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Human brucellosis from consumption of unpasteurized milk in a rural area of Manabí

Brucelosis humana por consumo de leche no pasteurizada en una zona rural de Manabí

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ABSTRACT

Brucellosis remains a significant zoonosis in various regions of the world and persists as a public health threat, especially in rural communities where the consumption of unpasteurized milk is still common. This practice increases exposure to infections caused by bacteria of the genus *Brucella*. The objective of this study was to analyze human brucellosis due to the consumption of unpasteurized milk in a rural area of Manabí. An observational, descriptive, and cross-sectional study was conducted among residents of the rural area of Olmedo. All participants underwent serological testing: agglutination of the febrile antigen for *Brucella* and an ELISA-based assay for the detection of IgG antibodies. One sample showed positive reactivity by agglutination, while all samples tested negative in the ELISA assay, indicating greater reliability and accuracy of the ELISA method for the diagnosis of *Brucella*. It was concluded that serological methods, such as the ELISA assay, should be used to prevent cases of brucellosis in rural areas.

Keywords: brucellosis, zoonosis, unpasteurized milk, rural areas, serological testing, *Brucella* diagnosis.

RESUMEN

La brucelosis es constantemente una zoonosis relevante en distintas regiones del mundo y persiste como una amenaza para la salud pública, especialmente en comunidades rurales donde aún es común el consumo de leche sin pasteurizar. Este hábito incrementa la exposición a infecciones por bacterias del genero *Brucella*. El objetivo de este trabajo fue analizar la brucelosis humana por consumo de leche no pasteurizada en una zona rural de Manabí. Se efectuó un estudio observacional, descriptivo y transversal en los residentes de la zona rural de Olmedo. A todos los participantes se les realizó pruebas serológicas: aglutinación del antígeno febril para *Brucella* y un ensayo basado en ELISA para detección de anticuerpos IgG. Se evidenció que una muestra presentó reactividad positiva por aglutinación, mientras que todas las muestras en el ensayo ELISA fueron negativas, lo que indica una mayor confiabilidad y precisión en el método ELISA para el diagnóstico de *Brucella*. Se concluyó que se deben aplicar métodos serológicos como el ensayo de ELISA para prevenir casos de brucelosis en zonas rurales.

Palabras clave: brucelosis, zoonosis, leche no pasteurizada, áreas rurales, pruebas serológicas, diagnóstico de *Brucella*.

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INTRODUCTION

Human brucellosis is one of the most significant bacterial zoonoses worldwide and represents a serious public health problem, especially in developing countries where sanitation, epidemiological controls, and prevention measures are limited. Its main route of transmission to humans is through the consumption of unpasteurized dairy products, including raw milk and its derivatives, which can act as vehicles for the transmission of *Brucella* spp. (Hurtado, 2017). According to the World Health Organization (WHO), hundreds of thousands of cases are registered each year, primarily in vulnerable regions where inadequate practices in the handling and consumption of animal products persist.

In rural areas of Olmedo in Manabí, Ecuador, the production and consumption of raw milk are part of the daily lives of many families, who consider it a natural, economical, and readily available food. However, these risky practices in local customs carry a risk to human health, since unpasteurized milk can contain pathogens capable of causing acute or chronic infections. Human brucellosis can manifest through nonspecific symptoms such as prolonged fever, fatigue, excessive sweating, general malaise, and muscle and joint pain, which in many cases delay its diagnosis and timely treatment (Mondragón-Lenis et al., 2020).

Studies conducted in Latin American countries have demonstrated a direct relationship between the presence of brucellosis in farm animals and its transmission to humans through the consumption of unpasteurized dairy products (Rivadeneira, 2022). Research carried out in Ecuador has shown the persistence of this disease, especially in rural areas linked to livestock farming, where sanitary control measures, epidemiological surveillance, and health education programs are insufficient (Mondragón et al., 2020). The population remains in constant danger, particularly those who regularly consume unpasteurized milk or are involved in tasks of care, raising, herding, and direct handling of livestock.

Similarly, factors such as limited knowledge about this infection, the absence of truly effective preventive measures, and limited intervention by health entities contribute to *Brucella* infection persisting as a discreet but present problem within these communities. In many cases, people are unaware of the origin of the infection and continue to practice habits that promote the spread of the disease within their environment. This situation highlights the urgent need to

strengthen health education and promote safe practices in the handling and consumption of milk, especially in vulnerable rural populations (Rivadeneira, 2022).

