

Prevalence of the *Haemonchus sp.* parasite in Oregon Cattle

by  
Kayla Castle

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of  
the requirements for the  
degree of

Honors Baccalaureate of Science in Zoology  
(Honors Scholar)

Presented August 11, 2016  
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AN ABSTRACT OF THE THESIS OF

Kayla Castle for the degree of Honors Baccalaureate of Science in Zoology presented on August 11, 2016 Title: Prevalence of the *Haemonchus sp.* Parasite in Oregon.

Abstract approved: \_\_\_\_\_

Claudia Häse

*Haemonchus sp.* is known to be present in geographical regions of Oregon that are more arid and warm, which has been the preferred climate of this parasite. However, it was not detected in Western Oregon bovine until recently. *Haemonchus sp.* was first detected in Western Oregon bovine from a fecal sample submitted to the Oregon Veterinarian Diagnostic Lab on August 6<sup>th</sup>, 2014. This study was aimed to determine whether there are significant numbers of cattle from Western Oregon affected with *Haemonchus sp.* as well to obtain a geological understanding of where the parasite is located throughout the state. It was found that *Haemonchus sp.* is no longer isolated to eastern arid and warm regions of Oregon. This parasitic worm is currently being detected in noteworthy numbers in many different areas around Oregon year round. The rising prevalence of *Haemonchus sp.* is a concern due to the economic losses that can result from Haemonchosis. One hundred bovine fecal samples from Oregon, with a focus on those from Western Oregon, were examined during the duration of this study; thirty-eight tested positive for *Haemonchus sp.*, six tested negative, and fifty six did not contain a significant number of Trichostrongyle eggs to be tested.

Key Words: *Haemonchus controtus*, *Haemonchus placei*, Peanut Lectin, geographic range, Oregon Bovine

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Kayla Castle, Author

**1. Introduction:**

### 1.1 *Haemonchus* sp.

There are many distinct species of *Haemonchus*. *Haemonchus contortus* and *Haemonchus placei* are the two species of the genus *Haemonchus* demonstrated to infect cattle in the United States (Blouin, M. S., Yowell, C. A., Courtney, C. H., & Dame, J. B. 1997). Though they have similar phenotypes, behaviors, and host interactions, *Haemonchus contortus* and *Haemonchus placei* are different species based on mitochondrial DNA (mtDNA) evidence (Blouin, Yowell, Courtney, & Dame, 1997). *Haemonchus contortus* primarily infest small ruminants whereas *Haemonchus placei* are hosted by cattle and other large ruminants. Both species however, can survive in the other's respective host (Blouin et al., 1997).

*Haemonchus sp.* is a gastrointestinal nematode of the family Trichostrongylidae, commonly known as a “Barber Pole” or “Large Stomach” worm. Of the nematodes in the family Trichostrongylidae, *Haemonchus contortus* is usually the most damaging species to food production animals (Jurasek, Bishop-Stewart, Storey, Kaplan, & Kent, 2010). The worm attaches to the stomach lining of the fourth stomach, the abomasum, in ruminant animals through the use of a single dorsal tooth (Sendow, 2003). During the first two stages of the parasite's life cycle, the larvae feeds on bacteria in manure; it is during the later stages that the organism parasitizes the host and feeds on the blood from the lining of the abomasum. *Haemonchus sp.* ova are secreted through feces and transmission occurs through animals feeding around infected fecal pats. *Haemonchus sp.* is very well adapted; it can survive in various environmental

conditions, and has the ability to migrate distances up to one meter from feces to the tips of the blades of grass (Rickard, 1989).

This parasitic worm has been the cause of serious economic loss for many food animal producers. It is especially a concern for younger animals, due to the nature of this parasite. The nematode obtains nutrients from feeding on the blood of its host. As animals age, immunity tends to decrease. However, each animal has its own unique immunities which can account for the presence of *Haemonchus sp.* in older animals. Haemonchosis, the disease caused by *Haemonchus sp.*, often causes severe anemia in animals. Clinical signs specifying Haemonchosis include anemia, indicated by pale eyelids due to blood loss; hypoproteinemia; edema, also known as bottle jaw; lethargy, weight loss, diarrhea, and/or death (Sendow, 2003). The *Haemonchus sp.* parasite makes weight gain and growth challenging and can be fatal to the host. Clinical symptoms of *Haemonchus sp.* mimic those of many other parasite infections. It is important to know which parasite is present to properly treat an animal. *Haemonchus spp.* are potentially very harmful parasites because they have been found to be resistant to many standard deworming methods.

