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MECHANISM OF IMMUNITY IN HOSTS TO CHRONIC INFECTIONS IN WILD CONDITIONS

KATHLEEN E. CREPPAGE

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Reviewed and approved* by the following:

Isabella Cattadori
Associate Professor of Biology
Thesis Supervisor

James Endress Howell
Coordinator of Undergraduate Advising
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

BACKGROUND: The aim of this study was to examine a pool of samples (one year, specifically) for evidence of arrested development of *Trichostrongylus retortaeformis (TR)* in wild rabbit guts. Prior studies have indicated that a variety of parasites in rabbits, sheep, and cattle move into this arrested state as a method of protection from both the host immune environment and the ecological environment. Patterns in arrested development can yield descriptive data about host immunity, population dynamics, and local climatic conditions over time.

RESULTS: There were a number of trends among arrested larvae and host characteristics. When comparing the numbers of recovered larvae over a period of 12 months, there was a steady increase in fall and winter, a decline in the spring, a sharp increase in April before the new offspring are born, and then another decline in the summer months. Arrested larval load rose with increasing age until about age 7, where it began to decline as the rabbits reached late adulthood and their immune systems weakened.

CONCLUSION: The results suggest two underlying mechanisms for arrested development of *T. retortaeformis* in the rabbit host. The first is likely a seasonal response; in the later fall and harsher winter months, the larvae arrest their development as a means of protection. The second is an immune mechanism, indicated by the large recovery of stage 4 larvae (L4s) in April when the majority of female rabbits are pregnant and probably shed larvae more rapidly. These results support prior research and beliefs concerning hypobiosis in rabbits, ruminants and other grazing animals. Further research with several consecutive years of samples could unearth more descriptive patterns and associations.

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INTRODUCTION

Complex host-parasite interactions are influenced and altered by the host, the parasite, and the environment. Like most organisms, parasites are able to respond to a variety of stimuli from their surroundings. (Grant and Viney, 2001) Nematodes with both free-living and parasitic stages have evolved to adjust to particular threatening conditions in a unique way known as *arrested development,* or hypobiosis (Taylor and Michel, 1973). Michel's definition of this state was once "the temporary cessation of development of nematodes at a precise point in early parasitic development, where such an interruption contains a facultative element, occurring only in certain hosts, certain circumstances or at certain times of the year and often affecting only a proportion of the worms," (Michel, 1974). More simply, it is a state of metabolic depression that allows the parasite to survive until the harsh conditions are removed. (Nisbet et al, 2004). Watkins and Fernando have also investigated the cessation of arrested development in parasites, but that will not be discussed or studied in this short study. (Watkins and Fernando, 1986). While there has been a sequence of studies on arrested development of stomach worms in rabbits, there have been few studies on the arrested development of the common intestinal parasite, *Trichostrongylus retortaeformis,* in wild rabbit host. (Watkins and Fernando, 1984; Watkins and Fernando, 1986; Anderson, 2000)

It is believed that there are a variety of factors that can induce the hypobiotic state in parasites; the two most significant are host immunity and environmental changes. (Michel, 1974; Schad 1977). Hypobiosis has been induced by changes in local temperature, indicating the link between environmental signals, or "seasonality", and the parasitic response. (Gibbs, 1986). There have been multiple studies generating evidence in cows, goats, and sheep with Trichostrongylids. One study in cows demonstrated a cold-temperature induced arrested development of the Trichostrongylid *Ostertagia ostertagi,* which is a similar phenomena to that proposed for rabbits. (Armour and Bruce, 1974). Experimental evidence also supports an immune-related mechanism for induction of arrested development. Fox examined the phenomena of arrested development as triggered by the immune system in stomach parasites in host rabbits, specifically *Obeliscoides cuniculi,* that are typically found in the Northern Hemisphere. He found that after active immunization with infective larvae, the majority of the parasitic population was arrested as parasitic stage L4. Another interesting discovery was that of a positive association between arrested L4 density and the initial dose of infective larvae. His findings were in accordance with previous studies for other rabbit strongylids, including *T. retortaeformis* and *Graphidium strigosum*. (Fox, 1976; Michel, 1952; Martin et al 1957).

Although there have been descriptions of the ability of TR to elicit a strong immune response in its host, there has only been a bit of discussion on the general life cycle and morphology of TR. (Cornell et al, 2008; Cattadori et al, 2005). Audebert et al. provides a lengthy but descriptive account about the evolution and general biological characteristics of *Trichostrongylus* *retortaeformis* (TR) and follows its movements throughout the small intestines of the New Zealand white rabbit host (*Oryctolagus cuniculus*). TR has both a free larval stage and a parasitic larval stage, the latter including the arrested L4 stage. Pathological examination of sections of the small intestine showed clustering of the majority of TR in the frontal sections. In a later paper, Audebert et al. study the movements of TR in the intestinal tract more closely. Here he demonstrated the ability of the parasites to bury beneath the mucosa of the intestinal lining, an important finding with regards to the mechanism of arrested development within the intestine. (Audebert et al, 2003). It is thought that the infective TR larvae hide beneath the mucosa and then subsequently enter into the state of arrested development.

Hypobiosis, or arrested development, of parasites is an important characteristic that when followed over time can indicate changes in climate and host/population characteristics. There have been previous studies on arrested development of parasites in the guts and stomachs of sheep and ruminants, but there are still uncertainties about rabbit parasites. (Michel, 1974; Dunsmore, 1960; Taylor and Michel 1953, Gatongi et al, 1998; Madsen, 1962) The aim of this study was to investigate a small pool of New Zealand wild rabbit intestines for evidence of arrested *T. retortaeformis* larvae, stage L4, in the mucosa and to find any trends between the recovered L4 and the host characteristics. This particular rabbit host was chosen because samples were easily obtained and considered representative of a natural host in Northern climates. If temporal and immune function play a role in arrested development, then it can be hypothesized that there will be observable differences in arrested L4 density over the span of a year and across host demographics (age, sex, etc.). Using a Pepsin/HCl solution, wild rabbit small intestines were digested and the washings collected in order to recover any arrested larvae. The larvae were counted for each sample and overall comparisons made for larval numbers against host age, sex, infection load, and sacrifice dates. Information about arrested development in specific parasites that infect large populations of both animals and humans can be applied so as to provide better anti-helminth treatments and control strategies. Temporal data showing trends in hypobiosis can alert us to potential alterations in host populations or slight variations in regional climates.

MATERIALS & METHODS

Samples. All small intestine segments were retrieved from wild white rabbits shot and killed in Scotland. All samples were bagged and labeled with the animal identification number and date of killing. The samples were shipped to the U.S. on ice and subsequently frozen at either -80° C or -20° C upon arrival at Penn State. All descriptive data for the samples, including age, sex, weight, pathology, prior infections with various agents, and organ weights, were collected and displayed in one file.

Digestion Solution. The digestion solution was prepared by trial and error to find the most efficient ratio of HCl to pepsin while preserving the morphology of the worms. The solution was based off of a similar one used by Watkins and Fernando, 1984. 100 ml of de-ionized water was combined with 2.0 g J.T. Baker powdered pepsin and 4.1 ml concentrated HCl per sample. HCl was measured and poured within a chemical hood. Typically preparations were scaled up to 1000 ml, or 1 L, at a time and stored at 4° C until needed. Solution was removed from the refrigerator and left to sit at room temperature for 30 minutes prior to digestion.

