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HOST IMMUNITY AGAINST GASTROINTESTINAL HELMINTH EGGS

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ABSTRACT

Host immunity comprises multiple defense systems that have evolved to clear unwanted pathogens, including gastrointestinal helminths. While immune mechanisms against larval and adult stages of worms have been broadly studied in the laboratory and in the field, immunity specific to the egg stage of the parasite has received less attention. For soil-transmitted parasites, eggs produced within the host are shed via host's feces into the environment. In the environment, egg hatching and larval survival are strongly modulated by climatic conditions. Also possible, the host might influence the ability of eggs to hatch and survive. We hypothesize that prior to shedding eggs within the host may be targeted by humoral immunity, influencing their hatchability. We examined this hypothesis using two gastrointestinal helminths *Trichostrongylus retortaeformis* and *Graphidium strigosum* common to the European rabbit, *Oryctolagus cuniculus*. Changes in egg hatching rate and volume were examined in relation to egg-specific antibodies in the serum and bound to egg shells over a 15 week laboratory experiment. Hatching rate was consistently higher for *T. retortaeformis* over *G. strigosum*. Over the course of infection, egg volume increased for *G. strigosum* but decreased for *T. retortaeformis*. Evidence of egg-specific antibody responses was observed with fewer antibodies bound to eggs of *T. retortaeformis* compared to *G. strigosum*. Little to no associations were found between antibodies, hatchability or volume for both helminths, suggesting that host antibodies play a minor role in regulating egg hatchability in the environment for these helminths.

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Chapter 1 : Background

1.1 Global Parasitic Burden

Parasites are a major source of infectious disease burden worldwide (De Silva et al., 2003; Brooker, 2010). Causing considerable morbidity and mortality in human, livestock and wildlife populations, gastrointestinal helminths reduce the nutritional status, growth, development and health of their hosts (Stephenson et al., 2001). Helminthic infections also cause comparable economic damage and financial loss in the public health sector and the livestock industry, where anthelminthic drugs necessary to contain infection are a steep burden, around $1.5 billion annually, and costly to develop (Artis et al. 2006). Studies of helminth infections of the gastrointestinal tract in animal models can contribute to the understanding of the epidemiology of these infections and provide the foundation necessary for improving public and veterinary health. In this context, research into parasitic infections of the European rabbit can yield understanding of host immunity, parasite survival strategies and the mechanisms of host–parasite interactions, which ultimately influence disease dynamics.

1.2 The Parasites

The gastrointestinal nematodes, *Trichostrongylus retortaeformis* and *Graphidium strigosum*, are common to the European rabbit (*Oryctolagus cuniculus*). Each nematode has a direct life cycle, where parasites infect *O. cuniculus* without vectors or intermediate hosts (Audebert et al., 2002; Massoni et al., 2011). The first species, *T. retortaeformis*, inhabits and matures to adulthood in the small intestine where it is immune regulated by the host (Murphy et al., 2011; Murphy et al., 2013). In contrast, the other nematode, *G. strigosum*, develops to

maturity in the stomach, where its growth, fecundity and survival are weakly mediated by host immunity (Murphy et al., 2011; Murphy et al., 2013). Both species shed their eggs to the outside environment through the host feces. Eggs expelled onto the herbage mature to free-living, third-stage larvae (L3). In the wild, new helminthic infections occur through consumption of L3 by feeding on contaminated pastures.

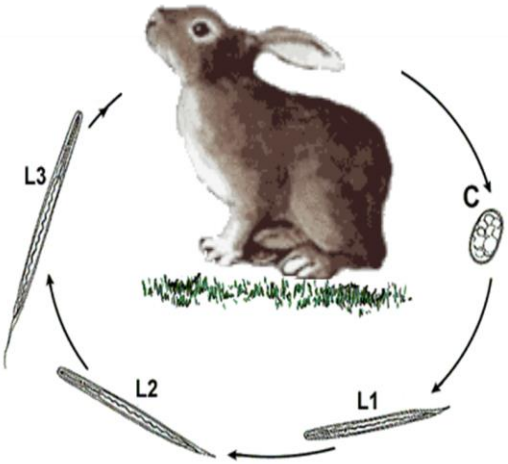

Figure 1- Helminth Lifecycle in European Rabbit. Both gastrointestinal helminth species, *G. strigosum* and *T. retortaeformis*, exist in free living stages on pasture. While grazing, the rabbit consumes third stage larvae that initiate helminthic infection. Inside the rabbit, larvae develop and mature into adult worms. Adult females in the gastrointestinal (G.I). tract of the rabbit generate offspring, shedding their eggs via the host feces to the environment.

1.3 Critical Effectors of Host Immunity: Antibodies

Previous studies in animal models as well as surveys on human infections have clearly shown that antibodies are important contributors to host immunity against parasitic helminths (Sotillo et al., 2007; Schallig, 2000; Nisbet et al., 2010; Kanobana et al., 2002; Balic et al., 2006; Henderson et al., 2006; Balic et al., 2000). During an infection different subsets of antibodies are produced and tailored to different parasites and pathogens. For helminth infections we commonly observe the activation of the "Th2' arm of the immune system characterized by increases in IgA, IgE and IgG antibodies (Pearce et al., 2004; Anthony et al., 2007; Allen et al. 2011). The relative contribution and level of these molecules varies based on the organ infected, the stage of infection (acute or chronic) and the presence of other concomitant infections.

