Journal of Hunan University

(Natural Sciences)

Vol. 52 No. 3

March 2025

Available online at

https://jonuns.com

Open Access Article

https://doi.org/10.55463/issn.1674-2974.52.3.6

Seroprevalence of Parvovirus B19 in Immunocompromised ICU Patients in Western Colombia

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Article History:

Received: December 7, 2024
Reviewed: January 16, 2025
Revised: February 9, 2025
Accepted: March 25, 2025
Published: April 30, 2025

Abstract: Human parvovirus B19 (B19) is a pathogen that primarily infects erythroid and other cell types due to its binding to the P globoside antigen. The seroprevalence of anti-B19 IgG varies with age, ranging from 2-15% in children to over 85% in older adults. B19 can cause a wide range of clinical manifestations, including erythema infectiosum (fifth disease) in children and pure red cell aplasia in immunocompromised individuals, particularly those with HIV, oncology or autoimmune diseases. The aim of this study was to determine the seroprevalence of IgM, IgG, and IgG avidity against B19 in immunocompromised patients hospitalized in two health institutions in Valle del Cauca, Colombia. A total of 104 immunocompromised patients hospitalized in intensive care units at two institutions in Valle del Cauca, Colombia, were enrolled in this study. Blood samples were collected and analyzed by immunoassay to determine the seroprevalence of IgM, IgG, and IgG avidity specific for B19. Statistical analysis was performed to assess the relationship between B19 seroprevalence and variables such as age, chronic non-communicable diseases, acquired immunodeficiencies, and severe anemic syndrome. The study showed a seroprevalence of 66.35% for IgG anti-B19 in immunocompromised patients. A statistically significant correlation was found between B19 seroprevalence and the presence of chronic non-communicable diseases (p = 0.000) and

acquired immunodeficiencies (p = 0.018). There was also a significant association between seropositivity and severe anemic syndrome (p < 0.000) and patient age (p = 0.009). This study is the first to document B19 seroprevalence in immunocompromised patients in Colombia and highlights a significant association with specific immunosuppressive conditions, such as chronic non-communicable diseases and acquired immunodeficiencies. The findings highlight the importance of monitoring B19 infection in these vulnerable populations, particularly given its association with severe anemia and other critical conditions.

Keywords: Seroprevalence; immunocompromised patients; anemia; human parvovirus B19.

哥倫比亞西部免疫功能低下 ICU 患者中細小病毒 B19 的血清陽性率

摘要：人類細小病毒 B19 (B19) 是一種由於與 P 球苷抗原結合而主要感染紅血球和其他細胞類型的病原體。抗 B19 IgG 的血清陽性率隨年齡的變化而變化，從兒童的 2-15% 到老年人的 85% 以上不等。 B19 可引起多種臨床表現，包括兒童傳染性紅斑（第五種疾病）和免疫功能低下個體（特別是患有 HIV、腫瘤或自體免疫疾病的個體）的純紅血球再生不良性貧血。本研究的目的是確定哥倫比亞考卡山谷兩家衛生機構住院的免疫功能低下患者中針對 B19 的 IgM、IgG 和 IgG 親和力的血清流行率。哥倫比亞考卡山谷兩個機構的重症監護室住院的總共 104 名免疫功能低下患者參與了這項研究。收集血液樣本並以免疫分析進行分析，以確定 B19 特異性 IgM、IgG 和 IgG 親和力的血清陽性率。進行統計分析以評估 B19 血清盛行率與年齡、慢性非傳染性疾病、後天免疫缺陷和嚴重貧血症候群等變數之間的關係。研究顯示，免疫功能低下患者的 IgG 抗 B19 血清陽性率為 66.35%。 B19 血清盛行率與慢性非傳染性疾病（p = 0.000）和後天免疫缺陷（p = 0.018）之間有統計學顯著相關性。血清陽性與嚴重貧血症候群（p < 0.000）以及患者年齡（p = 0.009）之間也有顯著相關性。這項研究首次記錄了哥倫比亞免疫功能低下患者中 B19 血清陽性率，並強調了與慢性非傳染性疾病和後天免疫缺陷等特定免疫抑制疾病的顯著相關性。研究結果強調了監測這些易感人群中 B19 感染的重要性，特別是考慮到它與嚴重貧血和其他危急情況的關聯。

