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Review Article

Protozoal infections in Ecuador, public health surveillance and research priorities, 2016-2023: a scoping review

Infecções protozoárias no Equador, prioridades de vigilância e pesquisa em saúde pública, 2016-2023: uma revisão de escopo

Infecciones por protozoos en Ecuador, vigilancia de la salud pública y prioridades de investigación, 2016-2023: una revisión del alcance

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ABSTRACT

Background and Objectives: Protozoal parasitic infections continue to impact public health in Ecuador, as reflected in reports indicating increases in malaria cases, a moderate presence of leishmaniasis, and the continued endemic status of Chagas disease. This underscores the need to focus policy and research efforts. The objective of this review was to synthesize recent evidence on the detection, diagnosis, and epidemiology of protozoal infections in Ecuador (2016-2023) to guide surveillance and research priorities. **Content:** Scoping review with searches in PubMed, SCOPUS, and SciELO (Mar-Jul 2024), following PRISMA-ScR and Joanna Briggs Institute (JBI) steps. We included studies conducted in Ecuador, in humans, animals, and the environment, that employed laboratory tests. We extracted species/genus, provincial location, sample type/population, and diagnostic technique. The base protocol was registered in OSF (https://osf.io/8vuc3). Of 1,180 records, 98 studies met the criteria. Most focused on *Leishmania* spp. and intestinal protozoa, with an increase in agent diversity since 2020 and inclusion of environmental samples. Molecular and serological methods were frequently used, with emphasis on vulnerable populations. Pichincha and Guayas concentrated the widest variety of genera studied, whereas Amazonian provinces had fewer studies. Nationally, *Leishmania* spp. and *Plasmodium* spp. were reported in 23 and 17 provinces, respectively. **Conclusion:** Studies on vectors and reservoirs, spillover, and zoonotic transmission must be strengthened. Research should be expanded in rural and Amazonian areas, and active surveillance should be maintained, incorporating sequencing and integrated "One Health" approaches.

Keywords: *Endemic Diseases. Epidemiology. Parasitic Diseases. Public Health. Zoonoses.*

RESUMO

Justificativa e Objetivos: As infecções parasitárias por protozoários impactam a saúde pública no Equador, como refletido em relatos que indicam aumento de casos de malária, presença moderada de leishmaniose e a persistência do status endêmico da doença de Chagas. Isso ressalta a necessidade de concentrar os esforços em políticas públicas e pesquisas. O objetivo desta revisão foi sintetizar as evidências recentes sobre a detecção, o diagnóstico e a epidemiologia de infecções por protozoários no Equador (2016-2023) para orientar as prioridades de vigilância e pesquisa. **Conteúdo:** Revisão de escopo com buscas nas bases PubMed, SCOPUS e SciELO (mar-jul 2024), seguindo PRISMA-ScR e as etapas do Instituto Joanna Briggs (JBI). Incluíram-se estudos realizados no Equador, em humanos, animais e ambiente, que empregaram testes laboratoriais. Foram extraídas as variáveis espécie/gênero, localização provincial, tipo de amostra/população e técnica diagnóstica. O protocolo foi registrado na OSF (https://osf.io/8vuc3). De 1.180 registros, 98 estudos atenderam aos critérios. Predominaram trabalhos sobre *Leishmania* spp. e protozoários intestinais, com aumento da diversidade de agentes desde 2020 e inclusão de amostras ambientais. Métodos moleculares e sorológicos foram frequentes, com foco em populações vulneráveis. Pichincha e Guayas concentraram a maior variedade de gêneros estudados, enquanto as províncias amazônicas apresentaram menos estudos. Em nível nacional, *Leishmania* spp. e *Plasmodium* spp. foram relatados em 23 e 17 províncias, respectivamente. **Conclusão:** É necessário fortalecer estudos sobre vetores e reservatórios, spillover e transmissão zoonótica. Ampliar a pesquisa em áreas rurais e amazônicas, e manter a vigilância ativa, incorporando sequenciamento e abordagens integradas de "Uma Saúde".

Descritores: *Doenças Endêmicas. Doenças Parasitárias. Epidemiologia. Saúde Pública. Zoonoses.*