Chapter 2 : Host Antibodies Have a Minor Effect on Helminth Egg Hatchability

INTRODUCTION

Host immunity against helminths target the incoming infective stages and act to distress established adults (Thomas et al., 2002; Maizels et al., 2004; Else, 2005; Moxon et al., 2010; Bourke et al., 2011; Hewitson et al., 2011; Viney and Cable, 2011; Morgan and van Dijk, 2012; Viney and Dia, 2012; Goncalves et al., 2012; Van Kuren et al., 2013). Research in the field has focused on immunity to larval and adult stages and its consequences on helminth survival. In contrast, the immune response to the egg stage of gastrointestinal helminths has not yet been well studied.

Gastrointestinal helminths**,** *Graphidium strigosum* and *Trichostrongylus retortaeformis* have free-living stages, such as eggs, that are central to their life cycles. Transmission success or the ability to spread infection to new individuals relies on the ability of eggs shed into the environment to survive and develop, as well as infect new hosts. Climate, including temperature and humidity, is often implicated in having an effect on egg development and hatching while in the environment (**Moinuddin et al., 2011**). Recent studies with *G. strigosum* and *T. retortaeformis* showed that seasonal changes in intensity, frequency and duration of daily temperatures influenced egg development and rate of hatching (Hernandez et al. 2013). Even before environmental cues can influence the outcome of parasite eggs and disease transmission though, interactions between the immune system and eggs occur within the host**.** We consider that immune responses mounted against other parasite stages might also prime eggs as they are

expelled from the host, modulating their development and hatching. Previous studies by Jorgensen et al. demonstrated that different constraints such as immunity might impact egg development and hatching (Jorgensen et al., 1998; Jorgensen, 2000). Yet, how immunity contributes to egg maturation and hatchability outside the host is still not clear. We hypothesize that antibodies of the immune system may attack eggs, affecting their later viability once they are in the environment. This research aims to address whether humoral immunity affects the fecundity of gastrointestinal helminths and egg hatchability, central to transmission and the dynamics of disease.

MATERIALS AND METHODS

2.1 Experimental Design and Parasite Egg Collection

This study was performed in the laboratory using two New Zealand white, two month old male rabbits which were orally infected with a single dose of third stage larvae of either *T. retortaeformis* (L3: 1500 worms/mL) or *G. strigosum* (L3: 750 worms/mL) diluted in mineral water (Murphy et al. 2011). Animals were housed individually and kept on food and water *ad libitum* on a 12 hour day, 12 hour night light cycle. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of The Pennsylvania State University, USA.

Following the prepatent period for *T. retortaeformis* (~12 days) and *G. strigosum* (~43 days), rabbits started shedding parasite eggs in feces. Given the different prepatent periods for each parasite, *G. strigosum* infection was anticipated so that both rabbits started shedding eggs at the same week 0 (Audebert et. al 2002; Massoni et al. 2011). Rabbit cage trays were cleaned every Monday and fecal samples systematically collected Tuesday mornings for 15 weeks following a.m. defecation of the rabbits. This practice ensured fresh samples containing eggs of similar developmental stage. Feces were soaked in water for 15 minutes, homogenized in a

commercial blender, filtered through a 212µm sieve, and the supernatant evenly distributed in 50 mL tubes and centrifuged for 10 minutes at 1500g at 4°C. For each tube, the supernatant was removed and the pellet vortexed and mixed with ~0.02g of kaolin clay powder and 20 mL of saturated NaCl solution. The resulting supernatant was then filtered through a 36µm sieve and kept under cold running water for 30 minutes to clean debris, including residual salt and clay off of the eggs. Finally, sieve contents were collected into a 50 mL tube, adjusted to 30 mL with tap water. Egg estimates per volume of solution collected determined the yield available for experimental set up with climatic chamber regime.

2.2 Egg Hatching Rate

To estimate the rate of hatching, eggs were placed into a climatic chamber (VWR International, USA) with programmable temperature (Series 982 microprocessor, Watlow Controls, USA). Eggs of both helminth species were exposed to the same environmental conditions, same variance in humidity and cyclic temperature regime, ranging between 7°C - 15°C. The temperature cycle was based on a 12 hour day, 12 hour day-night cycle (Hernandez et al. 2013). Based on data collected in the month of May in our field study site, the temperature regime was selected because it coincides with an increase of infective parasite stages on the pasture. At the same time, the month of May also corresponds with the emergence of newborn rabbits or naïve hosts for helminth infection. Established as a critical time of the year for disease transmission, the month of May provides an estimate of the potential seasonal force of infection (Cattadori et al. 2005; 2008; Hernandez et al. 2013). Day-night cyclic regime was selected over a more realistic day-night stochastic scenario to facilitate experimental execution (Hernandez et al. 2013).

