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RESEARCH ARTICLE

Impact of Yellow Fever Outbreaks on Two Howler Monkey Species (*Alouatta guariba clamitans* and *A. caraya*) in Misiones, Argentina

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Two yellow fever outbreaks (YFOs) ocurred in northeastern Argentina between November 2007 and October 2008, seriously affecting populations of two howler monkey species: the brown howler *Alouatta guariba clamitans* and the black howler *Alouatta caraya*. Both howlers live syntopically in El Piñalito Provincial Park, Misiones, where four groups (36 individuals) were studied since January 2005. The first dead howlers were found on January 20, 2008, in El Piñalito. Systematic searches found 14 dead howlers within the area (12 from the study groups and two from neighboring groups), with only two young seen on January 25, 2008, and none found since up to December 2008. In October 2008, another YFO hit howler monkey populations from El Soberbio, Misiones. Overall, 59 howlers were found dead in Misiones from November 2007 to December 2008. Thanks to the alert of the howler's death in El Piñalito, a prompt human vaccination campaign started in the area. Wild howler monkey populations from both species are in a delicate situation in Misiones, especially the brown howler, an already endangered species in Argentina and endemic to the Atlantic Forest. If we add the recurrence of YFOs to the reduction of suitable habitat to small fragments, it could be only a matter of time until howler populations disappear from the Upper Paraná Atlantic Forest in Misiones. Am. J. Primatol. 72:475–480, 2010.

Key words: disease; yellow fever outbreak; population decline; primate conservation

INTRODUCTION

Yellow fever, one of the most feared human diseases before the development of an effective vaccine, is caused by a flavivirus and transmitted by hematophagous mosquitoes [Aitken, 1988; King, 1977; Monath, 2001]. This African virus came to America during the slave trade, and still affects thousands of people in tropical Africa as well as Central and South America [Monath, 2001].

All Neotropical primates are susceptible to yellow fever [Ott-Joslin, 1986; Richter et al., 1984]. Probably because they evolved in the absence of the virus, New World monkeys are more vulnerable to the disease than are African primates [Ruch, 1959]. Infection in *Ateles* and *Alouatta* is almost always fatal [Ruch, 1959]. Other yellow fever outbreaks (YFOs) have been observed sporadically, affecting particularly *Alouatta* populations in Panama, Colombia, Trinidad and Tobago and Brazil [Bryant et al., 2003; Collias & Southwick, 1952; Elton, 1955; Ministerio de Salud e Instituto Nacional de Salud de Colombia, 2002; Viégas Sallis et al., 2003].

Two howler monkey species occur in Argentina: the black howler (*Alouatta caraya*) and the southern brown howler (*Alouatta guariba clamitans*). Black howlers inhabit the Chaco and Pantanal ecoregions in Brazil, Paraguay, Bolivia and North-Northeastern Argentina [Rumiz, 1990], and a small portion of the Atlantic Forest in Misiones (Argentina) and the state of Rio Grande do Sul in Brazil [Di Bitetti, 2003; Leiroz Codenotti et al., 2002]. Brown howlers are

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endemic to the Atlantic Forest ecoregion of Brazil and Argentina [da Silva, 1981; Kinzey, 1982] and are being threatened as a result of habitat loss and fragmentation (only 7.4% of the original Atlantic Forest is left; [Brown & Brown, 1992; Myers et al., 2000]), hunting pressure, and susceptibility to epidemic diseases and parasites [Crockett, 1998]. In Argentina, the brown howler is an endangered species [Barquez et al., 2006] present only in the Upper Paraná Atlantic Forest of Misiones province and little is known in regards to its current distribution and population status. In Misiones and areas of southern Brazil these two howler species' distributions overlap in a relatively narrow zone of sympatry [Aguiar et al., 2007; Bicca-Marques et al., 2008; Di Bitetti et al., 1994; Gregorin, 2006]. In at least one protected area of Misiones, El Piñalito Provincial Park, both howler species live syntopically [Di Bitetti, 2003].