## 1.2 Previous geographical range of *Haemonchus sp.* in Oregon

*Haemonchus sp.* is known to be present in Oregon; however, it was not previously seen in western regions of the state. The Epizootiology of Gastrointestinal Nematodes of Cattle in Selected areas of Oregon, a thesis written by Lora G. Rickard in 1989,

tested various study sites during multiple seasons for the prevalence of differing nematodes in various group bovine. The study tested four sites in Oregon that were ideal representations of differing environments throughout the state, including a site near Fort Rock, and a site southeast of Klamath Falls, Corvallis, and Langlois. Each site had twenty-four to thirty-two tracer calves introduced in sets of three to four in different areas around the site. The study was performed over a two year period, 1985 to 1986, and recovered eight nematodes in Oregon cattle including *Ostertagia sp.*, *Cooperia sp.*, *Nematodirus sp.*, *Trichostrongylus sp.*, *Trichuris sp.*, *Oesophagostomum sp.*, *Haemonchus sp.*, and *Capillaria sp.* *Haemonchus sp.* was found to be present in cattle at Klamath Falls and the site near Fort Rock only.

At Klamath Falls, one *Haemonchus sp.* worm was recovered in the summer of 1985, eight were recovered in the spring of 1986, and thirteen were recovered in the summer of 1986. The study indicated that at Fort Rock, fourteen *Haemonchus sp.* worms were observed in the summer and two were observed in the spring of 1985. None were recovered in 1986 at Fort Rock. No *Haemonchus sp.* parasites were observed at either the Corvallis or Langlois sites (Rickard, 1989). *Haemonchus sp.* was not recovered at any study site during the fall or winter seasons, and was first detected in bovine fecal matter from western Oregon at the Oregon Veterinary Diagnostic Lab in Lane County on August 6<sup>th</sup> 2014.

### 1.3 Identification of *Haemonchus sp.* ova

Historically the standard method for identifying the *Haemonchus spp.* parasites required them to be cultured for identification in the third stage larvae, which delayed diagnoses. D.G. Palmer found, through testing the use of 10 different lectin stains on Trichostrongylid nematodes in sheep, that the staining of ova using peanut lectin correctly identified the percentage of *Haemonchus spp.* eggs present in infected samples (Palmer & McCombe, 1996). Peanut lectin was determined to bind exclusively to the membrane of *Haemonchus spp.* ova (Palmer & McCombe, 1996) and not to other eggs of the family Trichostrongylidae.

A more rapid identification of *Haemonchus spp.* ova using peanut lectin was found through the study titled “Modification and Further evaluation of a fluorescein-labeled peanut agglutinin test for identification of *Haemonchus contortus* eggs”. The use of sugar centrifugation methods was found to reduce the time and labor required for the purification and collection of eggs used for identification of percent *Haemonchus* ova present using peanut agglutinin to bind to the membrane of positive ova and stain it. The presence of *Haemonchus sp.*, when in significantly high numbers, can be detected through the standard operating procedure “Rapid differentiation of *Haemonchus* Ova in Fecal Exams” set by the Oregon Veterinary Diagnostic Lab (OVDL). The method can also be reproduced by following procedural guidelines set by WJ Foreyt in the Veterinary Parasitology Reference Manual (Foreyt, 1997). The procedure uses peanut lectin to bind to, and cause, the outer-membrane of ova positive for *Haemonchus sp.* to fluoresce.

