larvae were combined with grams iodine and viewed under the microscope at 40X to identify *Haemonchus* versus non-*Haemonchus* nematodes. It was learned from the University of Georgia Parasitology Laboratory that larval identification cannot be used to determine the difference between *Trichostrongylus* and *Ostertagia* as was previously believed.

*Fluorescent-labeled lectin agglutination test results: West Virginia University (WVU) sample*s

It was found that fresh ova treated with PNA-FITC fluoresced. The ova was first visualized using visible light, as depicted in Figure 9A, and then the same ova was visualized using fluorescent light, as depicted in Figure 9B. Since the whole egg fluoresced (Fig. 9B), this means that the FITC-PNA bound to the entire surface of the egg, indicating the egg belongs to the species *H. contortus*. This result was expected

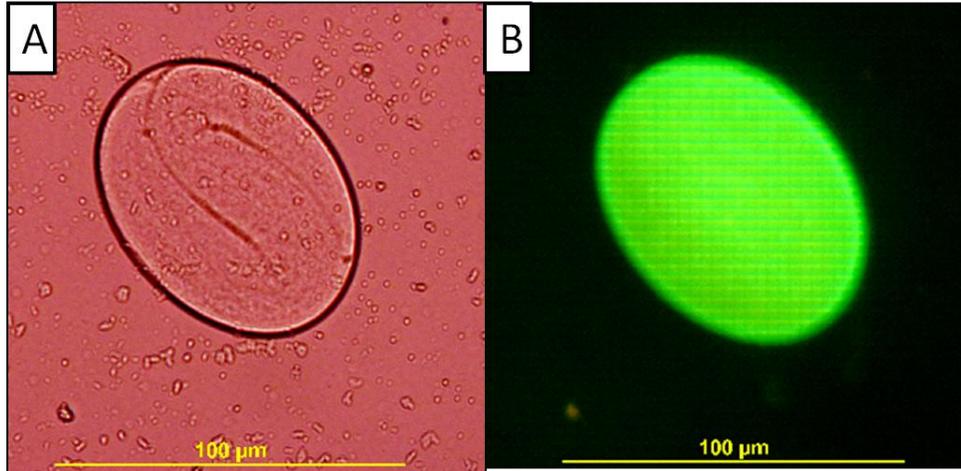since the sample was known *H. contortus* positive, and PNA binds specifically to *H. contortus.*

Fig. 9. Fresh FITC-labeled *H. contortus* ova without (A) and with (B) UV light. Image by Amanda Chaney

The fresh ova sample that was treated with galactose did not display fluorescence. No fluorescence indicated that the galactose saturated the PNA, meaning the negative control worked and that the PNA-FITC binding was specific for galactose residues on the surface of the *Haemonchus* ova's "egg shell."

The *H. contortus* ova that were preserved in 5% formalin fluoresced. While it was expected that the *H. contortus* ova would fluoresce, it was beneficial to determine that these eggs would fluoresce even after treatment with formalin. This discovery is beneficial since it may be required to preserve eggs to prevent hatching before diagnostics can be completed. The 5% formalin ova that contained galactose did not fluoresce, except for one egg in the sample showed slight fluorescence. The egg that fluoresced could indicate that the galactose did not completely saturate the lectin in this sample.
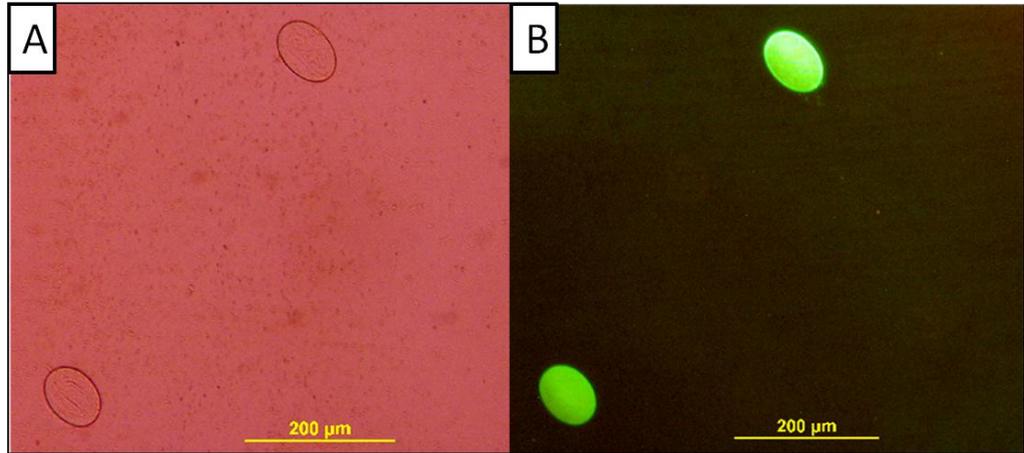
Fig. 10. Formalin FITC-labeled *H. contortus* ova without (A) and with (B) UV light. Image by Amanda Chaney

