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Overview of the genus *Paracoccidioides* in environmental areas of South America

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Resumo

Os fungos do gênero Paracoccidioides são agentes causadores de micoses sistêmicas consideradas endêmicas na América Latina, principalmente no Brasil. Estudos científicos têm investigado a distribuição deste fungo na natureza e sua associação com outras espécies, como o Tatu (Dasypus sp.) com a finalidade de fornecer estratégias para vigilância desta infecção em áreas de risco. Neste contexto, foi realizada a análise do panorama do fungo Paracoccidioides spp. em áreas ambientais da América do Sul por meio da análise sistemática de artigos publicados durante o período de 1963 a 2022, com a utilização dos termos Paracaccidioides, América do Sul, solos, áreas ambientais associadas com cada país desta região. Foram selecionados artigos em inglês, português e espanhol. Esta analise permitiu a identificação de 65 publicações que detectaram estes fungos em seis países da América Latina, predominantemente no Brasil (86,16%). Com relação as amostras utilizadas, os cães domésticos e bovinos, foram os mais investigados em 36,69% e 24,94% dos artigos, respectivamente. Além disso, a ocorrência também foi relatada em equinos, ovinos e tatu. As técnicas mais utilizadas nestes estudos foram ELISA (66,40%), Testes intradérmicos (25,95%), PCR e Nested PCR (4,14%) e Cultura (1,21%). Estas analises fornecem, portanto, informações fundamentais para as estratégias de vigilância da doença, indicando áreas ambientais com risco potencial para a disseminação do fungo Paracoccidioides ssp.

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Palavras-chave: América Latina; Micose sistêmica; Solo, Tatu.

Abstract

The fungi of the Paracoccidioides genus are causative agents of systemic mycoses considered endemic in Latin America, primarily in Brazil. Scientific studies have investigated the distribution of this fungus in nature and its association with other species, such as the Armadillo (Dasypus sp.), with the aim of providing strategies for monitoring this infection in at-risk areas. In this context, an analysis of the panorama of Paracoccidioides spp. fungus in environmental areas of South America was conducted through a systematic analysis of articles published from 1963 to 2022, using the terms Paracoccidioides, South America, soils, and environmental areas associated with each country in this region. articles in english, portuguese, and spanish were selected. This analysis led to the identification of 65 publications that detected these fungi in six Latin American countries, predominantly in Brazil (86.16%). Regarding the samples used, domestic dogs and bovines were the most investigated in 36. 69% and 24.94% of the articles, respectively. Furthermore, occurrences were also reported in horses, sheep, and armadillos. The most commonly used techniques in these studies were ELISA (64,60%), Intradermal Tests (25.22%), PCR and Nested PCR (4.13%), and Culture (1.58%). These analyses therefore provide crucial information for disease surveillance strategies, indicating environmental areas with potential risk for the dissemination of Paracoccidioides spp. fungus.

Keywords: Latin America; Systemic mycosis; Soil; Armadillo.

1. INTRODUCTION

In recent years, there has been an observed rise in the incidence and dissemination of emerging fungal infections (Fisher et al., 2020). It is estimated that over 200 fungal species have been identified as agents responsible for severe diseases in humans (Fisher et al., 2020). Among these fungi, the order Onygenales stands out due to its extensive diversity of pathogenic species, and it is responsible for triggering approximately 650,000 infections per year (Dyke; Teixeira; Barker, 2019).

Among these fungi, the genus *Paracoccidioides* stands out, which is the etiological agent of paracoccidioidomycosis (PCM), a prevalent systemic granulomatous mycosis in South American countries (Shikanai-Yasuda et al., 2017). Despite not being a compulsorily notifiable disease, it is known that PCM affects individuals engaged in soil-related activities (Martinez et al., 2017). Furthermore, it is estimated that in endemic regions, there are 1 to 3 new cases per thousand inhabitants per year, resulting in a mortality rate of 1.45 per million inhabitants. This classification ranks it as the eighth leading cause of mortality among infectious and parasitic diseases (Shikanai-Yasuda et al., 2017).

