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Evaluation of *Cruzia americana*, *Turgida turgida*, and *Didelphostrongylus hayesi* Infection in the Virginia Opossum (*Didelphis virginiana*) and Risk Factors Along the California Coast

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ABSTRACT: Three nematodes, *Turgida turgida*, *Cruzia americana*, and *Didelphostrongylus hayesi*, have been documented to cause morbidity and mortality in the Virginia opossum (*Didelphis virginiana*). The present study was designed to determine the frequency of infection of these nematodes in opossums at 2 study sites in California and to determine if there are risk factors associated with shedding of eggs or larvae in the feces. *Turgida turgida* and *C. americana* adults were found in 84.4% (stomach; n = 45) and 62.5% (intestinal wash and feces; n = 16) of sampled opossums. Eggs were present in opossum feces (n = 105) less frequently (40% *T. turgida* and 35.2% *C. americana*). *Didelphostrongylus hayesi* larvae were found in 79.0% of opossum feces examined (n = 105). Adult age and wet season (December through April) were significant predictive factors for the presence of *T. turgida* eggs, whereas the dry season (May through November) was significantly associated with the presence of *C. americana* eggs in feces. Adult opossums were more likely to have eggs and larvae from all 3 nematodes in the feces.

Several helminthological surveys have been conducted on the Virginia opossum (*Didelphis virginiana*) in the eastern United States and Mexico (Alden, 1995; Monet-Mendoza et al., 2005; Richardson and Campo, 2005). Three nematodes of high prevalence in these regions, namely, *Turgida turgida*, *Cruzia americana*, and *Didelphostrongylus hayesi*, have been reported to cause significant morbidity and mortality when present in large numbers. *Turgida turgida*, a nematode localized to the stomach of opossums, has been documented to cause granulomatous inflammation, ulcers, and fibrosis at the point of attachment. These lesions could potentially lead to gastric perforation, bacteremia, or sepsis (Nettles et al., 1975; Gray and Anderson, 1982; Alden, 1995). *Cruzia americana*, a parasite localized to the cecum of the opossum, does not appear to cause pathology in small numbers; however, in large numbers, this species can cause malabsorption (Nettles et al., 1975). *Didelphostrongylus hayesi*, a lung worm, has been reported to cause significant granulomatous inflammation and hyperplasia in the lungs and predispose the host to secondary bacterial infections, leading to significant morbidity and mortality in affected opossums (Duncan et al., 1989; Lamberski et al., 2002).

The prevalence and intensity of infection with these nematodes are unknown in opossums inhabiting the western United States. Furthermore, there have been no studies to determine if demographic or environmental risk factors, including gender, age, location, and sampling interval, are associated with infection by these helminths. The present study was designed to determine the prevalence of *T. turgida*, *C. americana*, and *D. hayesi* in the Virginia opossum sampled at 2 study sites in California, and to determine risk factors for infection. The mean worm burden of *C. americana* and *T. turgida* from Virginia opossums along the California coast was also determined.

Samples were collected from opossums obtained through trapping, opportunistic sampling, i.e., vehicle-killed opossums, and local wildlife rehabilitation centers. Live trapping of opossums was conducted at 2 study sites in central California—Rio Vista and Monterey Bay. At each site, forty 66-cm × 15-cm × 15-cm traps were set in multiple transect lines 50 m apart. Traps were baited with fresh fruit 1 hr before sunset and checked just after sunrise. Trapped opossums were weighed, sexed, aged, and feces were collected from the cage. Trapped opossums were ear tagged prior to release. All animals were humanely trapped and sampled in accordance with protocols established by a Department of Fish and Game collection permit and an animal-use protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California–Davis, which is accredited by the Association for the Assessment and Accreditation of Laboratory Care International.

Intestinal contents were obtained from 45 necropsied opossums. Stomach, intestine, and feces were flushed with saline into separate 50-ml vials. Stomach contents were sieved using a 500-μm sieve. Intestinal contents and feces (minus 5 g for fecal flotation) were sieved separately, each through a 150-μm sieve. After sieving, the stomach and intestinal contents were examined for worms. Adult worms were cleared with lactophenol and identified using described morphologic characteristics (Crites, 1956; Skrjabin, 1968; Chabaud, 1974, 1975; Skrjabin et al., 1982). The feces were then flushed from the sieve into a known volume of 70% ethanol, and 5 aliquots, each representing 10% of the total volume, were separated into 50-ml vials. Each aliquot was poured into a petri dish and examined using a dissecting microscope to determine the number and genus of the worms recovered.

Fecal flotation with double centrifugation and $ZnSO_4$ (specific gravity [sp. gr] 1.2), using the methods described by Alcaino and Baker (1974) and Dryden et al. (2005), was performed on feces collected from 105 opossums to detect the presence of *Physaloptera* sp. and *Cruzia* sp. eggs, and *Didelphostrongylus* sp. larvae. Morphological criteria were used to identify *D. hayesi* larvae and *C. americana* eggs (Crites, 1958; Prestwood, 1976). Additionally, a few adult female *T. turgida* and *C. americana* worms were incised, and their eggs were compared microscopically to those observed on fecal flotation.

