FULL ARTICLE

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The role of pacas of captivity as a potential reservoir of zoonotic fungi in Acre, Western Amazon, Brazil

O papel de pacas de cativeiro como potencial reservatório de fungos zoonóticos no Acre, Amazônia Ocidental, Brasil

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ABSTRACT

Wild animals can be natural reservoirs of different microorganisms, essential for monitoring these pathogens for the generation of knowledge and creation of tools aimed at programs for the prevention and control of infectious diseases, including zoonoses. The objective was to report the fungal diversity in the skin of pacas in captivity in Acre, Western Amazon, Brazil. Twenty-six animals were evaluated, from which skin samples were collected by superficial scraping, hair avulsion, and sterile plastic brush. The samples were seeded on Mycosel agar, and the phenotypic characteristics of the colonies were analyzed. In 80.8% of the samples, different fungi were isolated, from the genera *Candida*, *Microsporum,* and *Trichophyton*, among others. This is the first description of the identification of fungi in the skin of pacas and suggests that these animals can be considered essential reservoirs of saprophytic or pathogenic microorganisms with zoonotic potential in the Western Amazon.

Keywords: Wild rodent. *Candida*. *Microsporum*. *Trichophyton*. One Health.

RESUMO

Animais silvestres podem ser reservatórios naturais de diferentes microrganismos, sendo fundamental o monitoramento destes patógenos para a geração de conhecimento e criação de ferramentas direcionadas a programas de prevenção e controle de enfermidades infecciosas, incluindo as zoonoses. Assim, objetivou-se relatar a diversidade fúngica da pele de pacas criadas em cativeiro no Acre, Amazônia Ocidental, Brasil. Foram avaliados 26 animais, dos quais amostras cutâneas foram colhidas por raspagem superficial, avulsão pilosa e escova plástica estéril. As amostras foram semeadas em ágar Mycosel e as características fenotípicas das colônias foram analisadas. Em 80,8% das amostras houve isolamento de diferentes fungos, dos gêneros *Candida*, *Microsporum* e *Trichophyton*, dentre outros. Esta é a primeira descrição da identificação de fungos na pele de pacas e sugere que estes animais podem ser considerados importantes reservatórios de microrganismos saprófitas ou patogênicos, de potencial zoonótico, na Amazônia Ocidental.

Palavras-chave: Roedor silvestre. *Candida*. *Microsporum*. *Trichophyton*. Saúde Única.

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Introduction

Developing systems for rearing wild animals has aroused producers' interest in many Brazilian regions. Among the appreciated species, the paca (*Cuniculus paca* Linnaeus, 1766) has been used in meat production, gaining ascension in the consumer market, especially in the Amazon (Ribeiro et al., 2016). However, some factors may threaten the productivity and conservation of these animals, as well as a general concern for One Health (Rahman et al., 2020).

Subsistence hunting is a fundamental activity among traditional populations of the Amazon region, considering that the consumption of wild mammals is the primary nutritional protein source (Souza et al., 2021). However, the lack of health education and sanitary control programs increases the risk of contamination and disease transmission. In this context, paca consumption is essential in the cycle of infectious diseases, such as neotropical echinococcosis (Souza et al., 2022).

Implementing and regularizing paca farms in the Amazon is a relevant action to reduce hunting pressure and, consequently, collaborate with the conservation of these animals (Chaves et al., 2018). However, the cultural issues intrinsic to the region, the high cost of meat from these productions, the lack of environmental public policies, and the deficiency in inspections still favor the maintenance of illegal trade in these localities (Mendes, 2020).

In addition to the above information, the proximity between humans and wildlife enables diverse ecological changes, such as the dispersal of fungi, which are microorganisms all over the planet. About 400 species are considered pathogenic to animals and humans, especially in geographic areas with tropical climatic conditions (Chaturvedi et al., 2018). The Brazilian Amazon is the primary biome holding a variety of fungi and reveals factors propitious for the emergence and reemergence of fungal zoonoses (Zanella, 2016).

Wild animals, in general, can be natural reservoirs of infectious agents. Some wild rodents have already been reported as carriers of fungi (Losnak et al., 2018), but epidemiological data are still scarce. Thus, monitoring the fungal microbiome of pacas is critical for knowledge generation and creating tools for fungal zoonosis prevention and control programs.

In this context, we aimed to identify the fungal diversity in the skin of pacas raised in captivity in Acre, Western Amazon, Brazil.

Material and Methods

This study was approved in the Biodiversity Authorization and Information System, under registration number 58122, and authorized by the Ethics Committee on Animal Use of the Federal University of Acre (Ufac), under registration number 47/2017.

The animals came from the Wild Animal Breeding Facility of the Catuaba Experimental Farm of Ufac, located in Senador Guiomard, state of Acre (10º09'08" S and 67º44'21" W), Western Amazon, Brazil. The accommodation consisted of sheds with stalls to accommodate one male and three females, in a semi-covered area, with a feeder, swimming pool, and burrows made of logs. The food consisted of fruits, grains, tubers, vegetables, and berries (Figure 1).