#### 1.4 Initial Screening for Trichostrongyle eggs.

*Haemonchus sp.* is a member of the Trichostrongyloidea family. For diagnostic purposes, routine OVDL fecal screening exams are used to identify the presence of Trichostrongyle ova before running the *Haemonchus* Identification test. For the purpose of this study, both the “McMaster’s Fecal Floatation” (Foreyt 1997), and the “Sugar Centrifugation Fecal Parasite Examination” (Cebra C. K., 2008) were used for the identification of Trichostrongyle ova in samples prior to further testing. The McMaster’s exam is the common test for bovine samples and Sugars Centrifugation is primarily for camelid and companion animal samples submitted to the OVDL. These tests are fecal floatation methods used to allow various parasites to rise in the solutions on microscope slides and be identified by the reader.

C. K. Cebra and Bernadette V. Stang performed a study on the comparison of different methods to detect gastrointestinal parasites in camelids, and found that both the “Sugars Centrifugation Fecal Parasite Examination” and the “McMaster’s Fecal Floatation” yielded accurate results. It was found that Sugars Centrifugation yielded slightly more positive results for Trichostrongyle ova than the McMaster’s exam. However, the “McMaster’s Fecal Floatation” was also found to be a fairly accurate method and yielded the most positive results for coccidia.

#### 1.5 Statement of Purpose

The goal of this study was to test one hundred bovine fecal samples to determine the prevalence of *Haemonchus sp.* in cattle of western Oregon. Another aim of this project is to get a better geographical idea of where the parasite is located throughout the state of Oregon. We hypothesized that *Haemonchus sp.* is becoming more prevalent in western Oregon, which has the potential to cause significant problems due to lack of awareness and sufficient prevention techniques.

## **2. Approach/Methodology**

### **2.1 Sampling**

One hundred bovine fecal samples were examined throughout the duration of this study. Bovine fecal samples submitted for routine examination to the OVDL from various locations in Oregon were the primary source of samples tested. Many samples submitted were submitted to the OVDL for diagnosis of cause of illness; therefore, the sample population of bovine is somewhat skewed compared to the general population of Oregon. Samples were also submitted for “wellness” checks on apparently healthy animals. However, submissions forms do not always disclose that information and it was not recorded for this study. Other samples submitted directly for testing associated with this study were obtained through requests by e-mail and connections with the OVDL.

Bovine fecal samples submitted to the OVDL throughout the duration of the study were first examined using a quantitative fecal floatation (McMaster's or Sugar Centrifugation) as requested by the client. If a sufficient amount of the sample was submitted, the feces were included in this study. The typical test run for routine bovine fecal screening is the McMaster's Fecal Floatation Exam. If Trichostrongyle eggs were seen in the original floatation examination, samples were then subjected to the *Haemonchus* ID test. If no Trichostrongyle eggs were evident from the McMaster's fecal screening test, fecal samples were concentrated and examined using the Sugars Centrifugation Fecal Parasite Examination.

## 2.2 Data analysis

The data was categorized and analyzed based on the county of origin, presence and number of Trichostrongyle eggs, whether or not the *Haemonchus* test was performed, and the percentage of Trichostrongyle eggs found positive for *Haemonchus sp.* in each sample upon test conclusion. Location, age, sex, whether the feces were of a beef or dairy breed, other parasites found, egg counts, and whether a sample required fixing or was tested fresh were also recorded.

## 2.3 McMaster's Fecal Floatation

McMaster's Fecal Floatation parasitic examination procedures following those set by W. Foreyt (Foreyt 1997) were used. Briefly, four grams of feces were mixed with 26

mL of saturated salt solution using a mortar and pestle to break up the feces. The saturated salt solution consisted of a mixture of NaCl and H<sub>2</sub>O with a specific gravity of 1.18 to 1.21. Volumes were adjusted for sample sizes containing fewer than four grams of feces. The mixture was filtered through a funnel with gauze, loaded into one chamber out of three chambered counting slides, and the eggs were allowed to rise for fifteen minutes. The slides were then examined under 40X magnification for the determination of all common parasites.

