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Assessment of Rabies and Canine Distemper Viruses in Road-Killed Wildlife Mammals From the Semiarid Region of Northeastern Brazil

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Abstract

Road mortality due to collision with vehicles can affect many species, increasing the risk of local population decline or extinction. Infectious diseases that affect the central nervous system of wild animals may also promote an increase in road mortality due to the debilitating effects of blinding, neurologic disturbance, or behavior alteration. Roads in the surroundings of three conservation units in the Caatinga of Rio Grande do Norte, Brazil, were surveyed to identify which mammal species are being impacted by these anthropogenic structures. In addition, collected animals that were recently killed were also examined to determine their health status for diseases that affect the central nervous system (rabies and distemper virus) which cause neurological disorders. Between November 2014 and November 2015, 124 mammals from four potential reservoir species were found road-killed in the surveyed roads. Despite reports of these viruses circulating in Northeast Brazil, in both domestic and wild animal populations, none of the 18 road-killed mammals tested were infected by rabies or canine distemper, although some of the species identified are considered reservoirs for both diseases. This suggests that the animals most likely came from healthy populations or that the prevalence of these diseases is so low that it remained undetected. Furthermore, the high number of road-kills during this 1-year period indicates that mammal species from these conservation units are being negatively impacted, regardless of their health status. Hence, further studies must be conducted to identify other factors contributing to the road-killing of these species and implement the adequate mitigation measures to reduce or eliminate road mortality in the vicinities of these three conservation units.

Keywords

collision, vehicles, free-ranging, infectious diseases, mitigation

Introduction

Roads, similarly to other anthropogenic linear structures, cause negative impacts on species' distribution and genetic diversity, increasing the risk of local population decline or extinction (Canal, Camacho, Martin, de Lucas, & Ferrer, 2018; Ferrer & Hiraldo, 1991, 1992; Grilo et al., 2018). For wildlife species, mortality associated to collision with vehicles (e.g., road-kill) is often the most visible impact of road traffic, with mammals usually being one of the most frequently encountered groups (Canal et al., 2018). Furthermore, native populations are also directly affected by habitat loss and fragmentation, pollution and other factors. In addition, populations

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may also suffer from indirect effects, such as increased susceptibility to infectious diseases due to immunosuppression caused by a more stressful environment (Baskaran & Boominathan, 2012; Hollings, Jones, Mooney, & Mc Callum, 2013; Namroodi, Gholami, & Shariat-Bahadori, 2016). Conversely, the debilitating effects of infectious diseases, in particular those that affect the central nervous system and cause neurological disorders, may also increase the likelihood of species being road-killed (Greene & Appel, 2006; Namroodi et al., 2016). This highlights the importance of determining the health status of wildlife populations, particularly regarding diseases such as rabies and canine distemper and their respective reservoirs (Avendaño et al., 2016; Carnieli et al., 2006; Gomes et al., 2012).

Both rabies and canine distemper are diseases known to affect the nervous system of animals and have been shown to occur in several mammalian groups, particularly carnivores, that include wild and domestic animals (Beineke, Baumgärtner, & Wohlsein, 2015; Deem, Spelman, Yates, & Montali, 2000; Megid et al., 2009, 2010). However, whereas the domestic dog is considered the major canine distemper reservoir (Greene & Appel, 2006), rabies cycles are usually more associated with wildlife species. In Brazil, 1,703 aerial wild mammals and 460 terrestrial wild mammals were reported with confirmed rabies between 2002 and 2012, including the Rio Grande do Norte state (Rocha, de Oliveira, Heinemann, & Gonçalves, 2017), with the wild cycle of rabies being maintained active mainly in the Order Chiroptera, wild canids, and small primates (Antunes et al., 2018). Apart from several bat species, the crab-eating fox (*Cerdocyon thous*) and the hoary fox (*Lycalopex vetulus*) are thought to be the two main wildlife species to present the rabies virus (Carnieli et al., 2006; De Araújo et al., 2014; Gomes et al., 2012; Kotait et al., 2007). Transmission of rabies by nonhuman primates is largely attributed to *Callithrix sp.*, whose importance in the wild cycle of rabies is increasing (Antunes et al., 2018; Sousa et al., 2013). Moreover, both the crab-eating fox and the hoary fox have also been reported as infected with canine distemper in several Brazilian states (Megid et al., 2009, 2010). The expansion of human settlements and the increase in connectivity between them may promote a greater contact between domestic and wildlife animals and eventually facilitate the exposure to and the spreading of such infectious diseases (Hollings et al., 2013; Megid et al., 2009, 2010).

