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## POINT OF VIEW

# ON THE UNKNOWN ECOLOGICAL NICHE OF *Paracoccidioides brasiliensis*. OUR HYPOTHESIS OF 1989: PRESENT STATUS AND PERSPECTIVES

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## SUMMARY

In 1989, CONTI DÍAZ & RILLA published a hypothesis concerning the as yet unknown ecological niche of *Paracoccidioides brasiliensis*.

The hypothesis proposed a highly efficient paracoccidioidal ecological strategy based on an important natural reservoir of the parasite, probably in heterothermic animals from fresh water environments. Further, the hypothesis proposed, a transient and variable residence in the soil with a wide aleuriospore dispersion throughout the environment together with an elevated capability of infecting humans, and domestic and wild animals.

This paper analyzes scientific publications from the IX International Meeting on Paracoccidioidomycosis held in Águas de Lindoia, São Paulo, Brazil from 2-5 October 2005, providing a comparative study among this articles and with other recently published papers and the hypothesis' postulates.

Since various findings and observations appear to agree with the postulates, the pursuit of novel, specific research projects in the supposed reservoirs is recommended partially or fully to confirm the hypothesis using classical laboratorial methods and modern molecular biology techniques.

**KEYWORDS:** Ecological niche; *Paracoccidioides brasiliensis*; Animal reservoirs; Hypothesis.

## INTRODUCTION

The ecological niche of *Paracoccidioides brasiliensis*, the etiological agent of paracoccidioidomycosis (PCM) is still unknown. This lack of knowledge has hindered the adoption of preventive measures to avoid new infections[3], and efforts attempting to solve the problem, are more than welcome. There is a similar lack of information on other fungi such as *Blastomyces dermatitidis*[5] and *Lacazia loboi,* and also on *Rhinosporidium seeberi* currently considered to be an aquatic protist, and on *Phytium insidiosum,* now included in the Kingdom Straminipila. Curiously, the corresponding clinical cases in these latter diseases, and also in PCM, appear to be closely associated with sources of fresh water, neighboring vegetation and activities developed mainly in such rural environments.

Reports on the isolation of *P. brasiliensis* from soil samples are scarce and generally cannot be repeated in material from the same location, which signifies that soil is most probably not the true permanent habitat of the pathogen. Further, the only isolations of the pathogen from animals considered eco-epidemiologically significant are those obtained from armadillos[1,4]. Reports on the isolation of *P. brasiliensis* from a penguin, bats and a squirrel monkey have not been confirmed[1].

In 1989, we presented a hypothesis on the ecological niche of the fungus[12] that, while not corroborated, is supported by several findings and observations[1,4].

During October 2-5 2005, the IX International Meeting on PCM was held in Águas de Lindoia, Brazil when various interesting research papers were presented on ecological aspects of the disease and on the pathogen itself.

The main objective of this report is to provide a critical analysis of those findings, and of other recent publications on the subject, furnishing a comparative study among them and with the postulates of our original hypothesis, to evaluate future of the hypothesis with the aid of recent important technological advances in molecular biology.

### The hypothesis[12]

Our hypothesis is based essentially on several well-known

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observations, including the relationship of the probable habitat of the pathogenic agent with rivers and other humid locations[1,4,11,20,22,26]; the high rates of paracoccidioidin skin reactions among foresters felling native trees in Uruguay; the occupations of most PCM patients in the country[11]; the proven deleterious effects of drying and biological competition on *P. brasiliensis*[9], findings that render it difficult to accept the pathogen's remaining in the soil without some form of protection; and the dimorphic nature of the fungus, which may have been used by the pathogen to survive in nature.

The hypothesis postulates that *P. brasiliensis* would normally occur in nature in fresh water environments, such as rivers or streams lined with native riparian vegetation, protected by one or more species of heterothermic aquatic animals such as amphibians, mollusks, fish and arthropods, integrating with high efficiency into a special biocenosis or specific biota[28]. Such animals would provide the parasite with nutrients, humidity, limited biological competition, and so on, that are difficult to obtain from soils for example. Also, they would offer the fungus an appropriate temperature range considered to be remarkably constant[17] between 20 and 21 ºC (BORELLI)[7]. In these aquatic animal reservoirs, *P. brasiliensis* may perhaps adopt a yeast-like or a transitional (pseudohyphal) parasitic form.

To be capable of infecting humans and animals, *P. brasiliensis* must necessarily be released from such reservoirs and survive for variable periods of time in the natural environment (soil, vegetables, etc.) in its mycelial sporulating phase. Water birds often abundant around streams and rivers may be responsible for dispersing the pathogen throughout the environment after consuming the reservoir organisms and depositing the fungus on pastures, vegetables, nests, trees, other water reservoirs and coffee, sugar cane and tobacco plantations.

