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Review

Chlamydia trachomatis: The Long Road to Describe Its Association with Disease in the Amazon Region of Brazil

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Abstract: Sexually transmitted infections (STIs) represent a worldwide public health burden, but many infections and diseases continue to be neglected. Areas with a low human development index, including the northern areas of Brazil, particularly the immense geographic Amazon region, present a high frequency of STIs because of variables that contribute to disseminate the infection, including lack of access to education, prevention measures and treatment to these vulnerable population groups. This review describes the chronological investigation of the etiology of pathologies associated with infection by *Chlamydia trachomatis*, including its prevalence, distribution, and clinical, descriptive and molecular epidemiology in regard to STIs, trachoma and heart disease. Long-term investigations among urban and nonurban populations are discussed and show the need for and effects of continuous surveillance to diminish the burden among vulnerable populations (female sex workers, quilombos and indigenous peoples) and to define new etiological associations of diseases with infections by *C. trachomatis*.

Keywords: *Chlamydia trachomatis*; host susceptibility; Amazon region; Brazil

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1. Introduction

The discovery of *Chlamydia trachomatis* and later of *C. pneumoniae* included these two species among the most relevant human pathogens [1–3]. Severe human diseases associated with these bacteria are responsible for major public health emergencies [3–6]. Trachoma is a disease caused by infection of the eye, an affliction with a clear association with poor sanitation, precarious living conditions, low education and population vulnerability that is one of the most important causes of blindness resulting from an infectious agent. Genital infections by this species are the etiology of frequent sexually transmitted diseases, including nongonococcal urethritis, epididymitis, proctitis, lymphogranuloma venereum, salpingitis and mucopurulent cervicitis, which can progress to pelvic inflammatory disease, ectopic pregnancy and infertility [7–10]. It is estimated that more than 127 million cases of infection occur annually worldwide [11,12], causing thousands of women to become infertile [13–15]. *C. trachomatis* is transmitted mainly by the ocular and the sexual route, which makes the bacterium one of the most important sexually transmitted infections (STIs).

Among the 19 serotypes of *C. trachomatis*, A–C are causes of eye infections, D–K cause urogenital infections and L1–L3 cause cases of lymphogranuloma venereum [16,17]. *C. trachomatis* is worldwide distributed, but the prevalence of infection and diseases in most countries are not well defined. Although the bacterium is an important STI, it is usually regarded as a neglected infection. Difficulties are commonly attributed to the lack of laboratory diagnosis because of the historical need to use cell cultures for isolation, ignoring the most modern and rapid methods available. A direct consequence is the uncertainty of the actual figures of infection and disease because of the poor or absent notification of cases leading to the inappropriate use of antibiotics for the correct treatment. Equally

important are the poorly applied public health policies and measures to prevent the spread of *C. trachomatis*.

Since the second half of the 1980s, the group working at the the Virus Laboratory of the Institute for Biological Sciences has performed a chronological investigation with sequential levels of complexity to define the presence, prevalence, distribution and the etiological role of *C. trachomatis* in different pathologies within the Amazon region of Brazil.

2. Methods of Investigation

The Amazon region of Brazil (ARB) is an area of 5.1 million km^2 (60% of the country) inhabited by approximately 15% of the Brazilian population residing in nine federative states (Figure 1). In addition to being a unique geographical area, the region largely differs in its demographic, social, cultural, health, education, welfare and developmental characteristics from the rest of the country [18]. The initial approach for studying the genus *Chlamydia* and its impact on human population groups was to investigate antibodies to generate information on the serotypes circulating in the ARB, their geographical distribution and the possibility of establishing persistence in infected hosts.