Specifically, in Olmedo, the rural population is constantly exposed to unpasteurized milk due to local customs. Despite this reality, few studies evaluate human brucellosis in this area and its direct relationship with this practice, which limits the implementation of strategies for prevention, control, and timely treatment of the disease (Ormazábal et al., 2022).

Therefore, the objective of this research was to analyze the presence of human brucellosis associated with the consumption of unpasteurized milk in the rural area of Olmedo, Manabí province in Ecuador, through the application of serological tests, such as the *Brucella* febrile antigen agglutination and the IgG antibody test in ELISA, which allows establishing the relationship between this dietary habit and *Brucella* spp. infection, thus contributing to the generation of useful scientific information for public health decision-making to strengthen the knowledge of this problem and promote actions that improve the quality of life and well-being of the community.

METHODOLOGY

This research employs an observational, descriptive design, using a cross-sectional data collection approach. The study population consisted of residents of the rural area of Olmedo, Manabí, Ecuador, who had consumed unpasteurized milk in the past year. The sample comprised 70 individuals due to the limited population.

Participants who regularly consume unpasteurized milk were included in the study. Individuals with a prior diagnosis of brucellosis, those who do not consume unpasteurized milk or dairy products, and those who did not provide informed consent to participate were excluded.

This research employed a pseudo-anonymization process to safeguard the confidentiality of participant data, including the assignment of unique codes and the elimination of any reference to names or personally identifiable information. This process was carried out using data collected from each patient to ensure anonymous analysis. However, for the coding system, each patient received a structured alphanumeric code consisting of a letter (P: Patient), followed by three sequential numbers (001-070) assigned according to the order in which they were registered. The pseudo-anonymization process was then performed using the code P001.

Community outreach sessions were held in August 2025 to explain the study's purpose, procedures, and benefits to residents. Subsequently, data were collected based on inclusion and exclusion criteria designed to minimize issues during and after the analysis.

For this study, a venous blood sample was collected from the veins of the forearm. The most appropriate vein was selected for each patient, following biosafety and aseptic protocols. Subsequently, 3 to 5 mL of blood was drawn using a syringe and immediately placed in a yellow tube containing separator gel. This allowed for the separation of the serum from the blood, yielding the appropriate amount of serum for serological testing.

The samples were labeled with alphanumeric code, stored in refrigerated containers at 2–8 °C, and transported to the Koch Clinical Laboratory in the city of Manta, Manabí, for their processing. The Atlas Medical kit was used for the determination of antibodies (agglutinins) against *Brucella* in blood serum. This is a screening test, a slide agglutination assay. The principle consists of the interaction between the antibodies present in the patient's serum and the bacterial antigens of the reagent, generating a visible agglutination that indicates a positive reaction; it is negative.

For the ELISA, the Vircell S.L. kit for the quantitative determination of IgG antibodies against *Brucella* in serum or plasma was used. The assay principle is based on detecting antibodies directed against the *Brucella* lipopolysaccharide (LPS). According to the technical specifications of the Vircell S.L. protocol for the determination of IgG antibodies against *Brucella*, each serum sample was diluted in the corresponding buffer and dispensed into the antigen-coated wells. The plate was incubated for the specified time to allow antibody binding, followed by a series of automated washes to remove unbound fractions. Subsequently, the enzyme-labeled anti-IgG conjugate was added, and the plate was incubated again. After the second wash, the chromogenic substrate was applied, which reacted with the enzyme to generate a colored product proportional to the amount of antibodies present. The reaction was stopped with the stop solution, and the optical density was measured using a microplate spectrophotometer.

RESULTS AND DISCUSSION

Seventy participants who met the established criteria were evaluated. Regarding the distribution of participants by sex, 42 (60%) were male, while 28 (40%) were female. Table 1

shows that the majority of participants, exactly 90% (n= 63), reported consuming unpasteurized milk, compared to 10% (n= 7) who indicated they did not consume this type of product.