From January 2005 to January 2008, we carried out a study on the behavioral ecology of these two howler species in El Piñalito [Agostini et al., 2008]. Our regular monthly monitoring of howlers during this period allowed the detection of two YFOs between November 2007 and October 2008 that affected both species in Misiones, Argentina. Concurrently, these YFOs affected the same howler species populations in Brazil [Ministerio de Saúde do Brasil, 2008]. In Paraguay, yellow fever cases were confirmed for humans, but no official data on howler deaths were available for these YFOs [Ministerio de Salud Pública y Bienestar Social de Paraguay, 2008].

An outbreak has been defined as "the occurrence of a disease in excess of what would be expected in a defined community, geographical area or season. An outbreak may occur in a restricted geographical area, or may extent over several countries. It may last for a few days or weeks, or for several years. A single case of a communicable disease long absent from a population, or caused by an agent (e.g. bacterium or virus) not previously recognized in that community or area, or the emergence of a previously unknown disease, may also constitute an outbreak" [WHO, 2009].

In this article, we report the impact of recent YFOs on populations of black and brown howlers in Misiones, Argentina, estimate the minimum number of dead individuals, and discuss the implications of recurrent YFOs for the conservation of wild primate populations in Argentina.

METHODS

We obtained most information on wild howlers from our long-term study in El Piñalito Provincial Park (26°30′S, 53°50′W), where we monitored four groups of monkeys (two of each species) during three years. After we found the first four dead monkeys (on 20 January 2008), a team of 12 individuals, including biologists, park-rangers and veterinarians, intensively searched for monkeys in the area, especially within the home range of the study groups, for five days (between 24 and 31 January 2008) also trying to find monkeys carcasses fresh enough to determine the cause of death. We asked local residents for collaboration to search and report any dead monkey found in the area surrounding the park. On February 2, 2008, the same search procedure was carried out in Cruce Caballero Provincial Park (26°31′S, 53°59′W), a small protected area 30 km SW of El Piñalito, where a few groups of brown howlers are present [Di Bitetti et al., 1994]. Data on monkey mortality episodes from other localities of Argentina were gathered from reports of official sources and local residents.

On January 26, 2008, wildlife veterinarians of the government of Misiones performed a necropsy on an adult brown howler female (Penélope), found at El Piñalito within 24 hr of her death. The samples taken from Penélope, as well as all the other cases reported in this article, were analyzed by the INEVH (Instituto Nacional de Enfermedades Virales Humanas—Dr. Julio I, Maiztegui Institute, Pergamino, Buenos Aires, Argentina). The diagnosis of yellow fever was confirmed by isolation in cell cultures (*Aedes albopictus* C3/36), followed by direct immunofluorescence, RT-PCR generic for Flavivirus, and genome sequencing [INEVH, data not published].

We obtained data on rainfall and mean temperatures from meteorological stations located in Puerto Iguazú (25°35′S, 54° 34′W) and El Soberbio (27°17′S, 54° 12′W) for the 1999–2008 period. These data were used to explore whether the beginning of the YFOs could be associated with abnormal climatic conditions, as has been proposed [Vasconcelos et al., 2001].

All research reported in this manuscript adhered to the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates, and all research protocols were reviewed and approved by the Ministry of Ecology of the province of Misiones, Argentina. Moreover, all research reported in this manuscript complied with the protocols approved by the appropriate institutional Animal Care and Use Committee, and adhered to the legal requirements of Argentina, in which the work took place.

RESULTS

The temporal and geographical distributions of howler deaths reported in this study suggest the presence of two different YFOs: one occurring between November 2007 and April 2008 in northern and central Misiones, and another ocurring between October and December 2008 in southern and central Misiones (Fig. 1).