The sample that contained ova that were originally preserved in 5% formalin, but were subsequently transferred to a 50% EtOH solution, had inconclusive results because there were too many bubbles on the slide to see the ova. One explanation for why the EtOH slides were not usable is because alcohol has a different density than water or PBS, so the eggs may not float to the top as they do on the other slides. This makes it difficult to locate the ova using the microscope. Results of FITC-labeled lectin agglutination test for WVU ova is summarized in Table 2.

Sample	*Haemonchus contortus* Positive	*Haemonchus Contortus* Negative	Unsuccessful Test
Fresh Ova	X		
5% Formalin	X		
5% Formalin, 50% EtOH			X

Table 2. FITC-labeled lectin agglutination test results for WVU ova.

The final sample was the L3 larva, which did not fluoresce. This result was unexpected because the larvae should contain the same sugars on the surface as ova, and thus, the PNA-FITC should be able to bind to the larvae. Lack of fluorescence could be due to the inability of the lectins to penetrate the larvae cuticle, so no binding could occur. Aside from the PNA-FITC binding, the non-fluorescent result was also not expected because the L3 larvae actually auto-fluoresce using the fluorescent system at the University of Georgia (personal communication).

Fluorescent-labeled lectin agglutination test results: Maine sheep and goat farm samples

No fluorescence of the samples occurred, indicating that the sheep was infected with non-*Haemonchus* nematodes, either *T. axei* or *O. ostertagii.* However, it is possible that the sheep was infected with *H. contortus* based on clinical signs, but no eggs were being shed due to the season (hyperbiosis of *H. contortus* in the cold winter months).

HRP-labeled lectin agglutination test results:

The HRP-labeled lectin agglutination test was completed on fecal samples from WVU that had been preserved in 5% formalin for five weeks. To date, only one trial has been completed, and no negative control was used. For known *H. contortus* positive ova, at time zero after TMB was added, no, or patchy, precipitation occurred (Fig 7). At a time of 5 minutes, patchy precipitation occurred on the surface of the ova (Fig 7). At a time of 10 minutes, blue precipitate covered the surface of the egg. At a time of 20 minutes, the precipitate started to fan out from the ova surface (Fig 7). Similar results occurred for both concentrations of PNA-HRP, but the 400x concentration seemed to have more non-specific binding of enzyme and resulting precipitate. The reaction was

stopped at 10 minutes by a series of washes. However, the washes seemed to remove

