



Trematodes in Snails near Raccoon Latrines Suggest a Final Host Role for This Mammal in California Salt Marshes

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natural infection with *P. nucleophilum* has not been recorded in the blackcap (Bennett et al., 1982; Bishop and Bennett, 1992; Valkiūnas, 1997).

The data presented in our study are in accordance with information about the huge genetic divergence that is documented for avian malaria parasites and other hemosporidians recorded using polymerase chain reaction-based techniques (Ricklefs and Fallon, 2002; Bensch and Åkesson, 2003). More field and experimental transmission investigations, combined with a molecular approach, are needed to gain sufficient knowledge regarding the speciation of avian malaria parasites and the mechanism that accounts for their host restriction, particularly because of the large ranges of their vertebrate hosts and their immense geographic ranges in all parts of the world.

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Trematodes in Snails near Raccoon Latrines Suggest a Final Host Role for this Mammal in California Salt Marshes

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ABSTRACT: Of the 18 trematode species that use the horn snail, *Cerithidea californica*, as a first intermediate host, 6 have the potential to use raccoons as a final host. The presence of raccoon latrines in Carpinteria Salt Marsh, California, allowed us to investigate associations between raccoons and trematodes in snails. Two trematode species, *Probolocoryphe uca* and *Stictodora hancocki*, occurred at higher prevalences in snails near raccoon latrines than in snails away from latrines, suggesting that raccoons may serve as final hosts for these species. Fecal remains indicated that raccoons fed on shore crabs, the second intermediate host for *P. uca*, and fish, the second intermediate host for *S. hancocki*. The increase in raccoon populations in the suburban areas surrounding west coast salt marshes could increase their importance as final hosts for trematodes in this system.

Upstream host communities should strongly affect the composition of parasite communities in downstream hosts. As a consequence, parasite communities in downstream hosts should indicate the composition of upstream-host communities. We used this logic to indirectly determine whether raccoons serve as final hosts for trematodes in west coast salt marshes by studying associations between raccoon latrines and the prevalence of trematodes in snails at the Carpinteria Salt Marsh, California.

Trematodes in west coast salt marshes use *Cerithidea californica* (and closely related snail species in more southern latitudes) as first intermediate hosts. Birds are common predators on second intermediate hosts, e.g., polychaetes, snails, clams, crabs, and fishes, and all 18 trematode species that infect *C. californica* use birds as final hosts; none is known from wild mammals. Mammals are less common in salt marshes than birds, a potential explanation for why they have not been considered as final hosts. However, some trematodes that use *C. californica* have the potential to infect mammals.

Tracks of the raccoon, *Procyon lotor*, are common on mudflats and tidal channels, where their broad diet would expose them to several species of second intermediate hosts. The tendency of raccoons to defecate in communal latrines aided our investigation of the importance of raccoons as hosts. Some latrines occur on logs that are adjacent to habitat that supports populations of *C. californica*. Trematodes that regularly complete their life cycle within raccoons should be more common in first intermediate host snails occurring near latrines than in snails occurring away from latrines.

To avoid making this comparison for all 18 trematode species (which would increase the probability of a type 1 statistical error), we examined the life cycle of each trematode species to assess which ones might be viable in mammals. For example, *Probolocoryphe lanceolata* (glandu-

losa) in Florida parasitizes raccoons (Heard and Sikora, 1969), rice rats (Kinsella, 1988), and a wide variety of birds (Heard and Sikora, 1969), suggesting that *Probolocoryphe (Maritrema) uca* in California, might parasitize raccoons. *Probolocoryphe uca* uses crabs as a second intermediate host. Other trematodes from *C. californica* believed to primarily use birds as final hosts might also infect mammals, as demonstrated in experiments or from observations of closely related species. Adults of *Euhaplorchis californiensis* (which encyst on the brain of the second intermediate host, the killifish *Fundulus parvipinnis*) and *Phocitremonides ovale* (which encyst under the scales of several salt marsh fish species) can parasitize mammals in experimental infections (Martin, 1972). Although *Stictodora hancocki* (which encyst in the tissues of several salt marsh fish species) is only known to use bird as final hosts (Martin, 1972), adults of a similar species, *Stictodora cursitans*, from Florida, infect a wide range of mammals, including raccoons (Kinsella and Heard, 1974). *Mesostephanus appendiculatus* (that encysts in the musculature of several salt marsh fish species) occurs in shorebirds (Martin, 1972). However, Hutton and Sogandares-Bernal (1964) successfully infected a raccoon from Florida with *Mesostephanus appendiculatooides* by feeding it infected mullet. Finally, *Acanthoparyphium spinulosum*, a trematode that uses mollusks as second intermediate hosts, will develop to maturity in rats (T. Huspeni, pers. obs.).

