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RABIES VIRUS SEROSURVEY OF THE SMALL INDIAN MONGOOSE (*URVA AUROPUNCTATA*) ACROSS MULTIPLE HABITATS IN PUERTO RICO, 2014–21

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ABSTRACT: The small Indian mongoose (*Urva auropuncata*) is a rabies reservoir in Puerto Rico and accounts for over 70% of reported animal rabies cases annually. The presence of rabies virus–neutralizing antibodies (RVNA) is often used as a tool to measure exposure to rabies virus in wildlife populations. We conducted a serosurvey of mongooses at 11 sites representing six habitat types across Puerto Rico. We collected a serum sample from 464 individual mongooses during 2014–21. Overall, 80/464 (17.0%; 95% confidence interval, 14.1–20.9%; 55 male, 23 female, and two sexes not recorded) of individual mongooses sampled across all habitats were RVNA positive. The geometric mean (SD) RVNA titer for 80 unique seropositive animals was 0.58 (2.92) IU/mL. Our models indicated that the probability of mongooses being RVNA seropositive mostly varied by habitat, with some influence of sex in the individual-level analyses. Population-level RVNA seroprevalence is dynamic in mongoose populations, but these data may shed light on rabies virus transmission across regions to help inform rabies management activities in Puerto Rico.

Key words: Habitat, mongoose, Puerto Rico, rabies, rabies virus–neutralizing antibodies, serology, *Urva auropunctata*.

INTRODUCTION

The small Indian mongoose (*Urva auropuncata*; synonym *Herpestes auropunctatus*) was introduced throughout the Caribbean in the late 19th century, primarily to control rat (*Rattus* spp.) damage to sugar plantations (Hoagland et al. 1989). Mongooses are now considered pests throughout most of the introduced range and are a rabies reservoir on several Caribbean Islands, including Puerto Rico, Grenada, Cuba, and the Dominican Republic (Berentsen et al. 2017; Seetahal et al. 2018). In Puerto Rico, the first laboratory-confirmed rabid mongooses were reported in 1950 (Tierkel et al. 1952). Between 1986 and 1990, over 70% (236/330) of suspected rabid mongooses submitted for rabies diagnosis in Puerto Rico were rabid (Everard and Everard 1992), and mongooses accounted for over 70% of annually reported animal rabies cases in Puerto Rico during 2020 (Ma et al. 2022). In 2015, Puerto Rico documented the first human rabies fatality directly linked to a mongoose bite (Styczynski et al. 2017), highlighting a need to enhance rabies prevention and control strategies in Puerto Rico, and for applied research in support of an oral rabies vaccination (ORV) program targeting mongooses.

Use of ORV is the primary method to control and eliminate rabies virus in wild carnivore populations at a landscape scale globally and has been previously proposed for mongooses (Creekmore et al. 1994; Vos et al. 2013; Berentsen, Chipman, Nelson, et al. 2020). The presence of rabies virus–neutralizing antibodies (RVNA) is often used as a tool to measure natural exposure to rabies virus or to measure seroconversion rates in wildlife populations following vaccination as an index to population-level immunity (Moore et al. 2017; Moore 2021). In the absence of an oral biomarker to verify vaccine-bait uptake, it can be challenging to distinguish whether the presence of RVNA in wildlife populations managed with ORV resulted from vaccination or sublethal natural rabies virus exposure (Gold et al. 2020).

Surveys for RVNA among mongooses in Puerto Rico during 2011–14 had reported seroprevalence of up to 42.5% in some habitats (Berentsen et al. 2015). However, the Berentsen et al. (2015) study was conducted in only two sites representing two different habitat types on opposite sides of the island: El Yunque National Forest in the northeast and the Cabo Rojo National Wildlife Refuge in the southwest. Our objective was to conduct a broader survey of mongooses across multiple sites and habitats in Puerto Rico and to evaluate the probability of natural rabies virus exposure in mongooses at the individual and population levels. We sought to understand how RVNA seroprevalence might vary with habitat type or season and how any variability may relate to differences in rabies virus exposure and transmission among mongooses. We were interested in evaluating both individual-level and population-level dynamics associated with RVNA status within our study. Population-level analysis examines the relationship between seroprevalence (i.e., the proportion of the population that is seropositive) and landscape-level factors. Individual-level analysis examines more specifically factors that might influence an individual's probability of being seropositive. We hypothesized that seroprevalence would vary with habitat type, with seroprevalence higher in dry forest and grassland habitat types, as they tend to have higher population densities, thus, more potential for intraspecific interaction and rabies transmission than other habitat types (Johnson et al. 2016; Sauvé et al. 2022).

MATERIALS AND METHODS

We conducted this study at 11 sites (0.27–5.6 km^2) located on private, commonwealth, and federal land in Puerto Rico during 2014–21. We include 15 samples collected at the Cabo Rojo National Wildlife Refuge in August 2014 reported previously (Berentsen et al. 2015) as part of this broader study. Sites were located across six municipalities throughout the island (Fig. 1): Rio Grande, Manati, Isabela, Cabo Rojo, Lajas, and Salinas.

