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The relationship between the intensity of raccoon roundworm infection and egg production.

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Introduction

*Baylisascaris procyonis* is an intestinal nematode found primarily in raccoons. Over 130 species of mammals and birds can transmit the parasite, however the raccoon is the definitive host (“Baylisascariasis,” 2013). The common raccoon is often considered to be solitary (Ratnayeke et al., 2002), but individuals prefer to defecate in communal latrines (Gavin et al., 2005; Page et al., 2009). The social system of raccoons is more dynamic than previously thought and often revolves around the use of these latrines (Prange et al., 2011). These sites serve as markers for group territories (Prange et al., 2011) and often attract many intermediate hosts foraging for undigested seeds (Page et al., 1999). Roussere et al. (2003) concluded that the possibility of raccoon roundworm (RRW) transmission increases as latrine density increases. Millions of viable RRW eggs can be shed a day by a single infected raccoon and can survive for years in a latrine (“Baylisascariasis,” 2013). Shafir et al. (2011) attempted to establish parameters for egg viability and discovered that eggs were still viable after having been frozen at -15°C for six months. Eggs are also highly resistant to disinfectants (“Baylisascariasis,” 2013). The latrine behavior of raccoons allows for the accumulation of feces contaminated with RRW (Page et al., 1998) and ultimately, aids in the transmission of the parasite (Prange et al., 2011).

*B. procyonis* is transmitted through fecal-oral contact with an infected raccoon. Unembryonated eggs are shed into the environment by definitive hosts, where they become infectious in two to four weeks. The next life stage is completed when embryonated eggs are ingested by intermediate hosts. Once ingested, the eggs hatch and larvae penetrate the walls of the small intestine and enter the bloodstream where they are carried to the lungs. From there, the larvae are distributed throughout the body tissues where they continue to grow and eventually become encysted in muscle and connective tissues. Definitive hosts can contract the parasite by
either ingesting eggs directly from their environment, or more commonly, by consuming intermediate hosts (Center for Disease Control, 2010).

Humans have been known to contract RRW’s through contact with infected raccoons and after accidentally ingesting the eggs from water and soil (Gavin et al., 2005; Murray & Kazacos, 2004; “Baylisascariasis,” 2013). While maturing, the larvae often migrate to the tissues of the central nervous system (Center for Disease Control, 2010). Children are more frequently effected and have suffered from neural larva migrans, ocular larva migrans, and visceral larva migrans (Murray & Kazacos, 2004). The severity of infection is dependent on the number of eggs ingested and where the larvae migrate in the body (“Baylisascariasis,” 2013). Once RRW has invaded the central nervous system, the prognosis is often poor and without treatment options (Gavin et al., 2005).

The zoonotic potential of RRW has led to an abundance of publications. For example, the prevalence of RRW is commonly studied (Ingle et al., 2014). Page et al. (2005) found that prevalence is most reliably predicted through the necropsy of individual raccoons, followed by latrine sampling, and then fecal sampling. When factoring in time commitment, the most efficient method for predicting zoonotic potential is latrine sampling, however this method often underestimates prevalence (Page et al., 2005). Ingle et al. (2014) found a higher prevalence of RRW in raccoon populations found in closer proximity to fragmented landscapes, either by agriculture or urbanization. While the prevalence of RRW is commonly studied, there is a gap in our knowledge as to whether or not the intensity of infection can be used to predict the amount of egg production. In a blind study, we aim to quantify RRW eggs from fecal samples derived from raccoons with known intensities of RRW infection. We hypothesized there would be a positive correlation between the intensity of infection and the number of eggs found in fecal samples.
Methods

The raccoons used for this experiment were either collected from local fur buyers or found dead on the road in Ottawa, Kent, and Barry Counties—the majority being from Barry County. Raccoons were necropsied, with complete removal of the digestive tract, which was searched for RRW’s, and a fecal sample was obtained. RRW’s were preserved in 95% ethanol and fecal samples were frozen at -20°C, which does not harm RRW (Kazacos, 2001). This was a blind study in which two students independently counted eggs for each sample. The samples were prepared by mixing 1 gram of a fecal matter and 12 mL of Sheather’s Sugar Flotation Solution (specific gravity 1.27) in a disposable cup. The solutions were then strained through a double layer of cheesecloth. Samples were counted over a period of two months and those that were not being counted were refrigerated. When quantifying eggs, Modified McMaster slides were loaded with solution and allowed to sit for five minutes. Only eggs within the grid lines were counted. Eggs partially on grid lines were counted, but those found entirely within a grid line were disregarded. The data was statistically analyzed using SPSS software. We used regression analyses to determine if the intensity of RRW infection predicted RRW egg count. We performed a correlation to determine if our independent egg counts were similar or not.

Results

The average intensity of infection for the studied raccoons was 39 worms and out of a sample size of 49 raccoons, there were 12 animals that were uninfected (Figure 1). Student one had a maximum egg count total of 249 eggs (Figure 2), while the student two’s maximum egg count total was 83 eggs (Figure 3). Both student egg counts (Figures 2 and 3) yielded a positive correlation between the number of worms and the number of eggs. Further regression analyses presented an $R^2$ of 0.07 for Figure 2 and an $R^2$ of 0.09 for Figure 3. We found a statistically
significant correlation between the intensity of infection and the egg counts depicted in Figure 3. The p-value for this set of egg counts was 0.03. The relationship between the egg count and number of worms in Figure 2 was not significant, as determined by a p-value of 0.07. Figure 4 shows a comparison between the independent egg counts for each student. The R-value for this set of data is 0.68, suggesting a significant relationship between the two sets of data (p=0.00).

**Discussion**

We found a weak, positive correlation between the intensity of infection and the number of eggs counted in each sample. The intensity of infection (measured by the number of adult worms in an animal) only explained 7.0 and 9.9% of the variation in the number of eggs counted, respectively (data in Figures 1 and 2). The results were statistically significant only in Figure 2, with a p-value of 0.03. This said, our regression analyses lead us to the conclusion that our data is not strong enough to support using the intensity of infection to accurately predict the number of eggs present in its host.

In two cases, the raccoons had no RRWs, but a single egg was counted for each sample. We hypothesize that these eggs were ingested but not infective and therefore could not develop into larvae. Also unexpected were the extreme outliers present in Figure 3. We presume this discrepancy in results stems from counting the eggs for data sets one and two over a period of time, in which the samples were stored. Samples were stirred after refrigerated, but the slight difference in technique resulted in some noteworthy variation in data. Typically, the Modified McMaster procedure involves adding fecal solutions to slides immediately after being prepared (“Techniques for parasite…,” 2000). This poses a need to better standardize our experimental methods between individuals in the future.
We measured the intensity of infection through a holistic count of worms. By counting all worms present, as compared to counting only the adult female parasites, we potentially have undermined and weakened our correlation. In light of this, the intensity of infection should be modified to consider only the female worms present in a host, as each female can produce up to an estimated 179,000 eggs per day (“Baylisascariasis,” 2013).

The ability to predict egg production from intensity of infection could offer a new method for predicting prevalence. Current methods involve the sampling of latrines (Page et al., 2005), but by utilizing known information about geography and prevalence to predict the intensity of infection (Ingle et al., 2014), researchers would have an indication of the number of eggs shed in a geographic area. The prevalence of eggs in an environment undoubtedly contributes to the spread of this zoonotic disease, and therefore warrants further research. We will continue to investigate the relationship between the intensity of RRW infection and egg production, but we will modify our intensity of infection to include only adult female worms. We hypothesize that the intensity of infection will better predict egg production following this modification, and that more significant R² and p-values will result.
References