The genus *Paracoccidioides* is composed of two main species, *P. brasiliensis* and *P. lutzii* (Shikanai-Yasuda et al., 2017). Genotypic studies have revealed variations within *P. brasiliensis*, which have been attributed to cryptic species: *P. brasiliensis* (S1a and S1b), *P. americana* (PS2), *P. restrepiensis* (PS3), and *P. venezuelensis* (PS4) (Teixeira et al., 2020). The species S1a, S1b, PS2, and *P. lutzii* are widely distributed across the South American continent, whereas the species PS3 and PS4 are restricted to Colombia and Venezuela, respectively (Martinez et al., 2017).

Recently, the cryptic species *P. ceti* and *P. lobogeorgii* have been included in the phylogenetic complex of *P. brasiliensis*. *P. ceti* has been described in Brazil, Cuba, the United States, and Japan (Ueda et al., 2013; Esperon et al., 2012; Vilela et al., 2016; Minakawa et al., 2016; Sacristan et al., 2017), while *P. lobogeorgii* has predominantly

been identified in the state of Acre and in São Paulo, Brazil (Taborda et al., 1999; Vilela et al., 2023).

While there is a consensus regarding the widespread distribution of this fungus in the soil of the american continent, few studies have been able to demonstrate the isolation of this microhabitat, thus limiting our understanding of the ecoepidemiology of the genus *Paracoccidioides* (Arantes et al., 2016). Previous studies, utilizing molecular analyses, have indicated the presence of this fungus in soil, armadillo specimens, domestic animals, as well as in aerosols in different regions of Brazil (Arantes et al., 2016; Hrycyk et al., 2018).

The presented evidence indicates a broad dispersion of this fungus in the soil, along with its capacity for adaptation not only in humans but also in domestic and wild animals (Arantes et al., 2016; Martinez et al., 2017). Organizing this information, through the identification of risk areas and the description of cases involving accidental hosts, can provide an understanding of the dynamics of *Paracoccidioides* spp. fungus in the environment. Therefore, this study aimed to describe the landscape of the Paracoccidioides genus in environmental areas of South America.

2. METHODS

This is a quantitative and retrospective systematic review, conducted following an adaptation of the guidelines proposed in the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" guide (PRISMA) (Moher et al., 2015). The objective was to provide an overview of the genus *Paracoccidioides* spp. in environmental areas of South America.

Over a one-year period, two reviewers identified articles indexed in PubMed, Scientific Electronic Library Online (Scielo), Web of Science, and Google Scholar. The search was conducted using the descriptors "*Paracoccidioides*," "soils," "domestic and wild animals," "aerosol," "Argentina," "Bolivia," "Brazil," "Chile," "Colombia," "Ecuador," "Guyana," "French Guiana," "Paraguay," "Peru," "Suriname," "Uruguay," and "Venezuela." Only articles published in English, Portuguese, and Spanish between 1963 and 2022 were included in the review.

Inclusion criteria for this review encompassed studies investigating the detection of the genus *Paracoccidioides* in environmental areas within the South American countries. Conversely, exclusion criteria were established to eliminate studies conducted outside the specified geographic area and data related to human disease that did not align with the proposed objective of this review. Additionally, duplicate articles in the search platform and those outside the established timeframe were excluded.

Information from the selected articles was meticulously compiled and systematized in an Excel® spreadsheet to organize and facilitate data analysis. Additionally, using Prism-GraphPad® software, figures were created representing the frequencies of different variables, such as types of collected samples, regions of study origin, and techniques used for fungus detection. This approach provided a clear and concise visualization of patterns and trends related to the Paracoccidioides spp. genus in environmental areas and domestic and wild animals in South America.

3. RESULTS

A total of 3.007 records were identified, comprising 1.112 from Pubmed, 73 from Scielo, 142 from Web of Science, and 1.957 from Medline. Among these, 1.278 records were

duplicated across the databases, resulting in the identification of 1.878 unique documents. Following the application of selection criteria involving a thorough review of titles and abstracts, 500 documents were chosen for further assessment. Within this final selection, only 53 articles demonstrated alignment with the objectives of this study.