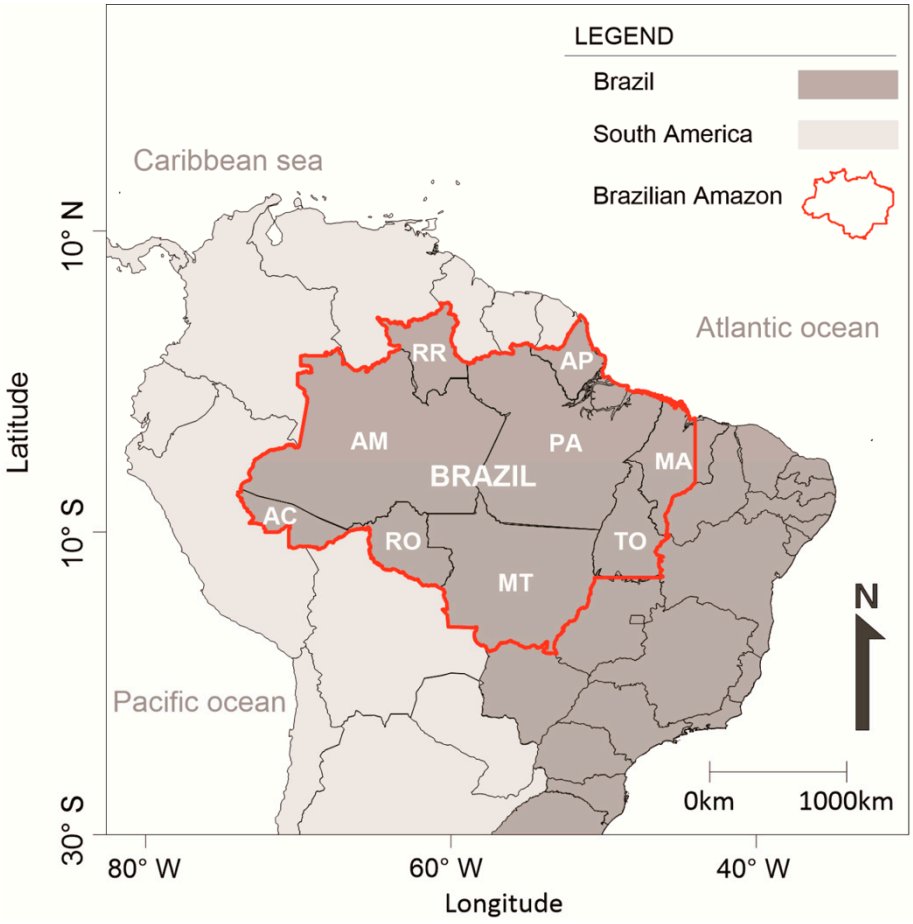

Figure 1. South America and Brazil map highlighting the Amazon region of Brazil (ARB).

The technical details of the methods used are fully described in the following references. The initial studies searching for *C. trachomatis* isolated the bacterium using McCoy cell cultures and detected short- and long-term antibodies (IgM and IgG) to identify recent and past infections using an indirect immunofluorescence assay with a substrate of serotype L2 of *C. trachomatis* [19]. The method was limited to describe the presence of antibodies to the genus *Chlamydia*. In order to detect specific antibodies, a microimmunofluorescence [MIF] assay was used to discriminate seroreactivity to *C. trachomatis* serotypes and *C. pneumoniae* [20], also allowing the chance to show the presence of genital and ocular

serotypes of *C. trachomatis*. Subsequently, specific antibodies were detected against both species using immunoenzymatic assays [21] when made commercially available. More recently, immunohistochemistry [22] and nucleic acid detection assays were used to confirm *C. trachomatis* infection [23,24].

The definition of exposure level indicates the percentage of persons with a previous contact with the bacterium, measured by the presence of antibodies to *Chlamydia*, as low exposure (up to 30%), medium exposure (from 31–70%), and high exposure (greater than 71%). Persistence level was defined as the percentage number of persons presenting antibody titers to *Chlamydia*, higher than 512, as measured by IFA as low persistence (up to 5%), medium persistence (from 5.1–10%) and high persistence (greater than 10%).

3. Results and Discussion

3.1. The Diverse Pathologies and Associated Diseases of C. trachomatis in the Amazon Region of Brazil

3.1.1. Clinical–Epidemiological Data and the Role of *C. trachomatis* as a STI

The presence of *C. trachomatis* in the ARB was determined to be a possibility only because of the detection of trachoma [25]. In the second half of the 1980s, the presence of the bacterium was confirmed to occur among population groups in this region [26]. Antibodies to the genus *Chlamydia* were detected for the first time, providing seroepidemiological information and the first clues of its prevalence and distribution in the city of Belém (53.6%), within mining areas (76.2%) and in the indigenous people of the Xicrin tribe (51.3%). This starting point to expand the investigation helped us to look further into urban groups attending prenatal clinics (42.2%), gynecological clinics (60%), STI clinics (33.3%), and vulnerable groups, including female sex workers (FSW) (94.6%) and the indigenous people of Parakanã (97.1%) and Kubenkokrê (35.7%) villages [19]. The large prevalence range from 33.3% to 97.1% served as motivation to look also for the incidence of *C. trachomatis*.