RESUMEN

Justificación y Objetivos: Las parasitosis por protozoarios siguen impactando la salud pública en Ecuador, lo cual es reflejado en reportes que indican repuntes en casos de malaria, una presencia moderada de leishmaniasis y el mantenimiento de la enfermedad de Chagas como endémica. Esto resalta la necesidad de orientar esfuerzos políticos y de investigación. El objetivo de esta revisión fue sintetizar la evidencia reciente sobre detección, diagnóstico y epidemiología de infecciones por protozoarios en Ecuador (2016-2023) para orientar prioridades de vigilancia e investigación. **Contenido:** Revisión de alcance con búsquedas en PubMed, SCOPUS y SciELO, siguiendo PRISMA-ScR y los pasos del Instituto Joanna Briggs (JBI). Se incluyeron estudios realizados en Ecuador, humanos, animales y medio ambiente, que emplearon pruebas de laboratorio. Se extrajeron las variables de especie/género, localización provincial, tipo de muestra/población y técnica diagnóstica. De 1180 registros, 98 estudios cumplieron criterios. Predominaron trabajos sobre *Leishmania* spp. y protozoos intestinales, con aumento de diversidad de agentes desde 2020 e inclusión de muestras ambientales. Se observaron frecuentes técnicas moleculares y serológicas y un foco en poblaciones vulnerables. Pichincha y Guayas concentraron la mayor variedad de géneros estudiados, mientras que las provincias amazónicas presentan menos estudios. A escala nacional, *Leishmania* spp. y *Plasmodium* spp. se reportaron en 23 y 17 provincias, respectivamente. **Conclusión:** Se requiere fortalecer estudios de vectores y reservorios, spillover y transmisión zoonótica. Ampliar investigación en zonas rurales y amazónicas, y mantener vigilancia activa, incorporando secuenciación y enfoques integrales "Una Salud".

Palabras Clave: *Enfermedades Endémicas. Enfermedades Parasitarias. Epidemiología. Salud Pública. Zoonosis.*

INTRODUCTION

Protozoal diseases comprise a broad clinical spectrum ranging from mild to potentially life-threatening infections.[1] To date, more than fifty thousand species of protozoa with both pathogenic and non-pathogenic characteristics have been identified, notable for their ability to adapt to different hosts and environments.[2] Pathogenic protozoa are particularly relevant due to their impact on human and animal health, as they cause complex infections that are difficult to diagnose and treat.

In recent years, the incidence of protozoal infections has increased significantly due to a combination of environmental, climatic, and socioeconomic factors. Anthropogenic activities such as deforestation, agricultural expansion, and mining have generated soil changes, altering natural ecosystems and favoring contact between humans, animals, and reservoirs. Additionally, factors such as the expansion of thermal niches, which modify the geographic distribution of biological vectors, as well as social conditions, including poverty, limited access to potable water, and exposure to water contaminated with fecal matter. These situations not only facilitate the spread of protozoa but also modify their capacity for adaptation and persistence in new environments.[3,4]

In addition to macro-environmental factors, protozoa exhibit intrinsic biological characteristics that determine their capacity for invasion, cellular adhesion, and tropism for specific host tissues.[5,6] For instance, protozoa relevant for their pathogenesis and prevalence include *Giardia* spp., which enter the intestinal epithelium through the consumption of contaminated water and food, which can compromise nutrient absorption and cause malnutrition, diarrhea, and dehydration.[7,8] Meanwhile, *Trypanosoma* spp. and *Plasmodium* spp., transmitted by hematophagous vectors, affect the circulatory and lymphatic systems, altering the immune response and oxygen transport.[9,10] This biological and clinical diversity limits the early identification of infections, underscoring the need to implement better diagnostic strategies within health systems.

Diagnosis of protozoal infections should not rely only on clinical assessments, as their manifestations are often non-specific. Confirmation requires laboratory testing, including microscopy-based techniques, cultures, and imaging studies in low-complexity settings, as well as molecular and serological techniques reserved for specialized centers.[11] In this context, early detection is crucial, as it prevents severe complications, assists in monitoring therapeutic responses, and allows for the identification of potential active transmission sources.[12] Improving research on the detection and diagnosis of protozoal infections is essential to strengthen public health systems, particularly in rural and vulnerable populations. This effort contributes directly to Sustainable Development Goals (SDG) 3 (Good Health and Well-being) and 6 (Clean Water and Sanitation), aligning with regional strategies to reduce the burden of Neglected Tropical Diseases (NTD) in the Americas.[13]

From a global health perspective, many of these parasitic infections belong to the group of NTDs, categorized by the World Health Organization (WHO) as a set of infections caused by bacteria, viruses, parasites, fungi, and toxins that impact public health systems, social and economic development, and the quality of life of over one billion people.[14] NTDs primarily affect vulnerable rural and peri-urban populations living in tropical and subtropical regions of the planet, notably Sub-Saharan Africa, Asia, and Latin America.[15] These diseases are associated with concerning situations of chronic malnutrition, delayed childhood development, and physical and social disabilities, perpetuating a significant cycle of poverty by limiting school and work productivity, even as the costs associated with managing these infections rise exponentially for governments year after year.[16,17]

Currently, Ecuador faces a complex landscape regarding the study of infectious diseases caused by protozoa. Due to their endemic nature, these parasitic diseases have become integrated into the regular health ecosystem of the country, disproportionately affecting children, pregnant women, agricultural workers, and rural and Amazonian communities, as well as animal populations distributed across the Coast, Highlands, Amazon, and insular regions.[18,19] It is estimated that millions of Ecuadorians live in conditions that increase their vulnerability to pathogens responsible for malaria, leishmaniasis, amoebiasis, and trypanosomiasis, within contexts associated with poverty, deficiencies in basic sanitation, limited access to potable water, and increased exposure to vectors or animal reservoirs.[20] In parallel, protozoal infections affecting livestock and wildlife, including babesiosis, neosporosis, toxoplasmosis, and cryptosporidiosis, generate a significant impact on the agricultural economy of the country, contributing to substantial annual economic losses and reinforcing the close interrelationship between human, animal, and environmental health.[18,21]