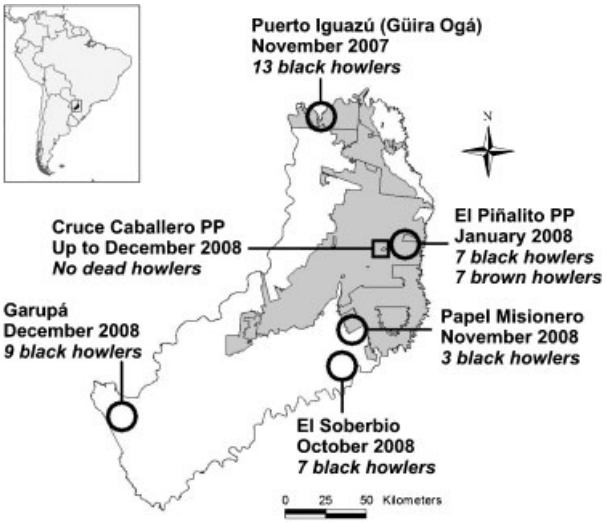

Fig. 1. Map of Misiones province, Argentina, showing localities, dates and number of dead howlers found during the two YFO reported in this manuscript (circles), and locality and date where no dead howlers were found in an intervening area (square).

In the spring of November 2007, 13 free-ranging black howlers died in the wildlife rehabilitation center 'Güirá-Ogá' (Puerto Iguazú, Misiones, 25°37′S,54°32′W; [A. Carvalho in litt., 2008], Fig. 1). This mortality event was not reported to the authorities and a confirmed diagnosis of yellow fever was obtained only after the findings of dead monkeys in El Piñalito led to the suspicion of yellow fever.

Between 20 and 31 January 2008, we found 14 dead howlers (seven from each species) at El Piñalito (Fig. 1). Twelve of them belonged to our study groups, which originally consisted of 36 individuals [Agostini, 2009]. All dead howlers, except for Penélope, were in advanced decomposition, and we estimated that these monkeys died between 10 and 25 January 2008. The bodies were not randomly distributed within their home ranges, but usually in clumps of several individuals with the same degree of decomposition, frequently at the base of a sleeping or feeding area, which suggests that their deaths were highly synchronous. With the exception of a 3-year-old and a 2-year-old-male juveniles of one of the brown howler groups observed together alive on 25 January, 2008, no howler monkeys have been observed or heard at El Piñalito since, despite efforts to locate them up to December 2008.

In the following six autumn–winter months, there were no further monkey mortality records in Misiones, until the spring of October 2008, when seven black howlers were found dead by a farmer at El Soberbio (27° 08′S, 54° 09′W, Fig. 1). All the dead monkeys were decomposed to a similar degree and dispersed within a range of 100 m, near a small river [J. Torresín in litt., 2008]. In November 2008, a park ranger found three dead black howlers in the Papel Misionero Reserve (26° 25′S, 54° 22′W, Fig. 1). In December 2008, at least nine black howlers were found dead in Garupá (27° 31′S, 55° 52′W, Fig. 1), 12 km from Posadas, the capital city of Misiones [INEVH in litt., 2009].

The presence of the yellow fever virus was confirmed by the INEVH from samples submitted from Güira Ogá, El Piñalito, El Soberbio and Garupá.

Other sites seemed to have been unaffected. No dead monkeys were found in Cruce Caballero Provincial Park, located between areas with confirmed YF howler casualties. On February 2, 2008, one group of seven brown howlers was seen near the park ranger's home. The same group was seen again on July 18, 2008 [A. Bodrati personal communication].

In total, 59 individuals of both howler species were found dead between November 2007 and December 2008 in Misiones. Taking into account that since the outbreak there have been no signs of howlers in El Piñalito, we also infer that all the individuals of the four groups under study (15 black howlers, 8 brown howlers and 1 putative hybrid [Agostini, 2009]) and many individuals from neighboring groups died during the mortality event in January 2008. The study and neighboring groups at El Piñalito were composed by adult males (5), adult females (10), subadult males (3), subadult females (3), juvenile males (6), juvenile females (5), infant males (4), infant female (1), and an infant putative hybrid (1). Since all presumably died during the YFO, we found no age–sex differences in susceptibility to the disease. Thus, we estimate a minimum of 83 casualties of both howler species in Misiones province. Six black howler monkeys were also found dead between October 2008 and February 2009 in the Argentine province of Corrientes, which borders Misiones to the South, and yellow fever was confirmed by the laboratory at least for one case [OPS, 2009].