In an effort to protect public health in Brazil, rabies and canine distemper cases are of mandatory reporting. However, the surveillance of these diseases is still predominantly based on reports of human and domestic animal cases, with an occasionally association of a wild animal to an exposure event. To bridge this knowledge gap, studies that assess the prevalence of these diseases in wild populations are badly needed, particularly regarding the species that can act as reservoirs. The traditional approach to investigating disease in wildlife presents several difficulties in collecting information about the health status of natural populations, since it requires capturing and sampling of live animals. Hence, opportunistic road-kill sampling represents an alternative to indirectly assess the prevalence of diseases in the wild (Case, 1978). By collecting road-killed animals, this study aimed to (a) determine which wildlife mammal species, that are potential reservoirs for infectious diseases, are being impacted by roads in three conservation units from the Brazilian semiarid region of the Caatinga and (b) assess the health status of the road-killed mammals regarding two infectious diseases (rabies and canine distemper). Hence, this work provides an estimation of the anthropogenic pressure caused by roads on the conservation units' fauna while also assessing whether the traffic-related mortality of these animals is solely due to their use of the roads (e.g., foraging, dispersal) or if they were suffering from illness-related debilitation caused by infectious diseases that affect the central nervous system (rabies and canine distemper).

Methods

The study was conducted in Rio Grande do Norte (RN) state, Brazil, encompassing several roads that are in the vicinity or go through three Federal Conservation Units: the National Forest (FLONA) of Açu (5°57′74.79″S, 36°94′7.14″ W; area: approximately 2.18 km^2), located in the city of Assu-RN, in the microregion of the Açu Valley; Furna Feia National Park (5°01′04.55″S, 37°28′18.97″W; area: approximately 84.94 km^2), located in the municipalities of Baraúna and Mossoró, in the western Potiguar mesoregion; and Seridó Ecological Station (ESEC Seridó; 6°34′44.63″S, 37° 15′6.78″W; area: approximately 11.66 km^2), located in northern Serra Negra-RN municipality, in the western Seridó microregion. The efficiency of the conservation efforts will depend on whether the populations are healthy and whether the impact of surrounding roads is minimal. These conservation units are located in the Caatinga, a biome that is highly sensitive to global changes, and where there are 136 species threatened with extinction by anthropogenic actions (agriculture and urban expansion), among them: the hoary fox (*Lycalopex vetulus*) a vulnerable mammal according to the *Brazil Red Book of Threatened Species of Fauna* (2016). This biome covers 9.92% of the national territory with a semiarid climate and is mainly composed of aesthetical savannah that are quite altered, with an estimated ~80% of the original ecosystems already changed by humans.

Surveys were performed between November 2014 and November 2015 (except for Furna Feia which ended in June 2015), with a periodicity of approximately 21 days. In each survey, roads were monitored by vehicle (speed <40 km/h) 2 times in a single-day round trip. Road-kill mammals were identified and their location was recorded using a GPS, after which the carcasses were removed. Recently, road-killed animals found were collected and transported to the Laboratory of Wildlife Ecology and Management of Universidade Federal Rural do Semi-Árido in Mossoró—RN under refrigeration.

In the laboratory, road-killed animals were maintained at −20°C until the brain samples were collected.

Table 1. Species and Number of Wild Mammals Found Road Killed in the Surveyed Regions of Rio Grande do Norte State, Brazil, Between November 2014 and November 2015 (Except for Furna Feia, Where Surveys Ended in June 2015).