We overlaid cell land class data (15×15 m) from the Puerto Rico Gap Analysis Project (PRGAP; Gould et al. 2008) onto satellite imagery of Puerto Rico. We extracted land class data within each site using the Extract by Mask tool in ArcGIS Pro (version 3.0.3, Environmental Systems Research Institute, ESRRI, Redlands, California, USA). We calculated the area of each site and the proportion of the site occupied by each habitat. We defined the dominant habitat type within each site by selecting the highest proportion of the "Puerto Rico Gap Analysis Project Land Cover Type" description in the ArcGIS attribute table for each site. We assigned each site to one of seven dominant land cover classes (hereafter habitats): 1) dry grasslands and pastures; 2) mangrove forest and shrubland; 3) mature primary Tabonuco and secondary montane wet noncalcareous evergreen forest; 4) hay and row crops; 5) seasonally flooded herbaceous nonsaline wetlands; 6) lowland dry limestone shrubland; and 7) moist grasslands and pastures (Table 1).

Capture and handling

We live captured mongooses in cage traps (Tomahawk Trap Company, Hazelhurst, Wisconsin, USA) baited with canned tuna (Quinn and Whisson 2005). During spring 2014 to spring 2016, we placed 100 traps >50 m apart in a 1-km^2 area and moved traps ≥30 m from any existing or previous trap location if a unique (unmarked) mongoose was not captured in the trap after 3–4 d. During autumn 2016 to autumn 2021, we arranged traps in a grid placed 100 m apart, and traps remained in place during a 10-d trapping period. Samples were typically collected within a 0.5- to 1.0-km^2 plot at each study site. We set traps in the morning and checked them every 24 h for 10 consecutive days, rebaiting as needed. We collected additional samples opportunistically during concurrent and related studies (e.g., Berentsen, Chipman, Nelson, et al. 2020).

Upon capture, we physically restrained mongooses either in a cone-shaped canvas bag (Berentsen et al. 2015) or by moving the animal to the rear of the trap using trap forks, for induction of anesthesia by intramuscular injection

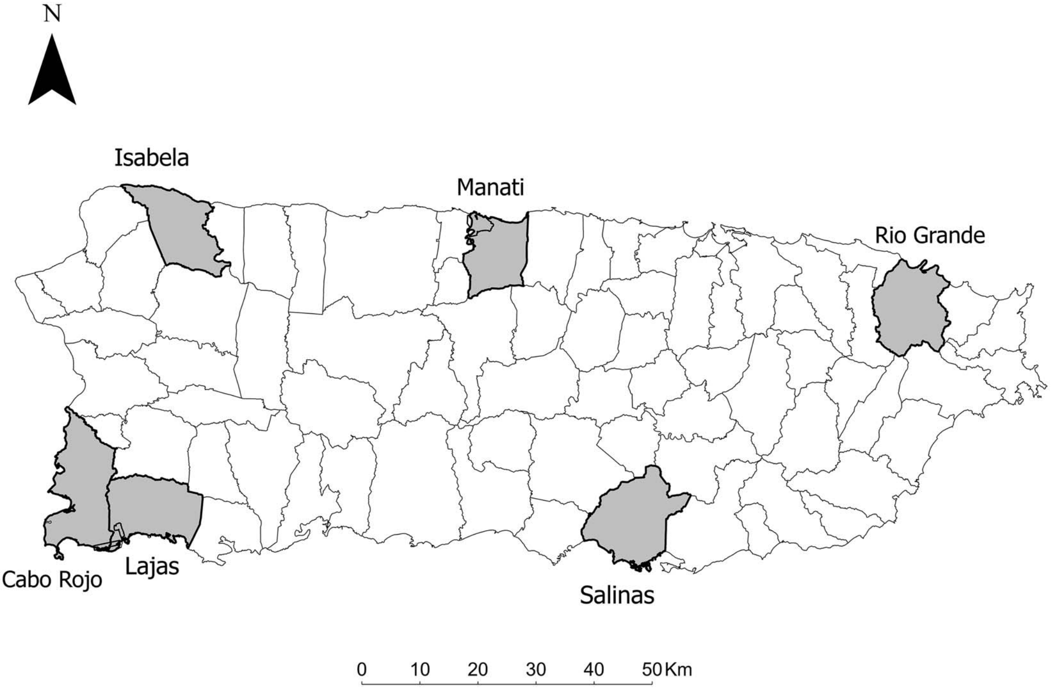

FIGURE 1. Location of six municipalities selected for rabies virus serosurveys in the small Indian mongoose (*Urva auropunctata*) in Puerto Rico, 2014–21.

of 5 mg/kg tiletamine-zolazepam (Telazol®, Zoetis Animal Health, Parsippany, New Jersey, USA; Kreeger and Arnemo 2012). Once the animal was anesthetized, we recorded its age, sex, weight, body length, and reproductive status. We collected 0.5–1.0 mL of whole blood by venipuncture of the cranial vena cava (Briscoe and Syring 2004) using a 1.0-mL syringe with a 1.6-cm (25-gauge) needle, transferring the blood into 3.0-mL serum separator tubes (Vacutainer, Becton Dickson, Franklin Lakes, New Jersey, USA) and injected a sterile passive integrated transponder tag (Avid Identification Systems, Inc., Norco, California, USA) subcutaneously between the shoulder blades for

TABLE 1. Dominant habitat class designation and proportion of each study site for small Indian mongoose (*Urva auropuncata*) occupied by the dominant habitat class on 11 sites in Puerto Rico: 2014–21.