No suspected deaths attributable to yellow fever were recorded for the other primate species widely distributed in Misiones, the tufted capuchin monkey (*Cebus nigritus*). A group of this species, composed of apparently healthy individuals, was observed at El Piñalito on 30 January 2008, when we were searching for howlers.

At the beginning of 2008, immediately after the first four howler monkeys were found dead in El Piñalito, we alerted the National Health Authority of Argentina. To avoid the spread of the epidemic to human population, a prompt and massive vaccination campaign was carried out in the area surrounding El Piñalito and the forested border with Brazil.

The weather in Puerto Iguazú and El Soberbio is, in general, very similar: rainfall since 1999 to 2008, show a positive and significant correlation ($r_s = 0.6319$, $P < 0.0001$), so does the average mean temperature ($r_s = 0.9133$, $P < 0.001$). We therefore, used only Puerto Iguazú records for comparison. For

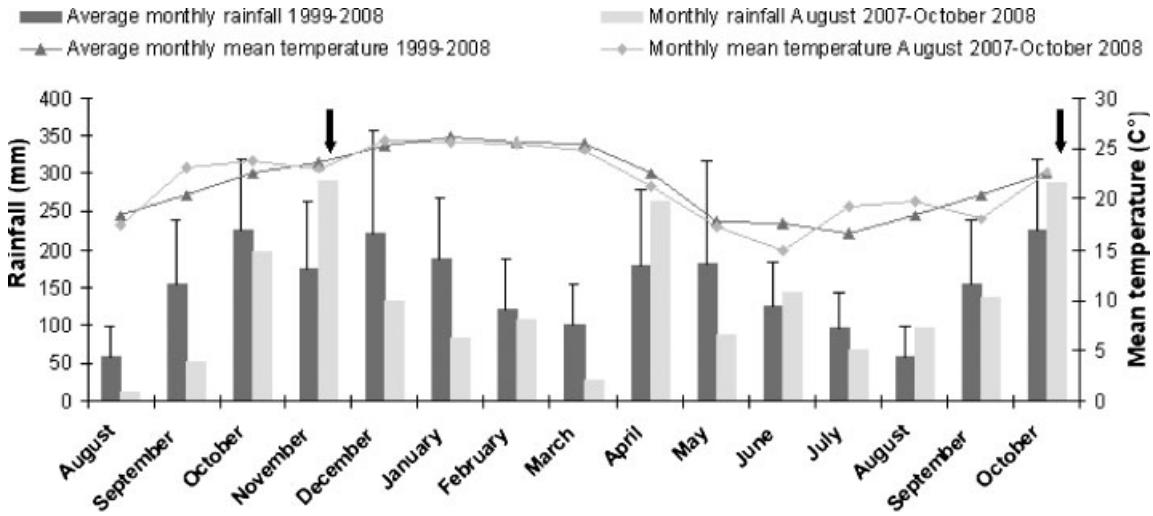

Fig. 2. Rainfall and mean temperature (+SD), during the YFOs (arrows) and average rainfall and mean temperature from 1999 to 2008. November 2007 and October 2008 show the highest rainfall with warm temperatures (over 20°C), but heavy rainfall for October 2008 was within the normal range of variation. April 2008 show similar rainfall and temperature values without YFO.

months in which YFOs started (November 2007 and October 2008), rainfall was higher than the Puerto Iguazú averages from 1999 to 2008 for those same months (Fig. 2). Also, these high values of rainfall were associated with warm mean temperatures (over 20°C). Rainfall in November 2007 was exceptionally high, however, for October 2008 rainfall was within the normal range of variation observed for this region.

DISCUSSION

In Argentina, 59 howler monkeys from two species, *A. g. clamitans* and *A. caraya*, were found dead within Misiones and another six *A. caraya* died nearby, in the province of Corrientes, during two YFOs that occurred in the spring–summer of 2007–2008 and again in the following spring–summer of 2008–2009.

Only two previous YFOs have been reported in Argentina. In 1966 (20 February–10 March), three brown howlers were found dead in Misiones and several yellow fever cases were reported in humans at that time [Crespo, 1974]. In 2001 (March–May), 80 brown howlers died of yellow fever near the Brazil–Argentina border [Viégas Sallis, 2003].