#### 2.4 Sugar Centrifugation Fecal Parasite Examination

Standard operating procedure for the Sugars Centrifugation Fecal Parasite Examination following guidelines established by CK Cebra (Cebra C. K., 2008) were used. Two grams of feces were crushed in a Wirl-Pak™ bag, mixed with ninety-eight milliliters of distilled water, and refrigerated overnight. Cold Storage was of importance because it was found to inhibit *H. contortus* eggs from hatching (Jurasek et al., 2010). Samples were left in bags in the refrigerator for longer durations of time. If samples contained few or no Trichostrongyle eggs when tested using the McMasters screening test they were occasionally concentrated using a higher ratio of feces to distilled water and then retested using the sugars centrifugation technique. When samples were kept for a period of longer than two weeks, formalin was added to fix any eggs the sample might contain. Formalin was found to preserve samples without affecting the results of the lectin staining (Jurasek et al., 2010).

The Sugars Centrifugation Fecal Parasite examination utilizes a series of centrifugation and decantation. Ten millimeters of the fecal suspension were decanted and centrifuged at 280 RCF for 5 minutes, and the supernatant was then decanted. Saturated Sugar, a solution of sucrose and water with the specific gravity of 1.27, was mixed with the fecal pellet and centrifuged at 280 RCF for 5 minutes. Enough saturated sugar solution was added to the tube to create a slightly bulging meniscus. A microscope coverslip was placed over the meniscus and left to set for an hour to allow the eggs rise to the underside of the coverslip. Slides were read for all common parasites with a specific focus on Trichostrongyle eggs.

### 2.5 *Haemonchus* Identification

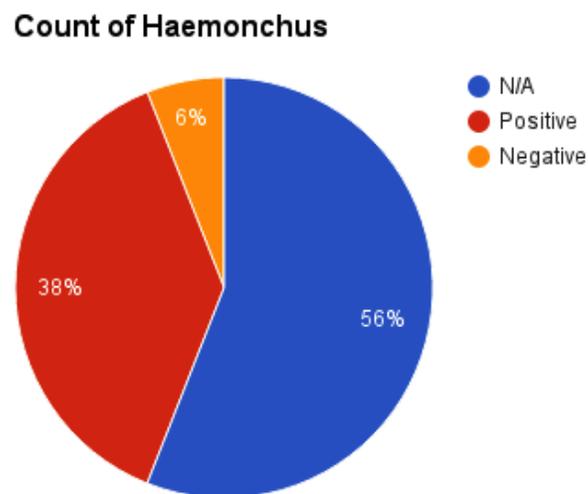
The procedure for the rapid differentiation of *Hamemonchus* ova in fecal using a modified sugars centrifugation technique for egg collection (Jurasek et al., 2010) and a peanut lectin staining procedure (Palmer 1996) for identification were used, with one modification; the concentration of peanut lectin was doubled to obtain a brighter ring around the eggs positive for *Haemonchus sp.* A positive control (*Haemonchus sp.* ova positive) and a negative control (Trichostongyle negative) were run alongside each group of samples tested for *Haemonchus sp.* identification. The same steps used for the preparation of the sugar centrifugation exam until the removal of the coverslip were used for the preparation of the *Haemonchus* ID test. If samples contained low numbers or no Trichostrongyle ova in the original screening test, they were

concentrated with an additional spin and decanting step. The original spin, where ten milliliters of fecal solution were poured into a centrifuge tube, centrifuged at 280RFC for five minutes, and then decantation was performed for all samples tested. For the samples that were concentrated, an additional ten milliliters of the fecal solution was added to the tube with the respected fecal pellet and then spun down for an additional five minutes at 280 RFC. After the concentration step, the normal operating procedures for the Sugars Centrifugation fecal exam were performed up until the removal of the coverslip.

For the collection of eggs, slides were rinsed into 1.5mL tubes with phosphate buffer saline (PBS) at pH 7.4. Tubes were then subjected to a series of rinsing, decanting, and centrifuging. Peanut Lectin with double the normal concentration used in routine *Haemonchus* ID test, was added to the tubes and left on a mixing plate for an hour to allow the lectin to bind to the outer-membrane of positive *Haemonchus sp.* eggs. The tubes were then subjected to another series of centrifuging, decanting, and rinsing in conjunction with the protocol. The small volume remaining after the final supernatant of the last rinse was removed, and contained the possible eggs for examination. This volume was pipetted onto slides covered with a coverslip and read under a fluorescent microscope to determine whether eggs were present and positive for *Haemonchus sp.*

### **3. Results and Discussion**

The procedure for rapid differentiation of *Hamemonchus* ova in fecal exams indicated that when Trichostrongyle ova were present in cattle of Oregon, *Haemonchus sp.* was the dominant genus. Figure 1 illustrates the percent of samples that tested positive and negative for *Haemonchus sp.* and those where not enough information was present for *Haemonchus sp.* identification.