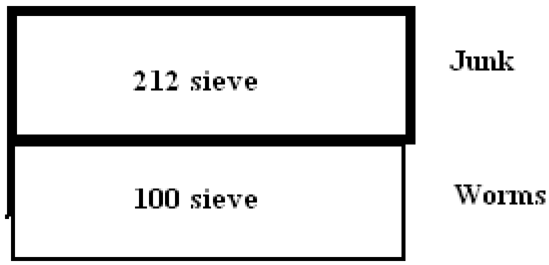
Digestion and Filtration Materials. Each sample required 1 200 ml glass bottle and 1 graduated cone for concentration. Digestion material was filtered and worms collected using 2 steel mesh test sieves (Endecotts), a 212 mesh and 100 mesh. Concentrated worm-filtrate was stored in 15 ml conical tubes labeled with the sample number and date in 5 mls of 10% formalin. An incubator kept at 37° C was used during digestion.

Counting Materials. Worms were counted using a LEICA brand microscope. Six 2.5 ml aliquots were counted per sample using a small, marked Petri dish.

Methods of Digestion. Each intestine was thawed the night before digestion in a 4°C refrigerator and washed to remove excess dirt and mucus. The intestine was cut into shorter sections, placed in a 200 ml bottle, and covered in enough digestion solution so as to immerse the entire sample. The bottle was shaken well for about 1 minute and then placed in an incubator (set at 37° C) for 2 hours, shaking the sample again with 20 minutes left in the incubation period.

The 212 mesh sieve was placed atop the 100 mesh sieve in a large sink and cool tap water poured over both. Once the incubation was up, the sample was emptied into the 212 mesh sieve and rinsed with tap water.

Figure 1. Sieve Set-up for L4 Collection



The 212 sieve was removed and the 100 mesh sieve rinsed thoroughly with tap water from top to bottom, tilting it so the contents collect along the bottom rim. This filtrate was poured into a cone. The sieve was rinsed 2 or 3 times to collect all worms. A small sample of this collected filtrate was checked under the microscope quickly for the presence of L4s.

The remaining undigested material in the 212 sieve was replaced back into the 200 ml bottle and filled with fresh digestion solution. It was shaken again and re-incubated for 2 hours to continue the digestion. These steps were repeated until all was digested (between 3-7 hours depending on the initial sample size).

Methods of Collection. The filtrate in the cone was allowed to sit, undisturbed, for 30 minutes before it was stirred to loosen any L4s from the grass and dirt on top. It then was left for 2 hours if digestions completed early in the day or overnight if completed later to allow the L4s to concentrate in the bottom 5 or 10 mls of liquid. The upper portion of liquid was removed down to about the 25 ml mark using a vacuum pump and large pipette tip keeping the tip right at the surface of the water. The remaining 15 – 20 mls was removed manually using a 10 ml pipette. The last 5-10 (approximately) mls of water and any present L4s were put into a 15 ml conical tube and topped off with 10% formalin. The samples were then stored at room temperature and NOT refrozen.

Methods of Counting. The sample in the 15 ml tube was inverted 5 times to thoroughly mix it, and then 2.5 ml were removed using a pipette and placed into a small Petri dish with clearly drawn section marks. Six 2.5 ml aliquots were counted for each sample, and the tube inverted between each aliquot. Each aliquot, once counted, was not immediately replaced in the tube but set aside in the top portion of the Petri dish until the rest of the tube contents was counted. The number of L4 larvae and adults found were recorded and subsequently entered into the file. L4s were identified after having consulted live samples and figures from prior literature. (Watkins and Fernando, 1984; Audebert, et al, 2000)

RESULTS

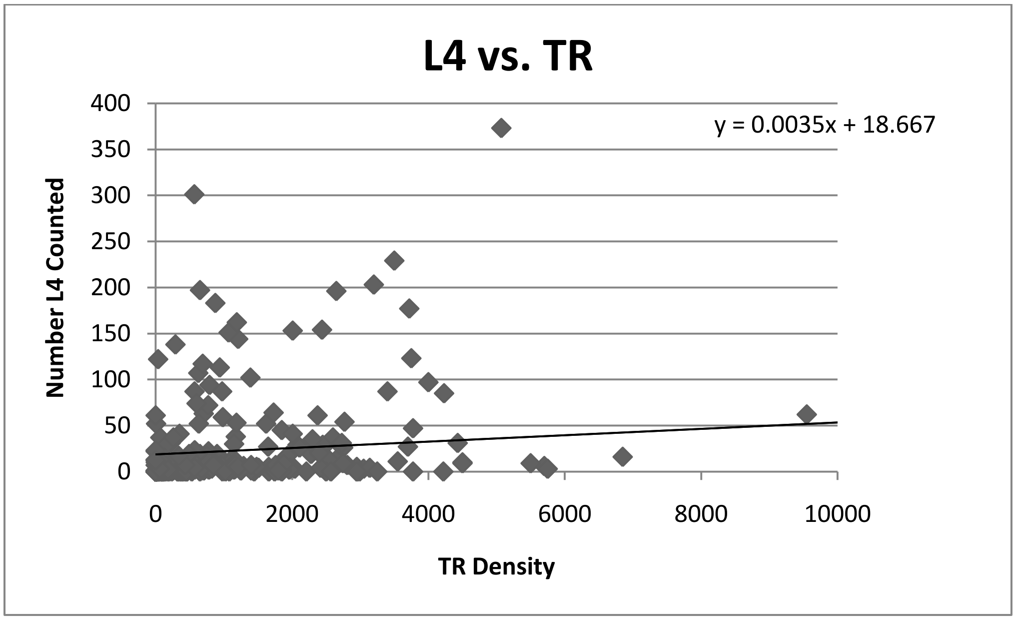


Figure 2. Arrested L4 vs. *T. retortaeformis* density. There was a positive relationship between recovered L4s and initial TR infection; the greater the infection with T.R., the more arrested larvae recovered. Regression line: y=0.0035x + 18.667

Figure 2 shows a positive trend between the arrested L4 and *T. retortaeformis* density; there is a greater number of arrested larvae seen with a greater TR density. This could indicate that as the TR density increases, it produces a type of "competition" for space between the developing larvae and the mature TR. This forces the larvae to arrest. The cluster of uninfected rabbits with little arrested larvae may be the younger kittens and juveniles.

Examining the data over a period of 12 months shows a seasonal variance in arrested L4, with the arrested L4 increasing steadily in the fall and winter months and declining sharply in the summer. There is a sharp rise in arrested larvae in April, with the average L4 recovered at 71.33. See Table 1 below for a complete list of average values and the curve in Figure 3.

Month	**Number of Samples**	**Average Arrested L4**
1	14	24.79
2	11	43.09
3	15	44.27
4	30	71.33
5	40	2.13
6	34	2.44
7	30	4.30
8	30	6.13

9	33	8.79
10	18	22.44
11	16	44.44
12	13	77.92

Table 1: Average Arrested L4 by Month. The average values were calculated for each month; highest in December (77.92) and lowest in May (2.13).