Approximately 100 eggs of *T. retortaeformis* were plated on fourty-five 6 cm Petri dishes prepared with a 10 mL solution of 1% agarose. Likewise, 100 eggs of *G. strigosum* were placed

onto forty-five 6 cm Petri dishes prepared with a 10 mL solution of 1% agarose. A total of 90 dishes were positioned in the incubator with initial conditions starting at 12.00 noon of the cyclic regime. For each parasite, five plates were removed from the incubator and analyzed at day 1, 2, 3,4,5,7, and 8. An additional five plates were also counted at day 0 that never entered the climatic chamber. Eggs and hatched larvae were counted in every plate randomly removed from the incubator. The proportion of larvae relative to the total number of eggs and larvae on the plate was estimated and provide information on the changes in the daily hatching rate of both parasites over the course of the 15 week experiment.

Figure 2-1 Climatic Chamber

Gastrointestinal helminth eggs were placed inside the chamber and exposed to cyclic, Day-Night-Day temperatures ranging from 7°C - 15°C. All eggs experienced the same environmental conditions. Hatching rate for the eggs was then determined upon removal from the chamber.

2.3 Egg Volume

To examine whether egg volume, as indicative of egg quality, changed over the course of infection and also whether egg volume affected egg hatchability, a random sample of 50 eggs was measured every week for each parasite species. Egg size (length and width) was quantified using a digitalized system and Image J software (version 1.45, NIH http://rsbweb.nih.gov/ij) (Chylinkski et al., 2009) and then used to estimate the volume of a prolate spheroid, approximated by $4/3\pi a^2 b$, where width is 'a' and length is 'b'.

2.4 Antibody Detection

To quantify the host immune response to parasite eggs two different approaches were used. First, blood serum from the rabbit was collected and anti-egg serum IgA and IgG quantified with ELISA weekly. Second, endogenous antibodies bound to the eggs' shells were visualized using immune-fluorescence and laser scanning confocal microscope methodology. All reagents and consumables were purchased from VWR International (Radnor, PA) unless otherwise noted.

For serum antibody responses to each parasite, whole egg homogenates from each species were used as a coating antigen in indirect ELISAs. About 10,000 eggs, collected as described in section 2.2, were stored at -80°C in 50 mL tubes with PBS supplemented with a protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). At the time of use, tubes were freeze-thawed seven times, eggs homogenized with 40 strokes in a ground glass homogenizer (Kimble-Chase, Vineland, NJ) and homogenization efficiency confirmed under a stereomicroscope. Homogenates were centrifuged at 5200g for 15 minutes to remove large debris. The resulting supernatants were used to coat 96-well ELISA plates (Greiner Bio-One, Monroe, NC). Plates coated with either *T. retortaeformis* or *G. strigosum* egg homogenates were blocked with 5% non-fat milk in 0.05% PBS-T at 37°C for 1 hour and then washed twice with PBS-T prior to incubation with sera from infected rabbits diluted in blocking buffer for 1.5 hours at 37°C. For *G. strigosum*, sera was diluted to 1:5 and 1:15 for detecting IgA and IgG, respectively while for T. retortaeformis, sera dilutions of 1:10 and 1:40 were used for IgA and IgG, respectively. Plates were washed four times with PBS-T and then incubated with HRP-conjugated goat anti-rabbit IgA (Abcam plc, Cambridge, MA) or IgG (SouthernBiotech, Birmingham, AL) diluted to 1:2500 in blocking buffer for 1 hour at 37°C. Reactions were then developed with ABTS as colorimetric reagent for 15 minutes and color intensities recorded on a spectro-photometric plate reader (Bio-Tek, VT, USA). Each plate incorporated positive and negative control sera which were pooled from single infected animals as part of a separate study

(Murphy et al. 2011). All dilutions and reaction conditions outlined here were optimized prior to the experiment in a checkerboard format as described previously (Murphy et al. 2011). Raw data were transformed into optical density (OD) indexes for the subsequent analysis (Murphy et al. 2011).

The level of antibodies bound to eggs' shells was measured using an immune-fluorescence based microscopy approach. After isolating and counting (section 2.2), 1500 eggs were aliquoted into three 1.5 mL tubes each and fixed overnight (~16 hours) at 4°C in 1.5 mL of 10% neutral buffered formalin. Optimal antibody dilutions were previously established from the inflection points obtained by incubating eggs with two-fold incremental dilutions of sera and corresponding fluorophore conjugates (Cy3 conjugated goat anti-rabbit IgG H+L, Jackson Immunoresearch, West Grove, PA) in a tube-based modified checkerboard format. Specificity of the fluorophore conjugates were initially confirmed in a preliminary test where eggs were stained with flurophore-conjugated rabbit-specific antibodies and no binding was observed with non-specific Cy3 conjugated goat IgG (Jackson Immunoresearch, West Grove, PA). Formalin-fixed eggs were washed by centrifuging the tubes at 17,000g for 1 minute, removing the supernatant and re-suspending the pellet in 1.5 mL of PBS. Eggs were washed a further two times in the same manner prior to blocking them in 1 mL of 10% goat-serum prepared in PBS-T for 30 minutes. All incubations were performed on a rocking platform shaker. Eggs in two of the three tubes as control samples were washed twice with PBS-T and re-suspended in 1 mL of PBS-T supplemented with positive or background control sera diluted 1:100 and incubated for an additional 60 minutes at room temperature. Eggs in the third tube or experimental samples were left un-disturbed in the blocking solution for this 60 minute period. Following incubations, all control and experimental samples were washed three time with 1.5 mL PBS-T and stained with 1 mL of PBS-T containing 1:400 diluted goat anti-rabbit IgG (H+L) conjugated to Cy3 fluorophore (Jackson Immunoresearch, West Grove, PA) and incubated for an additional 60 minutes at room.