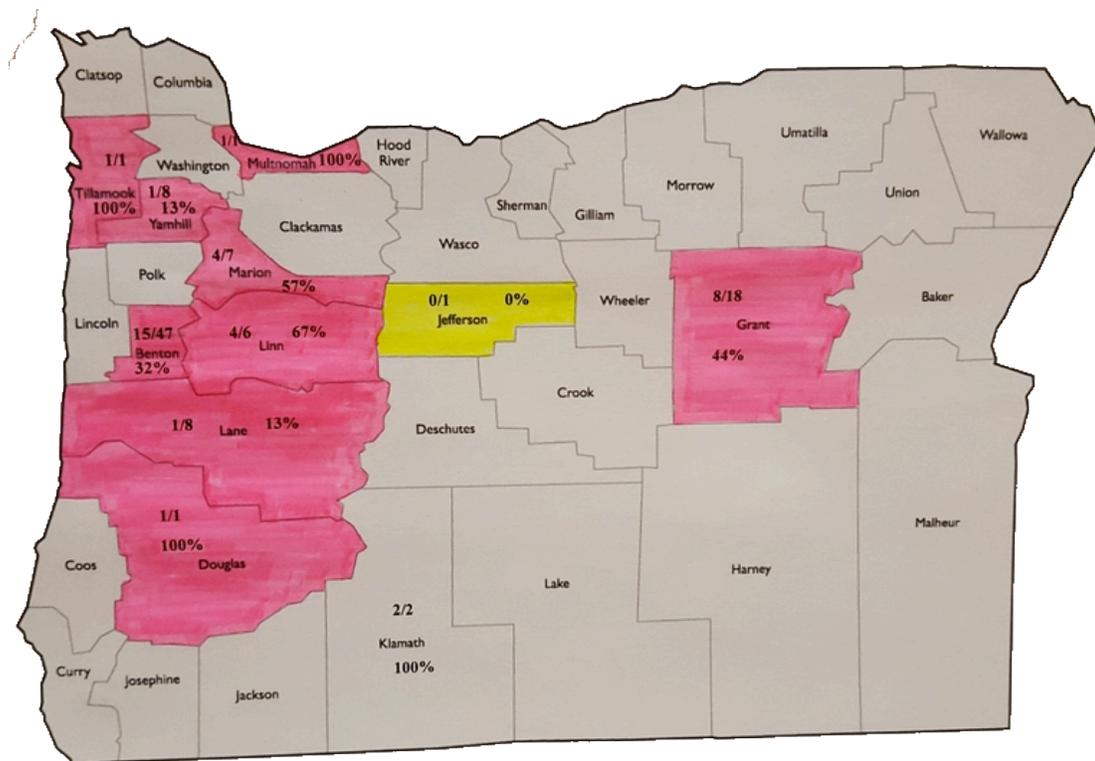


**Figure 1.** Percent of samples “Positive” (those that contained at least one positive *Haemonchus sp.* ova), “Negative” (those that contained only negative Trichostrongyles), and “N/A” (those that contained <5EPG Trichostrongyles and were inconclusive for *Haemonchus sp.* identification) out of the one hundred samples obtained for this study.