In mid-November, we collected 2 snail populations near substantial raccoon latrines in the Carpinteria Salt Marsh. From the first, we collected 306 snails from a pan that contained a large latrine on a stump. From the second, we collected 30 snails from a small tidal channel adjacent to a stump with a medium-size latrine. We found 3 other latrines, but their small size or lack of adjacent snail populations (or both) precluded their use in this study. Snails from 14 other sites distributed through the marsh were also sampled for trematodes during the first 2 wk of August 2004. Of these, 7 were from tidal channels (a habitat similar to the location of the snails adjacent to the medium latrine) and 7 were from pans or flats (habitats similar to the location of the snails adjacent to the large latrine).

We collected 10 fecal samples at the larger latrine between 13 and 20 March 2004. We chose samples so that each appeared to be from a separate defecation event, although it was impossible to determine how many raccoons contributed to the samples. Samples were mixed thoroughly with 15 ml of zinc sulfate solution by rotating the fecal insert inside an OVASSAY Plus fecal device. Cover slips (25 × 25 mm) were placed on the meniscus of the fecal device for 10 min, and the floating material adhering to the cover slips was inspected for the presence of trematode eggs under a compound microscope.

Where possible, 200 snails were collected from each of the sites and distributed evenly between 20–25 and 25–30 mm size classes. Snails were dissected and trematode infections were identified to species (Martin, 1972). The prevalence (Bush et al., 1997) of each trematode species was calculated. For species with the potential to use mammals and having a prevalence high enough to analyze (>1%), we investigated the effects of snail size class, habitat type, and the presence of a latrine on trematode prevalence.

Of the 11 collected fecal samples, 8 were sufficiently fresh to assess diet. Two of the samples contained egg-like items with filaments, but because there was no clear operculum, this prevented confirmation as trematode eggs. Five samples contained remains of large numbers of shore crabs, probably *Pachygrapsus crassipes*. One fecal sample included only fish bones, and 2 samples contained fish bones and crab carapaces. No mollusk remains was found in the fecal samples. These results suggest that raccoons ingest metacercariae of trematodes that use crabs and fishes as second intermediate hosts. Four fecal samples had larval nematodes of *Baylisascaris procyonis*, the common raccoon roundworm. Larvae of *Enterobius* sp. (nematode) and *Entamoeba muris* (protozoan) also occurred in the feces.

We collected 336 snails at latrine sites and 2,698 snails at nonlatrine sites. *Mesostephanus appendiculatus* and *P. ovale* were too rare (<1% prevalence) for an analysis to be completed, and it seems unlikely that raccoons play an important role in their transmission. The angular transformation (arcsine of the square root of the proportion infected) was used to help normalize the distribution of prevalence for statistical comparisons (to ease interpretation, we present the actual values of prevalence instead of the transformed values). A separate linear model was determined for each species by determining whether snail size class,

