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Control of a Ruminant Pathogen, Parelaphostrongylus tenuis, Using Poultry: Effects of Gastropod Diets on Ducks

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CONTROL OF A RUMINANT PATHOGEN, *PARELAPHOSTRONGYLUS TENUIS,* USING POULTRY: EFFECTS OF GASTROPOD DIETS ON DUCKS

by

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ABSTRACT

Parelaphostrongylus tenuis, referred to as "brainworm," is a parasite that originates in white-tailed deer (*Odocoileus virginianus*, WTD) and has the ability to spread and cause harm to livestock, particularly small ruminants. Larvae are shed in the feces of WTD and are picked up by gastropods (e.g. snails and slugs), where they mature to their infective stage. When livestock accidentally ingest the snails, the worms migrate through their spinal cord and around the brain, causing damage that can be fatal. Preventing brainworm infection is important to livestock owners, and a proposed method of mitigating risk is gastropod control. Snail populations can be controlled by introducing poultry (e.g. ducks); however, it is unknown whether the poultry are at risk or if they might even contribute to larvae dispersal.

The goals of this project were to determine a) whether ducks are an effective control for snails, b) whether ducks are at risk of harm when ingesting brainworm-infected gastropods, and c) whether *P. tenuis* larvae can survive the avian digestive tract to potentially go on to infect livestock. Ducks were fed infected snails in trials to monitor how many snails they eat, whether they exhibited any neurological signs, odd behaviors, or illness, and whether any parasites are present in the feces. The birds were not expected to be infected or to have larvae present in the feces. Evaluating poultry as a potential method for brainworm control could help inform livestock management decisions, potentially leading to lower risk of *P. tenuis* infection.

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Figures

Tables

BACKGROUND

P. tenuis: Life Cycle and Risk to Livestock

Parelaphostrongylus tenuis, more commonly referred to as "brainworm" or "meningeal worm," is a parasite that thrives in white-tailed deer (*Odocoileus virginianus,* WTD). The deer are the primary host, which means that the adult worms reproduce inside them. Larvae hatch in the brain of the deer and migrate to the lungs, causing the deer to cough them up. They are swallowed and move through the GI tract to be passed in the feces, from which they can be picked up by gastropods such as terrestrial snails. The L1 (first stage, noninfective to vertebrate animals) larvae mature inside the gastropods to their L3 infective stage. Animals such as moose, small ruminants, and the occasional cow or horse then ingest the gastropods and become infected. The worms migrate to the spinal cord and brain of the animal, damaging the nervous system and causing weakness, ataxia, circling, and other various neurological signs that can eventually lead to death.

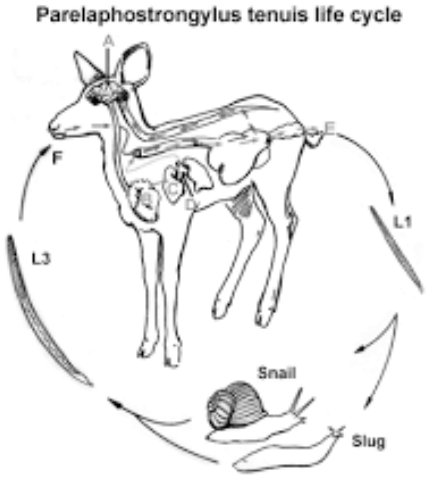

Figure 1. Brainworm Life Cycle
The life cycle of *P.* tenuis in the white-tailed deer is shown. If gastropods containing L3s are ingested by other animals, the *P. tenuis* life cycle ends, as anything but the WTD is a "dead-end" host. Courtesy of Tim McDermott DVM, and adapted from The Ohio State Sheep Team (2018).[1]

Risk Reduction

There are three main options to consider in reducing risk of infection to livestock: white-tailed deer or gastropods must be kept away from pastures, or livestock must be treated for worms periodically with a wormer that is effective against migrating *P. tenuis* larvae. By preventing deer from coming into contact with livestock grazing pastures, the amount of WTD feces deposited in that area that could potentially infect snails is limited. However, deer are common in the Eastern United States, and especially in the heavily forested state of Maine. Keeping WTD away from livestock areas can be difficult and could require a lot of effort.

Figure 2. WTD and *P. tenuis* Distribution in the US
The distribution of WTD (dotted line) and brainworm (shaded region) is shown. Courtesy of Pennsylvania Game Commission, adapted from Lankester (2001).[2]

Treatment for worms is also not without its drawbacks. Repeatedly treating animals with wormers can lead to other parasites building up resistance, causing more problems with your livestock. For example, excessive use of anthelmintics against *Haemonchus contortus*, another small ruminant parasite, have led to resistance in the worms to all major drug classes that were previously used against them.[3] Additionally, there are currently no wormers specifically designated for use against *P. tenuis*.[4] This means that farmers must work directly with veterinarians to work out a treatment plan, which adds to the farmer's costs. There can also be extra financial losses from production animals whose meat or milk must be withheld for a certain number of time after treatment. There is also no diagnostic test for livestock – because they are dead-end hosts, the worms do not reproduce within these hosts, and so infection cannot be confirmed via fecal evaluation, but only via necropsy. A serologic test for livestock is not available. Lacking confirmation of infection in the living animal makes treatment even more difficult.

The third option, gastropod control, could be done in several different ways. Monitoring pastures for long grass, wet areas, and leaf litter, and then moving your animals or altering the grazing terrain in response to these risks, has some potential. Molluscicides would also remove gastropods from the pasture, but could have the potential to harm fish, wildlife, and even livestock.[5,6] Finally, there is the option of introducing a predator: biological control.

Ducks as a Biological Control

According to many lay articles, ducks are exceptionally useful for controlling snail and slug populations in gardens. They are said to enjoy eating gastropods, being out in wet weather when snails are more active, and can offer fertilizer, eggs, or meat in return.[7] The use of ducks to control snail populations has already been studied to some extent: the birds were found to be an effective control for golden apple snails in rice fields.[8,9]

Golden apple snails are rice-field pests, and herding ducks through the fields was found to significantly lower snail populations. This is suggested to be a good way to circumvent problems related to a small labor force.[9] This would be ideal on a farm because animals could rotate between pastures, with the livestock following after the ducks, and it would be a mostly hands-free method of controlling snails. Different types of ducks might also have different affinities for snails – for example, Mallards were better than Pekins, which were better than Muscovy ducks. Additionally, only 5-10 ducks grazing over a period of 1-2 months were able to decrease snail populations dramatically – by over 80%.[8] If snail populations were to decrease on a livestock pasture in this way, the chances of an animal eating an infected snail would drop considerably.

Despite this promising research, there have been no studies thus far concerning the use of ducks in mitigating the risk of *P. tenuis* infection. However, Muscovy ducks have been assessed as a control for the aquatic intermediate host snails carrying schistosomes, which cause schistosomiasis, a disease that can harm humans. In the preliminary study, it was found that the ducks were an effective control for the snails, although further studies were suggested on a larger scale.[10]

These studies suggest that ducks have the potential to be an efficient biological control for snails on livestock pastures. However, it is uncertain whether controlling for the snails will actually prevent *P. tenuis* infection in livestock. Some birds, including mallard ducks, have the ability to pass live snails through their gastrointestinal (GI) tract. In one study, some species of aquatic snails passed through mallard GI tracts alive; thus, ducks might potentially actually disperse these snails. The aquatic snail *Hydrobia ulvae* was one of these. Interestingly, it was found that increasing the body mass of the ducks significantly decreased the amount of viable snails that survived passage through their GI tracts.[11] Additionally, another study found that some terrestrial snails can survive being digested by birds native to the Western Pacific.[12] While not specifically concerning ducks, this is relevant, because terrestrial and aquatic snails could potentially have qualities that allow for extreme environment survival (such as in the GI tract).