Despite this epidemiological relevance in the Ecuadorian context, protozoal infections have received limited attention compared to other high-impact infectious diseases, such as COVID-19.[22] This situation has been reinforced by issues including a lack of financial support and the low political and institutional visibility these parasitic diseases receive in national health agendas.[22] Although studies on protozoal infections in the country have been identified, they appear fragmented, addressing the situation of different geographical regions, parasitic species, and hosts in

isolation. Thus, the absence of integrative syntheses in the available literature limits a comprehensive understanding of the Ecuadorian reality, contributing to underreporting and inaccurate estimates of epidemiological patterns.[23] Consequently, these diseases continue to be perceived as secondary problems, strengthening their classification as NTDs and continuing cycles of low research, weak surveillance, and the implementation of control and prevention strategies lacking adequate scientific support.

Given this scenario, it is essential to develop studies that integrate and analyze available Ecuadorian research to understand the current landscape of protozoal infections and guide effective public health decision-making through a comprehensive "One Health" approach. This approach is fundamental because protozoal parasitic diseases simultaneously involve people, domestic animals, wildlife, and environmental determinants, whose understanding and control require real coordination among different actors in human health, animal health, and natural resource management.[24] Specifically, at the human-animal-environment interface, it is necessary to consolidate surveillance inputs that include a wide diversity of data, whether demographic, epidemiological, behavioral, meteorological, geospatial, or socioeconomic data, to better guide the identification of focal points of risk and territorial prioritization.[24]

Overall, this type of analysis would help identify knowledge gaps, diagnostic limitations, and territorial inequalities that condition the surveillance of these types of infections. Therefore, the objective of this scoping review was to analyze the production of scientific literature and research published in Ecuador associated with the detection of parasites and protozoal infections over the last eight years, between 2016 and 2023.

METHODS

Article selection criteria

The present scoping review was developed to map the available evidence and does not constitute a systematic review of efficacy or an integrative review. The search strategy was designed following the methodological principles for the formulation and execution of systematic searches (Cochrane Handbook), and the review was conducted according to the steps of the JBI Manual for scoping reviews (PCC framework) and reported according to PRISMA-ScR (https://www.prisma-statement.org/scoping).[25,26]

The decision to implement a scoping review protocol was based on the exploration of scientific production regarding protozoal infectious agents in Ecuador over time. This approach is not related to proposals for changes in diagnostic practice for these microorganisms or objectives associated with evaluating clinical efficacy. Additionally, it aimed to investigate the infectious agents studied, research by geographic area, and the populations studied, as well as the laboratory techniques employed, with the intention of identifying knowledge gaps and areas for potential prioritization.

In accordance with Ecuadorian legislation, public and private laboratories under the inspection of the Ministry of Public Health of Ecuador (MSP) can be divided into low- and high-complexity laboratories, depending on the type of tests they perform. In general, the included studies consisted of published articles on the use of laboratory tests, ranging from the basic use of a microscope to the implementation of high-complexity tests such as DNA/RNA sequencing in the field of molecular biology.

Study eligibility was established based on the Population-Concept-Context (PCC) framework described in the JBI guidelines for this type of review.[26] Thus, the population (P) consisted of humans, including the general population and vulnerable subgroups, domestic vertebrate animals, wildlife, and arthropod vectors relevant to protozoal epidemiology. The concept (C) was the detection, characterization, screening, or diagnosis of protozoa through laboratory tests. The context (C) comprised studies conducted in Ecuador across all provinces, clinical and community settings, and including environmental matrices with implications for human and animal health, such as abiotic components of the environment. These PCC elements guided the selection and synthesis of the evidence. The main inclusion criteria were the use of laboratory tests for the characterization, detection, screening, or diagnosis of infectious diseases and protozoal microorganisms associated with a relevant health problem in Ecuador. Pathogens affecting humans, vertebrate animals, and agents with zoonotic potential, whose detection was carried out from biological and abiotic samples such as water bodies, food, soil, and fomites, were included.

Exclusion criteria consisted of studies not focused on Ecuador or conducted without samples from the country. Studies based only on signs or symptoms without incorporating laboratory tests for the study or diagnosis of the disease, and those employing an approach primarily directed at treatment or the evaluation of prevention strategies rather than the characterization, detection, or diagnosis of diseases or pathogens. Furthermore, studies using Ecuadorian samples only as experimental controls were not included. Similarly, narrative and systematic reviews, book chapters, meta-analyses, letters, comments, grey literature, and preprints were excluded, as were databases not included in this review.