Site name	Area (km^2)	Dominant habitat type	Proportion of study site (%)
Aguirre	1.0	Dry grasslands and pastures	26.2
Escabi	0.94	Dry grasslands and pastures	88.5
Refuge	2.55	Dry grasslands and pastures	53.5
Salt Flats	1.55	Dry grasslands and pastures	52.5
Combate	0.27	Dry grasslands and pastures	64.7
Isabela	0.5	Hay and row crops	83.4
Manati north	0.45	Moist grasslands and pastures	65.4
Manati south	1.2	Seasonally flooded herbaceous nonsaline wetlands	47.5
Mateo	3.33	Lowland dry limestone shrubland	38.4
Pitahaya	1.75	Mangrove forest and shrubland	29.9
El Yunque	5.55	Mature primary Tabonuco and secondary montane wet noncalcareous evergreen forest	81.6

individual mongoose identification. We separated serum samples from whole blood using a centrifuge at 670 × G for 15–20 min and transferred serum into individually labeled cryovials. Sera were initially frozen at −20 C and then transferred to −80 C until analysis.

We shipped frozen sera to the rabies laboratory at Kansas State University (Manhattan, Kansas, USA) for RVNA analysis using the rapid fluorescent focus inhibition test (RFFIT; Yager and Moore 2015) with end-point determination. The threshold of detection reported by the laboratory for the modified RFFIT assay used was 0.05 IU/mL. We considered serum samples with titers $\geq$ 0.1 IU/mL RVNA to be positive, a threshold used extensively in previous related field and experimental studies with wild-caught mongooses (Berentsen et al. 2015; Berentsen, Ellis, Johnson, et al. 2020; Berentsen et al. 2021). We also evaluated a subset of the mongoose sera for rabies virus antibodies using a commercial blocking ELISA (BioPro ELISA, OK Servis, Prague, Czech Republic). A sample was considered positive for rabies virus antibody by ELISA if the percentage of inhibition was equal to or greater than 40% per manufacturer instructions.

Data analysis

We calculated geometric mean for $\log_{10}$ and geometric SD (GSD) for all individuals that were RVNA positive. For overall proportions, animals were counted only once, regardless of the number of recaptures. During the study, 64 mongooses were sampled more than once: 56 were sampled twice and eight were sampled three times. In cases in which individual animals were sampled more than once and both samples were positive, the mean titer value was used. In cases in which multiple samples were collected from an individual animal and only one sample was RVNA positive, that animal was considered positive, and the corresponding RVNA positive titer level was used in calculations. To compare the RFFIT and ELISA serologic results, we calculated sensitivity, specificity, and concordance, as well as the positive and negative predictive values of the ELISA against the RFFIT as the reference standard for comparison.

For individual-level analysis, we evaluated a set of factors that may be related to the probability that an individual mongoose would be RVNA seropositive, including sex, season (wet season=May–November and dry season=December–April; Miller and Lugo 2009), and habitat. We used a mixed logistic regression model with a binary response variable to represent RVNA seropositive or negative status. We included animal identification and year as random effects to account for potential individual and annual covariance in the data, whereas fixed effects were sex, season, and habitat.

We estimated population-level seroprevalence for mongooses based on mixed models with fixed effects of habitat, site, and season, including a random effect for year. We used a generalized linear model with a binomial response (the number of positives per site compared with the total number of samples per site). Sites were classified by habitat for sampling, but sampling was uneven, and there were five sites in the dry grassland and pastures habitat compared with other habitats represented only by a single site. We compared models with habitat to those with site predictor variables to determine the relative influence of habitat or site on seroprevalence.

We compared all models using the second-order Akaike Information Criterion corrected for small sample sizes (AICc; Burnham and Anderson 2002) using program R (R Core Team 2021). Lower AICc values were considered more parsimonious. We conducted pairwise comparisons of covariate factors using least-squares means using the emmeans package (Lenth 2022) in program R, version 1.8.2.

RESULTS

We collected 536 samples from 464 individual mongooses across all study sites and sampling years. The number of samples collected varied widely across habitats, from 14 in the hay and row crop site to 298 in the dry grassland and pasture sites (Table 2). Of the 464 unique animals sampled across all habitats, 259/464 (55.8%; 95% confidence interval [CI], 51.3–60.3%) were male, 201/464 (43.3%; 95% CI, 38.9–47.9%) were female, and for 4/464 (0.9%; 95% CI, 0.3–2.2%) the sex was not recorded. Overall, 93/536 (17.4%; 95% CI, 14.4–20.8%) samples were RVNA positive (63 male, 27 female, and three sexes not recorded), including recaptures. This equaled

Table 2. Number of site-specific unique small Indian mongooses (*Urva auropunctata*) sampled and proportion of mongooses with evidence of serum rabies virus–neutralizing antibodies (RVNA) across 11 study sites and seven dominant habitat classes in Puerto Rico during 2014–21.