At that time, the use of McCoy cell cultures was a technological innovation, and these cultures were used for the first time in the ARB to isolate the bacterium and establish its etiological role in diseased persons attending the STI clinic of the Universidade Federal do Pará, Belém, Pará, Brazil. *C. trachomatis* was the most commonly found etiological agent [30%] of nongonococcal urethritis [19], and it was also present among asymptomatic women attending a gynecology and obstetrics [3.6%] clinics [19] at a similar frequency described at that time in the northeast of the country [27].

The initial seroepidemiological and prospective investigations led us to focus on the impact of the involvement of the genus *Chlamydia* among vulnerable indigenous populations. A large seroepidemiological study was implemented to investigate 27 indigenous communities distributed across six states of the ARB and included 2086 individuals of both sexes. An average prevalence of antibodies to the genus *Chlamydia* of 48.6% was described, but no evidence of infection was shown in one village of indigenous people (Arára do Iriri) at the time of the investigation, although it was fairly close to another similar ethnic village (Arára do Laranjal/Kurambê) [20], probably because of the absence of contact between the two groups. Prevalence rates of up to 50% were found among 12 communities; four showed figures above 51% up to 70%, and ten showed prevalence rates from 71% up to 90.7% (Table 1). Recent infection was detected in 1.2% of the population (presence of IgM), while 6.1% showed persistent infections with *C. trachomatis* (antibody titers higher than 512). The evidence of persistence of the bacterium within epidemiologically closed communities indicates this is the possible source for its continuous transmission.

Table 1. Prevalence range of antibodies (IgG) to the genus *Chlamydia* among indigenous peoples from the Amazon region of Brazil.

Prevalence Rate	Indigenous People	Linguistic Group	Location
Absence of antibodies	Arára Iriri	Karib	PA
< or equal to 50%	Galibi-Uaça	Karib	AP
	Palikur	Aruak	AP
	Waiãmpi	Tupi-Guarani	AP
	Cinta-Larga	Tupi	RO
	Surui	Tupi	RO
	Karitiana	Arikém	RO
	Tiriyó	Karib	PA
	Assurini do Trocará	Tupi	PA
	Arára Laranjal/Kurambê Karib	Karib	PA
	Araweté	Tupi	PA
	Kikretun	Jê	PA
	Mundurukú	Tupi	PA
51% to 70%	Wayana-Apalai	Karib	PA
	Assurini do Kuatinemo	Tupi	PA
	Aukre	Jê	PA
	Kokraimôro	Jê	PA
71% to 90%	Urubu Kaapor	Tupi	MA
	Awá-Guajá	Tupi-Guarani	MA
	Yanomámi	Yanomámi	RR
	Yamamadi	Arawá	AM
	Parakanã	Tupi	PA
	Kubenkokrê	Jê	PA
	Pukani	Jê	PA
	Kararaô	Jê	PA
	Xicrin do Bacajá	Jê	PA
	Katwena	Karib	PA

PA = Pará; AP = Amapá; RO = Rondônia; MA = Maranhão; RR = Roraima; AM = Amazonas.

By the beginning of the 2000s, the most accurate methodology for addressing the seroreactivity to the most relevant species of *Chlamydia* infecting human beings was the MIF assay. The assay measured seroreactivity to *C. pneumoniae* and the serotypes of *C. trachomatis* associated with trachoma and STIs (urethritis and lymphogranuloma venereum). However, some of the samples derived from the native indians of the ARB were reactive by immunofluorescence, but nonreactive to any of the antigens used in the MIF assay. The most probable explanation was that it could be an infection with another species of the genus *Chlamydia* that could be circulating in their natural forest environment. Unfortunately, at the time, it was not possible to characterize such seroreactivity to any other *Chlamydia* species antigen that usually infect animals and are transmitted to human beings (such as *C. psittaci* and *C. pecorum*) because of their lack of availability. However, crossing of the interspecies barrier from animals to human beings is a strong possibility, as such transmission is responsible for the recent health emergencies caused by important infectious agents including Human immunodeficiency virus 1, HIV-1 [28], Human T-lymphotropic virus 3, HTLV-3 and HTLV-4 [29] and Severe Acute Respiratory Syndrome Coronavirus 2, SARS-CoV-2 [30].