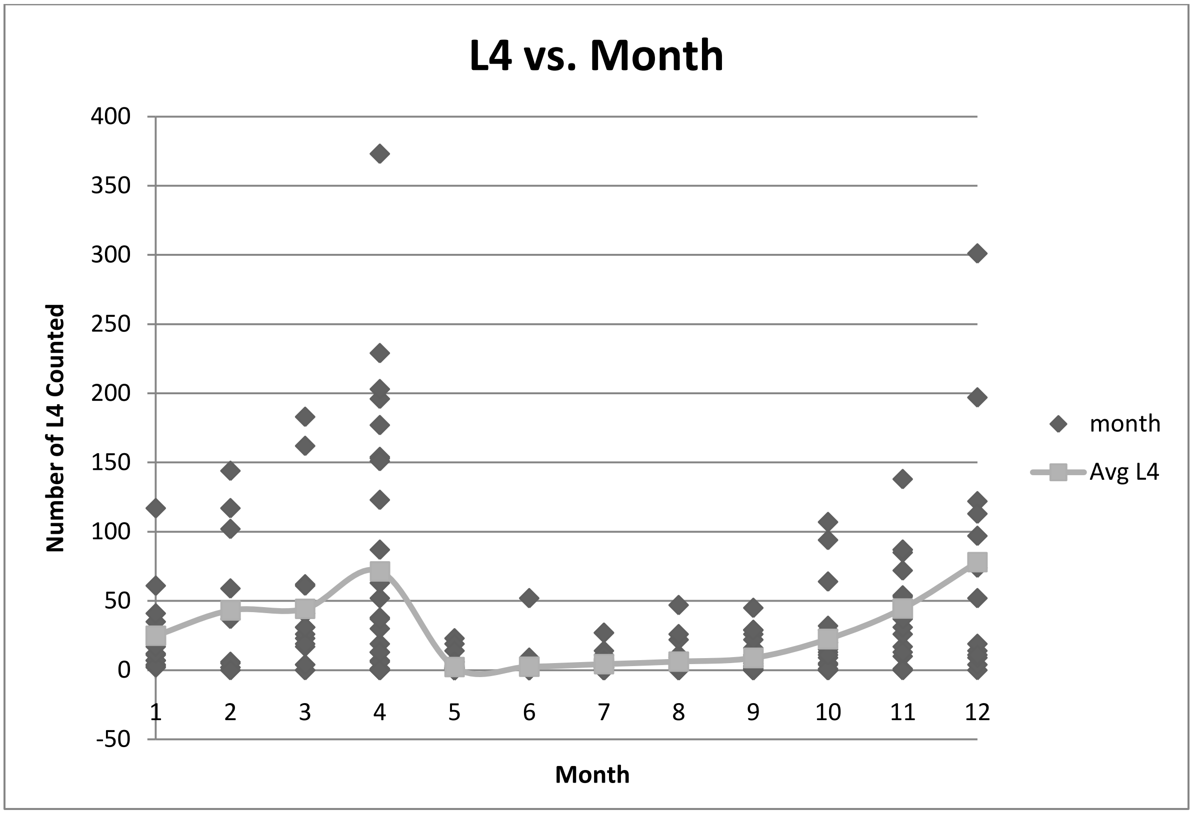


Figure 3. Arrested L4 vs. Month. Months range from February 2008 to January 2009. Dark circles indicate average L4 values and are connected with a line. Peak numbers of arrested larvae occur in December and April, and low numbers in May and June.

Comparisons for L4 and age showed a greater proportion of females with infected larvae, yet the average value of arrested L4 in males and females was very similar (22.99 and 23.03, respectively). Figure 4 shows the distribution of larvae between females (group 1) and males (group 2).

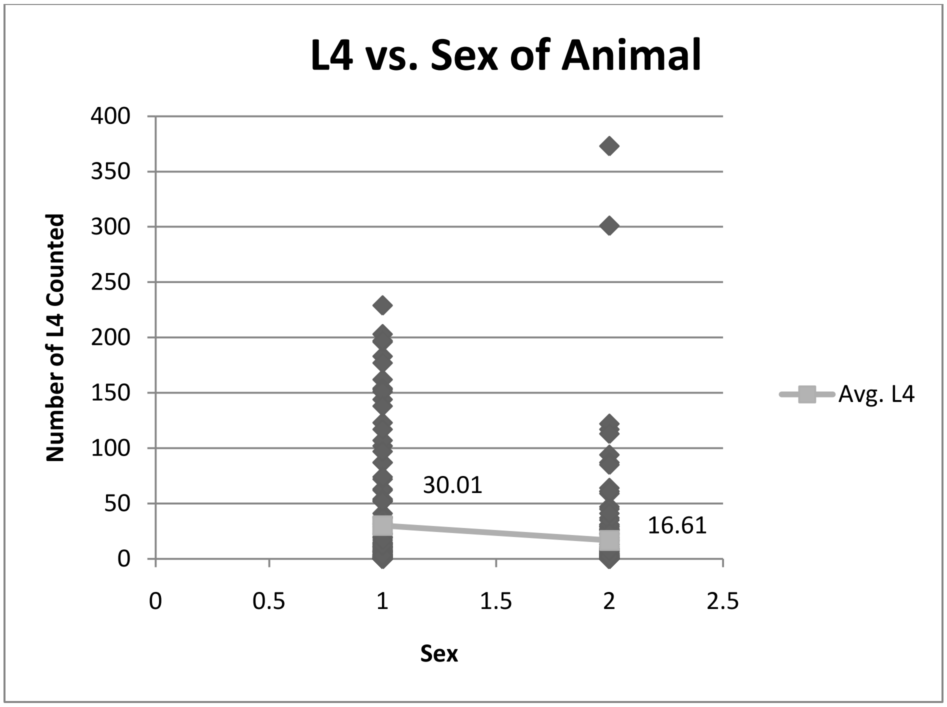


Figure 4. Comparison of Arrested L4 with Sex of Animal. Code for males is 2, code for females is 1. Average values are indicated by square points. Female: 30.01; Male: 16.61

When comparing the arrested L4 across 8 age groups, there were obvious differences between the youngest, middle, and eldest rabbits. The kittens, which comprised groups 1-3, had the fewest arrested larvae. The numbers increased with age and peaked between age 6 and 7, but then declined in the oldest adults. See Table 2 for a complete list of the average L4 counts for each age group. Age was determined using an established system based on weight (in grams) of each rabbit. Age Group Categories are as follows: 100-200 g = 1 (kittens); 201-480 g =2; 481-750 g =3; 751-1030 g = 4 (juveniles); 1031-1300 g =5; 1301-1580 g = 6 (adults); 1581-1860 = 7; 1861 or more = 8 (Cattadori et al, 2005).

