**Conservation letter**

# The role of monkeys, mosquitoes, and humans in the occurrence of a yellow fever outbreak in a fragmented landscape in south Brazil: protecting howler monkeys is a matter of public health

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**Abstract**

A recent (2008-2009) outbreak of sylvatic yellow fever caused the death of seven people and over 2,000 howler monkeys (black-and-gold, *Alouatta caraya*, and brown, *A. guariba clamitans*) in the state of Rio Grande do Sul, Brazil, spreading panic among the population. The fear of the disease and the misinformation about its relationship with howler monkeys led inhabitants of several regions to exterminate these primates from the forests near their homes. In this paper we describe the theoretical background supporting the idea that howler monkeys play an important role in fighting yellow fever via the surveillance of virus circulation and stress that they are not responsible for the re-emergence of this African infectious disease, its transmission, or its fast spread through the highly fragmented landscape of the state of Rio Grande do Sul. We also describe how this scientific information has been used in the campaign "Protect our Guardian Angels" that was launched to inform the public and the media about the actual relationship of these regionally threatened species to the disease. The campaign is run and supported by educational, scientific, governmental (health- and environment-related) and religious institutions, and NGOs, an alliance in favor of biodiversity conservation and public health that has been effective in changing the quality of the news media, but that still requires a great effort to achieve the necessary level of population awareness.

**Key words:** infectious disease, conservation, howler monkey, *Alouatta*, Rio Grande do Sul.

**Resumo**

Um surto recente (2008-2009) de febre amarela silvestre causou a morte de sete pessoas e mais de 2000 bugios (pretos, *Alouatta caraya*, e ruivos, *A. guariba clamitans*) no Estado do Rio Grande do Sul, Brasil, espalhando pânico na população. O medo da doença e a desinformação acerca de sua relação com os bugios levaram habitantes de várias regiões do Estado a exterminar os macacos das matas próximas às suas casas. Nesse trabalho descrevemos a base teórica que permite afirmar que os bugios desempenham um papel importante no combate à febre amarela através da vigilância da circulação do vírus e salientar que eles não são responsáveis pela re-emergência dessa doença infecciosa africana, sua transmissão ou seu rápido avanço na paisagem altamente fragmentada do Estado do Rio Grande do Sul. Também descrevemos como essa informação científica está sendo utilizada na campanha "Proteja seu Anjo da Guarda", lançada com o intuito de informar a população e a mídia sobre a real relação entre esses primatas ameaçados de extinção em nível estadual e a doença. A campanha é conduzida e apoiada por instituições educacionais, científicas, governamentais (relacionadas à saúde e à proteção ambiental) e religiosas e por ONGs. Essa aliança em prol da conservação da biodiversidade e da saúde pública foi eficiente na mudança da qualidade da informação divulgada pela mídia, mas ainda requer um grande esforço para atingir o nível necessário de conscientização da população.

**Palavras-chave:** doença infecciosa, conservação, bugio, *Alouatta*, Rio Grande do Sul.

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## Introduction

Primate diversity in the Neotropics is currently estimated at 199 species and subspecies [1]. According to the IUCN Red List of Threatened Species, 40 of the 56 platyrrhine species listed as either Vulnerable, Endangered, or Critically Endangered occur in Brazil. The major threats faced by these primates include habitat loss and fragmentation, hunting, and, to a lesser degree, the illegal pet trade [2]. Only five monkey species—*Cebus nigritus* (black-horned capuchin monkey), *Alouatta caraya* (black-and-gold howler monkey), *Alouatta guariba clamitans* (brown howler monkey), *Brachyteles arachnoides* (southern muriqui) and *Leontopithecus caissara* (black-faced lion tamarin)—are indigenous to the forests of south Brazil. The first three species occur in the three south Brazilian states (Paraná, Santa Catarina and Rio Grande do Sul), whereas the last two only occur in Paraná [3-5]. While *C. nigritus* is classified as Data Deficient in Rio Grande do Sul, both *A. caraya* and *A. g. clamitans* (Figure 1) are Vulnerable because of habitat loss and fragmentation, hunting, and the pet trade [4]. Howler monkeys hold the same conservation status in Paraná [6]. A similar assessment is lacking for Santa Catarina.