Figure 1 shows the large geographical distribution of the investigated communities in the ARB, which, together with Table 2, shows there was no evidence of an association between geographical location or linguistic group and the prevalence rate, because of the cultural differences among the indigenous peoples and the usual lack of contact among them. Another epidemiological pattern observed was that the relation of agent/host infection was not always modulated linearly between the prevalence and persistence rates in the communities, as previously observed with hepatitis B virus among indigenous people and urban communities of the ARB [31]. For instance, among the Tiriyó, the prevalence of *Chlamydia* infection was low, but the group maintained the bacterium at high levels

of persistence. It is possible that this is a result of the influence of the different genetic complexities of isolated and semi-isolated indigenous populations [21] and variation in immunological responses and degrees of outcome severity.

Table 2. Levels of exposure to and persistence of *Chlamydia* infection among isolated indigenous peoples of the Amazon region of Brazil (ARB).

Indigenous Community	Exposure Level (%) *	Persistence Level (%) *
Mundurukú	Low (20.4)	Low (3.3)
Arára Laranjal/Kurambê	Low (27.7)	Medium (7.0)
Tiriyó	Low (11.5)	High (33.3)
Cinta-Larga	Medium (47.1)	Medium (6.2)
Kokraimôro	Medium (55.9)	Low (1.9)
Asurini Kuatinemo	Medium (61.0)	Low (4.0)
Kubenkokrê	High (75.8)	High (10.1)
Yanomámi	High (87.6)	High (21.1)
Awá-Guajá	High (90.7)	Low (2.6)
Parakanã	High (81.0)	Medium (5.9)
Xicrin	High (81.5)	Medium (9.5)

* Definition of exposure and persistence levels are fully described under the section "Methods of Investigation".

High rates of prevalence and persistence of *Chlamydia* infection within small native Indian communities is a heavy burden, as *C. trachomatis* is also associated with infertility [13], a crucial factor for the decrease in population density, among other clinical outcomes of the urinary and genital system. Among epidemiologically closed groups, the transmission of STIs is rapid and includes routes of transmission other than sexual transmission, including sharing objects and scaring habits for ritual preparations [32,33]. The detection of STIs other than the genus *Chlamydia* commonly occurs, including *T. pallidum* infections, persistence and transmission of Hepatitis B virus (HBV), herpes simplex lesions and *Neisseria*-associated vaginal discharge in children [31]. Coinfections are detected particularly among *C. trachomatis*, *T. pallidum* and HBV.

The prevalence of antibodies against *Chlamydia* infection was investigated among HIV-1 infected persons (430), and 64.2% were positive; however, IgM antibodies were present in 12.6% [34]. A sample was tested with the MIF assay, and there was no reactivity other than to *C. trachomatis* serotypes. These rates of infection are commonly seen among population groups seeking diagnosis for STIs [19,35] and are a great cause for concern because of the widespread prevalence of *Chlamydia* infection, high frequency of recent or ongoing infections of another debilitating STI, occurring in a group who is eligible for basic preventive measures to diminish the continuing risk of behavior that promotes STIs. Although treatment is available for *C. trachomatis* infections, clinical and laboratory diagnosis are not commonly established. Anal sex and unprotected sex with multiple partners per week are risky behaviors observed in the same manner as commonly associated with other STIs elsewhere [36–41].

The Archipelago of Marajo is a very unique ecosystem in the ARB, with epidemiological characteristics that include low socioeconomic and education levels and precarious conditions that promote widespread STIs. Most travel within and out of the archipelago occurs by boat, crossing the network of rivers to reach urban centers. Consequently, the Marajo Archipelago is burdened with social, political and economic isolation, with poor access to health services, contributing to the increase in disparities affecting the human population. A large seroepidemiological investigation performed in four municipalities in the archipelago (Chaves, Anajás, Portel and São Sebastião da Boa Vista) showed a prevalence of antibodies to *C. trachomatis* of 30.9% among 1217 persons, with 6.7% having IgM antibodies, evidence of recent or ongoing infection [42].