Eggs were finally washed three times with 1.5 mL PBS-T and once with 1.5 mL PBS prior to microscopy. For each tube (controls and experimental), 50-150 stained eggs were visualized under a confocal laser scanning microscope and fluorescence intensity recorded at the equatorial perimeter of the ellipsoid egg. Images were obtained with a 10x objective (Olympus Fluoview® FV 1000) pre-calibrated at identical lamp intensities (485V) and acquisition parameters (38%) throughout the study, to allow weekly comparisons of staining intensities. Level of binding (i.e. intensity of staining) was quantified with the iVision software package (BioVision Technologies, West Exton, PA) and expressed as mean fluorescent intensity (MFI) using only positive eggs or ones that were fluorescing. Overall, from the shedding of feces onto the cage tray to the fluorescent reading, eggs were kept between 4°C and room temperature for 36-48 hours, depending on the procedure. While our approach could not halt egg development, we did standardize our procedures to maintain the processing time within 48 hours to allow data comparison.

2.5 Data Analysis

To determine significant changes in the egg hatching rate over the course of the infection (weeks) or the amount of time in the chamber (days), linear mixed effects models (LME-REML, package NLME in R, Pinherio and Bates, 2000) were applied. This approach was also used to investigate the relationship between the rate of hatching and the antibody responses, both serum and egg immunofluorescence data, in addition to the temperature changes (maximum and minimum) in the chamber. To take into account variability between Petri dishes, dish ID was initially considered as a random factor. However, this variable was excluded in subsequent analysis because of the low variability between dishes. Based on the combination of variables examined, experimental week or sampling day was included as a random factor. To consider the effect of pseudo-replication (i.e. sampling the same rabbit over the course of the infection) an

autoregressive function of order 1 (AR-1) was included. To examine changes in egg volume by sampling week generalized linear models (GLM with normal error distribution) were used while LME-REML was used to investigate the relationship between egg volume and immune responses. Egg hatching rate was arc sin transformed.

RESULTS

When comparing the two species of worms, the rate of weekly hatching was significantly higher for *T. retortaeformis* than for *G. strigosum* (coefficient ±standard error: -0.17±0.06, df= 1124, P<0.01) For each experimental week, daily egg hatching reached higher thresholds for *T. retortaeformis* as opposed to *G. strigosum* (two-way interaction species-sampling day, coeff±s.e.: -0.03±0.005, df=1140, P<0.0001).

For *T. retortaeformis,* egg hatching rate did not change significantly among the experimental weeks, starting with egg shedding at week 1 to the end of the 15 week trial (**Fig. 2-2a)**. Within the weeks, hatching rate increased with experimental day. Specifically, the longer the eggs were kept in the climatic chamber, the higher percentage of eggs that hatched (**Fig 2-2a**, coeff±s.e.: 0.18±0.004, df=544, P<0.0001). For *G. strigosum*, no significant differences were found in the hatching rate of eggs among the experimental weeks. Consistent with *T. retortaeformis,* hatching rate increased with the number of days spent in the climatic chamber (**Fig. 2-2b**, coeff±s.e.: 0.15±0.003, df=583, P<0.0001).