Study site	Dominant habitat type	Year sampled	Individuals sampled by site[a]	RVNA positive	Proportion RVNA positive (%)	95% confidence intervals	
						Lower	Upper
Aguirre	Dry grasslands and pastures	2021	29	0	—[b]	—	—
Escabi	Dry grasslands and pastures	2016–17	59	13	22	13	35
Refuge	Dry grasslands and pastures	2016–18	100	10	10.0	5.5	17.4
Salt Flats	Dry grasslands and pastures	2014–18	106	8	7.6	3.9	14.8
Combate	Dry grasslands and pastures	2015	5	2	40	12	77
Isabela	Hay and row crops	2018–19	14	2	14	4	40
Manati north	Moist grasslands and pastures	2019	52	16	31.8	20	44
Manati south	Seasonally flooded herbaceous nonsaline wetlands	2019	66	22	33	23	45
Mateo	Lowland dry limestone shrubland	2016	10	1	10	2	40
Pitahaya	Mangrove forest and shrubland	2014	15	0	—	—	—
El Yunque	Mature primary Tabonuco and secondary montane wet noncalcareous evergreen forest	2021	10	6	60	32	83
Total			466	80	16.9	13.7	20.6

[a] Two mongooses were captured more than once: one at the refuge and then twice at the Salt Flats; the other at the Pitahaya site and then again at the Mateo site.

[b] Not applicable.

TABLE 3. Number of unique small Indian mongooses (*Urva auropunctata*) sampled and proportion of mongooses seropositive for the presence of rabies virus–neutralizing antibodies (RVNA) at seven dominant habitat classes in Puerto Rico during 2014–21.

Habitat type	No. individuals sampled by habitat type[a]	No. RVNA positive	% RVNA positive	95% confidence intervals Lower	95% confidence intervals Upper
Dry grasslands and pastures	298	33	11.1	8.0	15.1
Hay and row crops	14	2	14	4	40
Moist grasslands and pastures	52	16	31	20	44
Seasonally flooded herbaceous nonsaline wetlands	66	22	33	23	45
Lowland dry limestone shrubland	10	1	10	2	40
Mangrove forest and shrubland	15	0	—[b]	—	—
Mature primary Tabonuco and secondary montane wet noncalcareous evergreen forest	10	6	60	32	83
Total	465	80	17.2	14.0	20.9

[a] One mongoose was captured once in the mangrove forest and shrubland habitat and again in the lowland dry limestone shrubland habitat.

[b] Not applicable.

80/464 (17.0%; 95% CI, 14.1–20.9%; 55 males, 23 females, and two sexes not recorded) of individual mongooses sampled RVNA positive at one or more points during the capture histories.

The raw RVNA seroprevalence varied by habitat from 0% in the mangrove forest and shrubland to 60% in the mature primary Tabonuco and secondary montane wet noncalcareous evergreen forest. Results by individual study site are detailed in Table 3. The geometric mean (GSD) RVNA titer for the 80 unique positive animals was 0.58 (2.92) IU/mL. Geometric mean (GSD) titer was 0.52 IU/mL (2.84; n=55) for males, 0.70 IU/mL (2.79; n=23) for females, and 2.17 IU/mL (7.17; n=2) for animals whose sex was not reported. A breakdown of geometric mean titers of seropositive animals by sex, habitat, and site is given in Figure 2. Overall, 58% (46/80) of all seropositive mongooses had titers ≥0.5 IU/mL across all habitats.

Among the subset of sera tested by both RFFIT and ELISA, 20% (27/132) of samples were RVNA positive, whereas 27% (36/132) of samples were considered positive for rabies virus binding antibody. There was 93% (123/132) agreement between the methods; none were false negatives (i.e., RFFIT positive, ELISA negative); nine cases of disagreement were false positives (i.e., RFFIT negative, ELISA positive; Supplementary Material Fig. S1). The BioPro ELISA kit demonstrated high sensitivity (1.0; 95% CI, 0.84–1.0%) and specificity (0.91; 95% CI, 0.84–0.96%) for detection of rabies virus antibodies in wild-caught mongoose sera compared with the RFFIT, with a positive predictive value of 0.75 (95% CI, 0.57–0.87%) and a negative predictive value of 1.0 (95% CI, 0.95–1.0%).

Mean (±SE) number of days between resampling events was 172 d (31.7), range 5–959 d. Of 64 mongooses recaptured at least once, 10 (15%) maintained RVNA-positive status between recaptures, five (8%) showed a change in RVNA status with respect to the 0.1 IU/mL cutoff (two from negative to positive, one from positive to negative, and one from negative to positive and back to negative), whereas 48 mongooses (75%) were RVNA negative upon initial capture and recapture(s). Finally, one mongoose (2%) was RVNA positive at initial capture and showed a fourfold increase in RVNA titer values upon recapture, suggesting virus exposure between the capture events (Supplementary Material Table S1).