Although neither primate belongs to the list of 54 threatened mammalian species that have an infectious disease (caused mostly by generalist viruses and bacteria) as a major threat to their conservation [7], disease outbreaks have impacted primate populations in several regions of the world [7,8]. A well-known example is the hemorrhagic fever caused by the Ebola virus that has significantly reduced some gorilla (*Gorilla gorilla*) and chimpanzee (*Pan troglodytes*) populations in West Africa [9,10]. In the New World, historical reports state that populations of both *A. caraya* and *A. g. clamitans* have been severely affected by yellow fever outbreaks [11,12]. More recently, outbreaks of this disease in 2001 and 2008-2009 caused the death of individuals of both species in Argentina [13] and the state of Rio Grande do Sul in Brazil [14]. Therefore, yellow fever outbreaks together with the aforementioned threats are likely to have a synergistic effect that may lead to an irreversible process of population erosion resulting in extinction, especially of populations isolated in fragments (see [7,14-17]).

Here we present a case study that shows how a yellow fever outbreak can compromise the conservation of howler monkeys in Rio Grande do Sul and how a fragmented landscape influences disease dynamics and the role of monkeys, mosquitoes, and humans on the spread and maintenance of the pathogenic agent. We also address the undesirable consequences that misinformation spread by the media can have on people's perception and attitudes toward monkeys. Finally, we describe the strategies adopted by a team of scientists in a campaign to release science-based information as a way of educating the public and promoting howler monkey conservation and public health.

**Fig. 1. Howler monkey species native to the state of Rio Grande do Sul. Top and bottom left: adult male and subadult female black-and-gold howler monkey (*Alouatta caraya*), respectively; top and bottom right: adult male and adult female brown howler monkey (*Alouatta guariba clamitans*), respectively. Photos by Júlio César Bicca-Marques.**

## Yellow fever outbreaks: the role of monkeys, mosquitoes, and humans

*Yellow fever*

Yellow fever is an arboviral disease originated in Africa whose infectious agent, a species of *Flavivirus* (family Flaviviridae), is transmitted by a mosquito vector [18-21]. The disease was brought to the New World in slave trade ships [18,20]. Georgiev [22] describes three types of transmission cycles: (1) the urban cycle, transmitted by the peridomestic mosquito *Aedes aegypti* (the same vector of dengue fever), which was eradicated from Brazil in 1942 [23]; (2) the intermediate cycle, characterized by the simultaneous occurrence of cases in separate villages in the African humid and semihumid savannas and low levels of death (this cycle is not mentioned in most publications); and (3) the sylvatic or jungle cycle, transmitted by *Aedes* and *Haemagogus* mosquitoes, respectively, in African and American (South and Central) forested regions (see also [18,23]).

Humans and monkeys are believed to be the major hosts [20,22], although a survey of 24 mammalian species in French Guyana found antibodies against yellow fever in seven non-primates (*Choloepus didactylus*, *Bradypus tridactylus*, *Tamandua tetradactyla*, *Dasyprocta leporina*, *Coendou* spp., *Eira barbara* and *Tayassu tajacu*) in addition to three primate species (*Alouatta seniculus*, *Pithecia pithecia* and *Saguinus midas*) via haemagglutination inhibition and seroneutralization, therefore increasing the array of species

that may be involved in virus maintenance [24]. Compared to Old World monkeys (infraorder Catarrhini), New World monkeys (infraorder Platyrrhini) are more susceptible to the disease [23]. Among platyrrhines, spider monkeys (*Ateles* spp.), squirrel monkeys (*Saimiri* spp.), capuchin monkeys (*Cebus* spp.), and titi monkeys (*Callicebus* spp.) are more resistant, whereas owl monkeys (*Aotus* spp.), marmosets (*Callithrix* spp.), tamarins (*Saguinus* spp.) and, particularly, howler monkeys (*Alouatta* spp. [21]) are more sensitive to the disease [23].