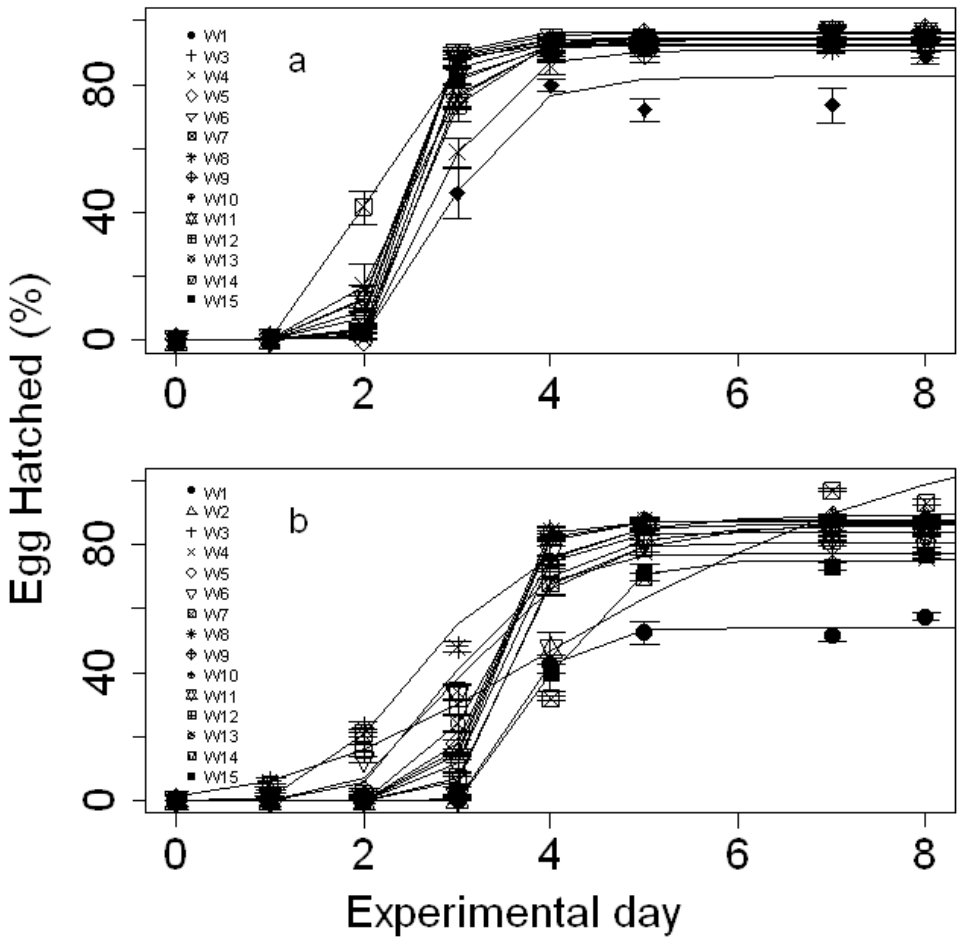

Figure 2-2- Hatching Success of *Trichostrongylus retortaeformis (a)* and *Graphidium strigosum (b).* For each experimental day of the 15 week infection, the number of hatched larvae and unhatched eggs were counted. Hatching rates for *T. retortaeformis* reached thresholds of 90% or greater (a), as opposed to *G. strigosum*(b) that reached thresholds of hatching around 85%. Additionally *T. retortaeformis* (a) achieved greater hatching success faster than *G. strigosum* (b).

No significant associations were observed between serum IgG levels and the mean weekly rate of hatching. Likewise, no significant associations were found between weekly mean rate of hatching and serum or egg bound antibodies for *G. strigosum*. Antibodies bound to eggs and IgA OD values decreased throughout the infection (MFI and IgA *vs* weeks, respectively, coeff±s.e.: -0.03±0.01, P<0.0001 and -0.02±0.01, P<0.05, for both: df=13) while IgG OD levels increased (coeff±s.e.: 0.07±0.01, df=13, P<0.0001) (**Fig. 2-3b**). For *T. retortaeformis*, both IgA and IgG OD indices, but not MFI, decreased with the course of infection; although, it is important to note that both the MFI and the species-specific serum IgA OD index rarely rise above background levels throughout the infection (**Fig.2-3a** , IgA and IgG *vs* weeks, respectively, coeff±s.e.: -0.04±0.01, P<0.05 and -0.04±0.01, P<0.0001, for both: df=12).

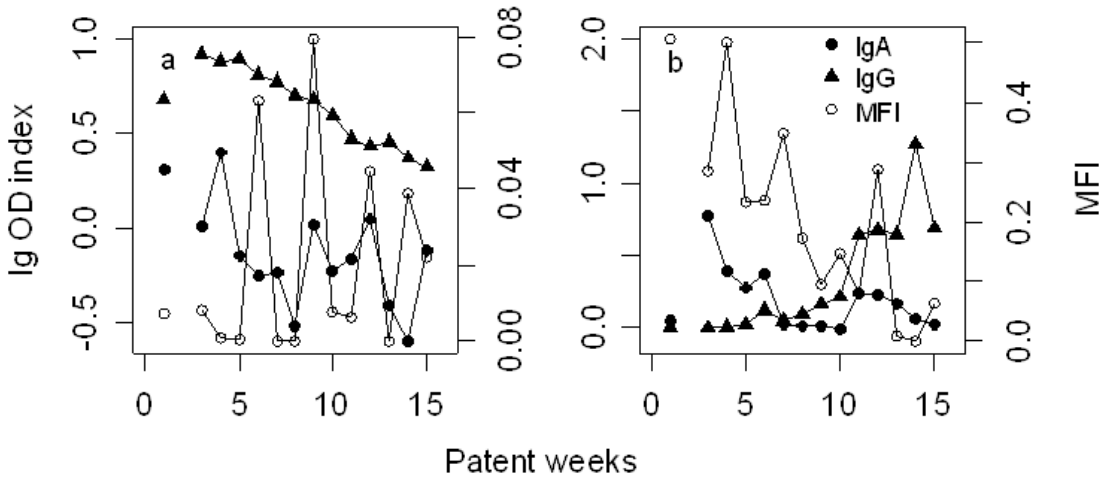

Figure 2-3- Serum antibody and egg-bound antibody concentrations of IgA and IgG in *T. retortaeformis (a)* and *G. strigosum* (b) infected animals. Optical density (OD) indices of serum IgA (●) and serum IgG (▲) against eggs and mean fluorescent intensities (MFI) of egg bound antibodies (○) over 15 weeks of infection.