It is important to emphasize that the exploration of the reference listings of these additional 53 articles led to the identification of an additional twelve documents that met the same pre-established selection criteria. Consequently, a cumulative total of 65 articles were selected for comprehensive review and subsequent systematization (Figure 1).

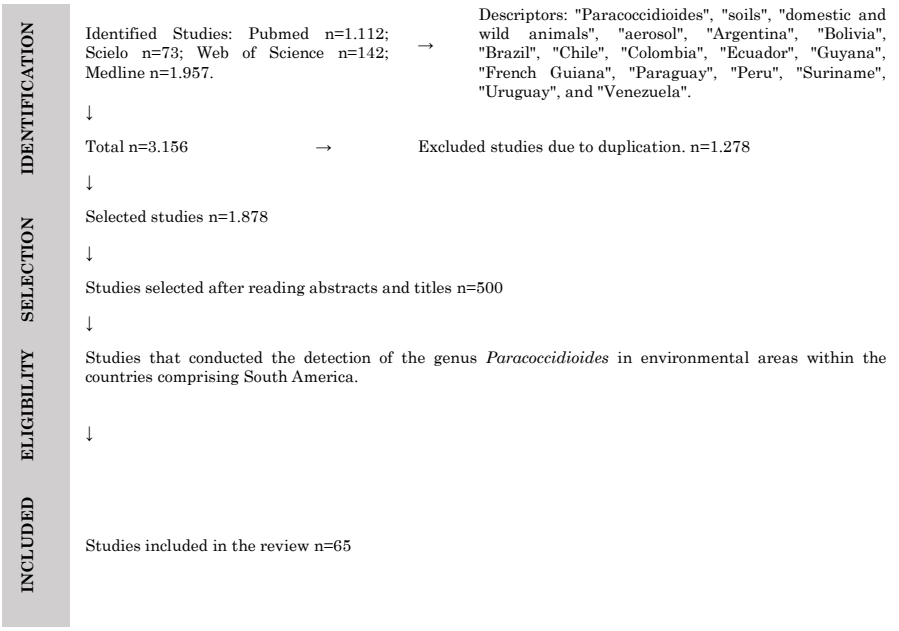


Figure 1 - Flowchart of identification and selection of literature review articles.

The systematic analysis of scientific articles detected the presence of *Paracoccidioides* spp. in six Latin American countries, namely Brazil, Colombia, French Guiana, Venezuela, Argentina, Uruguay, and Bolivia. A predominance of investigations was observed in Brazil, with 56 (86.16%) studies (Table 1).

Table 1 - Detection of genus *Paracoccidioides* fungi in Latin American countries, considering the types of samples used, techniques employed, total analyzed samples, quantity of positive identifications, and publication authorship.