In Brazil, sylvatic yellow fever is common in the north and center-west regions and part of the northeast region [23]. Recent epizooties of sylvatic yellow fever in the states of Bahia, São Paulo, Paraná, Minas Gerais and Rio Grande do Sul have alarmed the Brazilian Ministry for Health [25-27]. Although humans are accidental hosts in the sylvatic cycle [23], human infections have been reported in several Brazilian states (Goiás, Amazonas, Bahia, Distrito Federal, Mato Grosso, Minas Gerais, Pará, São Paulo and Tocantins [28,29]).

The state of Rio Grande do Sul, recently considered yellow fever-free, was reclassified by health authorities as an area of transition [30,31] after immunohistochemical exams confirmed the death of black-and-gold howler monkeys due to sylvatic yellow fever in the western border of the state in 2001 [27]. As of July 2009, the 2008-2009 outbreak beginning in October caused the death of seven people and over 2,000 howler monkeys. Because biological samples must be collected no more than about six hours after death for analysis [23], only a small proportion of the monkeys found dead was sampled. Laboratory analyses of 308 dead monkeys diagnosed 180 as positive for yellow fever, confirming the epizooty in 67 municipalities (M. A. B. Almeida, pers. comm., 2 October 2009) but also indicating that about 40% of these howler monkeys did not die of yellow fever and were likely killed by misinformed people. Considering that most cases in which people kill the monkeys are probably not reported to local health and environmental officers (this is an illegal activity in Brazil), we consider this to be a conservative estimate of both the number of monkeys that died and the proportion of animals likely killed by people.

Compared to howler monkeys, humans are much less sensitive to the disease. According to Vasconcelos [21], only 5-10% of people develop the malign (toxic) form that may lead to death, 10-20% develop the severe form that produces fever and jaundice, 20-30% develop the mild or moderate forms and only present fever and headache, and the disease is asymptomatic in 40-65% of people. Additionally, people can be immunized against the disease with a vaccine that is effective 10 days after administration and provides at least 10 years of protection [22]. The vaccine is contraindicated under a few particular circumstances and has been related to rare cases of death from general organ failure, but "*the risk to unimmunized individuals either living in or traveling to areas where there is known yellow fever transmission is far greater than the risk of having a vaccine-related adverse event, and WHO* [World Health Organization] *policy on yellow fever vaccination remains unchanged, strongly recommending vaccination against yellow fever*." ([22], p. 267, italics in the original).

### *Potential of howler monkeys, mosquitoes, and humans as virus reservoirs and carriers*

Howler monkeys and vector mosquitoes (*Haemagogus leucocelaenus* in Rio Grande do Sul) are diurnal arboreal species that prefer the upper layers of the forest [32,33], thus facilitating disease transmission. Howler monkeys cannot be considered reservoirs responsible for the maintenance of the yellow fever virus in their habitat because of their high sensitivity to the disease. They often die between three and seven days after infection [23], and if they do not die within this time, they develop permanent immunity [21]. Consequently, howler monkeys, like other monkeys and humans, only act as virus amplifiers during this short time [21].

On the other hand, mosquitoes are both vectors and reservoirs [21,32]. Female mosquitoes can become infected with the virus by biting an infected host, but they can also obtain it vertically from their mothers in

the egg [34]. Once a female mosquito is infected, it remains with the virus throughout its life [21]. Furthermore, mosquito eggs are resistant to drying [32], thus allowing interannual virus maintenance [22].