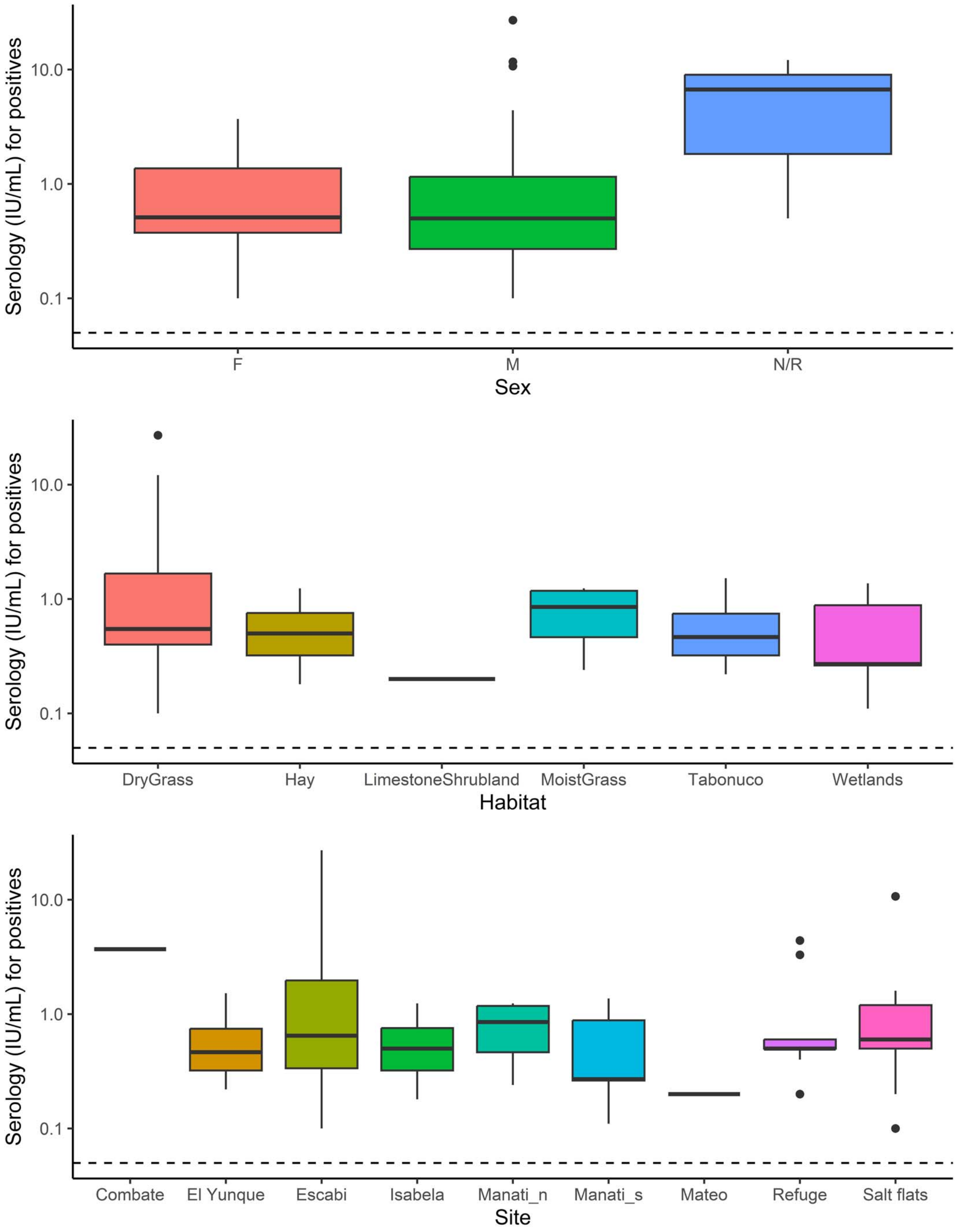

FIGURE 2. The geometric mean and geometric SD box plots of serum rabies virus–neutralizing antibodies, expressed in international units per milliliter among seropositive small Indian mongooses (*Urva auropunctata*), stratified by sex, habitat, and site in Puerto Rico, 2014–21. The y axis is on the $\log_{10}$ scale. The horizontal dashed line at 0.10 IU/mL represents the cutoff for being considered rabies virus-neutralizing antibody positive.

TABLE 4. Model selection results for the individual-level analysis of rabies virus–neutralizing antibody serostatus among small Indian mongooses (*Urva auropunctata*) in Puerto Rico during 2014–21.[a]

Model	*K*	AICc	Δ_AICc	AICcWt	LL
Habitat+Sex	9	455.38	0	0.47	−218.52
Habitat+Sex +Season	10	456.06	0.67	0.33	−217.82
Habitat	8	458.08	2.70	0.12	−220.91
Habitat+Season	9	459.05	3.66	0.08	−220.35
Sex	3	472.04	16.65	0	−233.00
Sex+Season	4	473.79	18.40	0	−232.86
Intercept only	2	476.78	21.40	0	−236.38
Season	3	478.73	23.34	0	−236.34

[a] All models include the random effect of year. Season levels are wet or dry, and *K* reflects the number of parameters in the model. Models are ranked using Akaike Information Criterion corrected for small sample size (AICc). Models within 2 Δ_AICc are considered competitive. AICcWt = the model weight given the model set; LL = the log likelihood of the model.