Risk factors for shedding *T. turgida*, *C. americana*, and *D. hayesi* were analyzed using logistic regression in SAS version 9.1 (SAS Institute, Cary, North Carolina). The event outcome was defined as the presence of *T. turgida* or *C. americana* eggs in feces, or the presence of *D. hayesi* larvae in feces. Risk factors considered in all models were age class (adult or juvenile), gender (male or female), season (wet or dry), and co-infection with any one of the other parasites. Wet season was defined between the months of December and April, and dry season as the months of May through November. The variables were first assessed singly, and those significant at $P \leq 0.20$ were selected for inclusion in a multivariable model using a forward selection procedure. If more than 1 risk factor was statistically significant in the selected model, then interaction between risk factors was checked. Variables were considered significant in the multivariate model if the coefficient had a P-value <0.05. Fit between the observed data and the model was assessed using the Hosmer–Lemeshow goodness-of-fit statistic.

Factors predictive for *T. turgida* and *C. americana* total worm burden in the stomach and large intestine, respectively, were also assessed using a negative binomial model in SAS version 9.1 for a subset of 45 opossums for *T. turgida*, and a subset of 16 opossums for *C. americana*. The negative binomial model is well-suited for overdispersed data, as would be expected for worm counts (Agresti, 2002). The suitability of the negative binomial model was confirmed by evaluating the distribution of worm counts in @Risk for Excel 4.5 (Palisade Corporation, Ithaca, New York). Predictive factors included in the model were the same risk factors listed previously (age class, gender, season, and co-infection).

Turgida turgida eggs were present in feces of 40.0% (42/105) of all opossums sampled. The frequency of *C. americana* egg detection was 35.2% (37/105), and the presence of *D. hayesi* larvae was 79.0% (83/105). *Turgida turgida* adults were found in 84.4% (38/45) of the opossum stomachs examined, whereas *C. americana* adults were found in 62.5% (10/16) of the intestinal washes and feces sieved and examined. No other helminths were noted.

According to the univariable analysis (Table I), adult opossums were

TABLE I. Risk factors for the presence of *Didelphostrongylus hayesi* larvae in feces, *Cruzia americana* eggs in feces or worms in the large intestine, and *Turgida turgida* eggs in feces using logistic regression.

Risk factor	Odds ratio	95% CI	*P* value
C. americana			
Age (adult vs. juvenile)†	4	1.1–14.7	0.037*
Season (dry vs. wet)	0.2	0.1–0.6	0.003*
Sex (female vs. male)	0.8	0.3–1.8	0.57
T. turgida (yes vs. no)	0.9	0.4–2.0	0.74
T. turgida			
	Adjusted		
Age (adult vs. juvenile)† ‡	24.9	2.04–12.2	<0.001*
Season (dry vs. wet)† ‡	9.3	1.68–5.58	<0.001*
Intercept = 1.22 (S.E. 0.40)			
	Unadjusted		
Sex (female vs. male)	2.3	1.0–5.4	0.047*
D. hayesi			
Age (adult vs. juvenile)†	5.7	2.0–16.6	0.001*
Season (dry vs. wet)	0.4	0.2–1.1	0.077
Sex (female vs. male)	1.1	0.4–3.2	0.82
T. turgida (yes vs. no)	0.8	0.3–2.2	0.7
C. americana (yes vs. no)	3	0.9–9.6	0.068

* Significant at $P < 0.05$.
† The only significant risk factor after multivariable logistic regression.
‡ Age-season interaction not significant, $P = 0.076$.

significantly more likely than juveniles to be infected with *C. americana* (as evidenced by observing eggs in the feces). Opossums were also more likely to shed *C. americana* ova during the wet season. Conversely, *T. turgida* eggs were shed significantly more often by adults during the dry season (OR-3.7, $P = 0.0024$), and females were more than twice as likely to be infected as males. The only significant risk factor for shedding *D. hayesi* larvae in the feces was age, where adults were more likely to shed than juveniles (Table I).

All risk factors for the presence of eggs or larvae with a *P*-value <0.20 from the univariable analysis were entered into the multivariable analysis (Table I). In the presence of other variables, only season was significantly associated with the presence of *C. americana* eggs. Both age and season were significant predictors of the presence of *T. turgida* eggs (Table I). The age-season interaction for *T. turgida* approached significance at $P = 0.076$. Age was the only significant predictor for detection of *D. hayesi* larvae. The Excel @RISK 4.5 program was used for *T. turgida* and *C. americana* worm burdens to determine the best-fit statistical distribution. For *T. turgida*, the negative binomial model was a good fit ($P = 0.92$). No significance was found for any of the risk factors analyzed using the negative binomial model. The mean intensity of *T. turgida* was 18.1, and the worm burden ranged from 0 to 131 worms. The distribution of adult worm burden of *T. turgida* in the stomach contents and washings from opossums is shown in Figure 1. The data set for *C. americana* did not show a good fit for any statistical distribution ($P < 0.001$), so these data were not analyzed. Worm burdens of adult *C. americana* varied from 0 to 437 in intestinal washings and feces, and 75% of opossums had 1–10 worms (Fig. 2).