Local de coleta	Tipo de amostra	Técnica para detecção	Total de amostras	Positivas (%)	Autor
Argentina	Domestic dog	Western blot	89	01 (1,12)	(Canteros et al., 2010)
	Soil	Culture	12	10 (83,33)	(Negroni, 1966)
Bolvia	Titi monkey	Direct exam	1	01 (100)	(Jhonson; lang, 1977)
Brazil	Soil	Culture	1	01 (100)	(Shome et al., 1993)
			887	05 (0,56)	(Montenegro et al., 1996)
			760	01 (0,13)	(Vergara et al., 1997)
		PCR and Nested PCR	4	3 (75)	(Theodoro et al., 2005)
			9	4(44,44)	(Terçarioli et al., 2007)
			27	07 (25,92)	(Arantes et al., 2013)
			44	23 (52,27)	(Arantes et al., 2016)
			11	05 (45,45)	(Hrycyk et al., 2018)
			30	08 (26,66)	(Mendes et al., 2019)
			18	03 (16,66)	(Macedo et al., 2020)
			16	07 (43,75)	(Mendes et al., 2020)
			15	09 (60)	(Teixeira et al., 2022)
	Armadillo[1,2,3,4]	Direct exam	21	01 (4,76)	(Vergara; martinez et al.,1999)
			16	02 (12,5)	(Vergara et al., 2000)
		Culture	20	04 (20)	(Naiff et al., 1986)
			4	02 (50)	(Bagagli et al., 1998)
			16	03 (18,75)	(Vergara et al., 2000)
			15	10 (66,66)	(Bagagli et al., 2003)
			7	06 (85,71)	(Hrycyk et al., 2018)
		Elisa	47	28(59,60)	(Fernandes et al., 2004)
			1	01(100)	(Albano et al., 2014)
			1	01(100)	(Albano et al., 2014)
		Double immunodiffusion	4	03(75)	(Bagagli et al., 1998)
			16	04(25)	(Vergara et al., 2000)
		Immunoblotting	4	4(100)	(Bagagli et al., 1998)
		PCR	4	03(75)	(Bagagli et al., 1998)
			16	03(18,75)	(Vergara et al., 2000)
		PCR and Nested PCR	9	1 (11,11)	(Bosco et al., 2005)
			1	01(100)	(Pereira et al., 2008)
			2	02(100)	(Pereira et al., 2008)
			7	06 (85,71)	(Hrycyk et al., 2018)
			18	4 (22,22)	(Costa et al., 2021)
			7	6 (85,71)	(Bagagli et al., 2021)
	Aerosol	PCR and Nested PCR	06	05 (83,33)	(Arantes et al., 2013)
			16	13 (81,25)	(Arantes et al., 2016)
	Primates[5,6,7,8]	Intradermal test	52	11 (21,15)	(Costa et al., 1995[a])
			33	9 (27,30)	(Costa et al., 1995[b])
		Elisa	39	25(64,10)	(Corte et al., 2005)
			93	45 (48,38)	(Corte et al., 2007)
	Equines	Intradermal test	109	84 (77,06)	(Costa; Netto,1978)
			234	147 (62,82)	(Costa et al., 1995[a])
		Elisa	100	30 (30)	(Corte et l., 2009)
			200	24 (12)	(Albano et al., 2015)
			200	26 (13)	(Mendes et al., 2017)
	Ring-tailed coati[9]	Intradermal test	37	35 (94,60)	(Costa et al., 1995[a])

