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Exposure to Rabies in Small Indian Mongooses (*Herpestes auropunctatus*) from Two Regions in Puerto Rico

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ABSTRACT: The small Indian mongoose (*Herpestes auropunctatus*) was introduced to several Caribbean Islands to control rat (*Rattus* spp.) damage to sugarcane plantations. Mongooses failed at suppressing rat populations and are now considered pests throughout most of their introduced range. Importantly, mongooses are rabies reservoirs on several Caribbean Islands. In Puerto Rico, mongooses have been implicated in up to 70% of reported animal rabies cases. There is no rabies vaccination program for wildlife in Puerto Rico, and data on rabies in mongooses are limited. We conducted a serosurvey of mongooses in two different ecologic environments in Puerto Rico: El Yunque National Forest and Cabo Rojo National Wildlife Refuge. We collected 119 serum samples from 112 mongooses, 44 (39.3%) of which were positive for rabies virus–neutralizing antibodies. We also collected oral swabs from 147 mongooses, including 88 from which we also collected serum. No oral swabs were positive for rabies virus RNA. Our data support previous research suggesting rabies virus is circulating within the mongoose population on Puerto Rico.

Key words: Caribbean, *Herpestes auropunctatus*, mongoose, Puerto Rico, rabies, wildlife disease.

The small Indian mongoose (*Herpestes auropunctatus*) was introduced to the Caribbean Islands in the 1870s, primarily to control rat (*Rattus* spp.) damage to sugarcane (Hoagland et al. 1989). Mongooses are now considered pests throughout most of their introduced range and are the primary rabies reservoir on several Caribbean Islands (Everard and Everard 1992). In Puerto Rico, mongooses account for up to 75% of reported rabies cases (Krebs et al. 1998; Dyer et al. 2014). In Puerto Rico, the first laboratory-confirmed rabies-infected mongooses were reported in 1950, although references to rabies date back prior to mongoose introduction (Tierkel et al. 1952). Between 1986 and 1990, 71.5% (236/330) of suspect mongooses tested in Puerto Rico were positive for rabies virus, as were 2.6% (4/152) of randomly trapped mongooses (Everard and Everard 1992). In comparison, Everard et al. (1981) reported rabies infection in 1.3% of >11,000 mongooses in Grenada between 1968 and 1977. Velez (1998) found 19.3% of mongooses sampled in portions of El Yunque National Forest (YNF), Puerto Rico, positive for rabies virus–neutralizing antibodies (RVNA) when tested by enzyme-linked immunosorbent assay (ELISA), although none were rabies virus positive. Few data are available on rabies exposure in mongooses on Puerto Rico, as most studies have occurred on other Caribbean islands, primarily Grenada (Everard et al. 1981; Zieger et al. 2014). We evaluate sera for RVNA and saliva samples for rabies virus RNA to investigate mongoose exposure to rabies in two ecologically different environments on Puerto Rico.

We conducted our study at YNF and Cabo Rojo (CR) National Wildlife Refuge (Fig. 1). The YNF is a subtropical rainforest approximately 40 km southeast of San Juan. In YNF, samples were collected from a 1.0-km^2 region of Palo Colorado and Sierra Palma forest types in October 2011 and March 2012. The CR is >7 km^2 of subtropical dry forest in southwestern Puerto Rico. We collected samples from CR in two ~1-km^2 regions, one each on the eastern and western sides of the