Because terrestrial snails can survive passing through the GI tract of some birds, and some aquatic snails have been proven to survive ducks, there is some concern about whether snails eaten on livestock pastures (and the infective brainworm larvae they are carrying) could survive and be dispersed throughout the grazing lands, going on to infect livestock. The potential for the ducks themselves to become infected by migrating *P. tenuis* could also be a concern; we are currently not aware of other studies investigating this possibility.

Objectives

This study seeks to evaluate the potential of ducks to be used as a biological control for terrestrial snails, and by extension *P. tenuis*, in order to lower the risk of brainworm infection to grazing livestock. There are three major goals: 1) to determine whether ducks will consume terrestrial snails, 2) to evaluate whether the ducks themselves may be at risk of *P. tenuis* infection and subsequent harm to their health, and 3) to evaluate whether snails or infective larvae can survive being eaten by ducks, and thus whether ducks contribute to dispersal of *P. tenuis* on pasture. We hypothesized that ducks would consume the snails, that they would not be harmed by ingesting *P. tenuis*-infected snails, and that viable larvae would not be present in the feces.

MATERIALS AND METHODS

Larvae Extraction from WTD Feces

Fresh WTD feces were collected from the area surrounding the J.F. Witter Teaching and Research Center in Old Town, Maine. Fecal Baermann tests were performed, which allowed for larvae collection from the feces. Fecal samples wrapped in filter paper were allowed to soak in water overnight, and liquid from the bottom of the funnel (suspected to contain larvae) was collected the next day. Once at least 50 mLs of liquid had been collected, it was centrifuged at 900 RPM for 10 minutes with no brake to concentrate the L1 larvae at the bottom. The supernatant was poured off, and the remaining portion with the suspected larvae was pipetted into 6-well plates (Fisher Catalog # FB012927).

These were examined with an inverted microscope for larvae. L1 larvae were morphologically identified to the best of our ability, as the larvae were moving quickly. We focused on the appearance of the posterior end (tail); *P. tenuis,* and its close relatives *P. andersoni* and *P. odocoilei*, have very similar tails with a steep slope followed by a short, tapered end.[13] In contrast, many other species of ruminant parasites have longer sheaths on the posterior end. Any larvae that were found that appeared to be reasonably close to *P. tenuis* morphologically were placed into microcentrifuge tubes (n=15-25 larvae) with a small amount of water.

Gastropod Inoculation

Amber snails (*Succineidae* genus) were collected from areas surrounding the J.F. Witter Teaching and Research Center in Old Town, Maine (Figure 3). They were collected by hand, placed into plastic bags, and later sorted by size. Those that were not immediately used were kept refrigerated at -4℃. A total of 72 snails, based on larger comparative size (~10mm total length), were selected for inoculation with L1 larvae using a protocol adapted from several sources.[14,15] The bottom of 24-well lidded well plates (like Fisher Catalog #14-380-865) were fitted with pieces of Kimwipe; then a microcentrifuge tube containing water and larvae was pipetted into each well. One snail was placed in each well and the plates were covered with the plate lid to prevent snail escape. The plates were left for approximately twelve hours in a light-neutral, room-temperature area, before snails were removed from the wells.

Snails were then placed in homemade terrariums for 14 days, estimated to be sufficient time for L3 larvae to develop. While this may differ between species and has not been extensively studied, it is estimated that it may take up to three weeks for all larvae in the snails to reach the L3 stage.[15] A subset (26 total, over three trials) of the gastropods inoculated were digested using pepsin and microscopically evaluated for L3 larvae prior to use for duck experimental trials to estimate whether infection was successful.

Figure 3. Gastropod Collection Location
Gastropods could be collected either from the sheep enclosure (labeled "1") or the deer grounds (labeled "2"). For this study, all snails were gathered from the deer grounds, which includes some marshy areas where the snails are readily available. Drawing courtesy of Tuuli Overturf (2021).[16]

Flock Care

All animal use in this project was previously approved by the Institutional Animal Care and Use Committee (approval # A2020-11-01). Ten Grimaud hybrid meat-type ducks, approximately 18 weeks old, were obtained from a local producer and kept indoors, in a climate-controlled room with natural light and appropriate ventilation. Birds were separated into two groups: five in Pen 1 (experimental) and five in Pen 2 (control). Ducks were identified with leg bands; birds 1-5 were the experimental group, and birds 6-10 were the control group. The pens were bedded with pine shavings, and feed and water were available ad libitum. A hanging nipple waterer was used in each pen, as well as feed troughs attached to the pen wall at approximately the same height as the ducks' shoulders. Commercial grower and layer feed pellets at a 50/50 ratio were provided, and the troughs were refilled as needed, usually once or twice a day. Ducks were allowed out

of their pens one pen at a time to enjoy a plastic wading pool of water in the morning while their bedding was cleaned. The control group was allowed water access first, then re-penned prior to the experimental group's water access, to prevent any potential contamination. Pool water was changed daily. Soiled bedding was transported to Witter Farm to be added to the manure pile for composting or routine manure management.

Ducks were trained to eat snails in preparation for the experimental trials. It was assumed that the ducks, which were purchased from a commercial facility, were unused to eating anything apart from a standard commercial feed. Each group was given a red dish in their enclosure that was left with them so they could become acclimated to it. They were trained to expect treats from the red dishes - for several days prior to the snail trials, dandelion greens were added to both, and ducks were observed for five minutes to see whether greens were eaten. After these trials, feeding trials were done to evaluate ducks' inclination toward eating snails. The ducks were separated into wire dog crates (Retriever brand, size medium, 36.5 L x 22.5 W x 24.75 H inches) within close proximity to their penmates. First, they were given dandelion greens to ensure that they would be willing to eat in the crates.

Once this was confirmed, they were then offered ten snails in their red dishes and were monitored for three minutes. The number of snails they ate, and their general behavior, was recorded. Because these snails were collected from around Witter Farm, there was approximately a 4% chance or less that these snails were infected with *P. tenuis*.[17] Additionally, the snails were collected just as they first started appearing in April, and therefore it is less likely that there would have been much time for larvae to develop if they had been picked up by the snails recently. Enough time passed between

these trials and the exposure trials for any issues from potential *P. tenuis* infection to have surfaced. We conclude that these snails were most likely not infected.

Behavioral and Health Data

Ducks were weighed prior to the *P. tenuis* exposure trials and again ten days after. They were observed twice a day throughout the project and any health concerns were noted on chore sheets. Due to the neurologic nature of *P. tenuis* infection, ducks were evaluated specifically for signs of weakness, lethargy, and behavioral changes once trials begin. Changes in the amount of feed or water consumed each day was noted. Additionally, a behavioral assessment protocol was adapted from a study on peafowl behavior.[18] All ducks were monitored one at a time for five minutes each during each data collection session. Ducks were assessed at the same time of day. They were observed in a randomized order to prevent any group behaviors, such as napping together, from influencing results, since ducks could only be evaluated one at a time. These evaluations occurred two times in the week prior to experimental trials and the results were averaged to provide a baseline of behavior for each bird. Behavioral data was not collected in the first week to allow the ducks time to adapt to their new surroundings.

