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**PRELIMINARY INVESTIGATION INTO THE USE OF LOGISTIC
REGRESSION TO PREDICT PARASITE INTERMEDIATE HOSTS.
CASE STUDY: *DUJARDINASCARIS WALTONI*
(NEMATODA: ASCARIDIDAE) IN THE AMERICAN ALLIGATOR
(*ALLIGATOR MISSISSIPPIENSIS*)**

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ABSTRACT

We present here a case study that illustrates the potential for the use of logistic regression to facilitate identification of a parasite's intermediate host. We used a full factorial logistic regression to estimate the probability that adult American alligators (*Alligator mississippiensis*) were infected with the ascarid nematode *Dujardinascaris waltoni* given the alligators' physical characteristics and stomach contents. The strength of association between a fish-based diet and infection predict fish as the intermediate host taxon. Significant but weaker associations with reptile-mammal and reptile-crustacean diets are likely due to the high percentage of alligator remains present in the stomachs examined, with infection likely the result of vertical transmission of the parasites, not the ingestion of these other prey categories. This case study strongly suggests logistic regression has the potential to determine parasite intermediate hosts if definitive host stomach contents can be routinely and adequately sampled and the food items identified to taxa.

Key words: *Alligator mississippiensis*, American alligator, ascarid nematode, diet, *Dujardinascaris waltoni*, gastrointestinal parasite, intermediate host, logistic regression.

INTRODUCTION

The study of parasitic nematodes is complicated by the appearance of heteroxeny (use of intermediate hosts). Knowledge of a complete life cycle is necessary in order to analyze parasite population structure. Furthermore, population structure is of significant importance in regards to control of

specific parasite infections because of the ability to generate a theoretical frequency distribution of the parasite among hosts. Without knowledge of intermediate hosts, strategies for interrupting the life cycle are incomplete and may be based on subjective rather than objective data. Because mathematical modeling has been used to formulate quantitative descriptions of parasite life cycles in regards to population structure (1), we postulated that other mathematical techniques, such as statistical applications, could help in elucidating further aspects of population structure. To test this hypothesis, we selected the American alligator (*Alligator mississippiensis*) and its nematode parasite *Dujardinascaris waltoni* (Phylum Nematoda, Family Ascarididae) as a case study in the predictive abilities of statistics to identify the unknown intermediate host.

Numerous accounts have documented the helminth communities of the intestinal tracts of American alligators (2-6) and other crocodilians (7-13). Most of these studies are systematic or taxonomic in nature and provide little insight into the factors that influence the likelihood of infection or the source of the infection. Clarification of relationships between infection intensity and the infected animal's physical characters (sex, age) (5) or diet (9) may be useful in identifying which individuals within a host population are most at risk of infection and subsequently predicting the population-level impacts of the infection. In addition, because infection with gastrointestinal parasites is primarily through consumption of infected prey, a relationship between presence of infection and diet may aid in identification of possible intermediate hosts (9) and improve our understanding of parasite life cycles.

In this investigation, we report the first use of logistic regression to predict the intermediate host taxon of *D. waltoni*, a common ascarid nematode of the intestinal tract of the American alligator. We used the alligators' physical characters (sex and length) and composition of stomach contents to estimate the probability that an individual was infected with at least one parasite. This statistical approach has applicability to the study of parasites in any animal species where the contents of the gut can be effectively evaluated.

MATERIALS AND METHODS

In conjunction with Louisiana Department of Wildlife's (LDWF) nutria management activities (14), 553 alligator stomachs were collected following harvest throughout Louisiana during the September hunting seasons of 2002, 2003, and 2004. In Louisiana, strict state and federal regulations control commercial alligator harvest and trade. Consequently, harvesting wild animals is only allowed under permit at certain times of the year. Trappers target alligators >1.83m in length, although there are no formal size restrictions.

Stomachs examined in 2002 were collected from trappers in Lafourche and Terrebonne parishes. In 2003 and 2004, stomach collections were expanded to include Cameron, Vermilion, and St. Charles parishes. All marshes from which alligators were harvested are classified as fresh marsh except those in Lafourche Parish, classified as intermediate (15). As a control, the

stomachs of 30 alligators raised in captivity by a commercial alligator farmer and fed a diet of pelleted commercial alligator feed and chicken parts were examined in the spring of 2006.