During the same project, cervical samples from 393 women were investigated for *C. trachomatis* infections and 4.1% were infected with five STI genotypes (D, E, F, Ia and J) and genotype B, which is commonly associated with eye infections [23]. This situation was

previously reported when seroreactivity specific to serotypes B and Ba was detected in other locations of the ARB [43]. The number of persons with active genital infections was similar to the frequency of persons having IgM antibodies in the Marajo Archipelago [42].

The situation in the archipelago commonly occurs among several other areas in the ARB. Social (illiteracy and poverty) and behavioral (alcohol abuse, multiple sexual partners) factors are some of the variables influencing the spread of STIs, including *C. trachomatis* [42], and the low educational level is a strong barrier preventing the development of the islands. Periodic seroepidemiological investigations commonly show low efficiency in controlling the spread of STIs [40,41,44]. The fact that only a small number of individuals of the population are capable of carrying out complex activities or reading and understanding disease preventive measures is a tremendous burden to face. As a repetitive cycle, some families are maintained with less than the minimum wage [42], and the archipelago continues its course as one of the most vulnerable regions in the ARB [45,46].

3.1.2. *C. trachomatis* and Trachoma in the ARB

Trachoma is a neglected disease and one of the most important causes of blindness resulting from an infectious agent [47]. Active or inactive trachoma has been reported in the ARB for more than 40 years, affecting at least 33% of the inhabitants evaluated [25]. Serotypes of *C. trachomatis* associated with the disease were commonly detected using indirect evidence of serological reactivity described in different geographical areas of the region, including urban population groups and nonurban isolated peoples [19–21,42]. Some *C. trachomatis* serotypes/genotypes are commonly reported as being geographically limited, and serotype A was once associated with the Middle East and North Africa. It was an interesting finding to detect serotype A in the ARB for the first time in a semi-isolated community originating from a massive contemporary human migration of a Portuguese colony from northern Africa resulting from religious persecution [48].

The finding of serotypes/genotypes associated with trachoma, including genotype B among cervical specimens [43,49], indicated the need to start an intensive disease surveillance in the Marajo Archipelago. The low hygienic measures were clear evidence of transmission of the bacterium among the general population [50], and a massive intervention effort was proposed to eliminate trachoma [51].

Following an initial attempt to define the epidemiological situation in one village of the archipelago (municipality of Soure), educational work began using the strategy proposed by the WHO after the detection and clinical diagnosis of cases. The SAFE (Surgery, Antibiotics, Facial Cleanliness, Environmental improvement) strategy was put into action. A follow-up study of eight years was efficiently conducted and allowed us to show a marked reduction in the clinical cases of disease and vectors of transmission associated with poor hygiene habits [51]. In 2008, 2054 children with an age range from 6 to 16 years, showed prevalence of clinically diagnosed trachoma of 3.4%, all of them presenting the follicular form of the disease (aged 6 to 14 years). In 2016, 1502 school children were examined and only three cases of trachoma (0.2%) were found presenting the folicular form of trachoma (ages from seven to eight years).

Infection with *C. trachomatis* results in various clinical outcomes; as the bacterium is well adapted to its host, pathogenesis in the eye results in a severe disease that is amplified when occurring in areas with low human development indexes. Trachoma was recognized to occur in the Marajo Archipelago and is consistent with the heavy burden on this highly vulnerable population in the ARB. Specific public health policies addressing trachoma and focused on this region are needed. In addition, experimental data presented in a long-term study stressed the importance of routine examination, clinical and laboratory diagnosis, immediate treatment, recognition of associated risk factors, campaigns in health education with accessible language adapted to the local population and a continued investment in school education [21,50,52].

3.1.3. *C. trachomatis* and Heart Disease

C. trachomatis is well characterized as a relevant STI agent causing genital diseases and eye infections. However, a new investigative experimental study has focused on its possible role in the etiology of heart disease (along with *C. pneumoniae* and other infectious agents associated with this pathology). Most of the information thus far obtained is related to the chronic inflammation observed in the arterial wall of patients with atherosclerosis that could possibly be associated with the continuous multiplication of infectious agents [53–55], and one of the main agents considered is *C. pneumoniae*.