Winds would probably not only favor the dissemination of aleuriospores, increasing the chances of infection of accidental hosts, but also induce reinfection of the animal reservoirs themselves.

The hypothesis also postulates that domestic animals such as cattle, horses and sheep[10,15] as well as armadillos[24] would become infected during their foraging activities in locations near riparian forests through the inhalation of propagules.

Finally, the hypothesis considers the possibility of paracoccidioidal infection in monkeys owing to their arboreal activities in riparian forests. One report of PCM disease in a monkey was registered prior to the publication of the hypothesis[21].

## The hypothesis and recent studies on *P. brasiliensis* ecology

### - Soil as a habitat of *P. brasiliensis*

Some papers presented at the IX International Meeting in Brazil insist on this notion[31,32]. However, the very small number of isolations of the pathogen from soil samples despite many attempts, usually not repeated in samples taken from the same localities, makes it difficult to accept soil as the pathogen's permanent habitat. Probably *P. brasiliensis* resides only transiently and for a variable time in the soil, an idea shared by FRANCO *et al.*[19].

Some types of soils would be more favorable than others for the production of conidia (aleuriospores) according to TERÇARIOLI *et al.*[32], thus increasing the efficiency of the parasite's ecological system in terms of persistence in the environment and the capacity to infect humans and animals.

### - Armadillos and *P. brasiliensis*

The recent isolations of the agent from *Dasypus novemcinctus* and the relationship of this armadillo with riparian forests[1,3,4] certainly confirm the hypothesis' premises in this regard.

However, armadillos should be considered as accidental hosts of *P. brasiliensis* and not as natural reservoirs as assumed in several papers presented at the meeting[1,4,29]. Properly speaking, "reservoirs" are living organisms able to harbor an etiological agent without manifesting the disease, and exhibiting the capacity to transmit it to humans or other animals either directly or indirectly[18]. Obviously this is not the case with armadillos and *P. brasiliensis.* On the contrary, humans and armadillos would be better considered as sentinels of the fungus.

### - Efficiency of *P. brasiliensis* ecological strategy

Our hypothesis postulates a highly efficient ecological strategy by *P. brasiliensis* which has allowed its survival for millions of years, and also for maintaining the paracoccidioidal human disease for more than 1000 years[26]. To attain such efficiency, the fungus would require important natural reservoir and an elevated facility for aleuriospore dispersion in the environment together with the capacity of infecting humans and animals.

Some of the papers presented at the last Meeting in Brazil refer to important and novel aspects of *P. brasiliensis* ecological efficiency, opening new and interesting avenues of discussion.

**a) The lengthy survival of the species:** SAN BLAS accepts the existence of *P. brasiliensis* on earth for at least 128 million years, spending 67 million years as a saprophyte in the mycelial phase, adding further that, in the Paleocene, the fungus may have shifted to its yeast phase owing to a rise in ambient temperature. It was then able, according to SAN BLAS, to infect armadillos, one of which *Dasypus novemcinctus,* is currently proposed to be the main reservoir of *P. brasiliensis* in Nature (sic)[29].

To me, it seems that the very well-known, labile characteristics of the pathogen, its susceptibility to drying and biological competence in the mycelial phase[9,26], make it very difficult to accept such a lengthy survival in nature exclusively under such a form. More probably, early in its evolution the pathogen may have procured some form of protection from adverse environmental conditions within other living organisms, possibly heterothermic aquatic animals as suggested in our hypothesis[12].

Interestingly, the relationship between species of *Chytridiomycetes*, aquatic fungi among the most primitive within the Kingdom Fungi, and amphibians is well known[23].

**b) Diffusion of *P. brasiliensis* and its aleuriospores into the environment and its capacity to infect humans and animals:**

- The discovery of clinical cases of PCM in patients not obviously exposed to the pathogen, one of whom was an 18 month old boy[6], as well as others in areas subjected to extremely hot, dry weather like

northern Bahia[8], agrees with the notion of wide dispersion of conidia, possibly from the supposed natural reservoirs during short periods of time. Also, according to our hypothesis, water birds might be responsible for transporting and depositing the propagules in such unexpected localities.

- The demonstration employing PCR techniques of the presence of *P. brasiliensis* in stools and soil samples from infected armadillo burrows[3], and from road-kill where animals from endemic areas of PCM are encountered[27] also corroborates the presumption of a wide dispersion of conidia.

- Similarly, the finding of new cases of PCM in armadillos, one of them on an island[3] supports this idea. The constant rooting behavior of these animals during their nocturnal activities also must favor the possibility of infection.