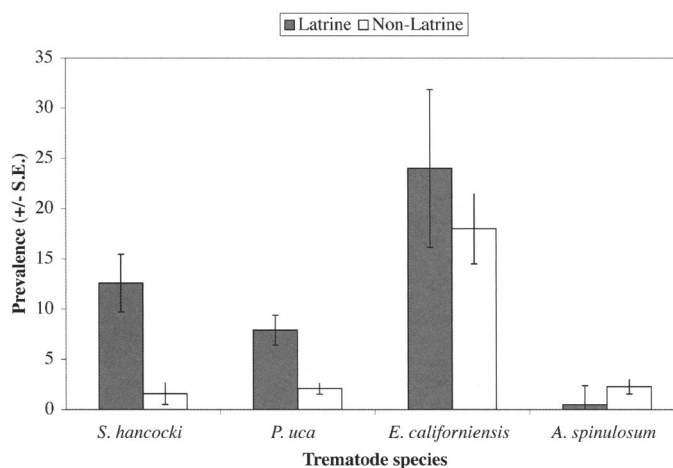


FIGURE 1. Comparison of prevalence of trematodes, by species, at sites near and faraway from raccoon latrines in Carpinteria Salt Marsh, California. Error bars are SEs. *Probolocoryphe uca* and *Stictodora hancocki* were significantly more prevalent near raccoon latrines.

habitat, or latrine explained a significant proportion of the variance in transformed prevalence.

Size and habitat, but not latrine, affected the prevalence of *E. californiensis*. Latrine sites (24%) did not have significantly different prevalence than nonlatrine sites (18%) (df = 1, F -ratio = 0.20, P = 0.67). Size did not affect the remaining 3 species, and data from the 2 size classes were combined. The prevalence of *A. spinulosum* did not differ among habitat or significantly between latrines (average = 0.5%) and nonlatrine sites (average = 2.3%) (N = 16, df = 1, F -ratio = 0.79, P = 0.39). The prevalence of *P. uca*, however, was significantly higher at latrine sites (7.1%) than at the nonlatrine sites (1.8%) (N = 16, df = 1, F -ratio = 8.37, P = 0.0126). This pattern is consistent with the prediction that raccoon latrines serve as sites of focal transmission for *P. uca* (Fig. 1). There was a marginally independent effect of habitat type on the prevalence of *P. uca*. Channel sites (1.7%) had a lower prevalence than flat and pan sites (3.4%) (N = 16, df = 1, F -ratio = 4.66, P = 0.050). The prevalence of *S. hancocki* was significantly higher in the latrine sites (14.6%) than at the nonlatrine sites (1.6%) (N = 16, df = 1, F -ratio = 14.08, P = 0.0024), an effect due greatly to a high prevalence of *S. hancocki* at the smaller latrine site (Fig. 1). There was an independent effect of habitat type on the prevalence of *S. hancocki*. Channel sites (5.4%) had a higher prevalence than flat and pan sites (1.0%) (N = 16, df = 1, F -ratio = 4.95, P = 0.0445).

Multiple statistical comparisons, such as for the 4 species cited above, require an adjustment of the critical P -value to assess significance. Application of the Bonferroni correction indicated that the equivalent of a 0.05 critical P -value for a set of 4 similar tests is 0.0127. The P -values for *P. uca* and *S. hancocki* were both below this adjusted level and, therefore, indicated statistically significant differences between latrine and nonlatrine sites for these species.

Raccoons forage on shore crabs and fishes in Carpinteria Salt Marsh. These prey serve as second intermediate hosts for some trematodes that use the California horn snail, *C. californica*, as a first intermediate host and birds as final hosts. Some of these species are able to use mammals as final hosts and, therefore, could infect raccoons that eat infected crabs or fish. Comparisons between trematode communities in snails at latrine and nonlatrine sites indicated a 4-fold higher prevalence of *P. uca* (a species using shore crabs as second intermediate hosts) and an 8-fold higher prevalence of *S. hancocki* (a species using fishes as second intermediate hosts) adjacent to raccoon latrines. These correlative data are consistent with the hypothesis that raccoons are likely to be hosts for *P. uca* and *S. hancocki* in California Salt Marshes. Direct support for this hypothesis was not found from the examination of raccoon feces. However, fecal examination for eggs is often an insensitive means of assessing parasitism. Necropsy of raccoons that forage in salt marshes would be necessary to conclude that raccoons serve as final hosts for *P. uca* and *S. hancocki*.