In a preliminary investigation, the first evidence of the causal relation between *C. trachomatis* with heart disease was the result of a seroepidemiological approach. Patients residing in a major urban area of the ARB (Belém, the state capital of Pará), with a heart pathology receiving treatment at a university hospital, showed a higher frequency of specific antibodies to the genus *Chlamydia* than that observed within the general urban population residing in the same geographical area, used as a comparison group [43].

The following step of the investigation was designed to produce stronger evidence for an etiological association of the bacterium among patients presenting two clinical heart conditions, namely, coronary artery disease (patients with an indication of coronary artery bypass graft) and heart valve disease (patients with an indication of valve prosthesis implantation, either mitral or aortic). Patients residing in Belém who were preparing for cardiac surgery in three hospitals went through clinical–epidemiological and laboratorial investigation.

Among the most relevant results obtained included the in situ detection of the cryptic plasmid of *C. trachomatis* in 7.4% of the investigated samples (four of the aorta samples and two of the mitral valves) [24]. Immunohistochemistry procedures were successful in showing the in situ presence of *C. trachomatis* antigens in the aorta, valves and atheromatous plaques [22]. The presence of genetic polymorphisms of proinflammatory cytokines indicating a systemic inflammatory reaction (high levels of C-reactive protein), which was confirmed by an intense inflammatory region within the microenvironment of the vascular and cardiac system characterized by the presence of proinflammatory cytokines (IL-6, IL-8) and acute phase mediators such as mannose-binding lectin [22,24,56]. The host mounts the immunological response to evade the infection of the atheromatous plaque, aorta and valves within the cardiac muscle. This is certainly another highly relevant pathology caused by *C. trachomatis* to be further explored.

4. Conclusions

The largest geographical area of the country, the ARB, is usually behind the rest of the country in terms of urban development, economics, welfare and health access. As a consequence, public health programs regarding the control of STIs are generally not effective in terms of convincing people to change for healthier sexual habits. Seroepidemiological studies are helpful for understanding the occurrence, frequency and dissemination of relevant agents, including HIV-1, HTLV-1/2, HBV, HPV, *T. pallidum* and *C. trachomatis* [57]. The necessary notification of diseases associated with *C. trachomatis* is not commonly mandatory, and seroepidemiological information among vulnerable population groups is sometimes the only available manner with which to recognize infections by the bacterium.

C. trachomatis infections among females is commonly asymptomatic, a major problem for detecting and treating infections, a simple procedure that could reduce further transmission. Screening for infection during gynecological examinations of sexually active women should be included in future government initiatives as a true effort for prevention and control of sexual transmission of *C. trachomatis*. There are several efforts from the health authorities and academic initiatives to reduce the impact of diseases caused by *C. trachomatis* (genital and trachoma) in both urban and rural communities, including among native peoples of the Amazon region of Brazil. Although they are of limited reach and commonly discontinued for several reasons, they are successful when appropriately applied.

Along this long period of investigation, the prevalence of infection was measured by a strong but cumbersome and fastidious method (indirect immunofluorescence assay), which was latter substituted by easier and automated procedures (enzyme immune assays), comparable both in sensitivity and specificity. The same could not be said about the challenge of the isolating and identifying of *C. trachomatis* (in cell cultures) but not *C. pneumoniae*. The indication of the presence of this bacterium consisted in showing seroreactivity to the antigen using an even more fastidious, but extremely specific method (microimmunofluorescence). Immuno histochemistry created a new possibility to detect antigens in situ in human tissues. Finally, nucleic acid detection performed by automated methods created a new window of possibilities to measure the presence of the bacterium (improving specific laboratory diagnostic methods) and confirming its frequencies observed since the initial gold standard method (cell culture isolation). It is possible that undercounting or overcounting in both prevalence and incidence could have happened, but the figures along the years were quite the same, particularly during new visits to some of the investigated communities.

The present report is an overview of the work performed by the Virus Laboratory of the Institute for Biological Sciences of Universidade Federal do Pará, since the 1980s and describes distinct clinical diseases investigated among different population groups, prevention policies shown to be effective for different pathologies, including trachoma, and new inflammatory pathologies that need future investigation in association with *C. trachomatis* to fill the gaps in knowledge of diseases in search of etiology.

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