关键词：血清流行率；免疫功能低下的患者；貧血;人類細小病毒 B19。

1. Introduction

Parvovirus B19 (B19) is a human pathogen and the only member of the *Parvoviridae* family, characterized by its non-enveloped, single-stranded DNA genome of approximately 5.5 kb. The virus forms an icosahedral capsid containing two structural proteins: VP1, which makes up 5%, and VP2, which makes up the remaining 95%, with a key difference of 227 amino acids in the N-terminal region [1]. The primary non-structural protein NS1 plays a critical role in viral replication and pathogenesis [2]. The cellular infection mechanism of B19 is mediated by the Gb4 globoside P antigen, which is abundantly expressed on a variety of cell types, including hematopoietic progenitors, erythroblasts, fetal myocyte, megakaryocytes, placental trophoblasts, endothelial cells, platelets, and smooth muscle cells [3].

Worldwide, the seroprevalence of IgG antibodies to parvovirus B19 varies considerably among different age groups. Seroprevalence ranges from 2% to 15% in children aged 1 to 5 years, increasing to 15% to 60% in individuals aged 6 to 19 years, and further increasing to 30% to 60% in adults. The geriatric population has the highest seroprevalence, exceeding 85% [4]. This widespread prevalence correlates with several clinical manifestations resulting from the B19-induced inhibition of erythropoiesis [5]. In children, B19 is the etiological agent of erythema infectiosum (fifth disease), whereas in adults it can cause erythroblastopenia and polyarthropathy [6]. In addition, B19 infection can cause thrombocytopenia, granulocytopenia, hemolytic anemia during acute infection, and severe aplastic crisis in cases of chronic persistent viremia [7].

The clinical manifestations of parvovirus B19 infection are closely related to the hematological and immunological status of the host. In immunocompromised individuals, such as those with hematological disorders, autoimmune diseases, cancer, transplant recipients or HIV/AIDS, B19 infection can lead to severe complications, including pure red cell aplasia and chronic anemia [8, 9]. In transplant recipients, B19 poses a significant risk, potentially leading to graft loss [10] and the

development of glomerulopathy, which can progress to chronic kidney disease [11]. B19 has also been implicated in the pathogenesis of systemic lupus erythematosus, Hashimoto's thyroiditis, and rheumatoid arthritis [12]. Neurological involvement has also been documented, with cases of Guillain-Barré syndrome, meningoencephalitis, acute encephalitis, and encephalopathy attributed to B19 infection [13]. In addition, the virus has been associated with chronic fatigue syndrome, further highlighting its diverse and potentially serious impact on human health [14].

In oncology patients, parvovirus B19 infection often results in persistent, generally non-specific symptoms [15]. While the infection is typically benign in healthy individuals, the natural history of acute B19 infection is characterized by an initial transient high-level viremia lasting approximately one week [16]. This phase is subsequently controlled by the production of neutralizing antibodies, leading to the appearance of specific IgM antibodies, followed by the lifelong persistence of specific IgG antibodies [4]. However, in immunocompromised individuals, particularly those with bone marrow disorders, the inability to mount an effective neutralizing antibody response prevents the clearance of the virus, resulting in chronic infection and carrier status [17]. Given the lack of data on the prevalence of B19 in immunocompromised patients in Colombia, this study aimed to determine the seroprevalence of anti-B19 IgG and IgM antibodies and to explore their correlation with the hematological status of these vulnerable patients.