In following the protocol, a stopwatch was used to measure the amount of time each duck spent on a given behavioral activity. The percentage of time each duck spent on each behavior was calculated, and differences between pre-trial and post-trial behavior were compared. Additional behavioral notes were added on the behavior data sheet. The data sheet (Figure 4) is an ethogram containing the behaviors that were measured, and it

was adapted from a similar ethogram created by Jones et al, with the addition of the note section and whether the pool was used on any given day.[19] This adapted ethogram is shown in Table 1.

Table 1. Ethogram

Behavior	Definition
Resting	Sitting down with eyes open or shut, without engaging in other activity such as preening
Standing	Standing without walking or engaging in other activities
Walking	Walking, including time spent socializing while moving
Socializing	Vocalizing, preening others, potential mating behaviors such as excessive tail wagging or head pumping
Feeding	Eating
Drinking	Drinking, excluding time spent collecting water for immediate use in preening
Pecking	Foraging behavior; digging in shavings, pecking at fencing or dishes
Preening	Grooming and collecting water for preening

Duck behavior was evaluated again ten days after the exposure trials. In moose and elk, clinical neurologic signs of *P. tenuis* infection typically begin between 10 and 60 days of infection; in young caribou, this number is 5-7 days.[20] It is suggested that in small ruminants it typically takes 10-14 days.[21] These numbers suggest that the time it takes for signs to appear may be proportional to body size. Therefore, it would be expected that signs in a duck, if there were any at all, would most likely appear within a week or two, which is why ten days was chosen for data collection.

BEHAVIOR SHEET Date:

	REST (s)	STAND (s)	WALK (s)	FEED (s)	DRINK (s)	SOCIAL (s)	PECK (s)	PREEN (s)	POOL (Y/N)	NOTES
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Figure 4. Sample Behavioral Data Sheet
This is a blank data sheet that was used to collect information about the amount of time each duck spent on each listed behavior. It was adapted from Jones et al (2008).[19]

A neurologic exam protocol[22] was also adapted for this project to evaluate the ducks ten days after exposure trials for any neurological signs that might result from *P. tenuis* infection. We evaluated the pupillary light reflex (PLR), which involved shining a light in the ducks' eyes to monitor pupil constriction, and the corneal reflex, which checked for an involuntary blink. The beak and ability to use the tongue were evaluated, as well as the vestibulo-ocular reflex (VOR), which monitored the ducks' ability to track and keep their gaze steady while their head was moved side to side. Then came the righting reflex, which involved tipping the bird sideways and seeing whether it could

keep its head steady, and testing placing ability, which involved lowering the bird toward a tabletop and seeing if it could correctly place its feet. Wing and foot withdrawal were measured by pinching lightly at the wings and feet to see if they pulled away. The menace reflex similarly checked whether the ducks would pull away, this time when a hand was moved toward each eye. Muscle symmetry of the keel was evaluated, as well as general alertness and stability in movement while walking.

P. tenuis Exposure Trials

Individual ducks were placed in the same wire dog crate as before in full view of the other ducks to prevent added stress. Trays were be placed underneath the crates and lined with trash bags (cut in half) for ease of feces collection. The experimental ducks were offered a dish of 7 infected snails on dandelion greens, while the control ducks were offered only the greens. The number of snails eaten was recorded to estimate the larval load. Ducks were then observed and feces were collected after two hours, which is estimated to be the GI transit time of a duck.[23] They were then be released back into their home pen, and the feces were collected into a Ziploc bag and labeled. Baermann tests were then performed within 24 hours, and any larvae collected was identified to the best of our ability following the same protocol as with the deer feces.

Data Analysis

The larval load was estimated based on the number of snails each duck ingested during the trials and the number of larvae found in the snails tested following inoculation (#snails ingested * # larvae/snail= larval load per duck). The number of uninfected snails

eaten in two feeding trials was recorded and these were statistically compared with t-tests. Ethogram data was collected and compared to assess whether there were any significant behavioral changes after the ingestion of infected snails. Two-tailed t-tests were used to determine the significance of behavioral changes and differences in the number of snails consumed, with significance set at a p-value of $p \leq 0.05$. Health data was collected throughout the project (see Tables 4-6) to compare pre- and post-trial differences and check for neurological signs. T-tests were also used to measure the significance of body weight changes. Finally, the number and viability of larvae found in the duck feces was evaluated. Data was entered into and analyzed with Excel.

Necropsy Protocol

Necropsy procedures were planned for ducks showing symptoms of neurologic or other abnormalities necessitating euthanasia, or for unexpected mortalities. Animals to be necropsied were immediately chilled, and necropsy was performed within 24 hours at the University of Maine Veterinary Diagnostic Lab (UMVDL). Animals with neurologic symptoms would be necropsied using both gross and histologic assessment, and tissue samples including the spinal cord and brain would be fixed in formalin, sectioned and stained with hematoxylin and eosin, and evaluated using light microscopy for damage and for evidence of parasite migration, per American Association of Avian Pathologists guidelines.

RESULTS

Unfortunately, Duck #3 was removed from the study after only one feeding trial, pre-*P. tenuis* infection, when it unexpectedly died. Health data we recorded prior to the event showed that the duck, while appearing to act normally, had soiled feathers in the vent area consistently since entry onto the study. It was necropsied at UMVDL, and results are reported in Appendix C.

Snail Consumption and Larval Load

In the initial trials to discover whether the ducks would voluntarily ingest snails, there were mixed results (Table 2). Some of the ducks ate most of the snails offered while others did not make any attempt to ingest snails. However, this data was collected during a training period. To the best of our knowledge, the ducks had not previously encountered snails in their lives. They were individually evaluated twice and the results were averaged. Data from the second trial was compared to the first to determine if there was a statistically significant difference – essentially, we were looking for evidence that the ducks were learning and that they could potentially improve their numbers.

In Trial 1, we observed that ducks which were less interested in the snails also tended to be less interested in the greens while in the crates. This could be due to personality differences or environmental distractions (other ducks), but it also might be possible that these ducks could have recently eaten and were not hungry. Ducks that ate more snails tended to immediately begin pecking at them, rather than waiting a minute or so like others. In Trial 2, ducks showed a trend toward improving or at least maintaining the percentage of snails eaten, but improvements were not found to be statistically

significant. Only one duck was found to perform worse in Trial 2. Ducks that did not consume snails in Trial 2 also refused them in Trial 1. As the ducks were still being trained at this stage, further testing could be done in the future to determine whether the improvement trend continues to a statistically significant level. The group on average ate 44% of the snails, with a standard deviation of 32.4, which illustrates variability among the ducks.

Table 2. Percentage of Offered Snails Eaten

Duck	Trial 1: % Eaten	Trial 2: % Eaten	Average % Eaten
1	20	40	30
2	0	0	0
3*	80	N/A	N/A
4	60	90	75
5	30	90	60
6	90	70	65
7	0	40	20
8	0	0	0
9	50	50	50
10	100	100	100

*This duck died unexpectedly; see Appendix C.