Twenty-six pacas, male (11/26) and female (15/26), young adults (between one and five years old), were selected and subjected to skin sample collection (Figure 2) by superficial scraping, hair avulsion, and application of sterile plastic brushes (Cubas et al., 2014). Three samples of each animal were collected from different anatomical regions (head, trunk, and limbs), totaling 78 specimens.

Figure 1 – Pacas were raised in captivity in Acre, Amazon, Brazil.

In duplicate, the samples were sent under refrigeration to the Laboratory of Infectious Diseases of Animals at Ufac for sowing in Mycosel agar (Ludwig Biotecnologia®, Alvorada - RS, Brazil). The plates were incubated in a B.O.D. (Biochemical Oxygen Demand) oven at 30°C for up to 30 days. The growth of fungal colonies was observed daily for analysis of phenotypic characteristics. Microscopy was performed with lactophenol blue dye and visualization in 40 and 100x magnification (Quinn et al., 2018).

Complementary tests were also applied, such as capillary perforation test, microculture on cornmeal agar, urease, germ tube and catalase tests, capsule research, chromogenic tests, and others (Brasil, 2013). The data obtained were analyzed by observational descriptive statistics, cross-sectional, with a quantitative approach.

Results and Discussion

As a result, in 80.77% (21/26) of the sampled animals, yeast and filamentous fungi were identified and classified taxonomically within seven genera (Table 1, Figure 3). The genera and species identified were *Aspergillus* sp., *Candida* sp., *C. albicans, Malassezia pachydermatis, Microsporum gypseum, Mucor* sp., *Penicillium* sp., *Trichophyton mentagrophytes and T. rubrum* (Table 1). *Malassezia pachydermatis* showed the highest isolation frequency (30% - 7/21). In 38.1% (8/21) of

Table 1 – Fungi isolated from the skin of *Cuniculus paca* (Linnaeus, 1766) were raised in captivity in Western Amazonia, Brazil

Fungal isolates	Frequency (n=21)
Aspergillus spp.	9.52% (02)
Candida spp.	14.29% (03)
Candida albicans	9.52% (02)
Malassezia pachydermatis	33.33% (07)
Microsporum gypseum	4.76% (01)
Mucor spp.	4.76% (01)
Penicillium spp.	9.52% (02)
Trichophyton mentagrophytes	4.76% (01)
Trichophyton rubrum	9.52% (02)

Figure 2 – Collecting skin samples from pacas by superficial scraping (A), hair avulsion (B), and applying a sterile plastic brush.

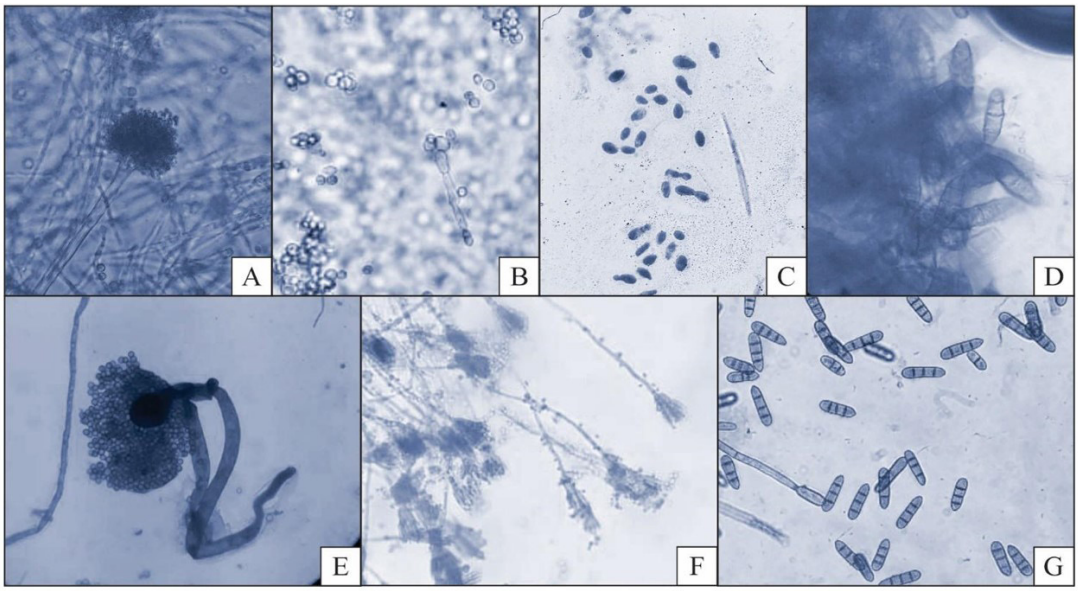

Figure 3 – Fungi isolated from skin samples of *Cuniculus paca* (Linnaeus, 1766) raised in captivity in Acre, Western Amazon, Brazil. (A) *Aspergillus* spp.; (B) *Candida* spp.; (C) *Malassezia pachydermatis*; (D) *Microsporum gypseum*; (E) *Mucor* spp.; (F) *Penicillium* spp.; (G) *Trichophyton* spp. Magnification 100x, lactophenol blue.

positive samples, two or more different fungi were isolated from the same animal.