Alligators harvested from the wild were brought to a processing shed where they were tagged, measured, and sexed. Most (>80%) stomachs were taken from alligators harvested during the first week of the September hunting season. Stomachs were removed and frozen after the carcasses were processed. Stomach contents were later thawed, weighed, and identified to the lowest taxon possible. Invertebrate exoskeletons and other body parts that are slow to be digested (toenails, turtle shells, fur, etc.) were the primary means of identification. However, contents of most stomachs were digested to the point where species identification was not possible.

All gastric parasites were sorted, counted and then preserved in 70% EtOH for species identification. Nearly all parasites were identified as *D. waltoni*. Fewer than ten stomachs contained other parasites, however, these few stomachs also contained *D. waltoni* as well.

For each stomach, we recorded presence or absence of *D. waltoni* and of major prey items (fish [all species combined], crustaceans [crayfish, crabs, and shrimp], reptiles [alligators, snakes, turtles, and frogs], and mammals [nutria and muskrats]). All variables were binary (1 = presence and 0 = absence of *D. waltoni* or of major prey items; 1 = male and 0 = female for sex) except for alligator length (recorded in cm).

We conducted two analyses using logistic regression (JMP IN 5.1.2, SAS Institute, Inc.) to determine which variables influenced the probability that a stomach would contain at least 1 *D. waltoni*. In the first analysis, we included sex, length, and their interaction as predictor variables. In the second analysis, we included the four prey categories and all interactions as predictor variables. For both analyses, if the “whole model test” was significant ($P < 0.05$), backwards stepwise elimination was used to eliminate non-significant effects ($P < 0.05$) from the model until only significant effects, or non-significant effects contained within significant interactions, remained (JMP IN 5.1.2) (16).

The stomachs of the commercially raised alligators were processed and examined in an identical fashion to that of the harvested animals.

RESULTS

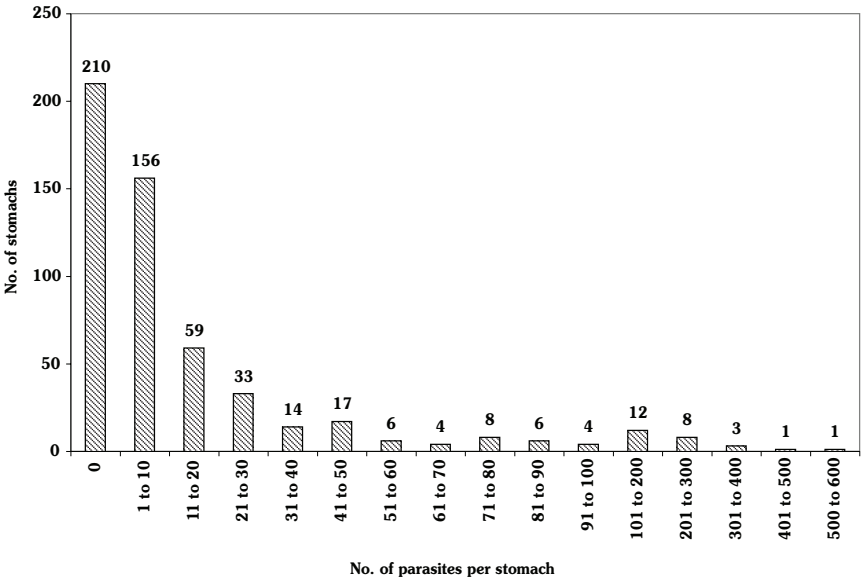
Over the 3-year study period, 553 harvested alligator stomachs were examined. We excluded 11 from analysis because of missing identification numbers or missing sex or length data. Males comprised 71% of the stomachs and averaged 28.3 cm longer than females (Table I). Sixty-one percent of all stomachs (332/542) contained at least one individual of *D. waltoni* (Table I). Of the 332 stomachs that contained *D. waltoni*, 215 (65%) contained between 1 and 20 individuals whereas 25 (8%) contained 101 to 600 individuals (Figure 1). The median number of *D. waltoni* in the 332 stomachs was 12.0; the maximum was 552. Eighty-eight of the 542 stomachs (16%) contained no food items; 24 (27%) of these contained at least one *D. waltoni*. None

of the 30 stomachs from commercially raised alligators contained *D. waltoni* and therefore these were excluded from the statistical analysis.

Table 1. Sample size, sex, proportion of stomachs containing >1 individual of *Dujardinascaris waltoni*, and mean length +/- 1 standard deviation of 542 alligators collected from alligator trappers during the 2002, 2003, and 2004 September trapping seasons in Louisiana.