Our knowledge of howler monkey ecology and behavior allows us to affirm that the monkeys are not responsible for the rapid spread of the disease through Rio Grande do Sul between the end of 2008 and the first half of 2009. Black-and-gold and brown howler monkey social groups may inhabit forest fragments or orchard forests as small as <1 ha and rarely use home ranges >15 ha [15,35,36]. In addition, compatible with the consumption of a highly folivorous, hard-to-digest diet [37], their activity budget is dominated by resting [15,33] and they travel on average only about 300 to 800 m a day within their small home ranges (day range rarely exceeds 1,000 m [15,35,36]). Moreover, although howlers may descend to the forest floor to cross canopy gaps [38,39], and dispersing individuals may even traverse open areas between isolated forests by travelling on the ground, data from mantled howlers (*Alouatta palliata*) living in a highly fragmented landscape in Mexico, similar to that found in Rio Grande do Sul, suggest a 200 m distance threshold for efficient dispersal [40]. This threshold is supported by findings of a survey of *A. guariba clamitans* in a fragmented landscape in Santa Maria, Rio Grande do Sul, that found four individuals out of 386 recorded in 21 forest fragments showing an abnormal, lighter pelage color [41]. All four individuals inhabited the same 20 ha fragment and belonged to three out of five resident groups. The distance between this fragment and the nearer five fragments ranged from 267 to 1,009 m. Fortes and Bicca-Marques [41] suggest that the fragment isolation distance, its high population density (2.2 howlers/ha), and the large size of resident groups (8.8 ± 2.4 howlers) may hamper successful immigration into this population, possibly leading to inbreeding and the expression of rare alleles. In addition to challenging isolating distances, dispersing individuals travelling on the ground may face hazards, such as roads, electric fences and water barriers, and are more vulnerable to humans, domestic animals, and wild predators. As a result, despite their capacity to travel on the ground, howlers appear to avoid it whenever possible. This dispersal limitation seems to be particularly strong for sick, febrile monkeys, whose locomotion skills are severely compromised by the yellow fever (F. E. Silva, pers. comm., 27 April 2009).

On the other hand, *Haemagogus* mosquitoes are much more likely to be responsible for the rapid dispersal of yellow fever in southern Brazil. Although these mosquitoes prefer the canopy in the forest interior [32], they can descend to ground level to bite humans (and other ground-dwelling mammals [24]) in forests disturbed by logging, road construction, and crop or pastureland establishment and when they reach high population densities [32]. *Haemagogus* mosquitoes can also cross 6 km (*H. leucocelaenus*) or 11 km (*H. janthinomys*) of open habitat [32]. This ability to leave the forest makes them eligible for passive long distance dispersal as adults, eggs or larvae inside trucks, buses, or airplanes, for example, as has been observed with *Aedes* mosquitoes [42,43]. Currently, however, we also cannot exclude the possibility, though unlikely, that a non-primate (still unknown) vertebrate host capable of dispersing through the fragmented agricultural landscape is responsible for the yellow fever spread.

Finally, our own species is the best candidate for having promoted the fast yellow fever spread through the state of Rio Grande do Sul because of the high proportion of undiagnosed asymptomatic people (and also of those developing the mild or moderate forms [21]) and because humans are highly mobile (see [44]). This is the main working hypothesis of the Brazilian Ministry for Health. Consequently, there is a real risk of disease reurbanization after 60 years or more, especially in cities showing high levels of *Aedes aegypti* infestation and low rates of human immunization. According to Beaty [42], the reurbanization of the disease in American cities infested with *A. aegypti* is a matter of when it will happen, not a matter of if it will. As seen above, howler monkeys do not qualify as virus reservoirs or disease carriers, but they do play a role of disease sentinel, as recognized by the Brazilian Ministry for Health. Because of their high sensitivity to the disease, the death of howler monkeys provides an early warning for health authorities of the need for vaccination campaigns to protect the human population ([45,46]; see also [47]). As such, howler monkeys play a critical role in helping to avoid the reurbanization of the disease and are essential for protecting people living in areas where the virus is circulating, but who are not or cannot get immunized. This public

health service provided by howler monkeys with no cost to humans is particularly useful considering the limited availability of yellow fever vaccine doses for promoting population immunization in large cities [42].