Wild felids[10]	Intradermal test	11	05 (45,45)	(Costa et al., 1995[a])
Fish[11]	Elisa	214	25 (11,68)	(Suguiura et al., 2020)
	PCR and Nested PCR	100	03 (3)	(Suguiura et al., 2020)
Nutria[12]	Elisa	4	1 (25)	(Albano et al., 2014)
Rodent[13,14,.15,16,17,18]	Elisa	3	1 (33,3)	(Sbeghen et al., 2015)
		7	1 (14,28)	(Sbeghen et al., 2015)
		8	1 (12,50)	(Sbeghen et al., 2015)
		12	6(50)	(Sbeghen et al., 2015)
	PCR and Nested PCR	4	1 (25)	(Losnak et al., 2018)
		12	1 (8,33)	(Losnak et al., 2018)
Wild mammals	Elisa	85	27 (31,76)	(Mendes et al., 2017)
Boar[19]	Elisa	106	40 (37,73%)	(Belitardo et al., 2014)
White-eared opossum[20]	Elisa	51	8 (15,68)	(Albano et al., 2014)
Crab-eating raccoon[21]	Elisa	2	2 (100)	(Albano et al., 2014)
Capybara[22]	Elisa	1	1(100)	(Albano et al., 2014)
Brocket deer[23]	Elisa	8	3 (37,50)	(Albano et al., 2014)
Lesser grison[24]	Elisa	2	1 (50)	(Albano et al., 2014)
Crab-eating fox[25]	Elisa	3	1 (33,33)	(Albano et al., 2014)
Pampas fox[26]	Elisa	9	1 (11,11)	(Albano et al., 2014)
Margay[27]	Elisa	3	1(33,33)	(Albano et al., 2014)
Geoffroy's cat[28]	Elisa	9	1 (11,11)	(Albano et al., 2014)
Guinea pig[29]	PCR and Nested PCR	1	1 (100)	(Pereira et al., 2008)
Grison[30]	PCR and Nested PCR	2	1 (50)	(Pereira et al., 2008)
Raccoon[31]	PCR and Nested PCR	2	1 (50)	(Pereira et al., 2008)
Porcupine[32]	PCR and Nested PCR	2	1 (50)	(Pereira et al., 2008)
Velvety-free-tailed-bat[33]	PCR and Nested PCR	95	2 (2,10)	(Paz et al., 2017)
Dolphin	PCR and Nested PCR	1	1 (100)	(Sacristán et al., 2017)
Crab-eating fox	PCR and Nested PCR	18	02 (11,11)	(Costa et al., 2021)
Paca[34]	PCR and Nested PCR	18	01 (5,55)	(Costa et al., 2021)
Ovine	Intradermal test	98	42 (42,8)	(Costa; Netto et al.,1978)
		98	40 (40,80)	(Costa et al., 1995[a])
	Elisa	262	97 (37,02)	(Oliveira et al., 2012)
Bovine	Intradermal test	254	114 (44,88)	(Costa; Netto et al.,1978)
		632	254 (40,18)	(Costa et al., 1995[a])
	Elisa	400	350 (87,50)	(Silveira et al., 2005)
		400	70 (17,50)	(Silveira et al., 2008)
Domestic dogs	Direct exam	01	01 (100)	(Farias et al., 2011)
	Culture	01	01 (100)	(Bosco et al., 2005)
		01	01 (100)	(Headley et al., 2017)
	Intradermal test	149	9 (6,04)	(Fontana et al., 2010)
	Complement fixation	145	109 (75,17)	(Môs; Netto, 1974)
	Elisa	305	85 (27,86)	(Ono et al., 2001)
		836	567 (67,82)	(Silveira et al., 2006)
		275	182 (66,18)	(Fontana et al., 2010)
		126	69 (54,76)	(Corte et al., 2012)
		196	58 (29,60)	(Teles et al., 2015)

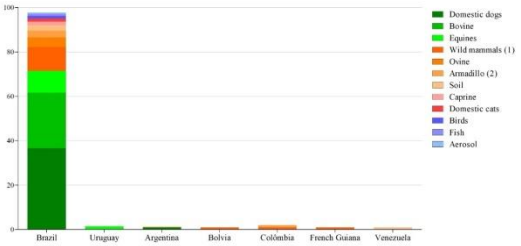
			196	52 (26,53)	(Mendes et al., 2017)
			300	22 (7,33)	(Petroni et al., 2017)
		PCR and Nested PCR	1	01 (100)	(Ricci et al., 2004)
		PCR	1	01 (100)	(Bosco et al., 2005)
			1	01 (100)	(Headley et al., 2017)
	Birds	Elisa	140	38 (27,14)	(Oliveira et al., 2011)
	Goats	Elisa	202	53 (26,23)	(Ferreira et al., 2013)
	Rabbit	Elisa	170	46 (27,05)	Belitardo et al., 2014
	Domestic cats	Elisa	136	43 (31,62)	(Oliveira et al., 2013)
Colômbia	Bat guano[35]	Culture	243	03 (1,23)	(Grose, Tamsitt, 1965)
	Armadillo[36,37]		2	01 (50)	(Corredor et al., 1999)
			1	01 (100)	(Corredor et al., 2005)
French Guiana	Two-toed sloth[38]	Direct exam	1	01 (100)	(Chavez et al., 2011)
Uruguay	Equine	Intradermal test	195	45 (23,07)	(Díaz et al., 1972)
Venezuela	Soil	Culture	84	03(3,44)	(Albornoz, 1971)

