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Etiological Diagnosis and Correlation of Chromoblastomycosis with Climatic Variables of Weather Stations in Villa Clara, Cuba

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ABSTRACT
In Villa Clara province, Cuba, microorganisms and climatic conditions coexist for the development of the infectious entity chromoblastomycosis. Besides, there is a high percentage of agricultural and handicraft workers exposed to acquiring this infection. The objective of the research consisted in describing the etiological diagnosis and correlating chromoblastomycosis with average climatic variables of the meteorological stations located in Villa Clara. Between 2018-2022, a descriptive, cross-sectional study was conducted in a population of 25 patients diagnosed with chromoblastomycosis by anatomopathological and microbiological studies. Absolute frequencies were used as a summary measure, and for correlation with climatic data from the five meteorological stations, inferential statistics. Of the total number of patients, 92% were diagnosed by anatomopathological studies and only 2/8% had mycological studies, in which the species *Fonsecaea pedrosoi* was isolated. There was a significant correlation between mean temperature ($p=0.045$) and mean predicted wind ($p=0.028$) and the number of cases. This was not the case with relative air humidity ($p>0.05$). It is concluded that in Villa Clara province, only the etiological agent *F. pedrosoi* was the cause of the disease during the period studied, with a significant relationship between the disease and the average climatic variables mean temperature and mean predicted wind.

Keywords: chromoblastomycosis, fonsecaea pedrosoi, climatic variables, villa clara

I. INTRODUCTION

The World Health Organization (WHO) considers 20 endemic infectious diseases to be neglected [1]. Of these, only mycetoma [2] and chromoblastomycosis (CBM) [3] are caused by fungi, because they affect the poor population worldwide, with high morbidity and discrimination [1,2,4]. They are curable and preventable, and can be controlled and even eradicated by implementing the strategies outlined by the Department for the Control of Neglected Tropical Diseases (NTDs) [4].

MBC is a chronic, progressive, granulomatous fungal infection of the skin and subcutaneous cellular tissue caused by multiple species of pigmented or pheoid fungi of the family *Dematiaceae*, which inhabit soil, plants, flowers, and decaying wood [5].

The main infectious agents are: *Fonsecaea pedrosoi* (Negroni, 1936) and *Cladophialophora carrionii* (Trejos, 1954). This disease has a worldwide distribution and is currently considered a cosmopolitan infection [4,6].

In Cuba, the microorganism and the climatic conditions for its development coexist. In addition, there is a high percentage of agricultural and artisanal workers exposed to acquiring this infection. According to the study conducted by Diaz et al. (1978) [7], in a ten-year study, with 72 patients with clinical, epidemiological and microbiological diagnosis of CBM, the prevailing isolated species was *F. pedrosoi*.

In Villa Clara, 47 patients with MBC were reported during the period 2003-2018 [8], a number that has been increasing in the five years since 2018.

The impact of climate change on MBC remains hypothesized. In any circumstance, dematiaceous fungi will have to adapt to varied niches to survive; estimating whether cases of this disease will increase or decrease depends on such

adaptations. Urbanization and desertification may lead to an increase in *C. carrionii* and a decrease in *F. pedrosoi*. Alterations in ecosystems will condition the disappearance of certain species, while others will thrive in new environments [4,9].

Fungal diseases are often regionally distributed, there is no doubt that climate change has a connotation on their delimitation, spread, and possibly etiological agents [9]. An understanding of the ecological changes of such niches, is only the first step in understanding the impact of climate change on opportunistic and medically important fungi [10].

Therefore, the aim of the study was to describe the etiological diagnosis and correlate the disease with average climatic variables of the meteorological stations of Villa Clara, during the period 2018-2022.

II. METHODOLOGY

A descriptive and cross-sectional study was conducted in Villa Clara province, Cuba, between 2018 and 2022.

The study population consisted of nine patients diagnosed with MBC in the Microbiology laboratory of the University Gynecological-Obstetric Provincial Hospital "Mariana Grajales", and the Department of Pathological Anatomy of the University Clinical-Surgical Provincial Hospital "Arnaldo Milián Castro".

The microbiological diagnosis consisted of scalpel scraping of the squamous lesions, a direct mycological examination with 20% potassium hydroxide (KOH). The parasitic form of the fungus in the form of clustered or single fumagoid or muriform cells was observed under the microscope after a 30-minute rest.