Literature search strategy

The bibliographic search was conducted in the PubMed, SCOPUS, and SciELO databases to ensure coverage of both international and regional studies. The search was performed between March and July 2024, considering publications from 2016 to 2023. 2016 was chosen as the starting year for the search due to the situation of Ecuador in 2015 regarding protozoal diseases relevant to the region, such as *Plasmodium* spp., *Leishmania* spp., and *Trypanosoma cruzi*. At the regional level, PAHO reported a spike in cases compared to 2014 and placed the country among those with an increase in malaria.[27] Regarding leishmaniasis, the 2015 country profile recorded 1,479 new cases, with an incidence of 14.9 per 100,000 and transmission classified as "moderate".[28] In the case of Chagas disease, it remains endemic and is recognized by PAHO as a neglected disease with vector-borne, congenital, and other routes of transmission.[29]

Specific search strings were developed iteratively for each database, following the recommendations of the Cochrane/JBI guidelines. Initially, a limited pilot search was conducted in PubMed to generate a seed set of clearly eligible studies associated with Ecuador. Subsequently, keywords and indexing (MeSH/DeCS) were analyzed to refine three blocks: 1) geography (Ecuador), 2) diagnostic/detection methods, in addition to epidemiological terms such as prevalence, incidence, risk factors, and publication types, and 3) broad concepts associated with protozoan genera and One Health. Similarly, the use of terms associated with taxonomic species was avoided to maximize retrieval. Each block was expanded with synonyms and spelling variants according to the instructions of each database. Searches were carried out using English terms for the PubMed and SCOPUS databases, while terms in English, Spanish, and Portuguese were used in the search strings for the SciELO database. The final strategies were peer-reviewed. The searches covered 2016-2023, and when a platform did not accept date limits in the query, year filters were applied directly in the interface. The search strings used for each database have been included in the Supplementary Material.

Selection process and extracted variables

For the selection of documents, the electronic publication year before printing was considered, as well as dates related to the first available documents. The multidisciplinary team assigned to review the documents during the PRISMA screening consisted of professionals from the areas of biomedicine, medicine, bioinformatics, and geographic information systems, whose roles are indicated in the authors' contribution section.

The search was conducted independently by five authors, who performed the initial screening by reviewing titles and abstracts, followed by an exhaustive full-text review. The variables taken into account were: the number of publications each year, the type of infectious agent studied, the type of host or sample source, the provincial geographic location of the studies, and the laboratory tools employed for the detection of the protozoa.

Synthesis and analysis of the results

The extracted data were consolidated into a Microsoft Excel (Windows) matrix and analyzed through descriptive synthesis. A numerical summary of the evidence was performed based on frequencies and percentages according to the reported protozoan, province, population/location of detection, and detection technique. Additionally, a narrative synthesis was conducted to contextualize epidemiological patterns and knowledge gaps. Since a single study could report more than one protozoan, the categories were not mutually exclusive. Therefore, when percentages were reported, they were calculated based on the total number of included studies, and their sum could exceed 100%. To explore temporal changes in scientific production, the studies were grouped into two time periods (2016-2019 and 2020-2023) to facilitate data interpretation. Cartography and spatial visualization by province were developed using ArcGIS Pro v3.2.0.

As this is neither a systematic review nor a meta-analysis, the present scoping review does not include bias analysis or assessment of the certainty of the evidence of the included studies.[26] The basis of this work protocol was registered on the Open Science Framework server (https://osf.io/), and is available at https://osf.io/8vuc3.

RESULTS AND DISCUSSION

The search identified 1,180 records. After removing 284 duplicates and 737 during the title and abstract screening, 159 full texts were evaluated, and 98 studies were included. The PRISMA diagram also includes the reasons for exclusion (Figure 1). Regarding scientific production, it increased from 40 publications (2016-2019) to 58 (2020-2023), suggesting an expansion of research lines in the country (Figure 2).

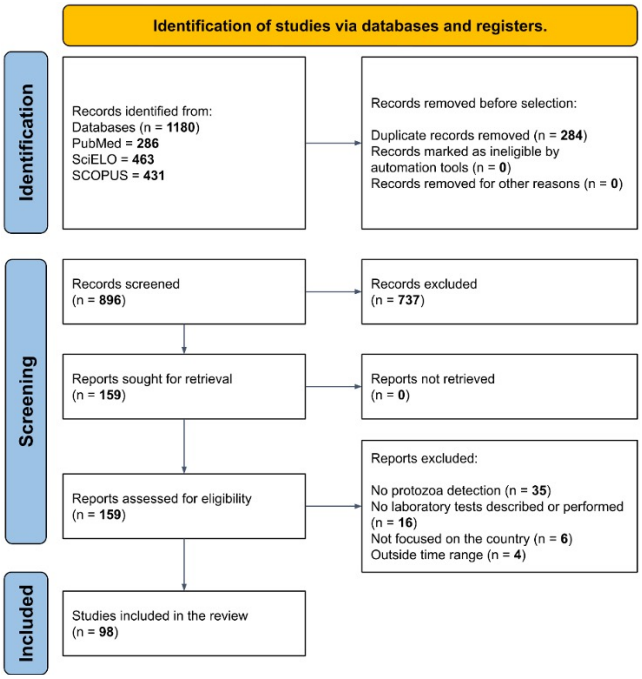

Figure 1. PRISMA flow and screening diagram of the retrieved and evaluated literature.