2. Methods and Materials

2.1 Immunocompromised Patients

This cross-sectional study was conducted among immunocompromised patients hospitalized in the intensive care units (ICUs) of two tertiary health care facilities in Valle del Cauca, Colombia, between 20 January and 23 November 2022. The study included 104 adult patients who voluntarily provided informed consent and completed a survey. Participants were immunocompromised due to chronic non-communicable diseases, including type II diabetes mellitus, chronic renal failure and chronic obstructive pulmonary disease.

The cohort also included oncology patients with diseases such as chronic lymphocytic leukemia, breast cancer, gastric cancer, prostate cancer, and cervical cancer. Hematology patients with non-autoimmune hemolytic anemia, sickle cell anemia and thalassemia were included, as well as those with acquired immunodeficiencies such as HIV, cancer in remission, cirrhosis and post-kidney transplant status. Patients with autoimmune immunodeficiencies including systemic lupus erythematosus, rheumatoid arthritis, autoimmune hemolytic anemia and collagen vascular diseases were also included.

The study focused on patients with anemic syndrome, defined by reduced hemoglobin levels (with a red blood cell count below 3x10^6/μL) and characterized by a normocytic, normochromic peripheral blood smear. The exclusion criteria included patients who were not immunosuppressed or without an anemic syndrome. The research flowchart is shown in Figure 1.

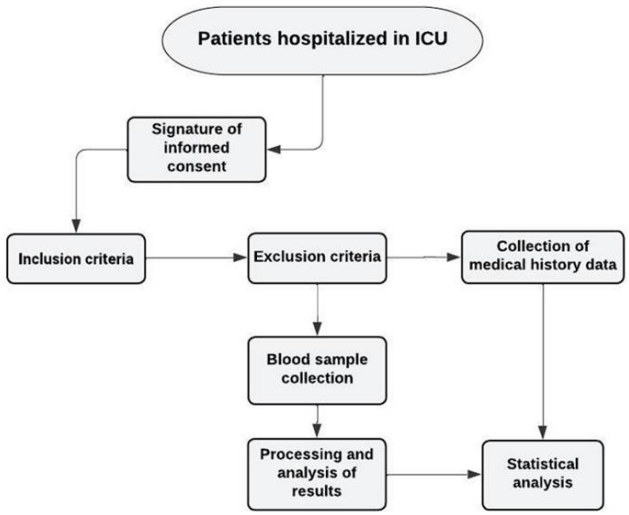

Figure 1. Flowchart for the research methodology (Developed by the authors)

This research was formally approved by the Ethics Committee of the Unidad Central del Valle del Cauca (UCEVA), Colombia, through an administrative act on July 30, 2019. The approval confirms that the study meets the scientific, technical and administrative standards outlined in Resolution 008430/93 of the Colombian Ministry of Health, as well as all applicable institutional bioethical requirements.

2.2 Blood Samples

A 5 mL sample of whole blood was collected from each participant using a sterile Vacutainer tube containing EDTA. This sample was used for hemoglobin determination, peripheral blood smear preparation, and serological analysis. Hemoglobin levels were measured using the Sysmex XN 1000-XN series analyzer using the sodium lauryl sulfate (SLS) method, a cyanide-free technique that forms a colored complex (SLS-HGB).

The severity of anemia was classified according to the World Health Organization (WHO) criteria. For males aged 15 years and older, anemia was classified as mild (hemoglobin levels between 11.0 and 12.9 g/dL), moderate (8.0 to 10.9 g/dL), or severe (<7.9 g/dL). In non-pregnant women aged 15 years and older, anemia was classified as mild (hemoglobin levels between 11.0 and 11.9 g/dL), moderate (8.0 to 10.9 g/dL), or severe (<7.9 g/dL).

2.3 B19 Seroprevalence in Immunocompromised Patients

The collected blood samples were centrifuged at 3,000 rpm for 15 minutes to separate the plasma, which was then aliquoted into cryogenic tubes and stored at -20°C for subsequent analysis. Anti-B19 IgM, IgG and IgG avidity were quantified using the recomLine Parvovirus B19 IgM (Cat. 4473) and IgG [avidity] (Cat. 4472) assays. These assays were developed using MIKROGEN DIAGNOSTIK's Western blot technology and were interpreted according to the manufacturer's guidelines to classify the infection status as past, active, or reactive.