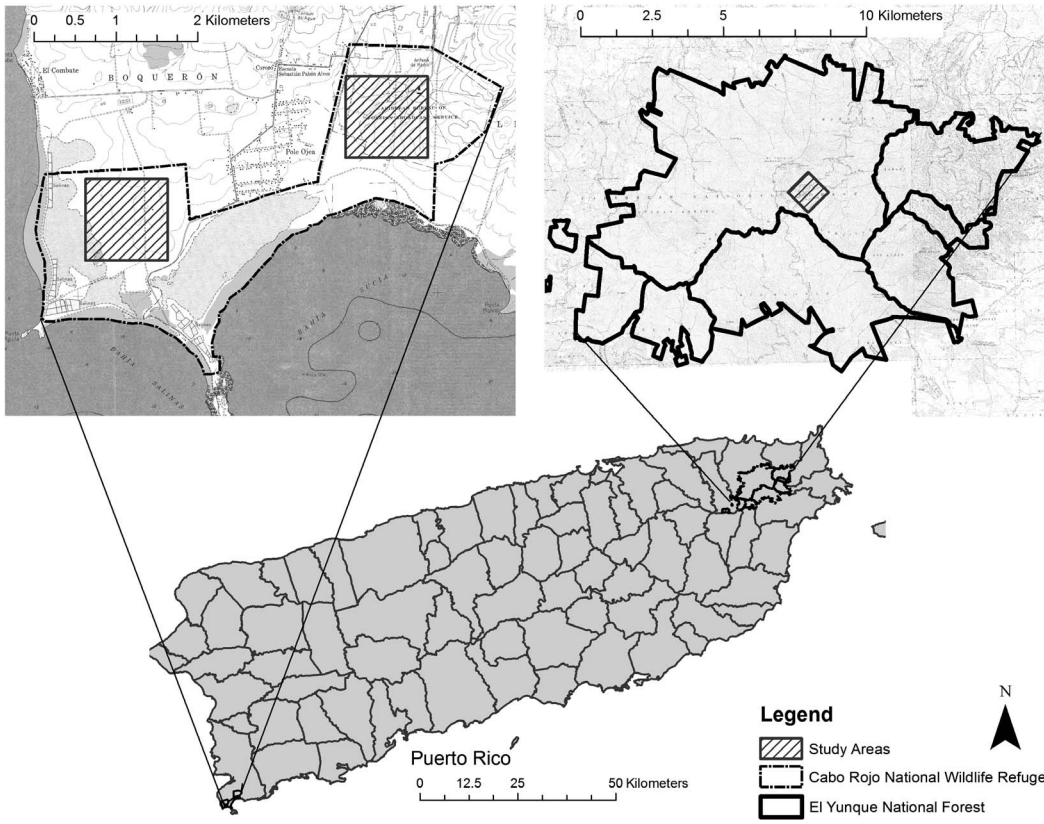

FIGURE 1. El Yunque National Forest and Cabo Rojo National Wildlife Refuge, Puerto Rico.

refuge, during September 2011, April–May 2012, and August 2014. Both sample sites are dominated by scrub-class vegetation (Weaver and Schwagerl 2008).

We used cage traps (Tomahawk Trap Company, Hazelhurst, Wisconsin, USA) baited with canned tuna to live capture mongooses (Quinn and Whisson 2005). Mongooses were hazed out of the trap into a cone-shaped canvas bag, physically restrained, and immobilized with an intramuscular injection of Telazol® (tiletamine-zolazepam, Fort Dodge Laboratories, Fort Dodge, Iowa, USA) at a dose rate of 5 mg/kg (Kreeger and Arnemo 2007). We collected 0.5–1.0 cc of whole blood by venipuncture of the cranial vena cava, as described for ferrets (Briscoe and Syring 2004), and injected a passive integrated transponder tag (Avid Identification Systems, Inc., Norco, California, USA) subcutaneously between the shoulder blades of each captured mongoose. We used a sterile cotton swab to collect saliva samples and stored swabs in a bovine albumin 1 viral transport medium to reduce potential bacterial or fungal contamination (Shriner et al. 2012). Blood samples were centrifuged, and sera were transferred by pipette into cryovials. Serum samples and oral swabs were frozen at −10 C for up to 14 d and transferred to −80 C until analysis.

Sera were analyzed for RVNA end-point titers by using the rapid fluorescent focus inhibition test (Smith et al. 1996) by the rabies laboratory at Kansas State University (Manhattan, Kansas, USA). Mongooses with sera >0.1 IU/mL were considered positive for RVNA. We compared prevalences by using the Logistic procedure in SAS (SAS Institute Inc., Cary, North

Carolina, USA). We accepted statistical significance at $\alpha=0.05$.

Oral swabs were processed by Atlanta Health Associates (Cumming, Georgia, USA). Total RNA was extracted by using TRIZol reagent (Invitrogen™, Ambion®, Carlsbad, California, USA). Heminested reverse-transcription PCR was performed for amplification of a partial sequence of the rabies virus nucleoprotein gene by using primers 1066:304 for the primary and 1087:304 for the heminested reactions. Positive (RNA from mouse brain infected with the mongoose-variant rabies virus) and negative (water) control samples were used with each PCR run. Reaction products were visualized by gel electrophoresis (Freuling et al. 2015).