The summary of the main results found regarding the most studied protozoan species, along with diagnostic methods and studies by province, is presented in the summary table of these aspects (Table 1; Supplementary Table 1).

During 2016-2019, research on *Leishmania* spp. predominated with 12 studies, followed by work on *Entamoeba* spp. (4 studies) and others, including *Giardia* spp. *Trypanosoma cruzi* (3) and *Plasmodium* spp. (4) were also reported (Figure 3A, Table 1). This pattern reflects the importance of leishmaniasis in the Coast, Highlands, and Amazon regions, where climatic conditions of 15-38°C and high humidity in peridomestic and forest environments favor its vectors.[30,31] In 2019, the MSP reported 1,108 cases, 1,086 of which were cutaneous and 21 mucocutaneous, primarily affecting individuals aged 20 to 49 years, and this trend has persisted for the years 2023-2024, with reports nearing 1,000 cases. [32,33] Despite this, scientific production on the subject decreased starting in 2020 (Figure 3B), which could be associated with the diversification of protozoan genera and species studied in the second evaluated time period. Knowledge gaps persist regarding vector ecology and wild reservoir dynamics, and there is a need to strengthen evidence on the role of canines as reservoirs of cutaneous and mucocutaneous forms in Ecuador, despite their relevance to the visceral form in the region.[34] These priorities coincide with those established in the 2023-2030 PAHO Action Plan, which include reinforcing applied entomology studies, reservoir studies, and systematic molecular diagnosis.[35]

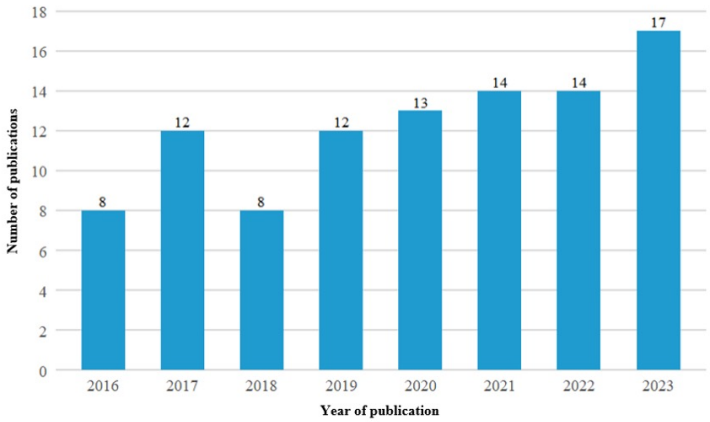

Figure 2. Scientific production associated with the detection and diagnosis of protozoal infections in Ecuador. Total quantification of studies is shown for each included year, obtained from PubMed, SCOPUS, and SciELO databases.

Intestinal protozoa constitute a significant group of studied infectious agents, primarily in human samples from children and indigenous populations (Figure 3, Table 1, Supplementary Table 1). National reports up to epidemiological week 30 of 2021 describe approximately 4,000 cases of food poisoning with a special emphasis on bacteria,[36] but without highlighting the potential role of protozoa in intestinal conditions. Globally, 1.5 billion people suffer from gastrointestinal parasitic infections, with a higher prevalence of *G.*

lamblia, *E. histolytica*, and *Cryptosporidium*.[37] Children under 5 years of age are the highest-risk group due to their immature immune systems and age-appropriate exploratory behaviors that increase the risk of exposure.[38] Although most studies employ PCR and other molecular techniques, further research into microbiota and microbial interactions is required, particularly the role of *Blastocystis* spp. in states of dysbiosis and general symptoms, which deserves greater exploration.[39] To complement this research, the WHO and PAHO have developed educational materials and recommendation manuals promoting basic measures focused on improving access to potable water, strengthening sanitation practices, and maintaining epidemiological surveillance.[40,41] However, scientific production in these aspects remains insufficient.

During the second period analyzed, a greater variety of studied infectious agents was evident, either individually or by simultaneously detecting different microorganisms in a single work (Figure 3B). Additionally, studies employing abiotic environmental samples are described starting from the year 2020 (Supplementary Table 1). As in the first period, several articles related to *Leishmania* spp. were identified. Furthermore, research focused on pathogens with affinity for the digestive system was presented, such as *Giardia* spp. and *Cryptosporidium* spp., both individually and in combination with the detection of other agents such as *Entamoeba* spp. and *Blastocystis* sp. In a lower proportion, studies were recorded on *Trypanosoma* spp., *Toxoplasma gondii*, *Babesia* spp., *Plasmodium* spp., and *Trichomonas vaginalis* (Figure 3B).