	Surveyed region			
Species	Seridó	Açu	Furna Feia	Total
Cerdocyon thous	79 (3)	30 (9)	10 (3)	119 (15)
Lycalopex vetulus	2 (1)	–	–	2 (1)
Challithrix jacchu	–	1 (1)	–	1 (1)
Euphractus sexcinctus	–	–	2 (1)	2 (1)
Total	81 (4)	31 (10)	12 (4)	124 (18)

Note. The number of animals tested is presented in parentheses.

These samples were then sent to the School of Veterinary Medicine and Animal Sciences of Universidade Estadual Paulista, in Botucatu—São Paulo (SP) state, were diagnosis of rabies and canine distemper infection was made using reverse transcription polymerase chain reaction (RT-PCR). Potential infection by the Canine distemper virus was determined by means of the nucleocapsid protein RNA, using the protocol described in Amaral (2007). To determine potential infection by the Rabies virus, RT-PCR and heminested RT-PCR techniques were used, as described by Soares et al. (2002).

Results

During the monitoring period, 124 wildlife mammals, known to constitute reservoirs for rabies and canine distemper, were found road killed in the surveyed roads, the vast majority of them belonging to the species *Cerdocyon thous* (Table 1). From the total of road-killed animals, 18 were recently deceased and therefore suitable for rabies and canine distemper analysis, including: 15 crab-eating foxes (*Cerdocyon thous*), 1 hoary fox (*Lycalopex vetulus*), 1 six-banded armadillo (*Euphractus sexcinctus*), and 1 common marmoset (*Callithrix jacchus*) (Figure 1). All other animals found road-killed were in a state of decomposition (ranging from mildly decomposed to only skin and bones) that did not allow for a proper

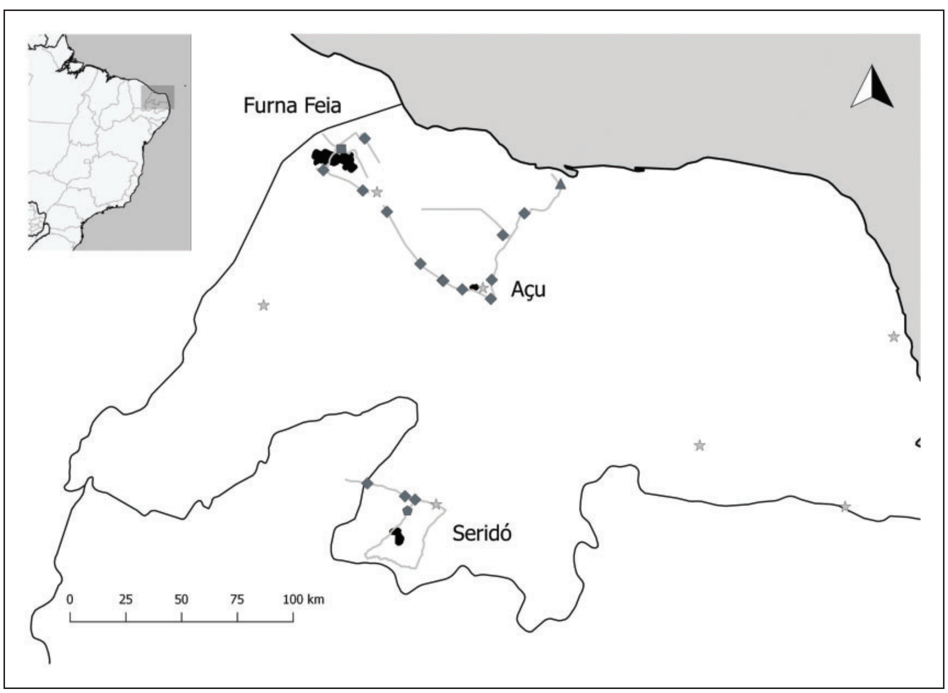

Figure 1. Map of the conservation units and sites were species found killed by traffic along of the surveyed roads between November 2014 and November 2015 (except for Furna Feia which ended in June 2015). Dark gray symbols represent collection sites: diamonds—*Cerdocyon thous*, squares—*Euphractus sexcinctus*, triangles—*Callithrix jacchu*, and pentagons—*Lycalopex vetulus*. Black polygons—conservation units; gray lines—roads; light gray stars—major cities.

assessment of the diseases. All animals collected in the surveyed roads, within the area of influence of the conservation units, that were tested for the presence of both diseases presented negative results.