[1,4] Armadillo (*Dasypus novemcinctus*); Armadillo[2] (*Dasypus hibridus*); Armadillo[3] (*Euphractus sexcinctus*); Primates[5] (*Callithrix jacchus*, *Cebus apella*); Primates[6,7] (*Cebus apella*); Primates [8] (*Cebus sp*; *Alouatta caraya*); Ring-tailed coati[9] (*Nasua nasua*); Wild felids[10] (*Panthera onca*, *Felis pardalis*, *Felis tigtina*, *Felis wiedii*, *Felis geoffroyi*); Fish[11] (*Oreochromis niloticus*); Nutria[12] (*Myocastor coypus*); Rodent[13] (*Oligoryzomys nigripes*), Rodent[14](*Euryoryzomys russatus*) , Rodent[15] (*Thaptomys nigrita*), Rodent[16] (*Akodon sp*), Rodent[17] (*Euryoryzomys russatus*), Rodent[18] (*Oxymycterus sp*); Boar [19] (*Sus scrof*); White-eared opossum[20] (*Didelphis albiventris*); Crab-eating raccoon[21] (*Procyon cancrivorus*); Capybara[22] (*Hydrochoerus hydrochaeris*); Brocket deer[23] (*Mazama guazoubira*); Lesser grison[24] (*Galictis cuja*); Crab-eating fox[25] (*Cerdocyon thous*); Pampas fox[26] (*Lycalopex gymnocercus*); Margay[27] (*Leopardus wiedii*); Geoffroy's cat[28] (*Leopardus geoffroyi*); Guinea pig[29] (*Cavia aperea*); Grison[30] (*Gallictis vittata*); Raccoon[31] (*Procyon cancrivorus*); Porcupine[32] (*Sphiggurus spinosus*); Velvety-free-tailed-bat[33] (*Molossus molossus*); Paca[34] (*Cuniculus paca*); Bat guano[35] (*Artibeus lituratus*); Armadillo[36] (*Dasypus novemcinctus*); Armadillo[37] (*Cabassous centralis*); Two-toed sloth[38] (*Choloepus didactylus*).

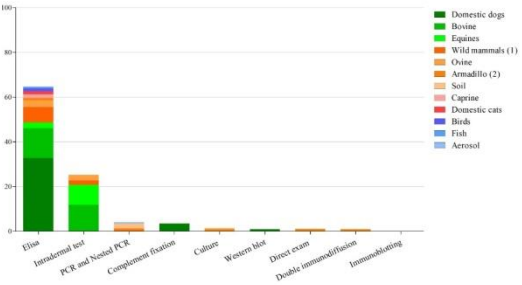
Additionally, variations were observed in the types of samples and techniques employed for the detection of *Paracoccidioides* spp. (Figure 2). Noteworthy among the utilized samples are domestic dogs (36.69%) and cattle (24.94%). The identification of this fungus was also reported in other domestic and wild animals, such as horses (11.27%), sheep (4.40%), and armadillos (3.07%). Furthermore, the presence of the fungus was identified in soil samples (2.82%), which is considered to be the natural reservoir of this fungus (Figure 2).

Figure 2 - Frequency of *Paracoccidioides* spp. detection in the analyzed studies. These records were correlated with the country of origin (A) and the technique used (B).

A



B



[1]Armadillo (*Dasypus novemcinctus*; *Dasypus hibridus*; *Euphractus sexcinctus; Cabassous centralis*); Wild mammals[1] (Primates - *Callithrix jacchus*, *Cebus apella*, *Cebus apella*, *Cebus sp*; *Alouatta caraya*); (Ring-tailed coati - *Nasua nasua*); (Wild felids *Panthera onca*, *Felis pardalis*, *Felis tigtina*, *Felis wiedii*, *Felis geoffroyi*); (Fish - *Oreochromis niloticus*); (Nutria - *Myocastor coypus*); Rodent - *Oligoryzomys nigripes*, (*Euryoryzomys russatus*, *Thaptomys nigrita*, *Akodon sp*, *Euryoryzomys russatus*, *Oxymycterus sp*); (Boar -*Sus scrof*); (White-eared opossum - *Didelphis albiventris*); (Crab-eating raccoon - *Procyon cancrivorus*); (Capybara - *Hydrochoerus hydrochaeris*); (Brocket deer - *Mazama guazoubira*); (Lesser grison - *Galictis cuja*); (Crab-eating fox - *Cerdocyon thous*); (Pampas fox - *Lycalopex gymnocercus*); (Margay - *Leopardus wiedii*); (Geoffroy's cat - *Leopardus geoffroyi*); (Guinea pig - *Cavia aperea*); (Grison - *Gallictis vittata*); (Raccoon - *Procyon cancrivorus*); (Porcupine - *Sphiggurus spinosus*); (Velvety-free-tailed-bat - *Molossus molossus*); (Paca - *Cuniculus paca*); (Bat guano - *Artibeus lituratus*); (Two-toed sloth - *Choloepus didactylus*).