In the *P. tenuis* exposure trials, ducks were fed snails placed on top of dandelion greens to encourage ducks to eat them, as all ducks have been observed eating the greens and the data in Table 1 showed that there is some variability in these ducks' willingness to consume snails alone. The larval load was calculated from the equation (# snails ingested * # larvae/snail= larval load per duck), where the number of larvae per snail was estimated from the number found in the 26 digested snails. Unfortunately, while some L2 stage larvae had been found in digested snails, showing that inoculation of the snails with L1 larvae was successful, no L3 stage *P. tenuis* larvae was found in this study.

However, because we had some L2s previously, and because we found plenty of other species of larvae in the digested snails, we decided to continue with the exposure

trials as planned – while the lack of L3s means that health data might not reflect actual infection of a duck that had consumed *P. tenuis,* dispersal risk would be interesting to know for any type of larvae. The larval load calculated in Table 3 does not differentiate between brainworm and other larvae species, but the average number of suspected *P. tenuis* L2s and all other non-*P. tenuis* larvae is recorded "# Larvae/Snail" column.

Table 3. Estimated # of Larvae Ingested

Duck	# Snails Ingested	# Larvae/Snail	Total Larvae
1	N/A	N/A	N/A
2	N/A	N/A	N/A
3	N/A	N/A	N/A
4	N/A	N/A	N/A
5	N/A	N/A	N/A
6	6	~ 1 *P. tenuis* ~ 6 of other species	~ 42 larvae
7	6	~ 1 *P. tenuis* ~ 6 of other species	~ 42 larvae
8	7	~ 1 *P. tenuis* ~ 6 of other species	~ 49 larvae
9	7	~ 1 *P. tenuis* ~ 6 of other species	~ 49 larvae
10	7	~ 1 *P. tenuis* ~ 6 of other species	~ 49 larvae

Behavior and Health Evaluations

The ducks were observed during two sessions; during each session, the amount of time each bird spent on each of eight behaviors was recorded, with five minutes of observation per duck in each session. These two sets of data for each duck were averaged to create a baseline, and the results are displayed in Figure 5. The ducks typically did not like to eat while being observed, unless they were given a treat such as the dandelion greens, which they consumed immediately. Other than that, the seven other behaviors were generally well-represented, but with a lot of variation among the ducks. For

example, Duck #3 spent about 60% of the time preening and did not spend as much time on other behaviors. It should be noted that this duck had a history of soiled feathers, which might have skewed its behavior. Duck #3 was removed from later observation sessions after its unfortunate death. Figure 6 displays the behavioral breakdown of ducks two weeks post-trial. The statistical significance of any behavior changes is displayed in Table 4. Pool use was also monitored.

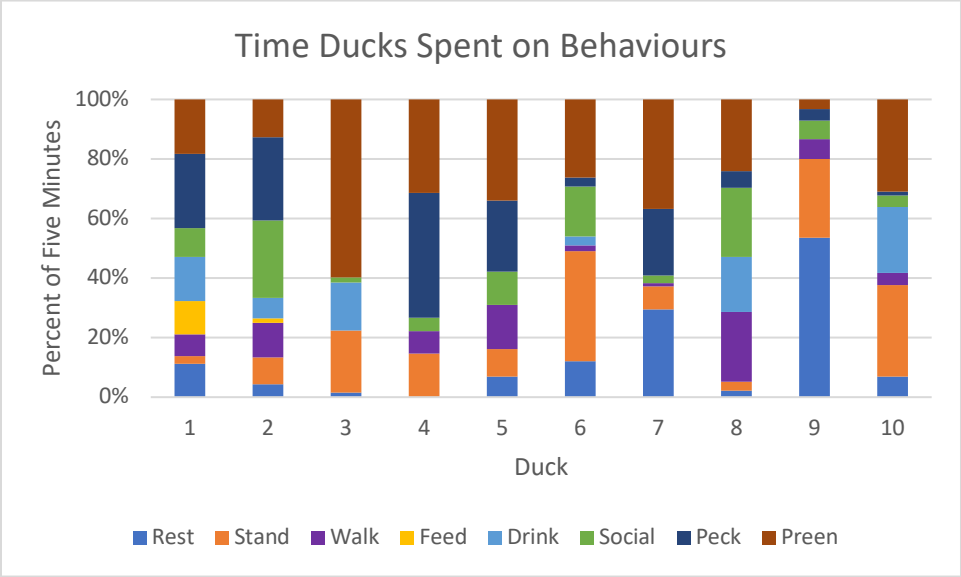

Figure 5. Duck Baseline Behavior
The percentage of time ducks spent on each behavior prior to exposure trials was recorded and is displayed here. Compare to Figure 6 below to see similarities and differences between this baseline and behavioral data collected 10 days after exposure trials were conducted.

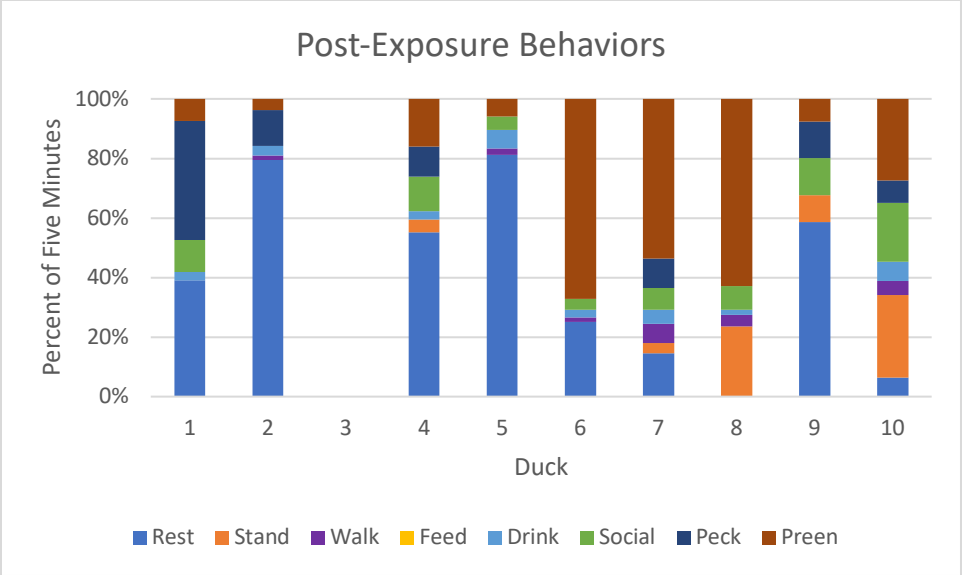

Figure 6. Post-Exposure Behaviors
The percentage of time ducks spent on each behavior on the tenth day following exposure trials is displayed here. Compare to baseline data in Figure 5 above. Note: Duck #3 was removed from the study prior to exposure trials and will be discounted from calculations in Table 3 below.