Discussion

Opportunistic sampling constitutes an important alternative as it allows an indirect assessment of wildlife diseases (Case, 1978). This type of sampling is becoming a staple in the study of diseases, either through road-kill assessments or other sources (Bourg et al., 2013), particularly in cases where traditional methods cannot or are yet to be employed. From all the animals collected by opportunistic road-kill sampling, approximately 15% were in adequate conditions (did not show any signs of tissue decomposition/degradation) to test for the presence of rabies and canine distemper and all of them yielded negative results for both diseases.

The 21-day interval between surveys is suitable for estimating wildlife road mortality (Canal et al., 2018) and the effect of carcass removal (runoff, scavengers, and rainfall; Teixeira, Coelho, Esperandio, & Kindel, 2013) can be estimated and used to correct mortality data. However, most of the animals collected were too degraded to allow for disease assessment through the methods used, either because they were road-killed several days prior to the surveys or due to the environmental conditions of the Caatinga (high temperatures and solar radiation). Hence, to increase the number of road-killed animals suitable for disease assessment, surveys should be conducted more frequently whenever possible. In addition, the use of serology methods could help confirm the results obtained, by using the immunological system to assess whether the animals had been previously exposed to these diseases, particularly regarding the canine distemper which does not necessarily lead to death. However, since the animals were already dead at collection, these techniques could not be implemented.

Results of the disease assessment suggest that these animals came from apparently healthy populations, where rabies and canine distemper are either not present or occur in very low frequencies that difficult its detection. This seems to be in agreement with data from the National System of Health Surveillance (NSHS, 2019) which shows that, from the 20 cases of rabies reported for Rio Grande do Norte state (in 2015, when this study was performed), only one corresponded to a wild canid and seven to Chiroptera (the remaining 12 were from domestic animals, including dogs and farm animals). However, these data from the NSHS may be biased toward the human/domestic reports, as studies that assess the prevalence of these diseases in the wild are still quite scarce for the region. Human settlements expansion has increased the pressure on wildlife, including in areas surrounding the studied Federal Conservation Units. Reports from the NSHS (2011) indicate that there has been an increase of rabies cases in Rio Grande do Norte, confirming the presence of an urban cycle, with cats and dogs acting as reservoirs for the virus, and a wildlife cycle, with some canids and several bats constituting the major reservoirs (Theimer, Dyer, Keeley, Gilbert, & Bergman, 2017). In addition, several cases of rabies were also reported for livestock from rural areas. The increase in reported cases of rabies may also be related to better surveillance methods, with the inclusion of rabies and other zoonosis in the list of compulsory notification diseases (cases must be reported to corresponding authorities). However, it is known that human cases of rabies due to contact with wildlife have been increasing in recent years (Rocha et al., 2017) as well as the death of wild carnivores due to distemper (Avendaño et al., 2016; Deem et al., 2000; Megid et al., 2009, 2010). This is of great concern, in particular considering that expansion of human settlements has promoted an increased contact between urban/domestic animals and the wildlife.

Regarding the road-killed mammals in this study, the *Cerdocyon thous* (crab-eating fox) has one of the highest numbers of road-kill records (Grilo et al., 2018). Furthermore, these animal species (crab-eating fox, hoary fox, marmoset, and armadillo) are susceptible to the diseases addressed in this study (Greene & Appel, 2006) and are among those most commonly diagnosed with rabies and canine distemper (Rocha et al., 2017; Suzuki et al., 2015). Since all animals tested negative, results suggest that none of the diseases examined was implicated in the road-killing of these animals. Additional ecological studies are then necessary to better comprehend why these species, in particular the crab-eating fox, are so susceptible to being road-killed and determine the spatiotemporal patterns of road mortality (Canal et al., 2018). For example, a relatively high number of road-kill may suggest that population density is high. However, when population density is low, home-range size is usually larger (Trewhella, Harris, Mc Allister, 1988) and animals move more in search of resources, increasing the chances of encountering a road. Low populational density would also be consistent with the absence or very low prevalence of the studied diseases, since it would be harder for the viruses to spread.