Of one hundred samples tested, forty-four contained Trichostrongyle ova when tested for the identification of *Haemonchus sp.* Of the forty-four samples, thirty-eight indicated there was ova positive for *Haemonchus sp.* in varying percentages and just 6 contained only negative ova. Fifty-six samples either did not contain high enough numbers of Trichostrongyle ova to run the test for the identification of *Haemonchus sp.* or did not yield ova for identification when tested. Through the examination of the test results and the counties from which the fecal samples were obtained, as we hypothesized, *Haemonchus sp.* is becoming more prevalent. In 1985 and 1986 they (Richards et al. 1987) found *Haemonchus* in Klamath and Lake County, but not in Benton or Curry County. Table 1 describes the current counties from which the samples were obtained and the corresponding results for *Haemonchus sp.*, and Figure 2 illustrates the counties in Oregon that tested positive for *Haemonchus* and the amount of positive samples over the total number of samples from that county that yielded Trichostrongyle ova for identification in the *Haemonchus sp.* Identification test. As is evident from Table 1 and Figure 2, *Haemonchus sp.* is now evident in many samples from Benton County. No samples were submitted from Curry County. Without obtaining a sample from the county, the investigators could not definitively state where the parasite is present but by looking at positive samples from surrounding counties the assumption that Curry County also contains infested animals is a logical one.

**Table 1.** Counties from which fecal samples originated and their corresponding results from the *Haemonchus sp.* Identification process.

County of Origin	<5 EPG Trichostrongyles	Only Negative Trichostrongyle ova detected	Positive for <i>Haemonchus</i> sp. ova	Total	% Samples in which at least 1 positive ova was detected
Benton	31	1	15	47	32%
Douglas	0	0	1	1	100%
Grant	6	4	8	18	44%
Jefferson	1	0	0	1	0%
Klamath	0	0	2	2	100%
Lane	7	0	1	8	13%
Linn	2	0	4	6	67%
Marion	3	0	4	7	57%
Multnomah	0	0	1	1	100%
Tillamook	0	0	1	1	100%
Yamhill	6	1	1	8	13%
<b>Total</b>	56	6	38	100	38%



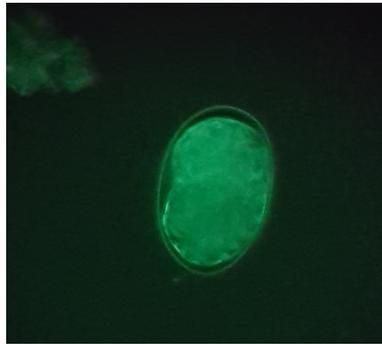
**Figure 2:** Pink shaded counties of Oregon indicate those positive for *Haemonchus* sp.; the yellow shaded county represents the one county that did not contain a sample

yielding positive ova for detection; the percentages indicate the percent of samples from the county that yielded a positive *Haemonchus sp.* ova for identification, and the numbers represent the amount of positive samples over the total amount of samples obtained from the corresponding counties.

Due to the nature of the testing method used, fecal sample must shed a significant number of eggs to test positive. Fecal samples are mixed with distilled water to release eggs from fecal matter. This step, however, dilutes the eggs per gram present. Samples with few or no Trichostrongyle ova seen in the original screening were occasionally concentrated using a greater concentration of feces to water to increase the opportunity for possible collection of eggs. If eggs were not seen in the original screening process the *Haemonchus sp.* Identification test was not performed; if ova were expected in low numbers the sample was concentrated and subjected to the *Haemonchus* Identification test. If feces were concentrated for the *Haemonchus* identification test and no ova were detected under the fluorescent microscope, samples were not considered negative for *Haemonchus sp.*, the samples were recorded as not applicable to the study. This is because *Haemonchus* identification could not be performed if Trichostrongyle ova were not present to examine. These samples potentially could be positive for *Haemonchus sp.*; however, a more extensive testing method would have been needed for definitive determination of these samples.

The two species of *Haemonchus* the investigators expect the samples to contain are either *Haemonchus contortus* or *Haemonchus placei*. *Haemonchus placei* has been found to be the dominant species of *Haemonchus* in bovine (Blouin, M. S., Yowell, C. A., Courtney, C. H., & Dame, J. B. 1997)., although *Haemonchus contortus* can also infest those animals. The methods used in this study do not distinguish between the two species. Peanut lectin stains the outer-membrane of both species. Due to the phenotypic similarities between the two species further testing would be needed for differentiation. PCR testing could be used to determine the species that were in the test sample; however, no test is available at this time. The pathology of both species in the host animal is virtually identical, and differentiation between the two species, for diagnosis of the presence of *Haemonchus sp.*, may not be of much significance.