No dead capuchin monkeys were found, most likely because these monkeys are more resistant to the yellow fever virus, usually only developing clinical signs such as fever, but seldom dying [Strode, 1951]. On the other hand, howlers are highly susceptible, and even when inoculated with low quantities of the virus they rapidly develop the disease and die [Strode, 1951]. Howler monkeys act as sentinels for public health: their mass mortalities caused by their high susceptibility to yellow fever warn human populations of the presence of the disease [Butcher, 1991].

Species that maintain specific endemic pathogens are little affected by the disease they carry [e.g. Hazel et al., 2000]. In howler monkeys, yellow fever occurs in the form of epidemics that kill most, if not all, individuals infected. Thus, howler populations are unlikely to play an important role in maintaining this virus in the continent, and there is no evidence that the virus can remain endemically in their populations. In fact, the mosquitoes could be the main reservoirs of yellow fever, as they are infected throughout their life, and can transmit the virus transovarially through infected eggs [Tomori, 2004]. Humans and monkeys, on the other hand, play the role of temporary amplifiers.

Changes in weather conditions, like heavy rainfall combined with warm temperatures, have been related to YFOs [Vasconcelos et al., 2001]. Heavy rainfalls create the optimal situation for vector mosquito breeding and, as a consequence, for the expansion of the virus [WHO, 1985; Shope, 1997]. Both YFOs were discovered because of the death of howlers in months with heavy rainfall, in association with warm temperatures (Fig. 2). Climatic conditions in April 2008 were similar to the months when YFO started, but no YFO was reported, suggesting that high rainfall and warm climate are necessary, but alone are not sufficient conditions to trigger YFOs in the study area. Our results thus do not support a simple model for a relationship between rainfall/temperature and epidemic outbreaks, but instead point to multifarious causation of YFOs.

Other factors, like invasive human activities, could also contribute to the generation of appropriate conditions favoring YFOs [Vasconcelos et al., 2001]. Deforestation for cattle ranching and wood trade, flooding of extensive areas for dam constructions, and construction of highways dissecting forests

disturb the natural dynamics of the ecosystem, and are related to the emergence and reemergence of dangerous viruses that affect humans and wildlife [Vasconcelos et al., 2001].

The current expansion of yellow fever in South America raises concern about the potential conservation problems for susceptible non-human primate species in the continent, as in some species it causes drastic population reductions and genetic erosion. Before the YFOs were reported, the size of the brown howler population in Argentina was unknown, but certainly small, given the rarity of both howler species in Misiones [Di Bitetti, 2003]. The relatively high frequency of YFOs in the region (four major outbreaks reported since the mid 1960s) might contribute to the rarity of the species. After these outbreaks, with the local death of most individuals (as in El Piñalito), and the possibility of recurrent episodes of the disease, the conservation status of howler monkeys in Misiones is particularly worrying. This small and fragmented population suffering habitat destruction, hunting pressure and cyclical events of yellow fever is at a serious risk of disappearing in the long term [Di Bitetti et al., 1994].

To ensure the survival of howler monkeys, actions should be encouraged at four different levels. First, since humans are involved in the amplification of the virus, human vaccination should constitute a priority. This will reduce both the number of human cases and the sources of virus for mosquitoes and the transmission of the disease to other species. Second, environmental education programs should be developed to involve the local communities surrounding the protected areas. Local residents sometimes capture monkeys (especially howlers because of their peaceful temper) to keep or sell them as pets. As a collateral effect of the recent YFOs in Misiones, Brazil and Paraguay, some people have killed howler monkeys because they are thought to be the cause of the disease, ignoring the importance of conserving them to have an early warning system for the human population [Bicca-Marques, 2009]. Third, collaborative work should be conducted between the national and provincial–state governments that make decisions about the management of the protected areas, timber extraction, health, and construction projects that are likely to have major ecological effects. Fourth, collaborative and trans-disciplinary research should be carried out to shed light on the determinants of virus circulation, on the role of primate species in the eco-epidemiology of the disease, and on the impact that the virus has on the population dynamics and conservation of non-human primates.

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