some of the precipitate from the ova surface. Therefore, more work needs to be

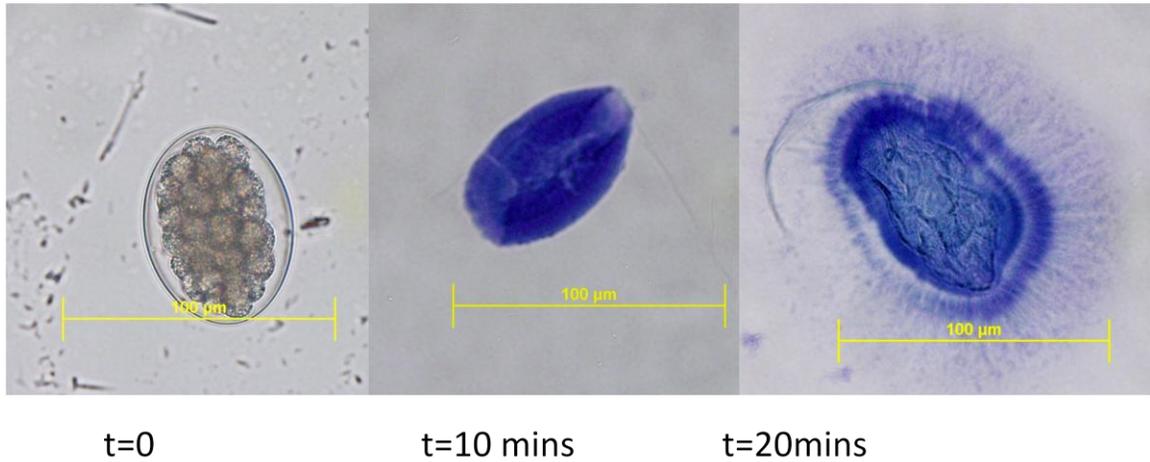completed to determine the best way to stop the reaction.

t=0   t=10 mins   t=20mins

Fig. 11. *H. contortus* ova treated with PNA-HRP 0, 10, and 20 minutes after TMB

was added. Image by Amanda Chaney.

Conclusions

The Modified McMasters technique, the larval identification technique, and the

fluorescent-labeled lectin agglutination test using PNA-FITC for *H. contortus*

identification were streamlined.

HRP-Labeled Lectin Agglutination test was developed, but more work needs to

be completed.

Implications

Identification of the most pathogenic nematode, *H. contortus*, is important in treating animals with a parasitic infection. The fluorescent-labeled PNA agglutination test is ready for use in the University of Maine diagnostic lab alongside the larval identification technique. In-house analysis must be completed on the sensitivity and specificity of the procedure before it can be used alone. As for the HRP-labeled lectin agglutination test, once the assay is fully developed, it will eliminate the need for an expensive fluorescent microscope. This will make the identification of *H. contortus* more available to Maine farmers and veterinarians. Overall, this research will benefit Maine sheep and goat producers by providing more available identification methods for internal parasites. Producers will then be better able to reduce parasitic disease and the economic losses associated with it.

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Appendicies

Appendix 1: Original budget

I am requesting funding to purchase the materials for lectin staining, to cover expenses of survey mailing, and to assist in travel expenses to attend the Sheep and Goat conference. I need several fluorescent-labeled lectins and galactose to complete the lectin-staining method of nematode egg identification. The lectins are needed to tag and therefore identify certain species of nematodes. Galactose is used to bind the lectins, preventing their attachment to the nematode eggs; galactose is an important aspect of the experimental control.

I hope to attend the UMaine Sheep and Goat Internal Parasite Seminar hosted at Kennebec Valley Community College. Kennebec Valley Community College is 70 miles from my house and will cost me $18.27 to drive the 140 miles round trip with gas prices being $3.55 and my Subaru averaging 27mpg.

I hope to receive funding that would enable me to mail a survey to 35 sheep and goat producers throughout the state of Maine. Such a survey would provide me with information such as flock or herd size, current parasite prevention and treatment protocols, current or previous parasite infections, and other information relevant to my project. Distributing a survey is also a good way both to inform sheep and goat producers of our project and to communicate which farms are interested in participating in our study.

Supplies—	$
Lectin from Arachis hypogaea (peanut) –FITC conjugate (1mg)	56
Lectin from Concanavalin A—FITC conjugate (1mg)	48
Lectin from Methyl alpha-D-mannopyranosidase	30
Galactose	30
Travel to attend Sheep and Goat Internal Parasite Seminar	18.72
Postage to mail 35 surveys	15.40
Total Costs	$198.12

Author's Biography

Amanda Lynn Chaney of Searsport, Maine, majored in animal and veterinary science with a concentration in pre-veterinary studies. She is also a student in the Honors College. Amanda has enjoyed her time at UMaine, particularly her involvement in the Witter Teaching and Research Center dairy and horse barns.

After graduation, Amanda plans to attend veterinary school. She then plans to return to the state of Maine to practice large animal veterinary medicine.