In the climatic chamber, *T. retortaeformis*, hatching rate increased with minimum temperature (interaction min. temperature-sampling day coeff±s.e.: 0.03±0.003, df= 371, P<0.0001) and was negatively associated with maximum temperature (two way interaction max. temperature-sampling day coeff±s.e.: -0.01±0.001, df=371, P<0.0001). Likewise, *G. strigosum* hatching rate was positively associated with minimum temperature (interaction min. temperature-sampling day, coeff±s.e.: 0.05±0.005, df=337, P<0.0001) and negatively related to maximum temperature (two way interaction max. temperature-sampling day, coeff±s.e.: -0.01±0.002, df=337, P<0.0001). In combination, this suggests that an increase in the minimum temperature stimulates egg development and hatching while higher temperatures have the opposite effect. This is consistent with prior knowledge that climate and environmental factors influence egg development. Moreover, it is indicative of an optimal thermal range for the development of both helminths, *T. retortaeformis* and *G. strigosum.*

The phenology of hatching, or the first day within each week when eggs hatched in at least one Petri dish, was about two days (mean±s.e.: 1.86±0.14) for *T. retortaeformis*. No significant relationship was observed between first day of hatching and antibody responses which is not surprising considering the narrow variability in the phenology of hatching observed during the 15 weeks of infection. In contrast, the mean first day of hatching for *G. strigosum* was 1.73 (±0.26). In keeping with the low variability in phenology of hatching over the trial, this trend was not related to antibody responses.

Egg volume or size of *T. retortaeformis* eggs extracted weekly from rabbits' feces slowly decreased with the progression of the infection through week 15. Some variation in egg volume was observed between the weeks (**Fig. 2-4**, coeff±s.e.: -0.08e-5±0.02e-5, df=716, P<0.001). No significant relationships were found between the weekly egg volume and antibody responses in

the serum or bound to the eggs' shell. Amongst the weeks, egg hatchability was not associated with egg volume.

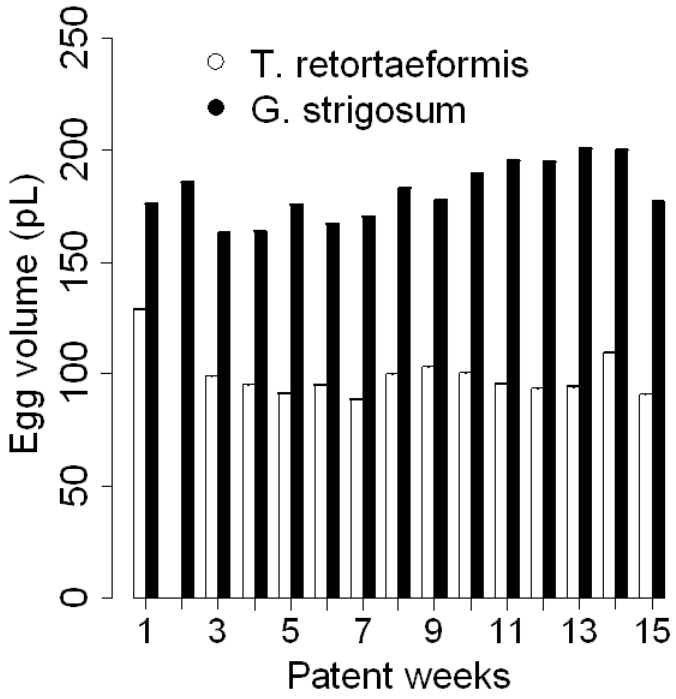

Figure 2-4 Egg volume of *T. retortaeformis* and *G. strigosum* over the course of an infection.

G. strigosum egg volume positively increased with sampling week (**Fig 2-4**, coeff±s.e.: 0.02e-4±0.03e-5, df=833, P<0.0001) with some variability observed between weeks. Egg volume was negatively related to MFI (coeff±s.e.: -0.04e-3±0.02e-3, df= 13, P<0.05) but positively associated to IgG (coeff±s.e.: 0.03e-3 0.06e-4, df=12, P<0.001). However, the weekly egg hatchability was not related to egg volume. The average volume of *T. retortaeformis* eggs (mean pL±s.e.: 98.86± 0.06) was consistently smaller than *G. strigosum* eggs (mean pL±s.e.: 181.29±0.93) (t- test, df= 1481.596, P<0.0001).