A possible alternative explanation for our results is that birds use

stumps for perches and, therefore, transmit trematode eggs at raccoon latrines more than at sites without stumps. Another possibility is that trematode prevalence was higher in November (when collected from the latrine sites) than in August (when collected from the control sites). Either alternative would predict that the entire trematode community (not just those suspected of using raccoons) would be more prevalent at the latrine sites. However, when averaged or summed, the other 12 species of trematodes were not associated with latrines. The summed prevalence of nonraccoon trematodes found in 20- to 25-mm snails was the same (34% vs. 33%) in latrine and nonlatrine sites, and the prevalence of nonraccoon trematodes found in 25- to 30-mm snails was lower (46 vs. 81%) at latrine than at nonlatrine sites. Data on the entire community of trematodes will be analyzed for future publications.

Other trematode species with the potential to use raccoons as final hosts were either too rare to evaluate or did not have a positive association with latrines. Of the relatively common trematode species with the potential to infect mammals that were not associated with latrines, one, *A. spinulosum*, uses mollusks as a second intermediate host that, according to the limited diet information available, may not be important prey for raccoons in this system. The other, *E. californiensis*, is specific to 1 species of fish (*F. parvipinnis*) that might not be commonly preyed on by raccoons.

Latrines result in a high degree of aggregation of raccoon feces, and this should lead to spatial structure in transmission of the trematode eggs/miracidia in these feces. Although the prevalence of *P. uca* and *S. hancocki* were much higher near latrine than at nonlatrine sites, latrines were relatively rare within the marsh, suggesting that, at the scale of the salt marsh, transmission of trematodes by raccoons contributed to a relatively small proportion of the total population of these 2 species in the salt marsh, relative to transmission by birds.

Urbanization may be changing the abundances of shorebirds and raccoons in this system. A decrease in the area of salt marsh habitat due to development could crowd shorebirds into remaining areas, whereas degradation of some habitats might reduce bird use. Either pattern could alter parasitism (Holmes, 1996). Urbanization favors raccoons by providing a convenient source of food and a dearth of top predators (DeLap and Knight, 2004). These factors may have changed and will continue to change the relative importance of raccoons and birds as hosts for trematodes in California Salt Marshes.

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Expression of P23 of *Cryptosporidium parvum* in *Toxoplasma gondii* and Evaluation of its Protective Effects

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ABSTRACT: In this study, P23 of *Cryptosporidium parvum* sporozoites, an immunodominant surface protein, was stably expressed in *Toxoplasma gondii* (Tg/P23) and its protective effects were evaluated in a mouse model. The molecular weight and antigenic property of P23 expressed by Tg/P23 were similar to those of the native P23. Mice immunized with lysed Tg/P23 tachyzoites produced specific neutralizing antibodies against *C. parvum*. These findings indicate that the *T. gondii* vector may provide a new tool for the production of a recombinant vaccine against cryptosporidiosis in animals.

Cryptosporidium parvum is a coccidian parasite that infects the intestinal epithelium in humans and animals (Fayer et al., 1997). Exposure of immunocompetent individuals to *C. parvum* results in a transient infection that may be asymptomatic or can result in self-limited diar-

rhea. In contrast, diarrhea persists and is life-threatening in immunocompromised patients, especially individuals with human immunodeficiency virus-acquired immune deficiency syndrome (Brasseur, 1997; Okhuysen and Chappell, 2002). The contamination of watersheds by cattle feces has been indicated as a primary source of outbreaks in human populations (Smith and Rose, 1990). Therefore, controlling *C. parvum* infections in cattle is important to resolve public health concerns. However, to date, there are no effective vaccines for cryptosporidiosis (de Graaf et al., 1999).

A 23-kDa glycoprotein (P23) of *C. parvum* was identified as an antigen with neutralization-sensitive epitopes, and, therefore, it is considered to be a good candidate for the development of an effective vaccine against cryptosporidiosis (Perryman et al., 1996, 1999). It was reported that immunization with a recombinant protein containing the amino acid