## The role of media in conservation biology and public health and the "Protect our Guardian Angels" campaign

The media can play an important role in conservation biology and public health by providing laypeople with science-based data. However, mainly because of its eagerness for speedy news delivery and sometimes a tendency to sensationalize news, it can spread incorrect and biased information to the audience, compromising biodiversity protection and public health (see [48,49]). This negative influence was evident during the first months of the yellow fever outbreak that afflicted the state of Rio Grande do Sul in 2008-2009. Because the news media were directly or indirectly linking howler monkeys to the occurrence and spread of the disease, people afraid of becoming sick started to kill them (probably by poisoning) in several localities (T. L. Codenotti, pers. comm., 2 April 2009; [14]). Powdering fruit eaten by the monkeys at orchard forests or forest edges near human habitations with poison, probably rodenticides or other easily obtained commercial poisons, is believed to have been used because it leaves no clues compared with shooting. Although illegal, this may have been the fate of about 40% of the howlers reported dead by local officers, hence aggravating their conservation status. The most affected monkeys are likely to be those belonging to small and isolated populations living near human habitations, which alone do not reach the minimum viable size theoretically required for long-term species survival, but whose extinction may increase isolating distances between subpopulations in an inhospitable matrix, further hampering the viability of a regional metapopulation dynamic ([14]; see [50]).

To stop the negative consequences of public misinformation on the conservation status of howler monkeys in Rio Grande do Sul, J. C. Bicca-Marques launched the internet-based campaign "Protect our Guardian Angels" on 3 April 2009. The name of the campaign is a metaphor intended to increase laypeople's understanding of the monkeys' role as disease sentinels. The campaign aimed at informing the population that howler monkeys do not transmit the yellow fever virus to people and that they are not responsible for the rapid spread of the disease in the state. In order to give the text of the campaign credibility it was signed by the author, and his academic position and professional affiliation details were included. The English translation of the released text, whose Portuguese version has been displayed in over 80 internet sites, is available on request from the senior author.

The first achievement of the campaign was the support received from 22 educational, scientific, governmental (health- and environment-related) and religious institutions, and NGOs. Only three weeks after its launch, the campaign had begun to be run by 12 local, regional, and national institutions, whose logos replaced the author's information released with the campaign's text [51]. The campaign also drew the attention of the Republic's Attorneyship from the Federal Prosecutor in the state of Rio Grande do Sul, who requested additional information both from J. C. Bicca-Marques and the state health surveillance center, with the objective of demanding that the appropriate governmental agencies take the necessary steps to enforce the environmental laws.

The activities of the "Protect our Guardian Angels" campaign include (1) the creation of a blog by David S. de Freitas (http://ameacafebreamarela.wordpress.com/), (2) radio, tv and newspaper interviews and public talks to different audiences by J. C. Bicca-Marques, (3) a theater piece titled "The howl, the buzz, and the yellow fever" created by Master's students Elenara Véras dos Santos and David S. de Freitas and voluntarily performed at several public places by graduate (PhD and Master's) zoology students and undergraduate biology students from the Pontifícia Universidade Católica do Rio Grande do Sul (Figure 2), (4) a movie produced by Med. Vet. Elisandro Oliveira dos Santos from the Zoológico Municipal de Canoas whose original and updated versions posted at YouTube® had over 20,000 views in the first three months (http://www.youtube.com/watch?v=b9VI10R8jkA&feature=related), (5) the production of posters by the

Secretaria Municipal do Meio Ambiente de Porto Alegre and Secretaria do Meio Ambiente from Rio Grande do Sul, (6) an exhibit at the science and technology museum (Museu de Ciências e Tecnologia) of the Pontifícia Universidade Católica do Rio Grande do Sul (Figure 2) and (7) the design and production of flyers by the Fundação Zoobotânica do Rio Grande do Sul (Figure 3, printed and handed-out to visitors of the foundation's botanical garden and zoo) and the Laboratório de Primatologia (Figure 4, released online only). The flyer aimed to warn of the need of continuing the campaign during the winter, a time when people tend to forget the disease because the lower temperatures cause the death of most mosquitoes and there is a significant decrease in virus circulation and disease-associated monkey death.