Sex	No. of Stomachs	% with parasites	Alligator length (cm) Mean +/- s.d.	Length range (cm)
Male	394	61	237.2 +/- 37.7	177.8-391.2
Female	148	61	208.9 +/- 17.1	172.7-264.2
Total	542	61	229.4 +/- 35.7	172.7-391.2

Figure 1. Frequency histogram of number of *Dujardinascaris waltoni* per alligator stomach.



The whole model test for a full-factorial logistic regression model of sex and length on the probability a stomach contained at least one *D. waltoni* was not significant ($X^2 = 0.43$, $df = 3$, $P = 0.93$).

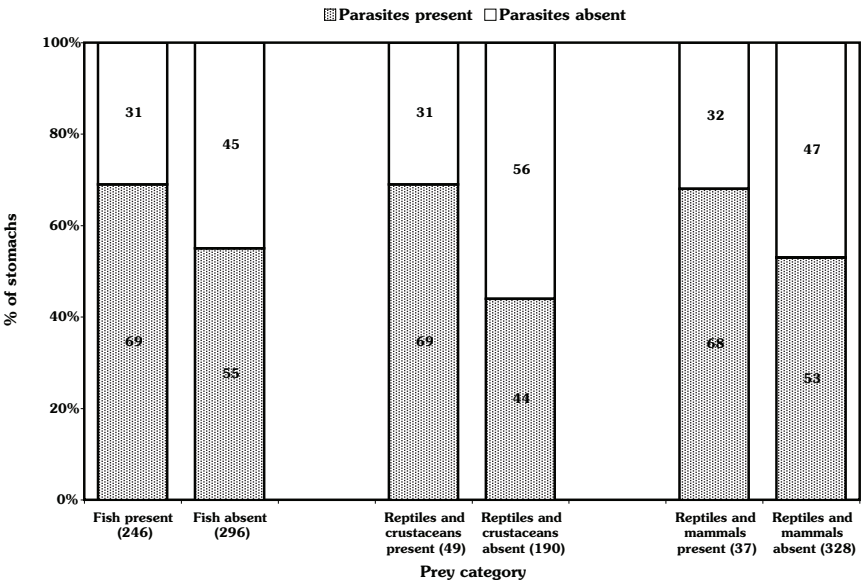
The whole model test for a full-factorial logistic regression model of fish, crustaceans, reptiles, and mammals on the probability a stomach contained at least one *D. waltoni* was significant ($X^2 = 69.4$, $df = 15$, $P < 0.001$). Backwards stepwise elimination of effects resulted in a significant model ($X^2 = 63.2$, $df = 6$, $P < 0.001$) retaining the fish main effect and reptile-crustacean

and reptile-mammal interactions (Table II). The $-2 \log L$ goodness of fit test was not significant ($X^2 = 6.18$, $df = 9$, $P = 0.72$), indicating that the model fit was appropriate. Stomachs containing fish, reptiles and crustaceans, or reptiles and mammals were about twice as likely to contain at least one *D. waltoni* than stomachs that did not contain those foods (odds ratios between 1.79 and 1.95, Table II; Figure 2).

Table II. Effects remaining in the reduced logistic regression model of presence of fish, reptiles, crustaceans, and mammals on the probability a stomach contained at least one *Dujardinascaris waltoni* parasite. Rows in bold text indicate significant effects.

Source	df	Wald Chi-squared value	Prob. >Chi-squared value	Odds ratio	Odds ratio 95% C.I.
Fish	1	9.9	0.0016	1.82	1.25-2.65
Reptiles	1	1.2	0.27	1.35	0.80-2.31
Crustaceans	1	3.4	0.065	1.61	0.96-2.68
Reptiles x Crustaceans	1	5.1	0.025	1.79	1.08-3.01
Mammals	1	1.5	0.22	1.39	0.82-2.36
Reptiles x Mammals	1	6.2	0.01	1.95	1.15-3.30

Figure 2. Proportion of alligator stomachs containing at least one *Dujardinascaris waltoni* and three groups of prey types (fish, reptiles and crustaceans, and reptiles and mammals). Numbers in parentheses indicate number of stomachs with each prey type present or absent.



DISCUSSION

We found no relationship between the probability of infection with at least one *Dujardinascaris waltoni* and the sex or length of American alligators. Scott et al. (5) also found no relationship between an alligator's sex and the intensity of infection with 12 helminth species including *D. waltoni*. However, they did find that immature alligators (<1.80m in length) were slightly more likely to be infected with nematodes (*D. waltoni* and 2 other species) than were mature alligators (>1.80m in length). Our sample of >540 alligators did not include any individuals <1.80m.