- The high paracoccidioidin skin incidences in horses evaluated using the ELISA technique[13], in cattle by the same method[31], in free-living monkeys with 64% seropositivity by ELISA[14] all confirming previous results of intradermal skin tests[10,15,16], and in dogs[30] also confirming previous studies[25] are consistent with the notable efficiency of the paracoccidioidal ecological system. While infection in horses, cattle and sheep[15] would be favored by their frequent grazing on areas near rivers or other water courses with native riparian vegetation, the likelihood of infection in monkeys may be increased by their frequent sojourns into trees and vegetation.

### Perspectives for corroboration of the hypothesis' postulates in the near future

Given the different aspects analyzed above, we believe in the need for and convenience of encouraging new research projects to corroborate or disprove the hypothesis proposed.

Considering this main objective, such projects should develop different laboratorial studies of susceptibility and a thorough search for the parasite should be made in some of the suspected heterothermal aquatic animals. Although this is a difficult undertaking, it might be facilitated by the use of modern molecular biology techniques, well-developed in laboratories throughout our region.

Such projects would open new means for improving our knowledge of *P. brasiliensis'* ecology so limited at present. Further, comparable projects on other fungi similarly or even less well understood today such as *Blastomyces dermatitidis* or *Lacazia loboi,* and fungal-related organisms such as *Rhinosporidium seeberi* or *Phytium insidiosum*, may be useful to decipher their respective ecological niches, apparently also associated with aquatic environments.

## FINAL COMMENTS

The ecological niche of *Paracoccidioides brasiliensis, the* agent of paracoccidioidomycosis (PCM) is unknown at present and this lack of knowledge has hindered the adoption of preventive measures to avoid new infections.

In 1989, a hypothesis was presented concerning the ecological niche of the fungus that has not been confirmed but which appears to be supported by recent findings.

This paper critically analyzes articles published on the subject, including those presented at the IX International Meeting on PCM held in Águas de Lindoia, Brazil, from October 2-5, 2005, providing a comparative study between them and the hypothesis postulated, attempting to ascertain the likelihood of confirmation in the near future with the aid of important modern molecular biology techniques.

The rare isolations of *P. brasiliensis* from soil samples generally not confirmed in repeat samples taken from the same localities together with the proved susceptibility to drying and biological competence of the fungus, make it virtually impossible to accept soil as the permanent true habitat of the pathogen. More likely, *P. brasiliensis* may reside only a transiently and for a variable period in the soil as suggested in the hypothesis.

Novel isolations of the agent from armadillos in humid, disturbed, riparian vegetation certainly confirm the hypothesis' presumptions in this regard. However, armadillos should be considered accidental hosts and not natural reservoirs as assumed by most authors.

The high efficiency of the paracoccidioidal ecological strategy postulated in the hypothesis mentioned is borne out by the survival of the agent for more than 130 million years, and the maintenance of the paracoccidioidal disease for more than 1000 years. To attain such efficiency, and according to the hypothesis, the fungus would require an important natural reservoir, probably in heterothermic aquatic animals, and also a high capability for the dispersion of aleuriospores in the environment with the capacity to infect humans and animals. Several recent observations seem to confirm this latter supposition. Among them, the demonstration of the fungus in different substrates, the finding of high rates of paracoccidioidal infection in various domestic and wild species, and the diagnosis of PCM in persons not obviously exposed and in others from areas showing extremely hot, dry weather (northern Bahia, Brazil).

The convenience of undertaking field and laboratorial studies employing classical and molecular biology techniques in the suspected animal reservoirs emphasized, in an attempt to solve the problem, may possibly allow the application of appropriate preventive measures.

## RESUMEN

### Sobre un desconocido nicho ecológico de *Paracoccidioides brasiliensis.* Nuestra hipótesis de 1989: situación actual y perspectivas

En 1989 CONTI DÍAZ & RILLA publicaron una hipótesis sobre el aún hoy desconocido nicho ecológico de *Paracoccidioides brasiliensis* proponiendo esencialmente en ella la existencia de una estrategia ecológica altamente eficiente del agente.

Tal estrategia estaría basada en una importante reserva del parásito en animales heterotérmicos de fuentes naturales de agua dulce, una estadía sólo transitoria y de variable duración en el suelo, con una amplia dispersión de aleuriosporos en el ambiente exterior con capacidad de infectar al hombre y a los animales.

El presente trabajo analiza comunicaciones científicas del último

Encuentro Internacional sobre Paracoccidioidomicosis celebrado en Aguas de Lindoia, San Pablo, Brasil, entre el 2 y 5 de octubre del 2005, comparando diferentes conceptos y resultados allí emitidos como también de otros trabajos al respecto, con los postulados de la hipótesis.

Desde el momento en que se comprueba una aparente clara correlación entre unos y otros, se insiste en la conveniencia y necesidad de llevar a cabo nuevos proyectos de investigación al respecto centrados fundamentalmente en la búsqueda del agente en los probables animales reservorios, mediante métodos micológicos clásicos y técnicas de biología molecular tan bien manejadas en varios laboratorios de la región.

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