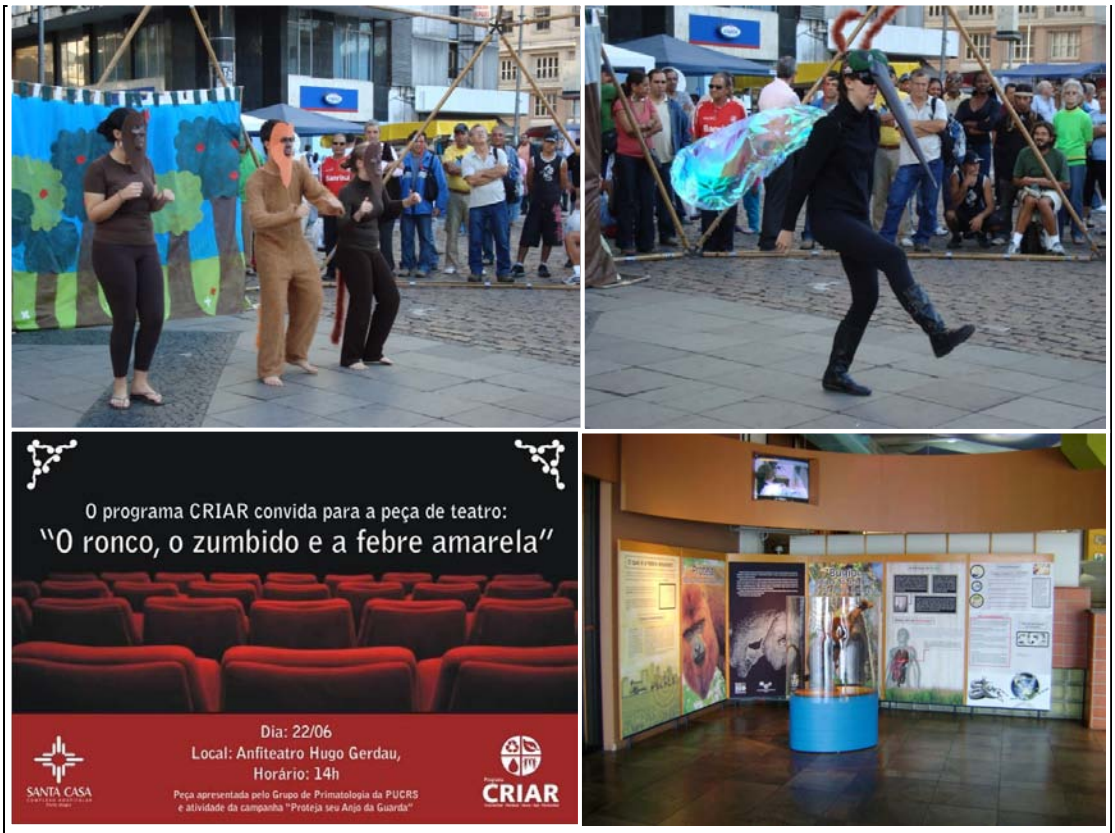


**Fig. 2. Top: scenes of the play "The howl, the buzz, and the yellow fever" (left - the adult female, the adult male and the young female brown howler monkey; right - the female mosquito); bottom left: public invitation for the play performed at a hospital theater; bottom right: exhibition at the Museu de Ciências e Tecnologia from the Pontifícia Universidade Católica do Rio Grande do Sul. Photos by Pedro Maria Abreu Ferreira (top) and Júlio César Bicca-Marques (bottom).**

The campaign partially achieved its goals by promoting a significant change in the way the media (at least in Rio Grande do Sul) represented the relationship between the howler monkeys and the yellow fever. Later news reporting the death of howler monkeys no longer treated them as responsible for the infection of the mosquitoes. But, as of July 2009 there were still reports of monkey harassment, indicating that part of the rural population was not reached or sensitized by the campaign or did not trust the "new," scientifically based information.