Animals may have collided with vehicles when trying to cross the roads or while performing other activities, such as opportunistic foraging. Hence, apart from acting as an ecological barrier, the road may also constitute an ecological trap, resulting in a relatively high number of animals found road-killed. This is particularly concerning since it has been demonstrated that species abundance is not necessarily related to high mortality rates in human linear structures; rather, they are more related to species traits and the local physical characteristics around these structures (Ferrer et al., 2012). Hence, although several mitigations measures can be used to reduce wildlife mortality on roads, the effectiveness of those measures depends on an understanding of species ecology and its relation with road characteristics, requiring information on a wide array of factors (species involved, adjacent landscape, magnitude, and patterns of road-kill; Rytwinski et al., 2016). There are several examples of such studies that have led to the implementation of mitigation measures and reduced the anthropogenic impact, providing positive results for species conservation (Ferrer & Hiraldo, 1991; López-López, Ferrer, Madero, Casado, & McGrady, 2011).

Implications for Conservation

Rabies and canine distemper are present in Northeastern of Brazil, albeit absent or with a very low prevalence in the studied areas. These diseases can be responsible for population reduction in wildlife species (Bingham, 2005; Wang, Tang, & Liang, 2014) and constitute important zoonosis (Cabello & Cabello, 2008). Reporting on the occurrence of rabies, canine distemper, and other similar diseases, regardless of a positive or negative identification (as in this study), is of extreme importance since it contributes to the surveillance effort of zoonotic diseases and allows to assess diseases' transmission dynamics between domestic and free-ranging animals (Kapil & Yeary, 2011). Opportunistic road-kill sampling provides an important tool for the assessment of wildlife diseases (Case, 1978). Assessing the health of road-killed animals may also contribute to a better understanding of the location of infectious diseases reservoirs and their dynamics (special and temporal), including the potential local vectors of transmission (Richini-Pereira, Bosco, Theodoro, Barrozo, & Bagagli, 2010). However, since most techniques used to determine the presence of a pathogen require relatively fresh samples, the frequency of the surveys must be adjusted accordingly to increase the number of samples adequate for disease testing. These considerations are important to improve surveillance programs for detecting disease and to further our understanding of the role of these diseases in population dynamics and local species extinction (McClintock et al., 2010; Nusser, Clark, Otis, & Huang, 2008).

As there was no previous assessment of road mortality in the surroundings of these three conservation units, the relatively high number of road-killed animals found, from apparently healthy populations (or with a low prevalence of the studied diseases), is quite concerning. Additional studies that determine the impacts of roads and other anthropogenic structures are needed to understand how wildlife is affected and to identify strategies to reduce road-kill. Particularly, if hot spots for road-kill can be determined, specific actions and mitigation measures can be used to reduce road mortality (Canal et al., 2018) and improve the efficiency of the conservation units. However, such studies should be conducted long term, considering also that the Caatinga is a relatively heterogeneous biome where conditions may change from year to year, and include a collaboration researchers and public administration. Road-kill data collected prior to the mitigation effort (such as that presented in this study) are also essential to assess the efficiency of the mitigation measures implemented (Rytwinski et al., 2015, 2016). Nevertheless, it is also recommended to employ general measures such as the installment of speed signs and warn drivers about the risks of road-killing wildlife species (Rincón-Aranguri, Urbina-Cardona, Galeano, Bock, & Páez, 2019), particularly in the vicinities of the conservation units, in order to reduce road mortality. Moreover, education programs must be conducted to emphasize the importance of wildlife and their role in the natural balance of the ecosystems.

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