Individual-level models, including the random effect for individual identity, often failed to converge. Therefore, we subsequently compared individual-level models, including only year as a random effect. We fitted all models with both the entire dataset and a dataset with only initial captures, to determine whether the inclusion of recaptures impacted the model results. The same two individual-level models were found to be competitive for both datasets (see Table 4 for full dataset and Supplementary Material Table S2 for the dataset without recaptures). A model with habitat and sex and another model with habitat, sex, and season were both supported by the data in the individual-level analysis (Table 4), with a random effect for year (Supplementary Material Fig. S2). The individual-level probability of a mongoose being seropositive varied by habitat, with a high of 0.71 (95% CI, 0.23–0.95%) in Tabonuco to a low of 0.00 (95% CI, 0.00–1.00%) in the mangrove habitat. The only habitats that were statistically different in their probability of being seropositive were the dry grass and Tabonuco (*P*=0.01; Fig. 3A). The probability of being seropositive was higher for males (0.08; 95% CI, 0.03–0.20%) than females (0.04; 95% CI, 0.01–0.13%; *P*=0.02; Fig. 3B). The probability of being seropositive was not different between the wet season (0.09; 95% CI, 0.03–0.21%) compared with the dry season (0.04; 95% CI, 0.01–0.13%; *P*=0.22; Fig. 3C).

There was greater uncertainty observed for habitats represented by fewer samples. The top model for the population-level analysis was the intercept-only null model, with moderate support for another model, including habitat (AICc weight for intercept only as 45% versus AICc weight of 42% for habitat only; Table 5), where both models include a random effect for year (Supplementary Material Fig. S3). Considering the model supporting habitat effects, the population-level estimates of mongoose RVNA seroprevalence by habitat are shown in Figure 4. The modeled population seroprevalence ranged from a high of 0.81 (95% CI, 0.41–0.96%) in Tabonuco to zero (95% CI, 0.00–1.00%) in the mangrove habitat (Fig. 4). The only pairwise comparisons of habitats that were statistically different for the population-level analyses were between dry grass and Tabonuco (*P*=0.003; Fig. 4) and between limestone shrubland and Tabonuco (*P*=0.02; Fig. 4).

DISCUSSION

Based on habitat-associated predictions of rabies virus circulation in mongoose populations in Puerto Rico (Sauvé et al. 2021), we expected to find higher seroprevalence in the grassland and pastures habitat, yet seroprevalence in this habitat ranked among the lowest from the seven habitat types we evaluated. In