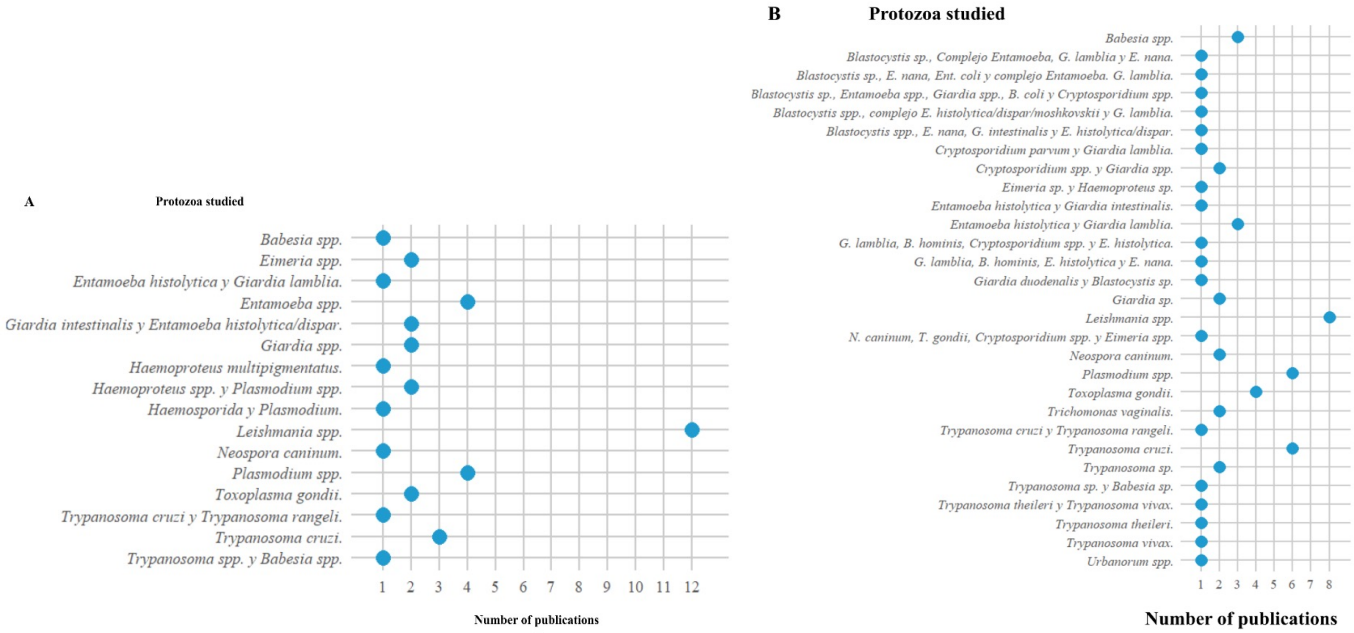

Figure 3. Classification of published articles on protozoan parasites in Ecuador. The number of articles in the two time periods and the species studied is shown. A) Articles published in indexed databases in the 2016-2019 period. B) Articles published in indexed databases in the 2020-2023 period.

Table 1. Summary of the main results from studies on protozoal detection and diagnosis in Ecuador (2016-2023).

Pathogen group	Frequency and percentage of appearance in studies*	Main diagnostic methods	Provinces with the highest N°. of studies	Characteristics of the predominant population studied.
Giardia spp.	21 (21.4)	Microscopy, PCR/qPCR, ELISA.	Pichincha, Esmeraldas.	Humans, children (and pediatric population).
Leishmania spp.	20 (20.4)	PCR/qPCR, microscopy, sequencing.	Pichincha, Santo Domingo de los Tsáchilas, Amazonian provinces.	Humans, vectors (insects).
Entamoeba spp.	18 (18.4)	Microscopy, PCR/qPCR, ELISA.	Chimborazo, Pichincha, Tungurahua.	Humans, Dogs.
Plasmodium spp.	13 (13.3)	PCR/qPCR, sequencing, NGS/Genomics.	Esmeraldas, Orellana.	Humans, wildlife.
Trypanosoma cruzi	11 (11.2)	PCR/qPCR, ELISA, microscopy.	Guayas, Manabí.	Vectors (insects), humans (congenital Chagas surveillance cases).
Toxoplasma gondii	7 (7.1)	ELISA, serology.	Chimborazo, Pichincha.	Humans, wildlife.

Abbreviation: A list of the most studied protozoan groups 2016-2023 is shown. *Values outside parentheses indicate frequency, and those inside indicate percentages of appearance. See Supplementary Table 1 for details of each included study.