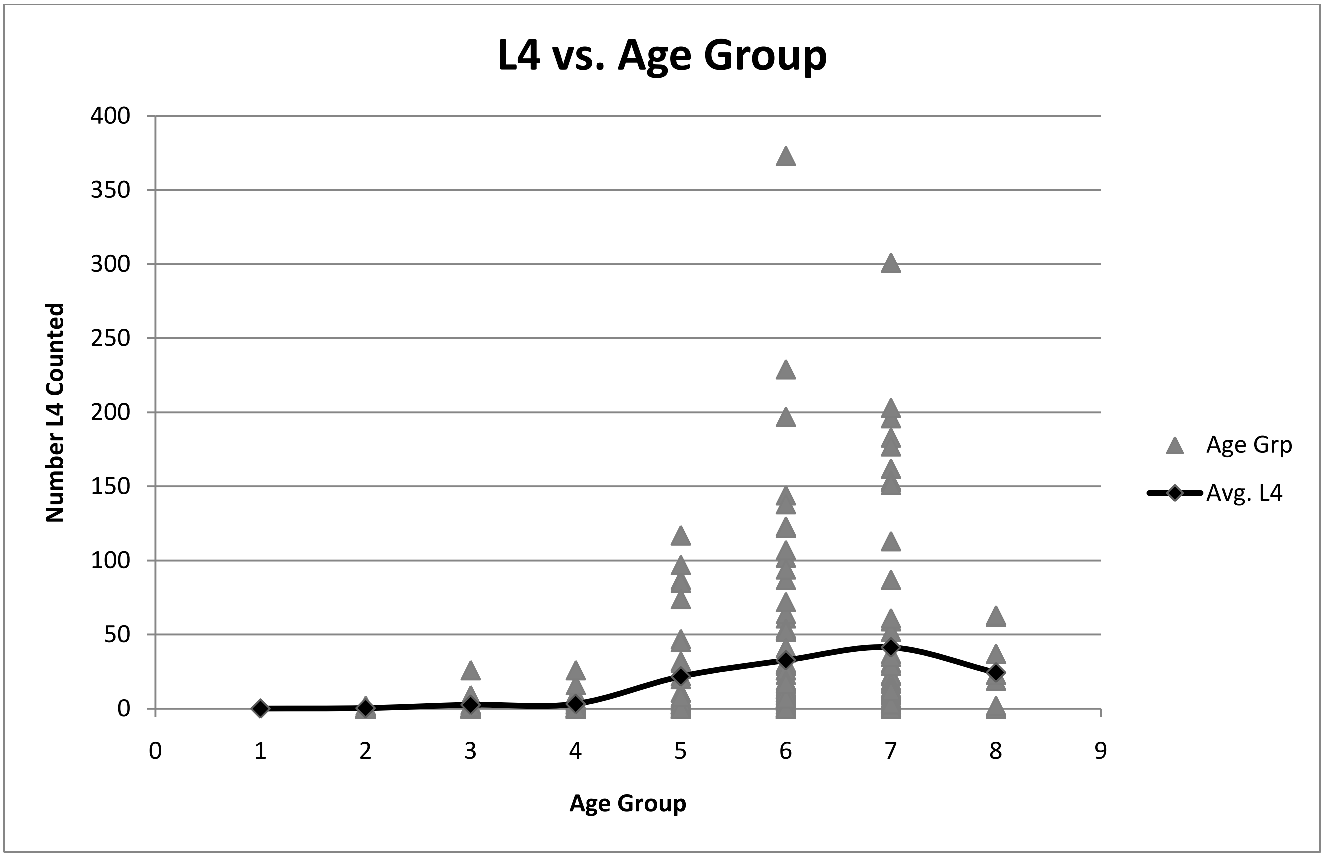


Figure 5. Arrested L4 vs. Age Group. Age groupings were based off the previously described weight classes (g). There were no rabbits in the youngest group (1), and the highest average L4 density was seen at age 7 (41.28 L4s).

Age Grp	Avg. L4
1	0
2	0.31
3	2.55
4	3.17
5	21.67
6	32.54
7	41.28
8	24.3

Table 2. Average L4 density per Age Group. Averages were calculated based off number off rabbits per weight class/age grouping. The highest average was seen for age 7 and the lowest for age 1.

A final comparison was made between arrested L4 density and initial *Graphidium strigosum* infection. *G. strigosum (GS*) is a common stomach parasite in rabbits. A similar positive trend to that of the L4 versus the TR infection was seen. See Figure 6 below for details.

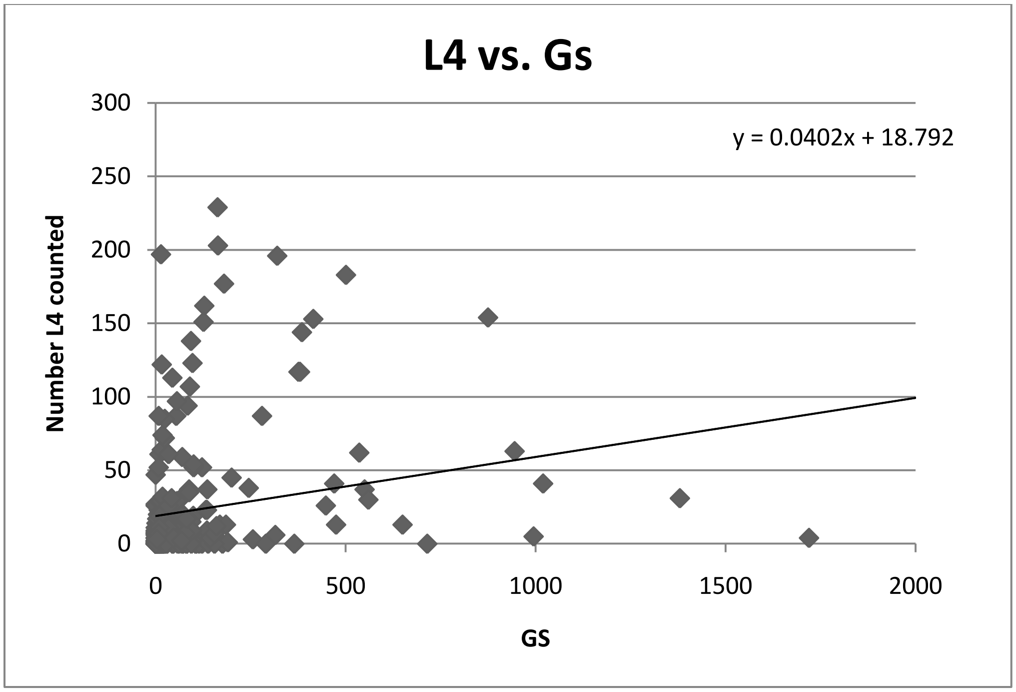


Figure 6. Arrested L4 vs. *Graphidium strigosum* density. Recovered L4 were compared to the initial G. strigosum (GS) infection density in the rabbits. Trend was similar to that of TR. Regression line: y= 0.0402x + 18.792

DISCUSSION

The results of this study support current ideas about arrested development in the rabbit model and suggest two separate mechanisms, host immunity and the environment, that can trigger the onset and termination of arrested development of *T. retortaeformis.* There are marked trends seen for comparisons of arrested L4 density across host age cohort, sex, sacrifice date, and TR infection density.

Figure 2 shows a positive trend when comparing recovered arrested L4 with initial TR density in the rabbit host. There are two points to notice here. The first is the large cluster of rabbits with almost no arrested larvae and few TR; these are likely the younger rabbits that are born in the early spring and summer months that have not yet been exposed to the parasites. Secondly, as the TR density increases, there is an increase in the number of arrested L4. This could be explained in terms of competition within the host for living environment. If more mature TR occupy the host gastrointestinal tract, there is less area for occupation by the L4. This situation might act as a trigger for arrested development. In the situation where there were few initial mature TR, there would be less pressure for the L4 to arrest and they would continue through their life cycle normally. However, another perspective on these data relates to the previous findings from Fox and Michel; those hosts with a high initial burden of infection (TR) seem to have a greater propensity for arrested larvae. This suggests that the TR present in the host system elicit a fairly robust immune response that in turn induces the infective larvae to arrest at the L4 stage. (Fox, 1976; Michel, 1952; Michel, et al, 1975; Cornell et al, 2008).

Because arrested development, or hypobiosis, is typically triggered by changes in environment or climate, it was important to examine the pattern of arrested development over time. For this particular study, samples from twelve consecutive months were processed and analyzed. The resulting pattern is illustrated in Figure 3; there is the expected increase in arrested numbers in the late fall and winter in response to the cold, Northern temperatures. There were also expected quick declines in early spring and summer, suggesting that the L4 no longer arrest. (Armour and Bruce, 1974; Cattadori et al, 2005). The sharp peak in arrested L4 in April is interesting in the context of the host environment. This is within the typical breeding season for the rabbits and as a result there is an abundance of pregnant females in April. These particular females have an altered, depressed immune system as a result of changing physical and hormonal states. One possible explanation for the large increase in recovered L4 could be that infective TR quickly take advantage of the depressed systems and begin to re-emerge at that particular time. Due to these drastic physical and hormonal changes, pregnant females remove considerable pressure from the larvae to arrest. These L4 likely arrest in prior fall or winter months and are seen at the end of it as April approaches. This suggests that there is some element of host immunity that plays a role in triggering the onset and lapse of arrested development, and that it is not only attributable to environmental changes.