Table 4. Significance of Behavioral Data

Ducks	**Behavior**	**Mean (s, Pre-Trial)**	**Mean (s, Post-Trial)**	**T-Value**	**P-Value**	**Significant (Y/N)**
1-5, Control (excluding 3)	Rest	16.73±14.02	199.67±58.39	-6.09	.001	Y
	Stand	26.38±14.76	3.91±7.825	2.69	.036	Y
	Walk	30.84±10.18	2.65±3.13	5.29	.002	Y
	Feed	9.39±16.12	0±0	1.16	.292	N
	Drink	16.32±22.44	11.72±4.93	0.422	.688	N
	Social	38.54±27.86	21.76±18.69	1.00	.356	N
	Peck	88.60±25.42	48.16±50.93	1.42	.205	N
	Preen	71.52±29.82	27.23±20.64	2.44	.051	N
6-10, Experimental	Rest	65.93±69.87	62.56±69.48	0.076	.941	N
	Stand	64.69±45.34	36.90±34.95	1.09	.309	N
	Walk	22.88±27.67	9.76±7.51	1.02	.336	N
	Feed	0±0	0±0	0	1	N
	Drink	26.1±31.89	9.08±7.13	1.16	.278	N
	Social	32.12±26.83	29.42±16.60	0.191	.853	N
	Peck	21.96±25.73	17.50±16.95	0.323	.755	N
	Preen	73.03±37.87	130.03±77.61	-1.48	.178	N

The pool was used by all ducks each day with the exception of #10, who refused to use the pool on the first day data was recorded. Any unusual health signs, such as soiled feathers or bouts of sneezing, were recorded to assess their relevance to *P. tenuis* infection. Only #3, which died before exposure trials began, exhibited any health problems. This duck had soiled vent feathers consistently. Duck weights were also recorded once prior to, and at 10 days after, behavioral and feeding trials (Table 5) for comparison. Feed intake was recorded as well to check for loss of appetite (Figure 7).

Table 5. Duck Weights

Duck	**Initial Weight (lbs)**	**Post-Exposure Weight (lbs)**
1	9.5	9.5
2	11.0	9.5
3	N/A	N/A
4	10.0	9.5
5	9.5	10.0
6	9.25	9.5
7	9.0	9.0
8	9.5	9.5
9	9.0	9.0
10	9.5	9.5

Control ducks averaged 10 lbs initially and decreased to 9.62 lbs 10 days after exposure trials ($P>0.05$). Experimental ducks averaged 9.25 lbs initially and 9.30 lbs 10 days later ($P>0.05$). Control ducks showed the greatest differences in weight, particularly #2, but this amount of weight change was not considered to indicate ill health; these ducks were close to a mature weight at the beginning of the study.

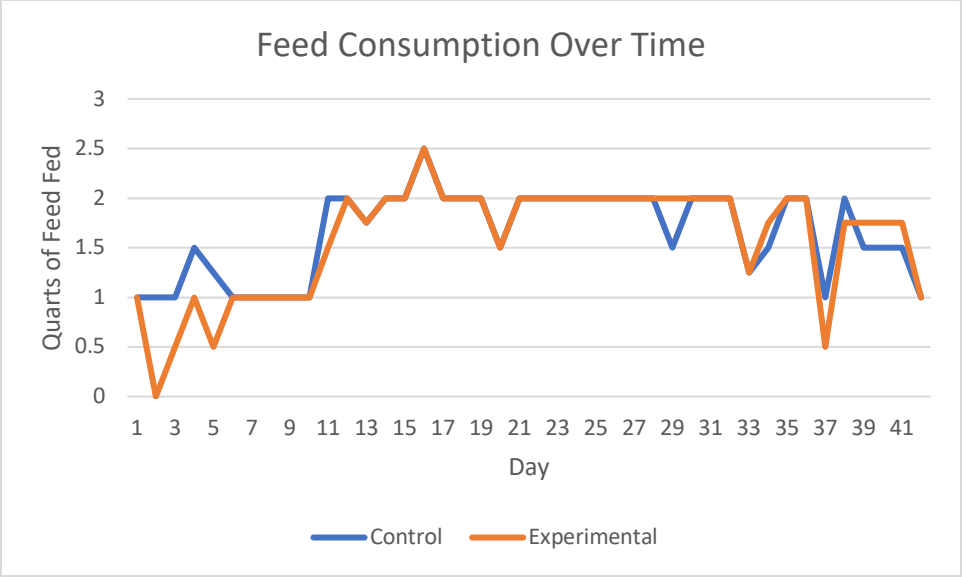

Figure 7. Feed Consumption
The control and experimental groups of ducks did not differ greatly in feed consumption. Both groups were relatively slow to start and eventually averaged about two quarts per day. Exposure trials took place on day 33, when they were not fed until after the trials. Consumption dropped after this point, but for both groups and sporadically – it is unlikely to be a result of the trials, and more likely is a result of suddenly much warmer weather noted starting around day 37.

Neurologic exams were performed on each duck ten days after the exposure trials. Results are in Table 5 below, where "N" is normal, "A" would be considered abnormal, and "*" would be considered abnormal if all birds in both groups had not reacted in the same way – therefore these were deemed normal for these particular birds. Duck #3, as before, was excluded from this data collection due to its demise. Ducks 1-5 were control and 6-10 were experimental. There was no difference between control and experimental ducks in these exams, and the only unusual result was that none of these ducks displayed an obvious wing or foot withdrawal when lightly pinched.

Table 6. Neurologic Results

Test	#1	#2	#4	#5	#6	#7	#8	#9	#10
PLR	N	N	N	N	N	N	N	N	N
Corneal Reflex	N	N	N	N	N	N	N	N	N
VOR	N	N	N	N	N	N	N	N	N
Righting Reflex	N	N	N	N	N	N	N	N	N
Wing Withdrawal	*	*	*	*	*	*	*	*	*
Foot Withdrawal	*	*	*	*	*	*	*	*	*
Menace Reflex	N	N	N	N	N	N	N	N	N
Muscle Symmetry	N	N	N	N	N	N	N	N	N
Alertness	N	N	N	N	N	N	N	N	N
Placing	N	N	N	N	N	N	N	N	N
Walking Balance	N	N	N	N	N	N	N	N	N

Fecal Evaluations

Feces collected during the exposure trials were evaluated using Baermann tests for the presence of larvae. We found larvae in all five of the experimental ducks' feces samples (Table 6). Notably, we did not find any larvae in the samples from the control group. None of these larvae were morphologically consistent with *P. tenuis*, which was expected as we could not confirm any mature infective form of *P. tenuis* (L3s) prior to the trials. None of the larvae found were consistent with typical duck parasites to the best of our knowledge. They appeared morphologically similar to larvae found in the

previously digested snails. These larvae were alive and remained able to move after being frozen for 24 hours.

Table 7. Larvae in Experimental Duck Fecals

Duck	Larval Load	Larvae in Feces
#6	~ 42	4
#7	~ 42	1
#8	~ 49	1
#9	~ 49	2
#10	~ 49	3

DISCUSSION

Efficiency

In this controlled setting, ducks varied wildly in their willingness to consume snails. None of them, to our knowledge, had been exposed to snails before; yet some ate most of the snails offered while others ignored them entirely. This could be a result of many things, such as personality differences in ducks. Some simply might not find snails palatable. There might also have been an issue with pecking order – maybe some of the ducks that went first were not used to eating before the others. Some might have been more prone to distraction from the other ducks. When we had finished a set of feeding trials, the released ducks were allowed to spend time in the pool, and this might have distracted the ducks still in the crates. For the exposure trials, ducks were immediately returned to their pens instead.