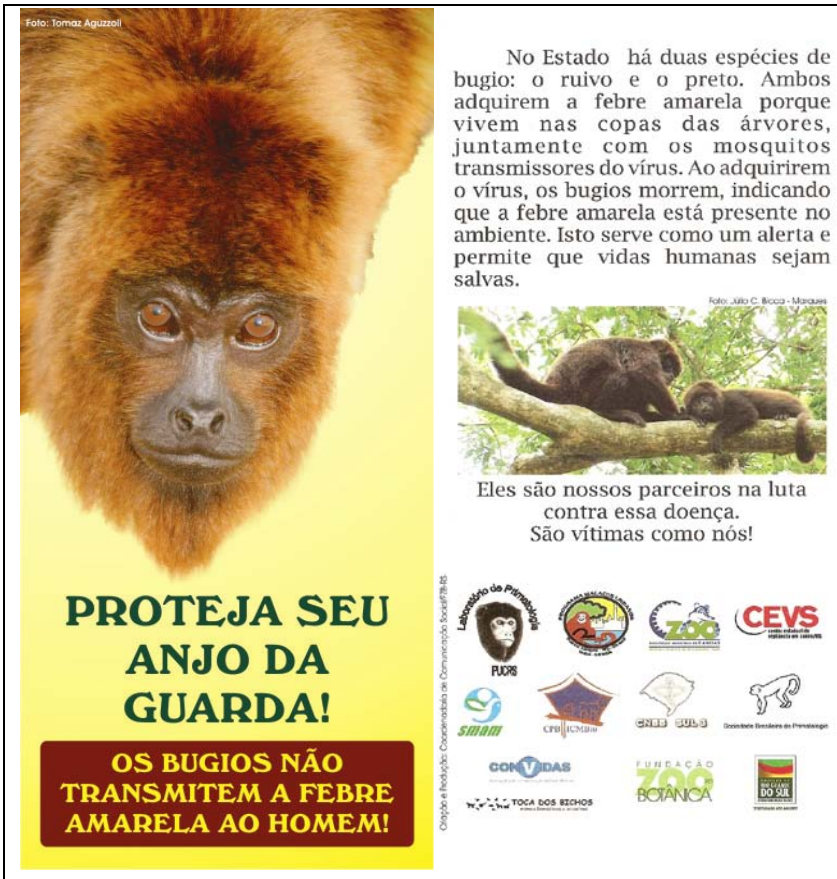


**Fig. 3. Flyer developed at the Fundação Zoobotânica do Rio Grande do Sul (text by Márcia Maria de Assis Jardim) including the logos of the 12 institutions that ran the campaign. The translation of the text is: Front - Protect our Guardian Angels! Howler monkeys do not transmit the yellow fever to people! Back - There are two species of howler monkeys in the state: the black-and-gold and the brown. Both contract the yellow fever because they live in the canopy together with the mosquitoes that transmit the virus. Howlers die when infected, showing that the yellow fever is present in the environment. This serves as a warning, enabling the saving of human lives. They are our partners in fighting this disease. They are victims like us!**

## Conclusions

The "Protect our Guardian Angels" campaign allied a public health problem to environmental conservation and transmited a science-based message to laypeople in an accessible language. Valuing the co-involvement of multiplier agents from different fields, such as academic institutions, governmental agencies, NGOs, veterinary clinics, and the Catholic Church, was essential to legitimize the information released, amplify its outreach, and improve media news.