We collected 41 sera from 39 mongooses at YNF. Fourteen sera were RVNA positive from 13 mongooses (nine males and four females), including one recaptured female whose titer decreased from 2.8 to 1.2 IU/mL between sampling periods (138 d). The geometric mean titer of positive mongooses at YNF was 0.52 IU/mL (range: 0.1–2.8 IU/mL), and antibody prevalence was 33% (13 unique positives and 39 unique captures; 95% confidence interval [CI]: 19–48%). At CR, 78 sera were collected from 73 mongooses. Thirty-two sera were RVNA positive from 31 individuals (20 males and 11 females). Five animals were recaptured during subsequent seasons: two females and one male, initially captured in 2011, were recaptured in 2012. Among recaptures, one male and one female seroconverted between captures (from <0.1 IU/mL to 0.4 and 1.4 IU/mL for the male and female, respectively), while the second female remained antibody negative. One male captured in 2012 (recaptured in 2014) seroconverted between captures (<0.1 IU/mL in 2012 and 10.7 IU/mL in 2014). A positive female captured in 2011 was recaptured in 2014, and the RVNA titer had decreased from 0.6 IU/mL to 0.5 IU/mL (1,073 d). Geometric mean titer of antibody-positive mongooses at CR was 1.62 IU/mL (range: 0.1–50.0 IU/mL), and antibody-prevalence was 42% (31 positive and 73 unique captures; 95% CI: 31–54%). Antibody prevalence (including recaptured animals) did not differ between sites ($\chi^2=0.53$; $P=0.46$) or seasons (wet vs. dry; $\chi^2=0.02$; $P=0.88$), or among years ($\chi^2=1.28$; $P=0.53$).

Oral swabs from 146 unique mongooses (72 from CR and 74 from YNF) were tested for rabies virus RNA. This included swabs from 88 individual mongooses whose sera were screened, and 33 of which were RVNA positive. All swabs were negative for viral RNA.

The RVNA detected in mongoose sera suggests prior peripheral virus exposure and adaptive immune response induction rather than an active nervous system infection at the time of sampling. A peripheral neutralizing antibody response may not be routinely induced or if it is induced, tends to be observed during late stages of clinical infection (Lafon 2013), which were not observed among mongooses in this study. Furthermore, viral shedding in saliva by infected rabid animals is intermittent and, though possible to detect several days in advance of clinical signs, is typically detected during clinical infection (Hanlon et al. 2007). Zieger et al. (2014) found that of 171 mongooses sampled on Grenada, only two (1.7%) were positive for rabies virus, whereas 33 (19.3%) had RVNA titers at the 0.1 IU/mL threshold. Rabies virus RNA was not found in saliva from either infected mongoose in the Zieger et al. (2014) study, and neither mongoose showed clinical signs of infection.

Overall, 39% of mongooses sampled in this study were RVNA positive. This is slightly higher than previous estimates from Puerto Rico (19.3%; Velez 1998) and Grenada (30.0%, Everard et al. 1981; 19.3%, Zieger et al. 2014), although caution must be exercised during comparison as Velez (1998) used an ELISA assay for antibody detection and Everard et al. (1981) used a lower cutoff than our study

to report positive RVNA results. The number of rabies-positive mongooses in our study is unknown because mongooses were not lethally sampled, but prevalence estimates from similar environments of Grenada are 1.7% (Zieger et al. 2014) and 1.3% (Everard et al. 1981). Rabies transmission routes among mongooses are poorly understood, but bite contacts during intraspecific aggression are likely the primary route.

Phylogenetic studies suggest the rabies virus circulating in mongooses of Puerto Rico is related to the North Central skunk variant and cosmopolitan dog lineages of rabies virus (Nadin-Davis et al. 2008). Virus strains from Puerto Rico (Nadin-Davis et al. 2008), Grenada (Zieger et al. 2014), and Cuba (Nadin-Davis et al. 2006) do not cluster in a monophyletic group, suggesting independent introductions to different Caribbean islands.

Rabies is maintained by mongooses as a reservoir species rather than a spillover host. Although domestic dogs (*Canis familiaris*) can account for up to 26% of rabies cases in Puerto Rico, infection is typically attributed to the mongoose variant (Dyer et al. 2014). It is not possible to distinguish whether multiple exposures occurred among recaptured animals in this study, but our findings support reports by Everard et al (1981), indicating potential for long-term maintenance of RVNA in mongoose. The seroconversion events detected among recaptured animals provides strong evidence of active rabies virus circulation among mongooses in Puerto Rico. Future research should include virus isolation from saliva of actively infected mongooses. With no wildlife vaccination program in place, mongoose rabies in Puerto Rico continues to pose a threat to domestic animal and human health.

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