DISCUSSION

As hypothesized, gastrointestinal helminth eggs shed directly into the lumen of the host stimulated an immune reaction. Serum levels of antibodies, IgA and IgG, were detected against eggs and found to vary over the course of infection. *T. retortaeformis* eggs prompted predominantly an IgG response, whereas *G. strigosum* eggs elicited both IgA and IgG responses. Immuno-fluorescence detected antibodies bound to the shells of *G. strigosum* eggs. In contrast, *T. retortaeformis* eggs had markedly low immunofluorescent signals, rarely above background levels, indicative of lower levels of antibody binding.

Rabbits infected with a single dose of *T. retortaeformis* or *G. strigosum* constantly shed viable parasite eggs and the rate of hatching did not significantly change over the course of the 15 week experiment. Within each week, hatching rate showed a characteristic logistic growth with the majority of eggs hatching between the second and third day of exposure to a cyclic 7°C - 15°C temperature regime. *T. retortaeformis* exhibited a more consistent pattern across the weeks while *G. strigosum* appeared to be more variable. Between the two helminth species, *T. retortaeformis* eggs developed faster by reaching higher rates of hatching compared to *G. strigosum*. This pattern was consistent over the course of the infection, both within and between weeks. Also, these trends were comparable with our previous studies based on the same temperature regime (Hernandez *et al.*, 2013). Higher thresholds of hatching for *T. retortaeformis* eggs were associated with lower concentrations of egg-bound antibodies. Likewise, slower hatching for *G. strigosum* was associated with greater antibody binding to eggs. Consistent with our hypothesis, greater antibody-mediated immunity may have rendered the eggs more susceptible to immune attack, resulting in reduced hatchability.

In general, the volume of *G. strigosum* eggs was twice that of *T. retortaeformis*, in agreement with previous reports on egg size (Taylor *et al.*, 2007). When also comparing egg biometry, *G. strigosum* eggs increased in size and *T. retortaeformis* eggs decreased in size over

the course of the infection. Despite these changes in volume over the course of the infection, hatching rate was comparable between weeks for both helminths. This suggests that the observed changes are probably insufficient to alter hatchability, or egg volume is, after all, not a critical constraint in the hatching success.. In general, no significant relationships were found between hatching rate and egg volume with antibody responses. This suggests that either the eggs can tolerate the host immune attacks or host immunity does not greatly affect egg development and hatchability. Alternatively, humoral immunity may preferentially attack or focus on larger and invasive adult and larval stages, as opposed to the smaller egg stage.

Although factors such as phylogenetic differences, contrasting evolutionary life history strategies or female worm qualities between the two parasite species, might be the primary forces driving the observed dissimilarities in rates of hatching, our findings indicate that humoral or antibody-mediated immunity might play a secondary role to the distinct dynamics of gastrointestinal helminths.

LIMITATIONS OF THE STUDY

Previous studies that observed IgG and IgA responses against somatic antigen from third stage infective larvae (L3) and adult worms found clear evidence of cross-reactivity between L3 and eggs (Murphy *et al*., 2011; Thakar *et al.*, 2012). Consequently, we do not exclude considerations of cross reactivity between the eggs and other worm stages within this study. Several notable differences exist however, between the level of IgA reactivity to the various stages of *T. retortaeformis.* For instance, we previously observed high IgA reactivity to the adult and larval stages of this helminth (Murphy *et al*., 2011; Thakar *et al.,* 2012); whereas, our current study shows little evidence of serum IgA reactivity to the eggs of *T. retortaeformis*. This suggests that distinct immune responses against eggs were observed and accurately quantified in this study.

Also, serum IgG and IgA responses were measured systemically in the host, causing concerns that the humoral immunity observed may not be indicative of responses at the actual site of infection in the gastrointestinal tract. However, our previous work indicates that similar antibody response profiles exist between the site of infection and systemic circulation (Murphy *et al.*, 2011 & 2013). Furthermore, an immuno-fluorescence approach was also utilized, in addition to serum ELISA, to quantify direct antibody binding to the eggs' shells at the site of infection. For example, the lack of IgA reactivity in the serum for *T. retortaeformis* is mirrored in the immuno-fluorescence data that also shows the shells of *T. retortaeformis* eggs devoid of any endogenously bound host antibodies.

While the methodology employed in the current study prevents us from discerning the isotype of antibody bound to egg surfaces (IgA or IgG), we do know that for the adult stages of *T. retortaeformis*, IgA may be the major isotype bound to their surface, and therefore a critical immune- mediator (AKP and IMC, unpublished observations). These observations are in line with other studies showing a negative role for IgA in regulating the fecundity of related nematodes in sheep (Stear *et al.*, 2009; Hein *et al.*, 2010). By inference then, the apparent lack of an egg-specific IgA response in this study suggests that the eggs of this parasite may be able to escape immune surveillance during its passage through the gastro-intestinal tract and thus maintain its consistently higher hatching ability.

The experimental design also prevented us from gaining any information on female worm quality or specific immune responses. While female conditions can affect number and quality of eggs, this may not be a critical factor to consider because these parasite traits show continual decline over the course of the infection, especially for *T. retortaeformis* (Murphy *et al.*, 2011 & 2013; Pathak *et al.*, 2012; Cattadori et al., 2014).