In the case of trypanosomatids, most studies focus on *Trypanosoma cruzi*, the causative agent of Chagas disease, and to a lesser extent, on species of veterinary interest such as *T. vivax* and *T. theileri* (Figure 3, Table 1). In Latin America, Chagas causes approximately 752,000 lost workdays and an annual cost of USD 627 million in healthcare.[42] In Ecuador, reported prevalences in the Coast and Amazon regions are below 1%. A key article addresses congenital Chagas surveillance, indicating that vertical transmission can occur in any trimester of pregnancy and that antiparasitic drugs are contraindicated due to teratogenic risk, which demands preventive and educational strategies in vulnerable populations.[43] Furthermore, studies on *T. cruzi* include detection in triatomines and dogs, important reservoirs in peridomestic cycles due to their proximity to humans. The analysis of arthropod vectors is essential for designing vector control programs and modifying at-risk housing.[44] The roadmap to control and eliminate *T. cruzi* includes triatomine eradication campaigns, separation of animal breeding areas from dwellings, community education, and attention to transmission through transfusions, oral routes, and migration events.[45]

Since 2020, research has increased regarding trypanosomatids of animal importance, such as *T. vivax*, which causes morbidity and mortality in cattle with an impact on host productivity,[46] while *T. theileri* is a cosmopolitan and opportunistic parasite in various hosts and vectors.[47] There remains a need to delve deeper into potential vectors, given the high density of horseflies in the humid tropical forests of Ecuador.[48] The underdiagnosis of diseases caused by these agents may be related to their low recognition compared to more prevalent pathologies like brucellosis and foot-and-mouth disease, which puts the economy of the farms at risk and favors the continuous presence of protozoa in extensive management systems.[49] Finally, surveillance is crucial due to its zoonotic potential, as human infections with *T. vivax*, especially in rural areas, have been documented in other parts of the world.[50] Recently, in 2025, another parasite of veterinary interest and zoonotic potential, *T. evansi*, was reported for the first time in the Ecuadorian Amazon, increasing the record of pathogenic protozoan species in the country.[51]

Similar to the trypanosomatids, studies for the Apicomplexa group have increased since 2020, highlighting articles on *Plasmodium* spp., *Toxoplasma gondii*, and *Babesia* spp., specifically using human and wildlife samples for detection (Figure 3, Table 1). Globally, approximately 28 million clinical cases and 405,000 deaths from malaria were estimated in 2018,[52] while in Ecuador, a decreasing trend in reported cases is observed, from 698 in 2023 to 340 in 2024, within the framework of disease elimination efforts.[53] A key component for contributing to malaria control and elimination actions is maintaining the detection of different disease-causing species via PCR, with special emphasis on detecting asymptomatic individuals and identifying natural reservoirs.[54] Likewise, efforts for global collaboration between countries to share genetic and epidemiological data, without neglecting the availability and distribution of treatment options,[54] highlight the need to maintain biomedical research on the disease in the country to achieve elimination goals.

On the other hand, the presence of *T. gondii* has been studied more extensively in the country since 2020, primarily employing serological tools with a routine screening focus across different populations (Figure 3, Table 1). However, for this pathogen, the need to strengthen biomedical studies has also been highlighted due to new facets associated with the infection. Despite the high global prevalence of 30% of the disease, *T. gondii* negatively impacts not only immunocompromised patients, but latent infections have also been associated with neuropsychiatric conditions and behavioral disorders, such as schizophrenia, bipolar disorder, epilepsy, and major depression.[55] The relevance of *T. gondii* to global health is significantly greater than commonly recognized due to its latent and long-term effects.

It is important to note that, during the years analyzed, the use of laboratory tests based on a combination of PCR targeting genetic regions of interest and DNA sequencing predominated, which allowed for the confirmation and in-depth study of the analyzed infections. To a lesser extent, the use of serological tools for antibody detection and basic laboratory analyses such as microscopy and smear observation, were also used (Table 1, Supplementary Table 1).

In the analysis of publication production by Ecuadorian provinces, the provinces of Pichincha and Guayas present the greatest variety of detected protozoan genera, with a total of ten, *Leishmania* spp. being the most researched (Figure 4). On the other hand, the province of Chimborazo presents studies on eight genera, with a particular emphasis on digestive system pathogens. At the national level, *Leishmania* spp. and *Plasmodium* spp. stand out for their wide detection in published studies, covering 23 and 17 provinces, respectively. Additionally, research involving between one and two protozoan genera has been conducted in 5 provinces, with a predominance of *Leishmania* spp. and *Plasmodium* spp., and occasionally *Trypanosoma* spp. Regarding the insular region, consisting of Galápagos, only works associated with *T. gondii*, *Haemoproteus* spp., and *Eimeria* spp. are recorded under the established methodological criteria, which are focused on animals (Figure 4, Supplementary Table 1). Further research into the ecological characteristics of each

region and their related risk factors is essential. This approach could help map high-risk areas and generate measures tailored to the most susceptible populations. Additionally, it is important to enhance research approaches in the country by formally incorporating training in molecular diagnostic tools in health-related degrees such as medicine and biomedical sciences, for both clinical service and research.[56]