Because an alligator can become infected with certain species of nematode by consuming infected prey, a relationship between parasite occurrence and stomach contents may be useful in identifying the prey species responsible for the alligator's infection. For parasite species for which intermediate hosts are unknown, such a relationship may also identify possible intermediate hosts. Our logistic regression shows that the presence of *Dujardinascaris waltoni* in an alligator stomach is more likely if the animal has a history of consuming fish, reptiles and crustaceans, or reptiles and mammals than if the animal had not consumed these prey types.

Several lines of evidence lead us to propose that fish alone are the likely source of infection for the American alligator and intermediate host for *D. waltoni*. First, the odds ratio associated with a fish diet was the second highest of the six effects in the final model.

Second, Hazen et al. (3) found *D. waltoni* in 67% of stomachs examined in South Carolina. Delaney (2) found *D. waltoni* in 73% of 113 alligator stomachs examined in Florida. Delaney and Abercrombie (21) reported that two species of parasitic worms (*D. waltoni* and *Brevimulticicum tenuicolle*) occurred in 82% of another sample of 350 alligator stomachs in Florida. These frequencies are similar to our overall frequency of 61%. Similarly, in both of these reports, fish was the most important food item as was the case in our stomach samples (present in 45% of our samples).

Third, infective stage larvae of *Dujardinascaris* spp. have been recovered from naturally infected fishes (*Lates* spp.) in Africa (17).

Finally, experimental evidence exists for the role of fish in the life cycle of a *Dujardinascaris* species in a crocodilian. *Tilapia* sp. was found to act as experimental intermediate hosts and *Cyclops* sp. as preintermediate paratenic hosts of *D. dujardini*, a parasite of *Crocodylus niloticus* (18). Also, experimental infection of two fish species (*Cichlasoma synspilum*, *Poecilia reticulata*), the Bullfrog (*Rana catesbiana*), and a copepod (*Diaptomus albuquerquensis*) with second stage larvae of *Dujardinascaris helicina* yielded larvae only from 1 of 5 *P. reticulata* but not from the other species (8). Although we detected potential relationships between occurrence of *D. waltoni* and reptile-crustacean or reptile-mammal diets (Table II), we suspect that these relationships are artifacts of cannibalism in alligators. Of the 49 stomachs that contained reptiles and crustaceans, 17 contained remains of other alligators. Ten of those 17 (59%) were positive for *Dujardinascaris*.

Of the 37 stomachs that contained reptiles and mammals, 18 contained remains of other alligators. Eleven of those 18 (61%) were positive for *Dujardinascaris*. The high incidence of *D. waltoni* infection in these alligators (21 of 86, 24%) in which no fish remains were found but which had a high cannibalism rate (41%) suggests that the association of parasites and a reptile diet may be due to vertical transmission of immature and mature nematodes from ingested alligators.

Our logistic regression, combined with other observational and experimental data, identified fish as the likely intermediate host in the *D. waltoni*-American alligator life cycle. However, we cannot state with certainty that this is the case for several reasons. First, relationships between nematode presence and stomach contents may be biased because different prey items are digested in the alligator intestines at different rates (19-21). Second, we collected our alligator stomachs during Louisiana's annual September trapping season; it is possible that alligators ingested the nematodes earlier in the year. Third, we were unable to investigate the occurrence of *D. waltoni* in fish inhabiting marshes from which the alligators were trapped. Finally, we were unable to identify the specific fish species (assuming fish are responsible) because most stomach contents were digested to the point where species recognition was not possible.

Although we were not able to positively identify the intermediate host species, our approach allowed us to reduce the number of potential intermediate hosts. Logistic regression, combined with other observations or data, may be a useful method for the prediction of intermediate and/or paratenic hosts of poorly known parasites when the definitive host has had ample dietary analysis performed. We recognize several deficiencies in our data that could easily be overcome by collecting alligator stomachs over several seasons, by finer-scale identification of prey items, and by adjusting stomach content data for differential digestion rates. Finally, identification of *Dujardinascaris waltoni* larvae within the tissues of the prey fish species most frequently consumed by alligators in southern Louisiana would confirm the utility of this investigative approach as a discriminating statistical tool in the study of parasite ecology.

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