Through testing it was found that when subjected to staining using peanut lectin *Haemonchus* ova in bovine stained dimmer than those in camelids. The reason for lighter staining is unknown. While it could be due to differences between the gut environments of the animal hosts, another possibility is that the two species of *Haemonchus* have different staining patterns. Due to the dimmer staining of ova in bovine, this study used peanut lectin at a concentration of two times that used in standard diagnostic testing. This allowed a brighter ring around the outer-membrane to be obtained (Figure 3).



**Figure 3.** Positive *Haemonchus* ova from test sample subjected to Peanut Lectin staining captured under 1000x magnification through a florescent microscope July 13, 2016

Age of Cattle	<5 EPG Trichostrongyle	Only Negative Trichostrongyle ova	Positive for Haemonchus sp. ova	Total Samples	% Samples containing at least 1 positive ova
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There was no noticeable correlation between the age of the bovine from which the sample feces was obtained and testing positive for *Haemonchus sp.* found. Of the study samples tested for bovine less than 6 months of age none tested positive for *Haemonchus sp.* ova (Table 2). However this is most likely due to the small sample size obtained. Determination of the age groups affected is notable because, the *Haemonchus sp.* parasite has a more severe affect on younger animals than on older animals. Due to time and financial constraints of this study the sample size of feces tested was fairly small. A larger study group would be needed to get a better understanding of whether there is a correlation between age and infestation with *Haemonchus sp.*.

<b>&lt;6 months</b>	5	1	0	6	0%
<b>6-12 months</b>	31	0	16	47	34%
<b>1 year</b>	2	0	0	2	0%
<b>2 years</b>	2	1	2	5	40%
<b>3 years</b>	0	1	1	2	50%
<b>5 years</b>	1	0	2	3	67%
<b>6 years</b>	0	0	1	1	100%
<b>7 years</b>	2	0	0	2	0%
<b>8 years</b>	1	0	0	1	0%
<b>9 years</b>	1	0	0	1	0%
<b>11 years</b>	0	0	1	1	100%
<b>17 years</b>	1	0	0	1	0%
<b>Recorded as an Adult</b>	1	0	2	3	67%
<b>Age Not Recorded</b>	9	3	13	25	52%
<b>Total</b>	56	6	38	100	38%

**Table 2.** Age of bovine from which samples were obtained and the corresponding results from the *Haemonchus sp.* Identification process

During the duration of the study other parasites were recorded when observed in the initial fecal screening. *Coccidia* oocysts were the most common ova seen, followed by *Trichostrongyle* ova. The focus of the screening process in this study was to determine whether *Trichostrongyle* ova were present for further examination; because of this, extensive focus was not placed on obtaining numerical data for other parasites in the feces. It was noted that *Strongyloides*, *Monezia*, Lungworm, and *Giardia* were seen in some samples of from Oregon bovine in addition to the more common Coccidian and *Trichostrongyle* ova seen.

*Haemonchus sp.* was found to be common in many bovine of Oregon, including those from Western Regions. Future studies could be done to determine a faster, more economically friendly test for the differentiation between *Haemonchus placei* and

*Haemonchus contortus*. The determination of the species the bovine is infested with could be useful for further drug treatment and prevention techniques. The differences in the mtDNA of the *Haemonchus* species could be of significance in the effectiveness of the drug. Currently *Haemonchus sp.* in general is resistant to many of the traditional deworming methods. Further testing is needed to determine a successful treatment and prevention method for *Haemonchus sp.* At the present time the awareness of *Haemonchus* in Oregon bovine is not where we would like it to be as seen through the low number of *Haemonchus* Identification test requested at the OVDL on bovine. An email to the cattlemen's association was sent requesting samples for use in this study at the start of the testing with no reply. A continuation of this study would be to notify the cattlemen's association of the occurrence of the parasite throughout the state as well as submitting a press release to notify the public and increase awareness. As previously noted *Haemonchus sp.* is the cause of significant economic losses for food animal producers. Expanding the study to obtain a larger sample size and geographic range would allow for more statistical relationships to be examined, allowing for a better understanding of the parasite. The resistance of the parasite to many traditional deworming methods as well as the lack effective treatments for *Haemonchosis* of serious concern due to the expanding range of *Haemochus sp.*

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