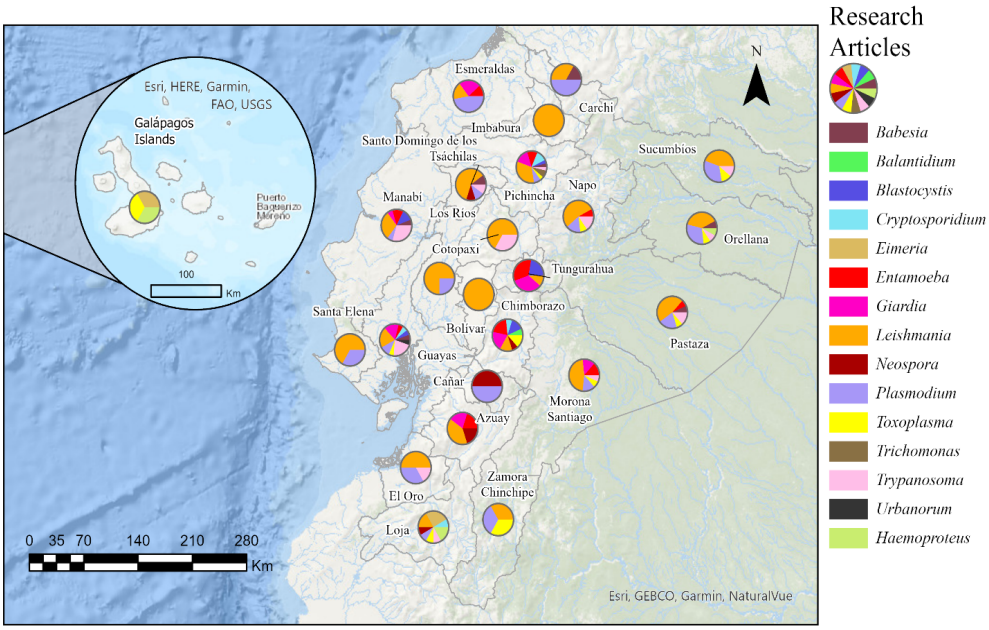

Figure 4. Proportion of publications including protozoal detection by Ecuadorian provinces. The map shows the detection of protozoa in Ecuador at the genus level by province, derived from the studies analyzed in the 2016-2023 period.

The primary limitations of this study are the bias associated with the databases used and the heterogeneity of methods and reports. This work used peer-reviewed articles that represent different aspects of the epidemiology and eco-epidemiology of the diseases described, but data on the general situation of these diseases in the territory are still needed. Therefore, to reinforce elements relevant to the public health of the country in the discussion, we used data and information on pathologies, based on electronic resources from national health institutions such as the MSP and international ones such as the WHO and PAHO. Similarly, the present study does not include analyses of other relevant aspects of disease management in general, such as prevention and treatment elements.

CONCLUSIONS

In conclusion, the present study determined the trend in scientific production of articles published in databases regarding protozoal detection in Ecuador. Studies on infectious agents transmitted by arthropods and through food and water contamination are highlighted. Furthermore, research focused on humans and the use of high-complexity laboratory tools stand out. These results represent the published evidence available in the consulted databases and do not necessarily reflect the actual diversity of protozoa present in the country. The absence or low frequency of reports for specific infectious agents, hosts, or sampling sites represents potential indicators of research gaps, underreporting, and diagnostic and territorial disparities. Based on the identified patterns, key research priorities for Ecuador include a greater number of investigations into other components of potential transmission chains, both biotic and abiotic, increased use of next-generation sequencing tools, directing efforts toward provinces lacking studies, and increasing research in rural areas and the Ecuadorian Amazon. Determining these patterns is useful for decision-making departments and surveillance systems in guiding the geographic and thematic prioritization of studies, as well as for universities and research centers in defining agendas and diagnostic capacities. These initiatives will support surveillance systems that integrate human, animal, and environmental health perspectives within the "One Health" framework.

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AUTHORS' CONTRIBUTIONS

Micaela Gómez-Hinojosa conceptualization, application of the PRISMA-ScR protocol, general drafting. **Agatha Sandoval** conceptualization, application of the PRISMA-ScR protocol, general drafting. **Gabriela Naranjo** processing, data analysis, application of the PRISMA-ScR protocol, and generation of figures. **Miguel Martínez-Fresneda** processing, data analysis, application of the PRISMA-ScR protocol, and generation of figures. **Grecia Victoria Vivas-Colmenares** processing, data analysis, and generation of figures. **Andrés Herrera-Yela** conceptualization and general drafting. **Juan-Carlos Navarro** conceptualization and general drafting. **José Ramírez-Iglesias** conceptualization, application of the PRISMA-ScR protocol, general drafting, processing, and data analysis.

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