In addition to continuing the public sensitization campaign, four main strategies need to be implemented to avoid the spread and the reurbanization of the disease. First, it is important to focus on targeting the infectious agent [52] by stimulating authorities to invest in yellow fever vaccine production and promote large-scale campaigns of human vaccination [21,53]. This option is not available for howler monkeys because of the risky, time-consuming, and expensive logistics that would need to be invested in their capture and their high sensitivity to the virus. Second, it is crucial to protect howler monkeys because of their important role as wild sentinel hosts. Third, there is a need to target the environment [54] by reducing the urban microhabitat available for *A. aegypti* proliferation (see [21]). Finally, researchers should investigate the role the dispersal of wild mosquito vectors play on the spread of the disease and, if appropriate, avoid it as proposed by Wobeser [55].

In sum, furtive killing of howler monkeys has harmful consequences to human health in both the short and the long-term. In the short term it gives a false alarm of virus circulation that misguides disease surveillance efforts and results in the inappropriate allocation of time and financial resources in urgent vaccination campaigns. In the long term it eliminates the species that first warn health officers about virus circulation.

The absence of these species from their habitat increases the likelihood of disease reurbanization and reduces the opportunities for protecting people who cannot get immunized. Therefore, protecting howler monkeys is not only a matter of biodiversity conservation, it is a matter of public health. By the same token, getting immunized is both a matter of public health and biodiversity conservation.

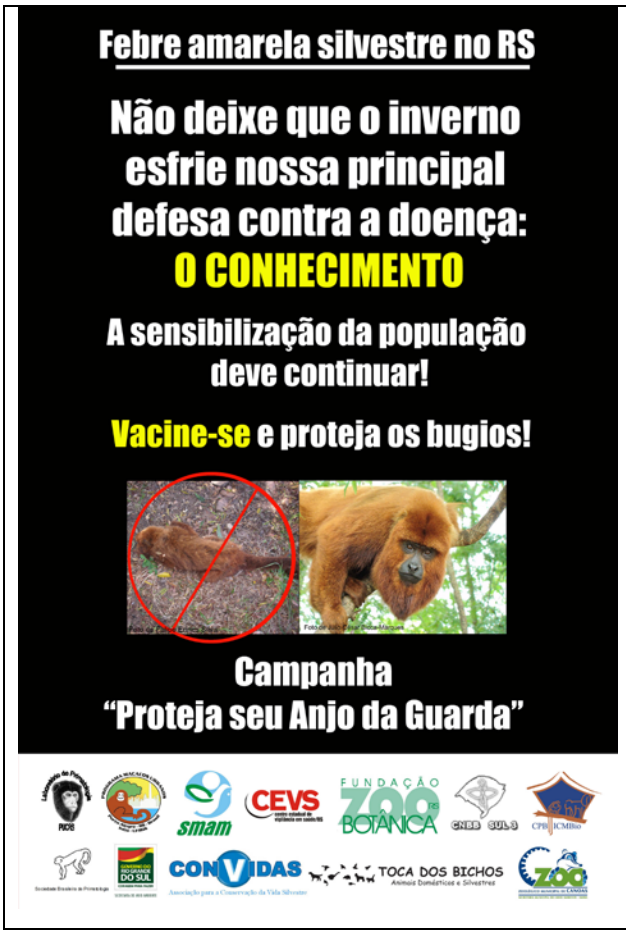


**Fig. 4. Flyer developed at the Laboratório de Primatologia from the Pontifícia Universidade Católica do Rio Grande do Sul for stimulating the continuation of the public sensitization (text by Júlio César Bicca-Marques, art design by David Santos de Freitas). The translation of the text is: Sylvatic yellow fever in the RS; Do not let the winter cool our main defense against the disease: the knowledge; Population sensitization must continue!; Get immunized and protect howler monkeys! Campaign "Protect our Guardian Angels".**

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