The average L4 density was similar between male and female rabbits (see Figure 4), but there appeared to be a greater amount of females with arrested L4s overall. This could be attributed to the cluster of breeding female rabbits with relaxed immune systems and relates back to the sharp increase in arrested L4s during the month of April. It is difficult to completely explain the mechanism without further months of data from prior and following years, yet it is interesting to find it occurring and supports prior long-term studies performed with the same host-parasite system by Cattadori et al (2005).

The local rabbit population's demographics, specifically age, vary over the course of a year. With changes in age comes change in overall health and immunity. Figure 5, shown above, illustrates the arrested L4 density per age group. The greatest arrested larval burden was seen at age 6, which is similar to the results from Cattadori et al, 2005 for parasite density among the same age groups. The youngest rabbits (ages 1-3) had very few arrested larvae as compared to the juveniles and younger adults (ages 4-7). There was a sharp decline in L4 density for the eldest adults (age 8). These results may be attributed to specific host immunity; that is, in the very young and very old rabbits, there is either a lack of acquired immunity as a result of young age or an overall weaker immune system, both resulting in less pressure for the L4s to arrest their development. Rabbits that are ages 4-6 are more likely to have been repeatedly infected over the course of their lifetime (attributed to the high incidence of re-infection in the wild) and so have built robust immunity against the infective TR. (Cattadori et al, 2005) Therefore, there is a greater pressure on the TR to bury themselves beneath the mucosa and arrest as a defense mechanism.

One substantial element that was not explored in this study was the effects of other host parasites on the arrested development of TR. To get a general idea about the potential relationship between the arrested L4 and other parasites, I compared the L4 density to the density of infection with a common rabbit stomach strongylid, *Graphidium strigosum*. (Cuquerella and Alunda, 2009). As illustrated by Figure 7, there was a positive trend similar to that seen with TR in Figure 2. As the GS infection density increased, the arrested L4 density increased. Although this explanation and discussion are beyond the scope of this paper, it could be that the infective TR still generate the strong host immune response and thus are forced to arrest while GS, which has been shown to elicit no real immune response by the host, remains unaffected. It is likely that there are a combination of factors, including host immunity and TR/GS co-infection that influence the infective larvae to arrest. (Cattadori et al, 2008).

There were some clear limitations to my study. Using only twelve months' worth of samples allows for analyses by month, but not year. If the patterns of development were slightly abnormal or the climatic conditions somewhat different, the resulting patterns of arrested development for the larvae may have been altered. Also, this made analysis difficult in that many of the trends are probably explainable with regards to prior exposure to TR for each rabbit, as well as the actual birth date of each rabbit. Variations in arrested L4 density may be attributed to complex interactions between the TR, host, environment, and *other parasites* that were not considered. Although the samples were washed thoroughly prior to digestion to remove any L4 or adults adhering to the mucus, there still may have been a small proportion of L4 recovered that were not arrested under the mucosa. In addition, samples that had to be recounted may have had slightly different counts from loss during transfer from tube to Petri dish. Samples from January of 2008 were never received in the lab and as such the January 2009 samples were used instead to complete the twelve months.

Host-parasite interactions are manipulated by a variety of factors attributable to the host, parasite, and surrounding environments. Over time, parasites have evolved to evade host immune responses and adapt to pressured situations. The ability of *T. retortaeformis* to arrest its development allows it to evade host immune defenses and to escape harsh conditions of the external, ecological environment. By following patterns in arrested development in well-studied host-parasite systems over time, valuable data on climate change and population dynamics can be obtained and analyzed. Key information about arrested development could also be used in the design and implementation of parasite-control strategies for agricultural and health purposes. (Nisbet et al, 2004).

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APPENDIX A

Table 3. Selected Raw Data (Experimental and Demographic)

AD	L4	ID	day	month	year	Burrow	Feeding	Sex	Age Grp	Grp Name
		1302	5	1	2008	grass	grass	1	6	Adult
		1303	5	1	2008	grass	grass	2	6	Adult
		1304	5	1	2008	grass	grass	2	7	Adult
		1305	5	1	2008	grass	grass	1	6	Adult
		1306	11	1	2008	grass	grass	1	6	Adult
		1307	11	1	2008	grass	grass	2	7	Adult
		1308	11	1	2008	grass	grass	2	7	Adult
		1309	11	1	2008	gorse	grass	2	6	Adult
		1310	11	1	2008	gorse	grass	1	7	Adult
		1311	11	1	2008	gorse	grass	1	6	Adult
		1312	11	1	2008	grass	grass	2	6	Adult
		1313	21	1	2008	grass	grass	1	7	Adult
		1314	21	1	2008	grass	grass	2	6	Adult
		1315	21	1	2008	grass	grass	1	7	Adult
		1316	21	1	2008	grass	grass	2	7	Adult
		1317	21	1	2008	grass	grass	1	6	Adult
2	6	1318	2	2	2008	gorse	grass	2	6	Adult
0	2	1319	2	2	2008	gorse	grass	1	6	Adult
2	2	1320	2	2	2008	grass	grass	1	6	Adult
0	0	1321	4	2	2008	grass	grass	2	7	Adult
1	37	1322	4	2	2008	grass	grass	1	8	Adult
		1323	4	2	2008	grass	grass	2	7	Adult
		1324	4	2	2008	grass	grass	2	7	Adult
		1325	4	2	2008	grass	grass	2	7	Adult
0	117	1326	9	2	2008	grass	grass	2	5	Juvenile
1	102	1327	9	2	2008	gorse	grass	1	6	Adult
2	144	1328	9	2	2008	gorse	grass	1	6	Adult
4	59	1329	9	2	2008	grass	grass	2	7	Adult
6	5	1330	9	2	2008	grass	grass	2	6	Adult
0	0	1331	9	2	2008	gorse	grass	2	6	Adult
0	41	1332	1	3	2008	hawthorn	grass	1	6	Adult
0	17	1333	1	3	2008	gorse	grass	2	6	Adult
0	0	1334	1	3	2008	gorse	grass	1	6	Adult
3	23	1335	1	3	2008	grass	grass	2	7	Adult
0	0	1336	1	3	2008	grass	grass	2	7	Adult
0.5	4	1337	2	3	2008	hawthorn	grass	1	7	Adult
2	62	1338	2	3	2008	grass	grass	1	8	Adult
1	26	1339	2	3	2008	grass	grass	2	6	Adult
0	4	1340	2	3	2008	grass	grass	2	6	Adult
0	183	1341	2	3	2008	grass	grass	1	7	Adult
0	19	1342	14	3	2008	grass	grass	2	7	Adult
0	162	1343	14	3	2008	gorse	grass	1	7	Adult
0	31	1344	14	3	2008	gorse	grass	1	7	Adult