Turgida turgida, *C. americana*, and *D. hayesi* all have the potential to cause significant morbidity and mortality in opossums. Results from this study indicate that risk factors such as age, season, and sex may be indicators of shedding of and, by implication, infection with, *T. turgida* and *C. americana* eggs and *D. hayesi* larvae in the feces.

The species found most frequently in the feces of opossums sampled was *D. hayesi* larvae (79%). Since *D. hayesi* has been documented to cause multifocal granulomatous pneumonia, resulting in morbidity and mortality (Lamberski et al., 2002). The empirical treatment for these worms and/or the associated secondary bacterial infections may warrant consideration in some cases. Adult opossums were significantly more likely than juveniles to shed all 3 of the nematodes. This is likely due to an increased exposure to infective stages with age.

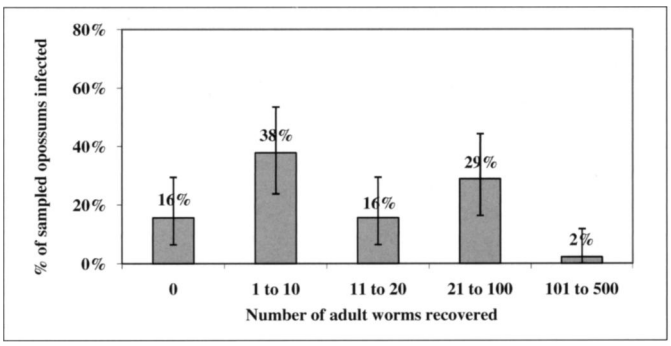

FIGURE 1. *Turgida turgida* adult worm burden in the stomach contents and washings from opossums sampled in Monterey and Rio Vista, California. Exact binomial 95% confidence intervals are from Epi Info™ version 3.3.2 (CDC, Atlanta, Georgia).

Risk factors most strongly predictive for the presence of *T. turgida* eggs were age and season. The interaction between these 2 risk factors was not significant, indicating that each independently influences shedding of *T. turgida* eggs. *Turgida turgida* ova were shed more frequently from adult opossums (possible explanations already presented). *Turgida turgida* was shed more in the dry season (May–November). The seasonal abundance of its arthropod intermediate hosts may account for the increased presence of *T. turgida* ova during this period (Gray and Anderson, 1982).

The most significant risk factor for the presence of *C. americana* was season—the higher prevalence of eggs was found during the wet season. *Cruzia americana* has a direct life cycle (Anderson, 2000); therefore, eggs shed during the wet season are likely to have greater survival probability and may, therefore, accumulate in the environment, providing a long-term source of infection.

Using the negative binomial program for *T. turgida* worm burdens, none of the risk factors analyzed was significant, which is likely related to the small sample size examined (n = 45). In the case of both *T. turgida* and *C. americana*, adult worms were found in the stomach and large intestine at approximately twice the frequency as eggs in feces. This could be due to the presence of immature worms that were not yet shedding eggs, the lack of sensitivity of fecal flotation, or the small sample number of whole feces examined. The original sampling protocol focused on examination of intestinal washes. It was later discovered that when whole feces were sieved and examined, they were a major source of adult *C. americana* worms. As this was late in the study, only 16 samples were examined for *C. americana* using this method. Therefore, none of the data set collected prior to the initiation of fecal examination for adults was included in the results due to the potential for false negatives and gross underestimation of worm numbers.

Due to financial and time constraints, the present study focused pri-

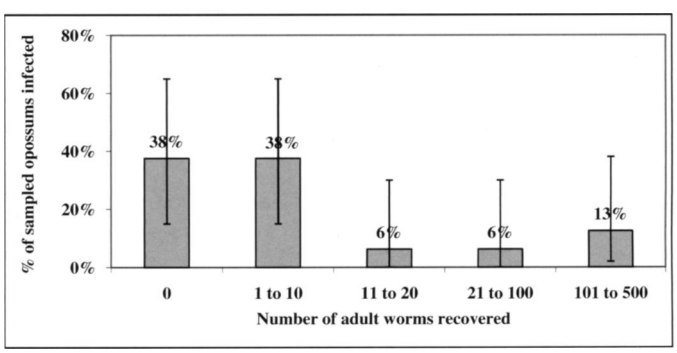

FIGURE 2. *Cruzia americana* adult worm burdens from intestinal washes and feces from opossums sampled in Monterey and Rio Vista, California. Exact binomial 95% confidence intervals are from Epi Info™ version 3.3.2 (CDC, Atlanta, Georgia).

marily on an evaluation of eggs shed in the feces of opossums. Nonetheless, a determination of total worm burden in dead animals at necropsy would be the preferred method for assessing worm prevalence and should be undertaken in future studies. The results of the present study indicate a need for further research in opossums to be conducted along the western coast of the United States, looking for adult nematodes to determine if intensity of infection is dependant on age, sex, season, or co-infection with other parasites. In addition, a survey of all opossum intestinal parasites needs to be conducted along the west coast to determine if other co-infections significantly affect the presence and/or intensity of infection by these nematodes.

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