In general, the ducks ate almost half of the snails offered, but we cannot make conclusions on ducks overall with just these short trials and ducks that were unused to eating snails. There are also still other factors to consider that were not tested, such as whether they would consume snails if offered a standard feed at the same time, or whether they would be more likely to eat them if they were secluded and not surrounded by other ducks trying to get their attention. This does not directly translate to the pasture, but does act as a simple test to see whether ducks would eat snails at all, so future studies could evaluate a similar question using ducks on a natural pasture setting.

Due to snail phenology, none could be offered to the ducks until the second week of April; therefore, we did not obtain ducks until early April, and they were adults at entry. Starting with ducklings could potentially yield better results, as they could have

been trained to eat snails over a longer period of time. The ducks used, Grimaud Pekins, were also not the original intended breed for this study. Khaki Campbells seem to be one of the more popular varieties for snail and slug control and are multi-purpose – they provide eggs and meat, which might make them a more popular choice for small farmers. A study conducted with different duck breeds could yield different results.

Behavior and Health

Behavior and health data, when pre- and post-exposure trials are compared, show that the ducks were not affected by the trials. Weights did not change significantly, and the neurologic exam data show that the ducks did not suffer from any damage to their nervous system. However, all of the ducks showed some abnormality in wing and foot withdrawal – but because all of them showed this, it can not be considered to be a result of the exposure trials. During the neurologic exams, the ducks were stressed (as they were unused to being handled) and did not react as would be expected. Behavior results were different in that there were a few categories that significantly changed, but only for the control group. For some reason, these ducks decided to rest more and stand and walk less. There were no differences in behavior, especially in the experimental group, that could be contributed to exposure to larvae.

It should be noted that, because we did not identify any L3 larvae, nor prove larvae to be *P. tenuis* larvae prior to the exposure trials, we did not optimize the chances of infecting ducks with *P. tenuis*. We did not expect the L1 or L2 larvae to cause any problems in the ducks, and we did not observe neurological signs in the experimental versus the control groups. We cannot conclude how ducks might be affected by *P. tenuis* infection because we cannot guarantee that they ingested any infective-stage larvae,

which means that this objective was not met and that further studies will be needed in order to draw firm conclusions.

We had expected to find L3 larvae in snails prior to exposure trials, and because we did not, we need to consider what might have gone wrong. First, we might have infected the snails with the wrong species of nematode. This is possible, because we did find L3 larvae of other, unidentified species in the snails. Some of these might already have been in the snails, but the quantity suggests that we might have infected them. Because the larvae were moving rapidly and because there were so many of them, it was difficult to draw firm conclusions based on morphologic assessment prior to inoculating the snails. In the future, a sample could be heat-killed and the percentage of *P. tenuis* could be evaluated. We are fairly certain that we found L2 stage *P. tenuis* in some snails, so it was odd that none fully developed in the snails that we digested. This might have something to do with the time of year, temperature at which the snails were kept, or a myriad of other potential reasons. More studies will be needed. As it was, we continued the trials with what we had.

We also had some issues with behavior data collection. Ducks are social birds. When one duck decides to preen itself, chances are that most of the other ducks will begin too. Unfortunately, this made recording behavior difficult. Because only one person was watching the ducks at a time, and only one duck could be observed at a time, behavior was skewed by what the group of ducks decided to do together. For example, while recording Duck #1, we might record data that says it drank a lot. All of the other ducks drank a lot, too, but by the time Duck #2's turn came, they had all moved on to resting together. The standard deviation for all behavior data points was large because it

appeared that there was a lot of variance in behavior among the ducks, when in reality they acted similarly but our recording system was flawed.

I randomized the order on each consecutive trial to attempt to account for this, but the most accurate way to record this sort of data in the future would probably be with a game camera. All of their behaviors would be recorded at the same time and video could be watched later and assessed. Alternatively, ten observers (or as many as there are birds in the study) could record data at the same time. However, this might make the ducks nervous and cause them to act unnaturally. These ducks in particular were not fond of humans, an unfortunate side effect of being raised in a commercial facility, and this made data collection difficult. The ducks preferred not to eat in front of us, and they often moved as far away as they could when we entered the room and their pens. They were difficult to catch and handle, making most tasks harder than anticipated. Raising them from a younger age would probably have solved this problem, but unfortunately we had several pandemic-associated setbacks in getting the project started and this was not an option.

Dispersal Risk

All of the experimental ducks passed at least one larva through their gastrointestinal (GI) tracts. These larvae remained alive even after 24 hours of freezing. They could not be identified with any accuracy, but they resemble the worms found in the digested snails. None were identified as *P. tenuis*, and none appeared to resemble duck parasites, to our knowledge. It appears that these larvae most likely were inside the infected snails that were fed to this group, which suggests that some larvae have the

ability to pass through the duck GI tract alive. We wanted to do these tests even without having confirmed infection with *P. tenuis* L3s, because we wanted to see if any kind of viable larvae could pass through the duck GI tract; therefore, we were surprised by this finding. Because these larvae were able to survive, it seems possible that *P. tenuis* L3s could also potentially survive the duck GI tract, which would imply that ducks can disperse larvae via their feces. However, it should be noted that the larval load ingested by these ducks was over 40 per duck, while the most larvae that passed through was four. Therefore, fewer than 10% of larvae passed through the duck GIT alive, which implies reduction of the risk of infection to livestock on shared pastures, even if it does not completely eradicate risk. Further studies are needed that can guarantee ducks' ingestion of *P. tenuis* L3-infected snails.

Overall Suitability of Ducks

The first goal of this project was to determine whether ducks would eat terrestrial snails. While they did not eat as many as we had hoped (for a variety of reasons), the ducks did eat snails. The second goal was to find out whether ducks could be harmed by ingesting *P. tenuis*. We cannot draw firm conclusions because we could not guarantee that the ducks actually ingested *P. tenuis*. However, they did ingest some sort of larvae, and on average may have ingested one *P. tenuis* larva per snail. None of the ducks displayed neurological signs, behavioral changes, or other health issues. Further studies are needed on this subject. The final goal was to discover whether ducks might contribute to dispersal of *P. tenuis*. For the same reasons as with the second goal, we cannot make conclusions. However, unknown types of larvae, most likely acquired from the infected snails, were found in the feces of experimental ducks. This was completely unexpected

and warrants further investigation. Overall, no conclusions on the suitability of ducks as a control for brainworm-infected gastropods can be made at this time.

While conclusions cannot be made, speculation on how ducks may be implemented could still be useful. The number of snails per pasture would dictate the number of ducks needed. We are unaware of any studies about the number of ducks needed on pasture, but if we consider Teo et al's rice field trials, each duck ate the most at a density of three snails per square meter, and plateaued after a density of five.[8] They found that optimum number of ducks was three per 7x7 plot, or nearly 250 per acre. This would likely be extremely unrealistic for a small farmer, but this is only the optimum over an extremely short period of time – not what would be used in a normal grazing situation. When they looked to a more realistic goal of decreasing snail density to less than one per square meter, it took four weeks for eight ducks to do this on a hectare – meaning that three might be a good number per acre. If we allow the time limit to increase to seven weeks, two ducks per acre would be sufficient.

Of course, this is on rice fields and not a pasture – which is why pasture studies with ducks would be useful. We would also need to consider risk of predation on the ducks as well as how to best implement a rotational grazing system with the farmers' other livestock. As far as predation goes, there will always be a risk on an open pasture. Keeping ducks in a poultry tractor or otherwise protecting them at night would be a good start. Introducing a few geese to the flock might be a good way to prevent predation from hawks or other animals looking to attack the ducks.