2.4 Data Analysis

Descriptive statistical analysis was performed on the basis of the characteristics of each variable. The Shapiro-Wilk test was used to assess the normality of the data, while the χ^2 test, Fisher's exact test and Mann–Whitney U test were used for inferential analysis. All statistical calculations were performed using STATA 14.0® software, with a p-value of <0.05 considered indicative of statistical significance.

3. Results and Discussion

3.1. Demographic Profile and Age Distribution of the Study Participants

A comprehensive socio-demographic analysis revealed a balanced gender distribution among the 104 participants, with a slightly higher proportion of women (52.88%) than men (47.12%). The median age was 55 years, with slight differences between the genders. Participants spanned a wide age range, with 48.08% classified as adults (30–59 years), 34.62% as older adults (≥60 years), and 17.31% as young adults (18–29 years). These findings provide a foundational understanding of the study population's characteristics (see Table 1).

Table 1. Clinical and demographic characteristics of immunocompromised patients stratified by the type of immunosuppression (compiled by the authors)

Type of immunosuppression	Sex		Age, mean (IQR)	Hemoglobin, mean (IQR)
	Female (%)	Male (%)		
Chronic noncommunicable diseases (NCDs)	23 (41,82)	22 (44,90)	60 (64-57)	8,5 (8,8-7,7)
Oncological	7 (12,73)	4 (8,16)	56 (61-45)	7,2 (8,4-5,7)
Acquired immunodeficiencies	2 (3,64)	17 (34,69)	32 (45-25)	6,3 (7,5-5,8)
Hematological Diseases	8 (14,55)	5 (10,20)	25 (30-23)	6,3 (6,9-5,5)
Autoimmune Immunodeficiencies	15 (27,27)	1 (2,04)	37.5 (52,5-32)	6,7 (8,25-6,05)

Note: IQR: Interquartile range. This table presents a breakdown of the demographic and clinical characteristics of immunocompromised patients, classified according to the type of immunosuppression. The dataset includes the distribution of sex, with a higher prevalence of females among those with chronic non-communicable diseases and autoimmune immunodeficiencies and a predominance of males among those with acquired immunodeficiencies. The median age exhibited notable variation between the groups, reflecting disparate immunosuppressive profiles. The youngest mean age was observed in patients with hematological diseases (25 years; IQR: 23-30), while the oldest was observed in those with chronic non-communicable diseases (60 years; IQR: 57-64). The mean hemoglobin levels were significantly lower in patients with oncological diseases (7.2 g/dL; IQR: 5.7-8.4) and hematological diseases (6.3 g/dL; IQR: 5.5-6.9), indicating a more severe degree of anemia in these subgroups. This stratification serves to illustrate the considerable heterogeneity in the clinical presentation and severity of immunosuppression observed within the study population.

3.2. Seroprevalence of Parvovirus B19 IgG and Infection Stages

The study identified an overall IgG anti-B19 seroprevalence of 66.35%, indicating that the immunocompromised cohort has been exposed to the virus on a widespread basis. The avidity testing of IgG-positive samples revealed distinct stages of infection, with 43.48% indicating prior infections, 34.78% consistent with active infections, and 21.47% corresponding to reactive infections. Notably, only two participants exhibited IgM anti-B19 positivity, which reinforces the predominance of the IgG-mediated immune response in this group.

3.3. Correlations Between Seropositivity, Clinical Parameters, and Immunosuppression

The results of the statistical analysis indicated significant correlations between IgG seropositivity and a number of critical factors, including age ($p < 0.009$), hemoglobin levels ($p < 0.000$), and specific causes of immunosuppression, such as chronic non-communicable diseases and acquired immunodeficiencies ($p < 0.000$ and $p < 0.018$, respectively). Patients with low hemoglobin levels (median 7.6 g/dL) exhibited a particularly strong correlation with IgG seropositivity. These findings highlight the complex interplay of immunological, clinical, and demographic variables in this vulnerable population (see Table 2).