Ultimately only one animal was used per species of worm to investigate the effects of host humoral immunity on gastrointestinal helminth eggs. Despite the inclusion of only one animal in the study, the results presented are still generally applicable for two reasons. First and foremost, the contrasting trends in hatching rates between the two parasites are congruent with previous studies based on the same temperature regime (Hernandez *et al.*, 2013) suggesting that any given cohort of eggs shed by a worm population of identical age will show similar hatching phenotypes for either parasite. Secondly, from a host perspective, the similarities in serum antibody response profiles are generally comparable between this and our previous studies with larger groups of rabbits (Murphy *et al*., 2011 & 2013). Although the inclusion of a larger host cohort and refined immunological methodology may enable us to better address the interaction between immunity and egg hatchability in the future, the current study still provides a possible mechanism for host-pathogen interactions. In this case specifically, we demonstrate for the first time that immunity may contribute to the different hatching rates of two gastrointestinal parasites' eggs.

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EDUCATION

The Pennsylvania State University, Schreyer Honors College, University Park, PA
B.S. Immunology & Infectious Disease | Minor and Honors in Biology 2014

Penn State College of Medicine, Hershey, PA - *Beginning July 2014*
M.D./Ph.D. Dual Degree Candidate

RESEARCH EXPERIENCE

Center for Infectious Disease Dynamics (2010-2014)
Penn State Department of Biology, Cattadori Lab Group

- Investigation of the host immune response's role in gastrointestinal helminth infection, disease dynamics, and parasite egg quality, fecundity, and survival.
- Mastery of parasitological techniques including helminth biometry, extraction and quantification of eggs and larvae, and digital imaging of gastrointestinal species.
- Determination of cytokine gene expression in helminth-infected host tissues using RNA extraction, purification and real-time quantitative PCR.

Penn State Milton S. Hershey Medical Center, M.D./Ph.D. Early Exposure Program
Department of Medicine, Division of Infectious Disease, Parent Lab Group

- Determination of host cellular proteins that interact with and facilitate retroviral assembly, using mammalian cell culture, immunofluorescence and confocal microscopy techniques.

CLINICIAL EXPOSURE

Penn State Milton S. Hershey Medical Center, Hershey, PA

- Allergy & Immunology Clinic Preceptorship (20 hours)
- Infectious Disease Clinic Preceptorship (20 hours)

Private Practice of T. Ramakrishnan, M.D., Easton, PA

- Allergy, Asthma & Immunology Preceptorship (15 hours)

PUBLICATIONS & PRESENTATIONS

- Lambert, K.A., Pathak, A.K., Cattadori, I.M. (2014) Does host immunity influence helminth egg hatchability in the environment. *Journal of Helminthology*. ***In press.***
- Lambert, K.A., Bann, D.V. and Parent, L.J. (2013) Role of P-body and RNA-Induced Silencing Complex Proteins in Retroviral Assembly. **Oral Presentation**. Penn State College of Medicine Summer Undergraduate Research Symposium, Hershey, PA.
- Lambert, K.A., Bann, D.V. and Parent, L.J. (2012) Role of Mov10 in Mouse Mammary Tumor Virus Replication. **Oral Presentation**. Penn State College of Medicine Summer Undergraduate Research Symposium, Hershey, PA.

HONORS & AWARDS

- National Science Foundation Research Experience for Undergraduates (**NSF-REU**) Grant
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- Schreyer Honors College Academic Excellence Scholarship
- John N. Adam Jr. Scholarship for Excellence in Agriculture
- Horace T. Woodward Scholarship in the College of Agriculture
- Theola F. Thevaos Honors Scholarship in the College of Agriculture
- Bernard and Beatrice Sandson Scholarship in Agriculture
- Paul M. Holder Music Scholarship in the Penn State Blue Band

EXTRACURRICULAR

Pennsylvania State University Marching Blue Band (2010-2014)

- Clarinet Section Guide, Squad Leader and Tunnel Lead

Penn State Chapter of GlobeMed (2012-2013)

- Global Health Equity Conference Committee
  - Developed agenda, recruited speakers, and employed community involvement to host a weekend-long conference to discuss socioeconomic barriers and disparities in healthcare.

Penn State Dance MaraTHON (THON) Benefitting the Four Diamonds Fund (2010-2014)

- Overall Chair of Blue Band For The Kids (2013-2014)
  - Oversaw our organization's yearlong involvement in commonwealth- wide dance marathon with effective communication of THON rules and logistics.
  - Led fundraising initiatives that raised $42,000 for the fight against pediatric cancer.
  - Facilitated the Blue Band's musical participation in THON events to raise awareness in the fight against pediatric cancer.
  - Danced for 46 hours without sleep or sitting in the annual Dance MaraTHON.

- Family Relations Chair of Blue Band For The Kids (2012-2013)
  - Acted as a liaison for the Blue Band in charity events with Four Diamonds Families affected by pediatric cancer.
  - Programmed events, prepared care packages, and maintained correspondence with adopted Four Diamonds Families paired with the Blue Band throughout the year.
  - Managed and maintained positive contact and relationships with our adopted Four Diamonds Families