Typically, a farmer would rotate poultry through after the livestock – cleaning up after them to reduce flies and generally clean up the pasture.[24] But what we would

propose is having ducks rotate through pastures for a few weeks prior to bringing ruminants onto the pasture. We are unaware of studies on rotating animals in this order at this time. A SARE study looked at use of poultry on pasture, rotated after ruminants, and found this to be ecologically sustainable and cost-effective.[25] However, using poultry prior to ruminants on pasture, or cograzing at the same time, might present additional things to consider, such as transfer of diseases between poultry and ruminants. *Salmonella* might be a concern, for example. I would suggest further studies on how ducks might be used on the pasture, and on the order of pasture rotation.

Future Directions

As stated above, use of younger ducks of the Khaki Campbell breed, on the pasture, could potentially be a more accurate study. An alternative to providing ducks with infected snails could be to gavage the ducks with L3 larvae to obtain more accurate results on the effects of *P. tenuis*, because there is no way to guarantee that every snail fed has the same amount of larvae. However, this would be less similar to how a field test in a pasture setting would work, because ducks on the field would be eating the entire gastropod, not simply the larvae. Potential alternative studies using other types of poultry such as chickens could be explored in the future. In the event that later studies show significant results either supporting or disagreeing with the potential for ducks to be used as a biological control for *P. tenuis*-infected snails on livestock pastures, this information may be useful for owners of grazing livestock and of ducks.

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APPENDICES

Appendix A: IACUC Approval

Maine's Public Universities
UNIVERSITY OF MAINE SYSTEM

Anne Lichtenwalner <anne.lichtenwalner@maine.edu>

Protocol A2020-11-01 - Approval

2 messages

Paula Portalatin <paula.portalatin@maine.edu> Mon, Nov 16, 2020 at 9:20 AM
To: Anne Lichtenwalner <anne.lichtenwalner@maine.edu>
Cc: Brenda Kennedy-Wade <brenda.kennedywade1@maine.edu>, Jess Majors <jessica.majors@maine.edu>

Protocol #: A2020-11-01
Title: AN INVESTIGATION INTO THE USE OF POULTRY AS A PASTURE-BASED CONTROL OF BRAINWORM-INFECTED GASTROPODS
PI: Anne Lichtenwalner
Approval Period: 11/16/2020 - 11/15/2023

Dear Anne,

The above referenced protocol has been approved by the University of Maine IACUC. As a courtesy the IACUC Office will generally send out reminders for annual and de novo reviews however, it is ultimately the responsibility of the PI to ensure that the protocol is renewed on time.

All of the proposed methods, procedures, and conditions have been approved AS STATED IN THE PROTOCOL APPLICATION. The IACUC must approve any changes or deviations from the approved protocol prior to being initiated.

University of Maine Animal Welfare Assurance #: A3754-01
The University of Maine is registered as a research facility in accordance with the U.S. Department of Agriculture Animal Welfare Act and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals. The University of Maine holds the Office of Laboratory Animal Welfare (OLAW) of the National Institutes of Health assurance for vertebrate animals used in research, teaching and outreach.

The Animal Welfare Assurance (1) confirms the commitment that the University of Maine will comply with the PHS Policy, with the Guide for the Care and Use of Laboratory Animals, and with the Animal Welfare Regulation; (2) describes the institution's program for animal care and use; and (3) designates the institutional official responsible for compliance.

Attached to this email is the final approved protocol and cage card.

Before initiating an approved protocol, please note that you must also submit a Research & Scholarly Activity Request. More information can be found on **COVID-19 Guidance for Researchers** page.

Let me know if you have any questions, thank you.

Sincerely,
Paula

Please check COVID-19 Guidance for Researchers for important updates.

IRB users - please view the new **FAQ page** on our website.

***Please note I am working remotely and actively responding to emails.*
If your message is urgent please indicate this in the subject line.

Paula Portalatin, M. Ed., CPIA
Research Compliance Officer III
University of Maine
Alumni Hall Room 311
(207) 581-2657
https://umaine.edu/research-compliance/