Table 2. Anti-B19 IgG seropositivity in immunocompromised patients: Distribution by sex, age, severity of anemia and type of immunosuppression (compiled by the authors)

	Anti-B19 IgG		
	Negative	**Positive**	***p* value**
Sex	***n* (%)**	***n* (%)**	
Female	17 (48,57)	38 (55,07)	0,53
Male	18 (51,43)	31 (44,93)	
Age (years)			
18-29	1 (2,86)	17 (24,64)	
30-59	18 (51,43)	32 (46,38)	0,009
>60	16 (45,71)	20 (28,99)	
Anemic syndrome			
Moderate	31 (88,57)	18 (26,09)	0,000
Severe	4 (11,43)	51 (73,91)	
Type of immunosuppression			
Chronic non-communicable diseases (n=45)	26 (74,29)	19 (27,54)	0,000
Oncological (n=11)	2 (5,71)	9 (13,04)	0,251
Acquired immunodeficiencies (n=19)	2 (5,71)	17 (24,64)	0,018
Hematological diseases (n=13)	2 (5,71)	11 (15,94)	0,136
Autoimmune immunodeficiencies (n=16)	3 (8,57)	13 (18,84)	0,170

Table 2 illustrates the anti-B19 IgG seropositivity rates in a heterogeneous cohort of immunocompromised patients and highlights notable correlations between seropositivity and specific clinical variables. It is noteworthy that seropositivity was significantly higher in younger patients (aged 18-29 years) and those with severe anemic syndrome ($p = 0.009$ and $p < 0.001$, respectively). Among the various forms of immunosuppression, a statistically significant correlation was identified between anti-B19 IgG seropositivity and acquired immunodeficiencies ($p = 0.018$). Conversely, no notable associations were observed in other categories, including chronic non-communicable diseases, oncological conditions, hematological diseases, and autoimmune immunodeficiencies ($p > 0.05$). These findings underscore the intricate relationship between age, the severity of anemia and underlying immunosuppressive conditions in influencing anti-B19 IgG seropositivity

3.4. Comprehensive Seroprevalence Analysis Across Diverse Immunosuppressed Groups

This study offers a pioneering evaluation of parvovirus B19 seroprevalence in five distinct immunosuppressed populations: patients with chronic non-communicable diseases, oncology, hematological disorders, acquired immunodeficiencies, and autoimmune conditions. Given that all participants exhibited moderate to severe anemia, characterized by normocytic and normochromic blood profiles, this research represents the inaugural documentation of B19 seroprevalence in an immunosuppressed cohort in Colombia. It offers invaluable insights into the epidemiology of the virus in this high-risk and understudied group.

3.5. High Seroprevalence of Parvovirus B19 in Colombian Immunocompromised Patients

The study revealed an overall anti-B19 IgG seropositivity rate of 66.35%, with a statistically significant correlation between the infection rates and the increasing age of the patients ($p < 0.009$). This prevalence is in close alignment with the findings of a Barcelona study, in which 66.7% of immunosuppressed patients, including those with HIV, cancer remission, and autoimmune diseases [18], tested seropositive. It is noteworthy that while the Barcelona study observed a higher seropositivity rate in individuals aged over 60 years, it did not find a significant age-related trend, which contrasts with the current research findings.

3.6. Comparison with General Population Studies: Amplified Risk in the Immunosuppressed Groups

A comparison with general population studies reveals that an increased risk is observed in immunosuppressed groups. In broader populations, such as pregnant women and blood donors, the seroprevalence rates of parvovirus B19 range between 53.9% and 69.5% [19-21]. These figures are notably lower than those observed in immunocompromised individuals, which highlights the heightened vulnerability of this group to B19 infections. These findings emphasize the critical need for targeted screening and tailored preventive measures for immunosuppressed patients, who face an increased risk of severe complications from B19 infection.