The culture was performed in tubes with dextrose sabouraud agar with chloramphenicol and dextrose sabouraud agar with chloramphenicol and cycloheximide; they were incubated at 28°C for six weeks, checked weekly.

The positive culture was microcultured in rice agar medium, incubated at 28°C for 10 days, with the objective of diagnosing the etiological agent according to its sporulation by microscopy, which could be: *F. pedrosoi* (phialophoric, acrothecia and short-chain cladosporic sporulation), *C. carrionii* (long-chain cladosporic sporulation), *Phialophora verrucosa* and *Exophiala dermatitidis* (phialophoric sporulation) and, finally, *Rhinocladiella aquaspersa* (acrothecia sporulation).

For anatomopathological diagnosis, the sample was fixed in 10% formalin for 24 hours. Subsequently, macroscopic description, technical processing and kerosene embedding were performed to perform the vertical microstamo cut.

The section was stained with hematoxylin eosin, mounted on the slide and the diagnosis was made by microscopy after the observation of an inflammatory response of the tissue and the presence of fungal cells.

The diagnosed cases of the disease were recorded in control books existing in these laboratories, which constituted sources for the analysis at the population and individual level. In addition to the data corresponding to the variables to be studied, a triangulation of information referring to the flow of patients in the different services was performed.

Description of the Study Area

Annual data corresponding to the period 2018-2022 were used, of the existing cases of CBM in the central province of Villa Clara, Cuba, in this region, which were correlated with some meteorological variables, according to the following stations: Yabú, Sagua la Grande, Caibarién, Santo Domingo and La Piedra.

For the study period, a climatic data base of the meteorological stations of Villa Clara province was prepared, which includes the following variables:

- Average maximum temperature (Tmax med)
- Average minimum temperature (Tmin med)
- Average temperature (Tmed)
- Average relative air humidity (Hr med)
- Mean maximum relative humidity of the air (Hrmax med)
- Mean minimum relative humidity of the air (Hrmin med)
- Wind direction (D wind)
- Predicted mean wind (Wind med pred km/h)
- Mean wind (Vto mean km/h)

III. RESULTS

Between 2018 and 2022, nine cases of MBC were diagnosed in Villa Clara province. Only in one patient was a comprehensive study performed by the two services, the rest were diagnosed indistinctly in one or the other medical service. Ninety-two percent of the patients were seen in the Anatomic Pathology Service without being previously evaluated in the Microbiology Laboratory. None of the patients seen in the Anatomic Pathology Department were preceded by a negative mycological examination. In this subgroup the existence of the disease was confirmed, but without concluding on the etiological agent.

Of the total number of patients, two (8%) underwent mycological studies and *F. pedrosoi* was diagnosed in both.

Descriptive statistics were calculated for the cases (Table 1), noting that the maximum of 2 corresponds to the year 2019. The mean of 0.36 cases per year, with a standard deviation of 0.64.

Table 1: Descriptive statistics of the CBM in Villa Clara, 2018-2022

	N	Minimum	Maximum	Media	Standard deviation
CBM	9	0	2	0.360	0.6377
N valid (by list)	9				

Source: Microbiology log books.

Correlations with climatic variables were calculated (Table 2), and it should be noted that there was a significant correlation between the number of cases (MBC) and the average mean temperature of the meteorological stations. As these increases, the MBC should decrease, and this correlation was significant at 95%.

Table 2: Correlations of CBM with temperature in Villa Clara, 2018-2022

Correlations

		CBM	Tmax med °C	Tmin med °C	T med °C
CBM	Pearson correlation	1	0.183	-0.250	-0.403*
	Sig. (bilateral)		0.381	0.228	0.045
	N	9	9	9	9

* The correlation is significant at the 0.05 level (2-tailed).

Source: Annual records of the Meteorological Center of Villa Clara.

Correlations with climatic variables of wind direction, mean wind and predicted mean wind were also calculated. Only the predicted mean wind was statistically significant at 95% (Table 3).

Table 3: Chromoblastomycosis correlations with wind in Villa Clara, 2018-2022

Correlations

		CBM	D wind	Wind med pred km/h	Wind medio km/h
CBM	Pearson correlation	1	-0.203	-0.438*	-0.371
	Sig. (bilateral)		0.330	0.028	0.068
	N	9	9	9	9

* La correlación es significativa en el nivel 0,05 (2 colas).