The yeasts predominated about the frequency of isolates (57.14%-12/21). As for the septate filamentous fungi, there was a predominance of hyalohyphomycetes (38.09%-08/21), with no record of phaeohyphomycetes. Only one genus was identified of the non-septate filamentous fungi (zygomycetes) (4.76%-01/21).

No other studies on fungi identified in the skin of pacas were found in the national or international literature to corroborate the present study for a better understanding of microbial dynamics. However, in Brazil, the studies by Losnak et al. (2018) and Scramignon-Costa et al. (2021) used molecular techniques to analyze biological samples from roadkill wild animals for the eco-epidemiological description of fungal infections. Among the results, in the work of Scramignon-Costa et al. (2021), one paca was positive for *Paracoccidioides brasiliensis* in lung tissue. Regarding rodents in general, it is known that they can be considered potential disseminators of fungi to other susceptible hosts, either by direct contact, fomites, or surfaces (Guimarães et al., 2014; Thomas et al., 2012).

A systematic review by Seyedmousavi et al. (2015) described the main neglected and potentially zoonotic mycoses to animals and humans. Among the fungi cited, *Microsporum* spp., *Trichophyton* spp., *Malassezia* spp., *Cryptococcus neoformans* and *C. gattii*, *Coccidioides immitis,* and *C. posadasii* were reported in wild mammals. For rodents, synanthropic and wild specifically, *Sporothrix schenckii* and *S. brasiliensis*, *Talaromyces marneffei*, *Emmonsia* spp., and *Pneumocystis carinii* have been identified in different biological samples. This fungal variability, associated with the data from the present study, reinforces the role of wild animals, including rodents, as potential reservoirs and their relevance in the health monitoring of these individuals.

M. gypseum, T. mentagrophytes, and *T. rubrum*, detected in this study, are geophilic fungi that cause dermatophytosis, a highly contagious zoonotic disease that is a burden to public health and requires special attention from veterinarians and animal caretakers. Kraemer et al. (2013) asserted that species of the *T. mentagrophytes* complex are the main dermatophytic agents affecting rodents, such as *Cavia porcellus* (Linnaeus, 1758), and are susceptible to adaptation in new hosts, including humans (Bloch et al., 2016).

C. albicans and *M. pachydermatis* are opportunistic yeasts commonly found in the cutaneous microbiota that may be associated with dermatopathies in immunocompromised animals and humans. The genus *Candida* is little investigated in domestic and wild animals (Alves et al., 2017) but quite incident in humans, with profound public health implications, mainly when it entails systemic infections, such as *C. auris*, which has been generating recent concern globally (Lone & Ahmad, 2019). *Malassezia* sp. has a high occurrence in domestic animals' skin and ear ducts but has rarely been reported in rodents and logomorphs (Galuppi et al., 2020). In the present study, this yeast was isolated at a higher frequency, raising the possibility of its physiological colonization in the skin of pacas, as in other species.

Aspergillus sp., *Mucor* sp., and *Penicillium* sp. are saprophytic fungi of low pathogenicity. However, their spores can eventually penetrate injured body surfaces or be inhaled, resulting in superficial or systemic mycoses, especially with risk factors (Carobeli et al., 2019). *P. marneffei*, for example, has been identified as causing severe systemic mycosis in wild rodents and humans in Thailand (Ajello et al., 1995).

It is observed that, in recent decades, there has been growth in morbidity and mortality rates associated with mycoses. Some of these diseases are zoonotic and present themselves in an emerging and re-emerging form, although still neglected in the context of One Health (Chaturvedi et al., 2018). Thus, studying fungal pathogens in wild populations, whether in situ or ex-situ, is fundamental to generating knowledge and creating tools for disease prevention, control, and monitoring programs and public and animal health policy-making (Cubas et al., 2014).

Conclusions

This is the first description of the isolation and identification of fungi in skin samples from pacas and suggests that these animals may be considered essential reservoirs of saprophytic or pathogenic microorganisms with zoonotic potential in Western Amazonia. It is recommended that attention be paid to sanitary measures in farms, aiming at the conservation of wildlife, as well as neutralizing risk factors, avoiding the active dissemination of infectious agents, and preventing diseases.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics Statement

This study was approved in the Biodiversity Authorization and Information System, under registration number 58122, and authorized by the Ethics Committee on Animal Use of the Federal University of Acre (Ufac), under registration number 47/2017.

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