3.7. Gender as a Non-Influential Factor in Anti-B19 IgG Seroprevalence

No statistically significant relationship was observed between patient sex and anti-B19 IgG seroprevalence ($p = 0.53$), which aligns with the findings of similar studies on healthy donors and women with adverse obstetric histories [22]. The

seroprevalence rates observed in this study (55.07%) were found to be closely aligned with those reported in global studies, including those by [23] (52.6%) and the Taiwanese cohort (42.85%) with PCR-confirmed B19 infections. These findings reinforce the notion that gender is not a determining factor in B19 seroprevalence, instead suggesting that other influencing factors, such as immunosuppressive conditions, may play a more significant role [24].

3.8. Immunosuppression and its Role in B19 Infection Susceptibility

The study underscores the substantial overlap between parvovirus B19 infection and immunosuppressive conditions, with 43.48% of the participants exhibiting acquired or autoimmune immunodeficiencies. These findings are consistent with those of previous studies, which have demonstrated an elevated risk of B19 infection in patients with immunosuppression. The correlation between immunosuppression and infection emphasizes the necessity of prioritizing these conditions in the assessment and management of B19 risks.

3.9. B19 Infection and Hemoglobin Levels: Linking Anemia to Seroprevalence

A notable correlation was observed between anti-B19 IgG seropositivity and severe anemic syndrome ($p < 0.05$) [25], emphasizing the considerable influence of B19 infection on hemoglobin levels. The case of the Colombian report by Martinez et al. [26], in which a male patient with HIV exhibited hemoglobin levels as low as 6.2 g/dL, serves to illustrate the severity of anemia induced by B19. However, discrepancies emerge when the findings are compared with those of Iranian studies on HIV-related immunosuppression, in which no clear link between seropositivity and anemia was established. These disparate outcomes suggest that the pathogenesis of B19 in immunosuppressed patients is complex and influenced by factors that have yet to be fully elucidated.

3.10. Complexity of B19 Pathogenesis in Immunocompromised Populations

The variability in the clinical outcomes of B19 infection among immunosuppressed individuals underscores the complex interplay between viral, host, and environmental factors. Although this study identified a robust correlation between B19 infection and anemia, other investigations have yielded conflicting results, underscoring the necessity for continued research in this area [27]. It is recommended that future studies investigate the underlying mechanisms that underpin these associations in order to gain a deeper understanding of the clinical challenges posed by B19 in vulnerable populations.

3.11. Regional Variations in B19 Infection: Insights from Colombia and Beyond

The contrasting findings on the association between anemia and B19 infection, as exemplified by the study conducted in Nigeria [5], highlight the influence of regional factors, including genetic and environmental variables, on B19 epidemiology. Our study is the first in Colombia to identify a significant association ($p = 0.018$) between B19 infection and acquired immunodeficiencies, which underscores the distinct epidemiological context of the region.

3.12. Chronic Non-Communicable Diseases as Critical Risk Factors

The findings of this research demonstrate a statistically significant correlation between B19 infection and the development of chronic non-communicable diseases in patients with HIV. These findings provide robust evidence of increased susceptibility to B19 infection in individuals with pre-existing chronic conditions, thereby necessitating enhanced surveillance and tailored management strategies to mitigate severe anemic outcomes [28-33]. There is considerable geographic, demographic, and clinical variability in B19 infection. Patients with conditions such as sickle cell disease, beta-thalassemia major, or leukemia demonstrate a range of infection outcomes, emphasizing the necessity to consider regional epidemiological trends and immunosuppression stages in the assessment and management of B19-related risks [21, 34, 35].

3.13. Study Contributions and Methodological Limitations

This study elucidates the intricate interrelationship between B19 infection, severe anemia syndrome and underlying chronic or acquired conditions in immunosuppressed patients. However, potential selection bias and the limited investigation of clinical manifestations may compromise the generalizability of these findings. Further research into regional B19 genotypes and clinical outcomes is imperative.