Appendix B: Additional Photos

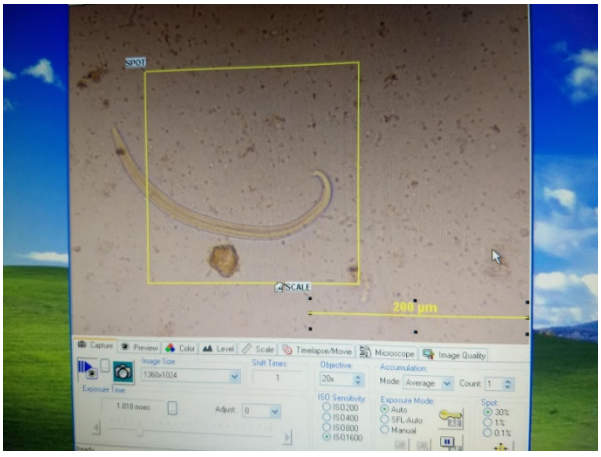


Photo 1: L1

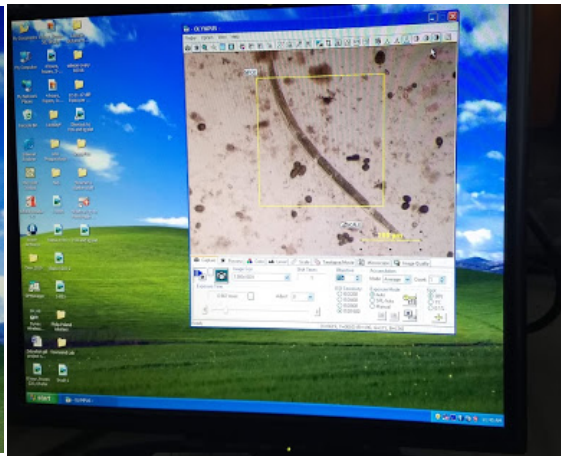


Photo 2: L2

Photo 3: Snails from Witter Farm

Photo 4: Fecal Baermanns

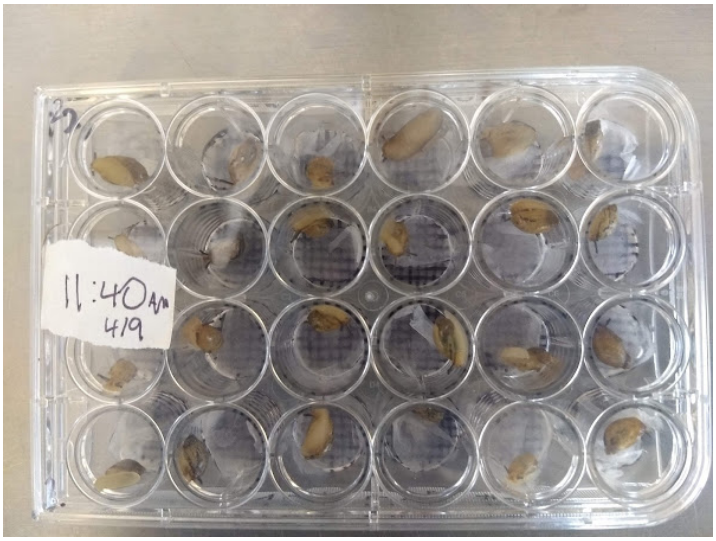


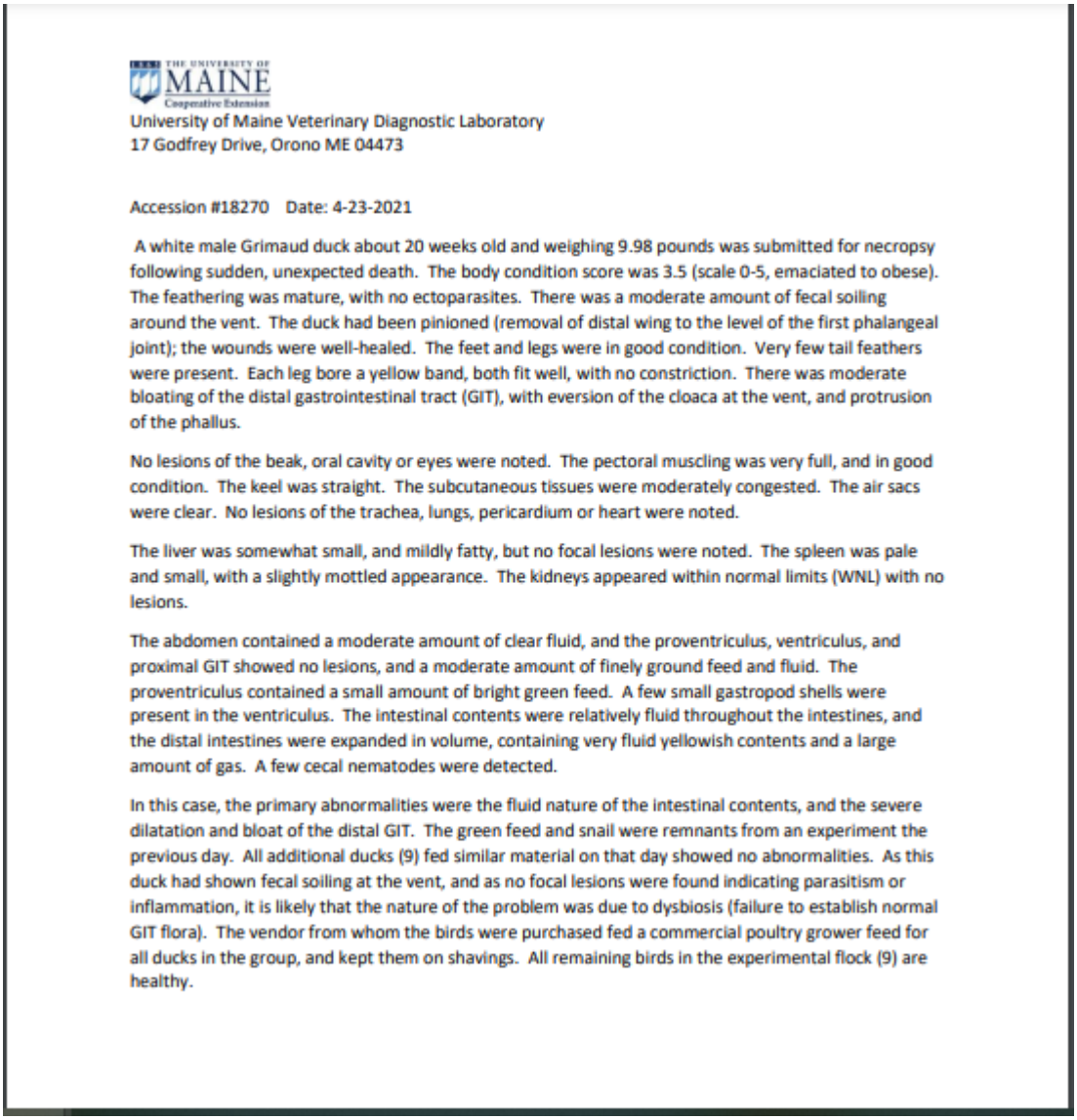
Photo 5: Snail Inoculation

Photo 6: Duck Pens

Photo 7: Feeding Trials

Appendix C: Necropsy Results

Duck #3 was found to have dysbiosis. It suffered from a constricted vent and distension in the abdomen from excessive gas and fluid. Its distal colon was severely distended and a culture is pending to determine whether an enteric infection had occurred. There was no obvious evidence of parasitism. It was concluded that the death of the bird was due to a chronic condition and was unrelated to the study. An official necropsy write-up is below.



THE UNIVERSITY OF MAINE Cooperative Extension

University of Maine Veterinary Diagnostic Laboratory
17 Godfrey Drive, Orono ME 04473

Accession #18270 Date: 4-23-2021

A white male Grimaud duck about 20 weeks old and weighing 9.98 pounds was submitted for necropsy following sudden, unexpected death. The body condition score was 3.5 (scale 0-5, emaciated to obese). The feathering was mature, with no ectoparasites. There was a moderate amount of fecal soiling around the vent. The duck had been pinioned (removal of distal wing to the level of the first phalangeal joint); the wounds were well-healed. The feet and legs were in good condition. Very few tail feathers were present. Each leg bore a yellow band, both fit well, with no constriction. There was moderate bloating of the distal gastrointestinal tract (GIT), with eversion of the cloaca at the vent, and protrusion of the phallus.

No lesions of the beak, oral cavity or eyes were noted. The pectoral muscling was very full, and in good condition. The keel was straight. The subcutaneous tissues were moderately congested. The air sacs were clear. No lesions of the trachea, lungs, pericardium or heart were noted.

The liver was somewhat small, and mildly fatty, but no focal lesions were noted. The spleen was pale and small, with a slightly mottled appearance. The kidneys appeared within normal limits (WNL) with no lesions.

The abdomen contained a moderate amount of clear fluid, and the proventriculus, ventriculus, and proximal GIT showed no lesions, and a moderate amount of finely ground feed and fluid. The proventriculus contained a small amount of bright green feed. A few small gastropod shells were present in the ventriculus. The intestinal contents were relatively fluid throughout the intestines, and the distal intestines were expanded in volume, containing very fluid yellowish contents and a large amount of gas. A few cecal nematodes were detected.

In this case, the primary abnormalities were the fluid nature of the intestinal contents, and the severe dilatation and bloat of the distal GIT. The green feed and snail were remnants from an experiment the previous day. All additional ducks (9) fed similar material on that day showed no abnormalities. As this duck had shown fecal soiling at the vent, and as no focal lesions were found indicating parasitism or inflammation, it is likely that the nature of the problem was due to dysbiosis (failure to establish normal GIT flora). The vendor from whom the birds were purchased fed a commercial poultry grower feed for all ducks in the group, and kept them on shavings. All remaining birds in the experimental flock (9) are healthy.

AUTHOR'S BIOGRAPHY

Tuuli Overturf was born in Bangor, Maine, on January 25, 1999. She was raised in Corinth, Maine, and graduated from Central High School in 2017. She then decided to pursue a major in Animal and Veterinary Sciences at the University of Maine, adding a Pre-Vet concentration her freshman year and a Neuroscience minor the year after. Tuuli has been a member of UMaine's track and field team and a seasonal employee of the animal science department's J.F. Witter Farm. She has accepted an offer to become a member of the Cornell University College of Veterinary Medicine class of 2025, where she hopes to specialize in large animal medicine to prepare her to return to rural Maine for her future career.