0	61	1345	14	3	2008	gorse	grass	2	6	Adult
0	31	1346	14	3	2008	grass	grass	2	6	Adult
0	52	1347	1	4	2008	grass	grass	1	6	Adult
2	19	1348	1	4	2008	grass	grass	2	6	Adult
0	13	1349	1	4	2008	grass	grass	2	6	Adult
0	0	1350	1	4	2008	grass	grass	1	2	Kitten
0	123	1351	1	4	2008	grass	grass	1	6	Adult
0	87	1352	1	4	2008	grass	grass	1	6	Adult
0	1	1353	1	4	2008	grass	grass	1	2	Kitten
0	196	1354	8	4	2008	grass	grass	1	7	Adult
0	373	1355	8	4	2008	gorse	grass	2	6	Adult
5	154	1356	8	4	2008	gorse	grass	1	7	Adult
2	153	1357	8	4	2008	grass	grass	1	7	Adult
0	0	1358	8	4	2008	grass	grass	2	6	Adult
8	38	1359	8	4	2008	grass	grass	1	6	Adult
0	0	1360	8	4	2008	grass	grass	1	2	Kitten
1	1	1361	8	4	2008	grass	grass	2	2	Kitten
0	151	1362	8	4	2008	grass	grass	1	7	Adult
1	177	1363	17	4	2008	hawthorn	grass	1	7	Adult
1	229	1364	17	4	2008	grass	grass	1	6	Adult
1	1	1365	17	4	2008	grass	grass	2	5	Juvenile
1	30	1366	17	4	2008	grass	grass	2	6	Adult
4	13	1367	17	4	2008	grass	grass	2	7	Adult
0	0	1368	17	4	2008	grass	grass	2	2	Kitten
0	0	1369	17	4	2008	grass	grass	1	2	Kitten
1	7	1370	17	4	2008	grass	grass	1	3	Kitten
1	203	1371	17	4	2008	grass	grass	1	7	Adult
0	37	1372	17	4	2008	grass	grass	1	8	Adult
3	6	1373	23	4	2008	grass	grass	1	7	Adult
		1374	23	4	2008	grass	grass	1	8	Adult
0	13	1375	23	4	2008	hawthorn	grass	2	6	Adult
2	63	1376	23	4	2008	hawthorn	grass	1	8	Adult
0	0	1377	23	4	2008	grass	grass	2	6	Adult
0	3	1378	3	5	2008	hawthorn	grass	1	6	Adult
0	1	1379	3	5	2008	grass	grass	2	2	Kitten
0	0	1380	3	5	2008	hawthorn	grass	1	2	Kitten
0	0	1381	3	5	2008	gorse	grass	2	3	Kitten
0	0	1382	3	5	2008	gorse	grass	2	2	Kitten
0	1	1383	3	5	2008	gorse	grass	1	3	Kitten
0	1	1384	3	5	2008	gorse	grass	1	3	Kitten
0	0	1385	3	5	2008	gorse	grass	1	2	Kitten
0	0	1386	3	5	2008	gorse	grass	2	2	Kitten
1	1	1387	3	5	2008	grass	grass	1	2	Kitten
0	0	1388	3	5	2008	grass	grass	2	2	Kitten
0	0	1389	3	5	2008	grass	grass	1	3	Kitten
0	2	1390	3	5	2008	grass	grass	2	6	Adult
0	0	1391	3	5	2008	gorse	grass	2	6	Adult
0	0	1392	3	5	2008	grass	grass	2	6	Adult

0	0	1393	3	5	2008	grass	grass	1	3	Kitten
0	23	1394	7	5	2008	deciduous	grass	1	6	Adult
0	0	1395	7	5	2008	grass	grass	2	2	Kitten
0	2	1396	7	5	2008	grass	grass	1	2	Kitten
4	19	1397	7	5	2008	grass	grass	1	8	Adult
0	1	1398	7	5	2008	grass	grass	2	3	Kitten
0	0	1399	7	5	2008	grass	grass	2	3	Kitten
0	1	1400	7	5	2008	grass	grass	2	2	Kitten
0	0	1401	7	5	2008	grass	grass	1	2	Kitten
0	0	1402	7	5	2008	grass	grass	1	8	Adult
0	14	1403	7	5	2008	grass	grass	1	7	Adult
0	0	1404	7	5	2008	grass	grass	1	4	Juvenile
0	0	1405	7	5	2008	grass	grass	2	2	Kitten
0	0	1406	7	5	2008	grass	grass	1	2	Kitten
0	0	1407	7	5	2008	grass	grass	2	6	Adult
1	6	1408	7	5	2008	grass	grass	1	7	Adult
0	6	1409	30	5	2008	hawthorn	grass	1	4	Juvenile
0	0	1410	30	5	2008	hawthorn	grass	2	3	Kitten
0	1	1411	30	5	2008	nettles	grass	1	3	Kitten
0	1	1412	30	5	2008	hawthorn	grass	1	4	Juvenile
0	0	1413	30	5	2008	hawthorn	grass	2	6	Adult
0	0	1414	30	5	2008	grass	grass	1	3	Kitten
1	2	1415	30	5	2008	grass	grass	1	4	Juvenile
0	0	1416	30	5	2008	grass	grass	1	2	Kitten
		1417	30	5	2008	grass	grass	1	3	Kitten
0	0	1418	30	5	2008	grass	grass	2	7	Adult
0	2	1419	4	6	2008	hawthorn	grass	2	3	Kitten
0	0	1420	4	6	2008	grass	grass	2	3	Kitten
0	4	1421	4	6	2008	hawthorn	grass	1	7	Adult
0	0	1422	4	6	2008	hawthorn	grass	1	2	Kitten
0	0	1423	4	6	2008	gorse	grass	1	3	Kitten
0	0	1424	4	6	2008	gorse	grass	2	4	Juvenile
0	5	1425	4	6	2008	gorse	grass	2	7	Adult
0	0	1426	4	6	2008	gorse	grass	1	2	Kitten
0	1	1427	4	6	2008	gorse	grass	2	6	Adult
0	0	1428	4	6	2008	gorse	grass	2	4	Juvenile
0	0	1429	4	6	2008	gorse	grass	2	3	Kitten
0	52	1430	4	6	2008	gorse	grass	1	7	Adult
0	0	1431	4	6	2008	gorse	grass	1	2	Kitten
2	1	1432	4	6	2008	bracken	grass	2	2	Kitten
0	0	1433	4	6	2008	bracken	grass	2	2	Kitten
0	0	1434	4	6	2008	grass	grass	2	2	Kitten
0	0	1435	4	6	2008	gorse	grass	2	3	Kitten
0	3	1436	4	6	2008	grass	grass	2	7	Adult
0	1	1437	8	6	2008	grass	grass	2	4	Juvenile
0	0	1438	8	6	2008	hawthorn	cereal	2	6	Adult
0	0	1439	8	6	2008	hawthorn	grass	2	2	Kitten
0	0	1440	8	6	2008	grass	grass	2	3	Kitten