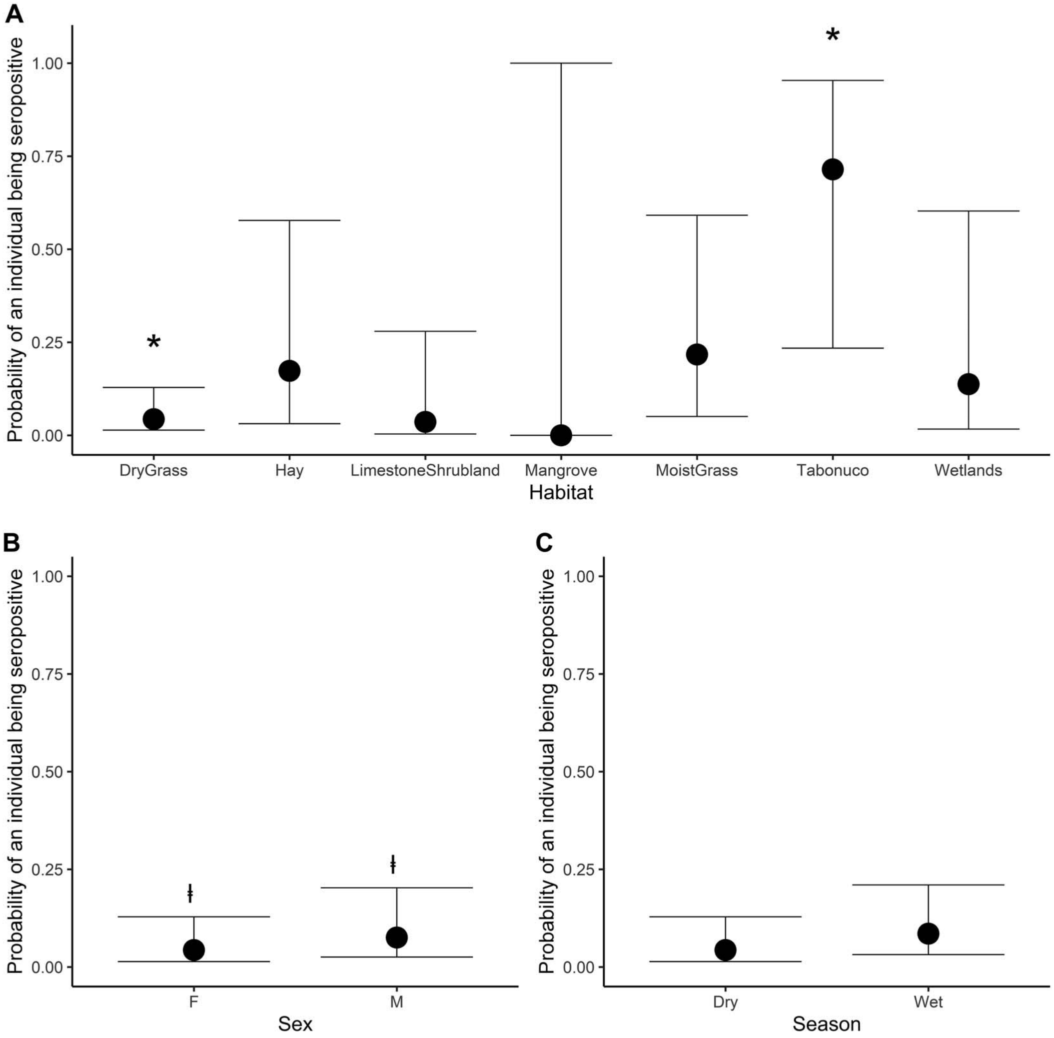

FIGURE 3. Probability of an individual small Indian mongoose (*Urva auropunctata*) testing positive for serum rabies virus–neutralizing antibodies across (A) habitats, (B) sex, and (C) season, in Puerto Rico during 2014–21. Vertical bars indicate 95% confidence intervals. Pairwise comparisons were made for each factor level within a covariate. Identical symbols displayed above a set of factor levels represent a significant post hoc contrast.

addition, compared to the estimated population RVNA seroprevalence of 39.3% during 2011–14 from 112 mongooses using the same laboratory and RFFIT assay method (Berentsen et al. 2015), our 2014–21 survey found lower estimated population seroprevalence. This difference may be related to increased sampling across multiple sites in the current study, rather than longitudinal resampling of individual sites over time, resulting in lower within-site recapture rates than in the Berentsen et al. (2015) study. Rabies virus circulation in mongoose populations is dynamic through space and time, and we demonstrated modest evidence of this based on two animals that were seronegative upon initial capture and RVNA positive upon recapture, plus one mongoose that was RVNA seropositive initially and then showed a fourfold rise in titer between capture events.

We found close agreement in results between the RFFIT and blocking ELISA

TABLE 5. Model selection results for the population-level rabies virus–neutralizing antibody seroprevalence analysis of small Indian mongooses (*Urva auropunctata*) in Puerto Rico during 2014–21.[a]

Model	K	AICc	Δ_AICc	AICcWt	LL
Intercept only	2	98.18	0	0.45	−46.71
Habitat	8	98.33	0.15	0.42	−33.96
Season	3	101.01	2.83	0.11	−46.71
Habitat+Season	9	104.84	6.66	0.02	−33.42
Site	11	115.11	16.93	0	−27.7
Site+Season	12	126.14	27.96	0	−25.07

[a] All models include the random effect of year. Season levels are wet or dry, and *K* reflects the number of parameters in the model. Models are ranked using Akaike Information Criterion corrected for small sample size (AICc). Models within 2 Δ_AICc are considered competitive. AICcWt = the model weight given the model set; LL = the log likelihood of the model.

assays across a subset of 132 sera representative of the overall population seroprevalence estimated in this study and providing continuity with our previous data on natural mongoose exposures to rabies virus in Puerto Rico (Berentsen et al. 2015). The probability of mongooses being seropositive varied primarily by habitat, with some influence of animal sex and season. Only the effect of habitat on RVNA seroprevalence was supported in both the individual and population models. Given that most habitats were only represented by a single site or year of sampling, the habitat associations reported should be considered with some caution. The RVNA seroprevalence was highest for moist grasslands and pastures (Manati north), seasonally flooded herbaceous nonsaline wetlands, and mature (Manati south) and primary Tabonuco and secondary montane wet noncalcareous evergreen forest (El Yunque), but, collectively, these three sites made up only 27% of all samples collected. The grasslands and pasture habitat provided 68% of the total samples but was among the lowest with respect to mongoose RVNA seroprevalence at the individual and population levels. Five sites shared "grasslands and pasture" as the dominant habitat type, yet the proportion of each site occupied by that habitat type ranged from 26.2–88.5%, suggesting substantial variability in habitat composition within some sites.