The detection of the *Paracoccidioides* fungus was predominantly in Brazil (97.91%) compared to other regions of Latin America (2.09%) (Figure 1A). These findings were established through the application of diverse methodologies, with the highest utilization of ELISA techniques (64.60%), followed by intradermal tests (25.22%), PCR and Nested PCR (4.13%), and culture (1.58%) (Figure 1B).

4. DISCUSSION

The results underscored the detection of *Paracoccidioides* particularly in samples collected in Brazil, aligning with previous studies describing the region as endemic to the disease, accounting for approximately 80% of the reported cases only in Latin America (Martinez, 2017). Climatic factors specific to this region, such as average temperatures and rainfall indices, also contribute to these findings by maintaining soil moisture conducive to the fungus's persistence (Mendes et al., 2020; Macedo et al., 2020).

Furthermore, the biodiversity present in Brazil offers a wide array of species susceptible to the disease, contributing to the fungus's dispersal within the environment. Among these species, armadillos stand out as significant indicators of *Paracoccidioides* occurrence in nature, given their close association with soils – the natural habitat for the fungus. Studies frequently document the infection of these animals, as indicated by the preceding results (Figure 2).

In this context, previous studies have also investigated the fungus's occurrence in other wildlife species to enhance our understanding of its natural dispersion. Consequently, additional cases have been detected in primates, such as the Common Marmoset (*Callithrix jacchus*) and the Capuchin Monkey (*Cebus apella*) (Costa et al., 1995[a]). Furthermore, newly identified potential hosts from the region, like the Crab-eating Fox (*Cerdocyon thous*) and the Paca (*Cuniculus paca*), have also shown evidence of infection (Costa et al., 2021).

Other studies have documented the presence of *Paracoccidioides* in domestic animals, further underscoring the potential of these species as indicative of the fungus's occurrence in the environment. Cases have been recorded in dogs (Petroni et al., 2017;

Farias et al., 2011; Ricci et al., 2004) and felines (Oliveira et al., 2013), as well as in production animals like cattle (Silveira et al., 2008) and pigs (Belitardo et al., 2014). Despite these analyses, the significance of these findings in relation to the occurrence of the disease in humans should be explored in subsequent studies.

The variation in techniques employed across the articles was also observed, serving as a pivotal factor for the accurate detection of cases within the environment. In this context, experiments predominantly utilized ELISA, especially for the analysis of both domestic and wild animals. Conversely, the detection in armadillos and soil samples was primarily conducted through Nested PCR and culture techniques (Figure 2B).

Despite some studies identifying the occurrence of *Paracoccidioides* through culture isolation (Figure 2B), this technique can be influenced by various factors, particularly in soil samples. Factors such as the presence of saprophytic fungi and the use of pesticides in these regions can potentially hinder the fungal mycelial growth (Oliveira et al., 2011).

This challenge encountered in isolation has spurred the development of more sensitive methods for the detection of *Paracoccidioides* spp. in the environment, including the Nested PCR technique. This methodology was employed in 33.33% of articles with positive samples, standing out as an efficient approach for fungus detection and identification. It enables the classification of new risk areas for the disease, thereby facilitating a more accurate assessment of its geographical spread.

5. CONCLUSIONS

These studies contribute to the understanding of the occurrence and distribution of *Paracoccidioides* spp. in Latin American environments, providing essential insights for disease control strategies within these areas. Moreover, recent findings, especially in new animal species, suggest the possibility of describing this fungus in previously unexplored regions.

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