0	1	1441	8	6	2008	grass	grass	2	4	Juvenile
0	0	1442	8	6	2008	grass	grass	2	7	Adult
0	1	1443	8	6	2008	grass	grass	1	4	Juvenile
0	0	1444	8	6	2008	grass	grass	2	4	Juvenile
0	0	1445	8	6	2008	grass	grass	2	7	Adult
0	0	1446	8	6	2008	grass	grass	1	3	Kitten
1	2	1447	8	6	2008	grass	grass	2	2	Kitten
0	9	1448	8	6	2008	grass	grass	2	3	Kitten
		1449	8	6	2008	grass	grass	1	6	Adult
0	1	1450	8	6	2008	grass	grass	1	7	Adult
0	0	1451	8	6	2008	grass	grass	1	8	Adult
		1452	8	6	2008	grass	grass	1	8	Adult
0	0	1453	8	6	2008	grass	grass	1	2	Kitten
0	0	1454	8	6	2008	grass	grass	2	2	Kitten
0	2	1455	6	7	2008	decidous	grass	2	5	Juvenile
1	8	1456	6	7	2008	hawthorn	grass	2	3	Kitten
0	2	1457	6	7	2008	gorse	grass	2	3	Kitten
0	6	1458	6	7	2008	grass	grass	1	4	Juvenile
0	0	1459	6	7	2008	grass	grass	1	7	Adult
0	0	1460	6	7	2008	grass	grass	1	3	Kitten
0	0	1461	6	7	2008	grass	grass	2	2	Kitten
0	1	1462	6	7	2008	grass	grass	1	3	Kitten
0	0	1463	6	7	2008	grass	grass	2	6	Adult
0	14	1464	6	7	2008	grass	grass	1	6	Adult
0	0	1465	14	7	2008	gorse	grass	2	6	Adult
0	0	1466	14	7	2008	gorse	grass	1	2	Kitten
0	2	1467	14	7	2008	gorse	grass	2	5	Juvenile
0	0	1468	14	7	2008	gorse	grass	2	7	Adult
0	2	1469	14	7	2008	gorse	grass	2	3	Kitten
1	11	1470	14	7	2008	gorse	grass	1	5	Juvenile
0	3	1471	26	7	2008	hawthorn	grass	2	5	Juvenile
0	7	1472	26	7	2008	grass	grass	1	5	Juvenile
2	0	1473	26	7	2008	hawthorn	grass	1	4	Juvenile
0	5	1474	26	7	2008	grass	grass	2	4	Juvenile
1	27	1475	26	7	2008	garden	grass	2	5	Juvenile
2	27	1476	26	7	2008	garden	grass	2	5	Juvenile
0	0	1477	26	7	2008	grass	grass	2	5	Juvenile
0	0	1478	26	7	2008	decidous	grass	2	6	Adult
0	0	1479	26	7	2008	decidous	grass	2	5	Juvenile
0	2	1480	26	7	2008	grass	grass	1	7	Adult
0	3	1481	26	7	2008	grass	grass	2	3	Kitten
0	4	1482	26	7	2008	grass	grass	2	5	Juvenile
0	0	1483	26	7	2008	grass	grass	1	3	Kitten
0	3	1484	26	7	2008	grass	grass	1	4	Juvenile
1	11	1485	1	8	2008	decidous	grass	1	5	Juvenile
0	10	1486	1	8	2008	decidous	grass	2	4	Juvenile
0	9	1487	1	8	2008	hawthorn	grass	1	3	Kitten
2	6	1488	1	8	2008	grass	grass	1	4	Juvenile

1	2	1489	1	8	2008	hawthorn	grass	1	5	Juvenile
2	6	1490	1	8	2008	hawthorn	grass	2	6	Adult
1	47	1491	1	8	2008	grass	grass	2	5	Juvenile
0	0	1492	1	8	2008	grass	grass	2	6	Adult
0	2	1493	1	8	2008	grass	grass	1	4	Juvenile
2	3	1494	1	8	2008	decidous	grass	2	5	Juvenile
0	0	1495	1	8	2008	grass	grass	1	5	Juvenile
0	0	1496	1	8	2008	grass	grass	1	5	Juvenile
0	0	1497	1	8	2008	grass	grass	2	4	Juvenile
0	4	1498	1	8	2008	grass	grass	1	4	Juvenile
0	22	1499	1	8	2008	conifer	grass	2	7	Adult
2.5	7	1500	1	8	2008	grass	grass	1	6	Adult
1	7	1501	1	8	2008	grass	grass	2	6	Adult
0	3	1502	3	8	2008	hawthorn	grass	2	4	Juvenile
1	0	1503	3	8	2008	gorse	grass	2	5	Juvenile
1	4	1504	3	8	2008	gorse	grass	1	4	Juvenile
0	0	1505	3	8	2008	gorse	grass	2	5	Juvenile
0	4	1506	3	8	2008	gorse	grass	1	5	Juvenile
0	0	1507	3	8	2008	gorse	grass	1	4	Juvenile
0	0	1508	7	8	2008	hawthorn	grass	2	5	Juvenile
0	0	1509	7	8	2008	grass	grass	1	5	Juvenile
4	9	1510	7	8	2008	hawthorn	grass	2	4	Juvenile
5	1	1511	7	8	2008	hawthorn	grass	1	3	Kitten
0	0	1512	7	8	2008	grass	grass	1	4	Juvenile
0.2	1	1513	7	8	2008	gorse	grass	2	4	Juvenile
0	26	1514	7	8	2008	gorse	grass	2	3	Kitten
0	10	1515	12	9	2008	gorse	grass	1	6	Adult
0	3	1516	12	9	2008	gorse	grass	2	7	Adult
5	7	1517	12	9	2008	gorse	grass	1	6	Adult
0	3	1518	12	9	2008	gorse	grass	1	5	Juvenile
0	0	1519	12	9	2008	grass	grass	1	5	Juvenile
0	8	1520	12	9	2008	grass	grass	1	5	Juvenile
0	3	1521	12	9	2008	grass	grass	1	5	Juvenile
0	0	1522	12	9	2008	grass	grass	1	6	Adult
0	9	1523	12	9	2008	gorse	grass	2	6	Adult
0	1	1524	12	9	2008	gorse	grass	2	6	Adult
0	45	1525	12	9	2008	gorse	grass	2	5	Juvenile
0.5	16	1526	17	9	2008	hawthorn	grass	2	4	Juvenile
5	26	1527	17	9	2008	grass	grass	2	4	Juvenile
0	0	1528	17	9	2008	grass	grass	2	7	Adult
1	29	1529	17	9	2008	grass	grass	2	6	Adult
0	0	1530	17	9	2008	grass	grass	2	4	Juvenile
1	11	1531	17	9	2008	decidous	grass	2	6	Adult
0	2	1532	17	9	2008	decidous	grass	1	7	Adult
2	8	1533	17	9	2008	grass	grass	1	6	Adult
4	29	1534	17	9	2008	grass	grass	1	7	Adult
0	5	1535	17	9	2008	grass	grass	2	5	Juvenile
0.5	7	1536	17	9	2008	grass	grass	1	6	Adult