The influence of sex may be similarly explained by over 68% of seropositive mongooses

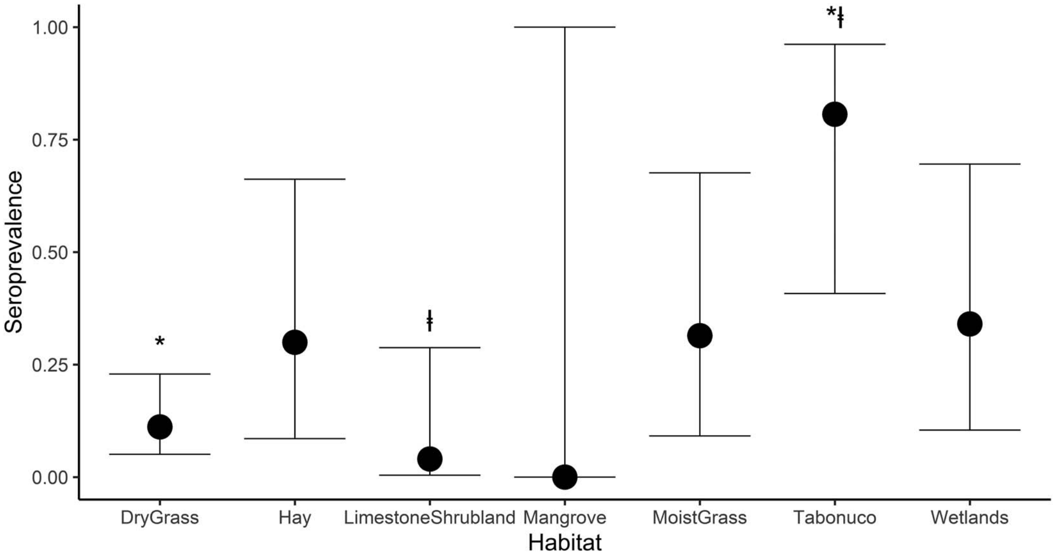

FIGURE 4. Population rabies virus–neutralizing antibody seroprevalence in small Indian mongooses (*Urva auropunctata*), stratified by habitat in Puerto Rico during 2014–21. Vertical bars indicate 95% confidence intervals. Pairwise comparisons were made for each factor level within a covariate. Identical symbols displayed above a set of factor levels represent a significant post hoc contrast.

sampled being males. This finding is important because male mongooses tend to have larger home ranges than females in Puerto Rico, particularly during the dry season (Berentsen, Rivera-Rodriguez, McClure, et al. 2020), which may impact sex-specific contact rates and the potential for disease spread.

Whether naturally acquired RVNA in mongooses are protective against rabies infection remains unknown, but research suggests RVNA titers ≥0.5 IU/mL induced by oral vaccination may provide some protection against rabies virus infection for mongooses (Moore et al. 2017). At the three habitats of greatest seroprevalence in this study, 30–60% of mongooses sampled had RVNA titers of ≥0.1 IU/mL, yet the seroprevalence estimates evaluated at a threshold of 0.5 IU/mL ranged from 9% to 30%, suggesting that approximately half (20/44, 45%) of the mongooses naturally exposed to rabies virus had RVNA titers greater than 0.5 IU/mL.

Some habitats and sites were sampled unevenly during our study. Research during 2011–14 found that 33 and 42% of mongooses were seropositive at El Yunque and the Salt Flats, respectively (Berentsen et al. 2015). At the El Yunque site in 2021, we found 60% of mongooses sampled seropositive, almost twice the seroprevalence reported by Berentsen et al. (2015) for the same region. In contrast, at the Salt Flats site, seroprevalence decreased from 42% in 2014 (Berentsen et al. 2015) to 7.6% by 2018 (this study). The El Yunque and Salt Flats sites are two sites for which serology data are available prior to Hurricanes Irma and Maria; these may have impacted mongoose populations in Puerto Rico during autumn 2017. Following these catastrophic events, mongoose capture success at the Salt Flats declined dramatically, suggesting a decrease in mongoose abundance (A.R.B., pers. obs.), as has been reported for other Caribbean regions during the same time period (Shiels et al. 2020). The precise reasons for the observed changes in seroprevalence at some sites remain unknown; simulated population disease models may be useful to evaluate the effects of landscape features with respect to mongoose, and thus potential rabies virus, movements (Sauvé et al. 2021).

The differences in population RVNA seroprevalence may reflect, in part, variation in mongoose population densities and contact rates across habitat types, but we caution readers that inferring infection pressure directly from raw seroprevalence requires careful consideration and a recognition that rabies virus transmission among wildlife may be a function of both frequency and density-dependent mechanisms (e.g., Morters et al. 2013; Pepin et al. 2017; Sauvé et al. 2021). Our research suggests that habitat type plays a role in the probability of whether mongooses are seropositive, but more even and systematic replication across types of habitats used by mongooses is warranted. We found only slight variability in the proportion of seropositive mongooses between wet and dry seasons, but additional research into seasonal variability may help to understand the dynamic disease process across habitats and to determine when rabies control efforts targeting mongoose populations may be most effective. Further investigation into the role population density may play in intraspecific contacts and the incidence of rabies infection among mongooses across multiple habitat types in Puerto Rico may provide additional information to help guide our understanding of natural infection dynamics and the development of habitat-based rabies control strategies.

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SUPPLEMENTARY MATERIAL

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