3.14. Future Directions: Genotypic Insights and Regional Epidemiology

To advance understanding, future studies should prioritize the genotypic characterization of circulating B19 strains in Colombia and neighboring regions. Such investigations could elucidate transmission patterns, refine diagnostic techniques, and inform targeted management strategies for immunosuppressed populations.

4. Conclusion

This study identified a seroprevalence of anti-B19 IgG of 66.35% in an immunocompromised Colombian population, offering critical insights into the epidemiological and clinical dynamics of parvovirus B19 in vulnerable groups. The statistically significant association between B19 seropositivity and severe anemic syndrome ($p = 0.000$) serves to underscore the pronounced erythroid tropism of the virus and its role in intensifying hematological disorders. Furthermore, there was a strong association between parvovirus B19 and chronic non-communicable diseases ($p = 0.000$) and acquired immunodeficiencies ($p = 0.018$), which highlights the increased risks posed by underlying health conditions and the heightened vulnerability of immunosuppressed patients.

These findings reinforce the necessity for comprehensive serological screening and enhanced surveillance in at-risk populations, particularly in regions with distinctive epidemiological profiles. Furthermore, the study highlights the necessity for region-specific research to elucidate the complex interplay between B19 infection, immunosuppression, and clinical outcomes. To address these challenges, it is necessary to integrate molecular diagnostics, genotypic characterization of circulating B19 strains, and tailored interventions aimed at reducing infection-related complications. It would be beneficial for future investigations to explore the broader clinical manifestations of B19 infection and its long-term impact on immunocompromised populations, in order to inform the development of more effective prevention and management strategies.

This study highlights the importance of implementing routine serological testing in immunocompromised patients, given the high prevalence of parvovirus B19 infection and its impact associated with severe hematologic complications. In addition, the scientific community is urged to continue to investigate in depth the clinical manifestations and long-term effects of the virus in these patients, actions that will allow the development of more effective intervention strategies, improving clinical management and epidemiological surveillance.

Declarations

Author Contributions

Conceptualization, D.F.L.M. and B.G.O.; methodology, D.F.L.M., B.G.O., H.G.M., J.C.L.P., M.I.H.M.,F.R.L.V. and I.A.L.C.; software, D.F.L.M., B.G.O.; validation, D.F.L.M., B.G.O., H.G.M., J.C.L.P., M.I.H.M.,F.R.L.V. and I.A.L.C.; formal analysis, D.F.L.M. and B.G.O.; investigation, D.F.L.M., B.G.O., H.G.M., J.C.L.P., M.I.H.M.,F.R.L.V. and I.A.L.C., resources, D.F.L.M. and B.G.O.; data curation, D.F.L.M. and B.G.O.; writing—original draft preparation, D.F.L.M. and B.G.O.; writing—review and editing, D.F.L.M. and B.G.O.; visualization, D.F.L.M. and B.G.O.; supervision, D.F.L.M. and B.G.O.; project administration, D.F.L.M. and B.G.O.; funding acquisition, D.F.L.M. and B.G.O. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Central Unit of Valle del Cauca (UCEVA) through Call 12 of 2019, in accordance with Administrative Act 1300-39.1-006-F.

Acknowledgements

We would like to express our gratitude to the leadership of the UCEVA and the two participating health institutions in Valle del Cauca, Colombia, for their invaluable support and collaboration in the successful conduct of this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

Institutional Review Board Statement

Rigorous ethical guidelines were adhered to throughout the study to ensure participant privacy and data confidentiality in compliance with institutional and national research standards.

Informed Consent Statement

Participation in the study was voluntary, and informed consent was obtained from all the individual participants included in the study.

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https://doi.org/10.1016/j.bjid.2016.11.002

Word count: 4,324 words, excluding references.

Peer review information:
Whether the manuscript was fast tracked? - No
Number of reviewer report submitted in first round: 3 reports
Number of revision rounds: 2 rounds
Final revised version submitted: March 25, 2025