Source: Annual records of the Meteorological Center of Villa Clara.

It was also correlated with relative air humidities, neither of which proved to be statistically significant (Table 4).

Table 4: Correlations of chromoblastomycosis with relative air humidities in Villa Clara, 2018-2022

Correlations

		CBM	Hr med %	Hrmax med %	Hrmin med %
CBM	Pearson correlation	1	0.251	0.234	-0.261
	Sig. (bilateral)		0.227	0.259	0.208
	N	9	9	9	9

Source: Provincial Meteorological Center anual records.

IV. DISCUSSION

In Cuba, Sordo Cuervo reported the first case of CBM in 1912, but his report was not well documented, and it was not until 1941 that the strain was identified as *F. pedrosoi* [11]. Since 1978, the training of dermatologists and mycologists has been suggested, as well as a place for consultations, and early diagnosis and treatment is emphasized [7].

In Villa Clara, since the 1960s, it was diagnosed by Dr. Serafín Ruiz de Zarate [12], and it was not until 2022 that he published an article with important results at the provincial level [8]. The authors of this article emphasized the lack of clinical, anatomopathological and microbiological integration for the diagnosis of MBC, a situation that is maintained in the period of analysis of this research.

Only 8% of the patients had mycological studies performed, results that coincide with the study by García et al. (2022) [8] and Wattiez et al. (2017) [13], in which the highest proportion of diagnoses of the disease is in the Anatomic

Pathology Service, when it is known of the importance of achieving a joint diagnosis by the three medical specialties. Medical situation already resolved since January 2023 in Villa Clara province.

F. pedrosoi was isolated as the causal agent in the two patients with microbiological studies, remaining among the most isolated in this central region of the country. These results coincide with those obtained by other authors [8,14] in their two publications; in the first one it was obtained together with *C. carrionii* as the main agents, and in the second publication on case presentation, as the causative fungus of the disease.

The results obtained in this research also coincide with those achieved by Simón et al. (1998) [15], who reported 49 cases in eight years of study, and isolation frequency in 26 cases (89.7%) of *F. pedrosoi*.

It is clear that climate change affects multiple areas of human health, probably most if not all, but from the point of view of infectious diseases, this is associated with the proliferation of environmental pathogens and regional redistribution of them. It also means a consequent increase in inocula and transmissibility, with its effect on greater severity and incidence of the diseases they cause [16].

The abundance and distribution "the potential to cause disease" of environmental fungi probably depends on climatic factors such as temperature and humidity [17].

The average mean temperature of the five meteorological stations in the province, in those years of study was 25°C; after analysis there was a significant correlation (p=0.045) with disease. This result coincided with that obtained by Bonifaz et al. (2022) [9], when they stated that most of the causative agents of CBM inhabit places with a temperature range between 25 and 30°C. They also agree with Sumba (2022) [18], when he stated that CBM is prevalent in Mexico, Brazil and Venezuela, due to the climatic conditions prevailing in areas with average temperatures at 24°C.

Of the average of the climatic variables related to wind in the province, there was significant correlation only with mean predicted wind (p=0.028). Its average value was 8.8 km/h in the five years of the study. No published international or national literature was found linking the disease with this climatic variable.

It is known that several experts suggest that fungal spores can be disseminated and spread by air currents. Spores comprise a large percentage of airborne particles, and spore composition shows seasonal fluctuations. The ability of intercontinental spread by air currents is of great concern for the occurrence of pathogenic fungi, as it implies that, such outbreaks are unlikely to be contained by the usual disease control measures such as quarantine and isolation [10].

The value of relative air humidities in Villa Clara during 2018-2022, ranged from 55% to 94.6%, with a mean value of 79%. None had statistical significance with the diagnosed cases of CBM. However, Queiroz (2015) [2] posited that *F. pedrosoi* predominates in humid areas, as did Bonifaz (2015 and 2022) [9,19].

Climate change could affect the ecology of pathogenic fungi in ways that are not yet fully understood. Minor or gradual changes in temperature, humidity, and wind patterns may affect fungal growth, distribution, and dispersal [17].

V. CONCLUSION

In Villa Clara province, only the etiological agent *F. pedrosoi* was the cause of the disease during the period studied, showing a significant relationship between MBC and the average of climatic variables, mean temperature and mean predicted wind, but not with the relative humidity of the air.

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