1.5	22	1537	17	9	2008	grass	grass	1	5	Juvenile
2	5	1538	24	9	2008	hawthorn	grass	2	5	Juvenile
0	0	1539	24	9	2008	grass	grass	2	6	Adult
0	0	1540	24	9	2008	grass	grass	2	5	Juvenile
0	0	1541	24	9	2008	gorse	grass	2	7	Adult
0	0	1542	24	9	2008	gorse	grass	1	6	Adult
0	3	1543	24	9	2008	gorse	grass	2	6	Adult
1	8	1544	24	9	2008	grass	grass	2	5	Juvenile
0	29	1545	24	9	2008	grass	grass	2	6	Adult
0	0	1546	24	9	2008	grass	grass	2	7	Adult
0	1	1547	24	9	2008	grass	grass	2	7	Adult
0	0	1548	1	10	2008	grass	grass	1	4	Juvenile
4	20	1549	1	10	2008	grass	grass	1	5	Juvenile
2	29	1550	1	10	2008	hawthorn	grass	2	6	Adult
0	32	1551	1	10	2008	grass	grass	1	5	Juvenile
0	0	1552	1	10	2008	grass	grass	2	6	Adult
0	107	1553	1	10	2008	gorse	grass	1	6	Adult
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0	9	1555	1	10	2008	gorse	grass	2	6	Adult
0	0	1556	20	10	2008	grass	grass	2	7	Adult
0	0	1557	20	10	2008	conifer	grass	2	7	Adult
0	4	1558	20	10	2008	hawthorn	grass	2	7	Adult
0	15	1559	21	10	2008	grass	grass	1	6	Adult
1	0	1560	21	10	2008	grass	grass	1	6	Adult
0	1	1561	21	10	2008	gorse	grass	2	6	Adult
0	13	1562	21	10	2008	grass	grass	1	6	Adult
0	64	1563	21	10	2008	grass	grass	2	6	Adult
0	94	1564	21	10	2008	grass	grass	2	6	Adult
0	11	1565	21	10	2008	grass	grass	1	6	Adult
0	10	1566	2	11	2008	grass	grass	1	7	Adult
1	87	1567	2	11	2008	bracken	grass	2	5	Juvenile
0	17	1568	2	11	2008	gorse	grass	2	7	Adult
4	85	1569	2	11	2008	grass	grass	2	5	Juvenile
0	26	1570	2	11	2008	gorse	grass	2	6	Adult
0	53	1571	2	11	2008	grass	grass	1	6	Adult
0	138	1572	9	11	2008	gorse	grass	1	6	Adult
0	31	1573	9	11	2008	gorse	grass	2	5	Juvenile
0	0	1574	9	11	2008	grass	grass	2	6	Adult
2	1	1575	9	11	2008	grass	grass	1	6	Adult
0	72	1576	9	11	2008	grass	grass	1	6	Adult
0	13	1577	15	11	2008	gorse	grass	1	6	Adult
2	87	1578	15	11	2008	grass	grass	1	7	Adult
1	37	1579	15	11	2008	grass	grass	2	7	Adult
0	0	1580	15	11	2008	grass	grass	2	6	Adult
0	54	1581	15	11	2008	hawthorn	grass	1	6	Adult
0	0	1582	1	12	2008	grass	grass	2	8	Adult
0	14	1583	1	12	2008	grass	grass	1	7	Adult
0	4	1584	1	12	2008	grass	grass	2	7	Adult

1	9	1585	1	12	2008	grass	grass	2	7	Adult
0	197	1586	2	12	2008	grass	grass	1	6	Adult
2	122	1587	2	12	2008	grass	grass	2	6	Adult
0	113	1588	2	12	2008	grass	grass	2	7	Adult
1	301	1589	2	12	2008	grass	grass	2	7	Adult
5	97	1590	6	12	2008	gorse	grass	1	5	Juvenile
0	19	1591	6	12	2008	grass	grass	1	6	Adult
1	74	1592	6	12	2008	gorse	grass	1	5	Juvenile
1	11	1593	15	12	2008	gorse	grass	1	6	Adult
0	52	1594	15	12	2008	gorse	grass	1	6	Adult
0	35	1595	7	1	2009	grass	grass	2	7	Adult
0	11	1596	7	1	2009	hawthorn	grass	1	7	Adult
1	7	1597	12	1	2009	grass	grass	2	7	Adult
0	7	1598	12	1	2009	grass	grass	2	7	Adult
0	4	1599	12	1	2009	grass	grass	2	7	Adult
1	61	1600	12	1	2009	grass	grass	2	7	Adult
0	23	1601	14	1	2009	gorse	grass	2	8	Adult
0	2	1602	14	1	2009	gorse	grass	2	8	Adult
0	7	1603	14	1	2009	gorse	grass	2	6	Adult
0	3	1604	14	1	2009	gorse	grass	1	7	Adult
0	117	1605	21	1	2009	gorse	grass	1	5	juvenile
0	12	1606	21	1	2009	grass	grass	1	7	Adult
0	41	1607	21	1	2009	grass	grass	2	6	Adult
0	17	1608	21	1	2009	grass	grass	1	6	Adult

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Kathleen E. Creppage

Home: 705 Ridge Avenue · Altoona, PA·16602

Email: kec2233@gmail.com

Phone: 814-215-8584

Education

Pennsylvania State University

Schreyer Honors College, University Park

B.S. Immunology & Infectious Diseases with Honors (candidate)

B.S. Toxicology (candidate)

Expected graduation date: May 2010

GPA: 3.94/4.00

Thesis Title:	"Mechanism of Immunity in Hosts to Chronic Infections in Wild Conditions."
Thesis Supervisor:	Dr. Isabella Cattadori

Research Experience

Apr. 2008 – present: **Undergraduate Research - Dr. Isabella Cattadori**

Pennsylvania State University, University Park

- Bled rabbits for 1 month and periodically assisted with preparations, tissue collection and clean-up during rabbit sacrifices
- Assisted in the development and use of an agar plate-based technique to study bacterial shedding rates of infected rabbits
- Performed enzyme-linked immunosorbent assays (ELISAs) with rabbit serum to determine rabbits' antibody responses to infection with *Bordetella bronchiseptica, Trichostrongylus* r*etortaeformis,* and/or *Graphidium strigosum*
- Digested wild rabbit small intestines to recover *T. retortaeformis* larvae buried under the mucosa in order to determine if their development was arrested

Jan. 2008 – Dec. 2008: **Undergraduate Research - Dr. Eric Harvill**

Pennsylvania State University, University Park

- Prepared lab reagents and cleaned equipment for general lab maintenance
- Assisted graduate students with mice dissections, plating, and other general procedures if needed

- Amplified housekeeping gene DNA for 96 *Bordetella bronchiseptica* isolates using Polymerase chain reactions (PCR)
- Used Multi-locus sequence typing (MLST) to identify sequence types of each isolate from sequence data

Publications in Process

Pathak, Ashutosk K., Kathleen E. Creppage, Jake R. Werner, and Isabella M. Cattadori. Immune regulation of a chronic bacterial infection and consequences for pathogen transmission. Presently under review by *BMC Microbiology*.

Awards/Honors:

Pennsylvania State University College of Agricultural Sciences
2009 Oswald Scholarship
2009 Gamma Sigma Delta Honor Society Inductee
2008 Rumbaugh Agricultural Leadership Award

Pennsylvania State University Schreyer Honors College
2008 Gateway Schreyer Scholar Selection

Pennsylvania State University
2007 President's Freshman Award
2006-present Dean's List, made list all 7 semesters attended so far

Volunteer/Community Service:

St. Vincent de Paul Soup Kitchen, Server and office worker
Nov. 2009 - Jan. 2010

Mount Nittany Medical Center, Patient Volunteer
Sept. 2009 - Dec. 2009

Mid-state Litcorps, ESL Tutor
Jan. 2009 - May 2009

Relay for Life, Team Captain and member
Jan. 2009 - Apr. 2009

Jan. 2008 - Apr. 2008

Second Mile, College Friend
Sept. 2007 - May 2008

Pan-Hellenic Dance Marathon/THON, Committee member
Oct. 2007 - Feb. 2008
Oct. 2006 - Feb. 2007

Organizations & Leadership

Association for Women in Science (AWIS), Penn State Chapter
Vice-President, Jan. 2009-Dec. 2009

- Maintained and updated chapter website with announcements, photos, and links
- Developed and implemented a new point system to determine membership (along with the other members of the executive board)
- Gained recognition from AWIS National in newsletter for both the website design and new point system

Fundraising Co-Chair, Jan. 2007-Dec. 2008

- Organized raffles for Relay for Life, club raffles, food sales, and business-sponsored fundraisers with the other co-chair
- Raised between $100 to $200 per fundraiser