Table 1. Overall average of consumers of unpasteurized milk and the result of *Brucella* febrile antigen agglutination in blood serum

Consumption of unpasteurized milk	Frequency	Percentage	Febrile *Brucella* antigen agglutination	Frequency	Percentage
Yes	63	90	Positive	1	1
No	7	10	Negative	69	99
Total	70	100	Total	70	100

On the other hand, the results of the *Brucella* febrile antigen agglutination test show that only one participant (1%) tested positive, while the remaining 69 participants tested negative.

Table 2 shows that 100% of the samples analyzed (n= 70) were negative for *Brucella* antibodies by ELISA, with values below 9 units. No positive or intermediate cases were recorded, as the prevalence was 0% in the Olmedo population, indicating the absence of this infection in the participants studied.

Table 2. Results of the ELISA test for *Brucella* in the population of Olmedo

ELISA result	Frequency	Percentage
Positive (> 11)	0	0.0
Negative (< 9)	70	100.0
Indeterminate (9 to 11)	0	0.0
Total	70	100.0

Table 3 shows a comparison of the results obtained with the febrile antigen agglutination test and the ELISA assay for the detection of antibodies against *Brucella* spp. The agglutination test detected only one positive case (1.43%), while 98.57% of the samples were negative. However, the ELISA method reported 100% negative results, with no evidence of a positive effect in any of the participants evaluated. The sensitivity and specificity of these methods indicate that the ELISA assay shows better results and greater reliability in detecting positive or negative outcomes.

Table 3. Results of agglutination and ELISA tests in the diagnosis of human brucellosis

Diagnostic test	Result	Frequency	Percentage	Sensitivity (%)	Specificity (%)
Agglutination (Febrile antigen)	Positive	1	1.43	76	71.2
	Negative	69	98.57	76	71.2
Subtotal agglutination	Total	70	100	-	-
ELISA anti-*Brucella* spp.	Positive	0	0.0	98	95
	Negative	70	100.0	98	95
Subtotal ELISA	Total	70	100.0	-	-

Conversely, the *Brucella* febrile antigen agglutination test does not provide specific values for specificity and sensitivity; however, different manufacturers report a sensitivity of 76.47% and a specificity of 71.2%. This corroborates the argument made for the ELISA assay.

In this study, determining the presence of brucellosis among those who consume unpasteurized milk using the agglutination test (febrile antigen) reveals a possible constant exposure in the rural population, which is consistent with epidemiological studies that identify raw milk consumption as a significant zoonotic risk factor (Garrido-Haro et al., 2023). For example, a study in Ecuador identified a human seroprevalence of 1.88% using multiple serological tests, finding contact with livestock and handling of fetuses as the main risk factors, suggesting that traditional practices and consumption of unpasteurized animal products are associated with infection (Ibarra et al., 2021).

Despite their limited sensitivity and the fact that, in people with frequent exposure to *Brucella*, they can produce results that are difficult to interpret, serological tests remain essential for diagnosis, especially in resource-poor regions. Thus, they represent the key or most relevant method in nations where brucellosis has been eliminated, and the disease has faded into obscurity (Loubet et al., 2024).

At the same time, applying ELISA to detect antibodies against *Brucella* in a representative sample from Olmedo could yield a more precise and reliable estimate of the prevalence of serological exposure, since this technique allows for the identification of antibodies (IgG, IgM) and tends to be more sensitive than agglutination tests. This approach is especially relevant in endemic contexts. In Ecuador, studies on cattle have used ELISA to determine bovine prevalence and warn that the consumption of raw milk without sanitary control represents a threat to public health (Chamorro, 2022).

Finally, comparing the usefulness of agglutination and the ELISA assay in the diagnosis of human brucellosis in Olmedo is fundamental for defining local surveillance and control strategies. The literature suggests that, although agglutination (such as SAT or febrile agglutination tests) is a classic and quite specific technique, it does not discriminate between recent or past antibodies, whereas ELISA offers greater sensitivity and allows profiling the type of antibodies, although it may sometimes have slightly lower specificity depending on the cutoff point (Xu et al., 2020).

Currently, a study conducted in China mentions the use of agglutination-based serological testing, similar to bacterial culture. The positive reaction rate of this method tends to decrease as the infection progresses. Xu et al. (2020) indicated that research shows the ELISA method offers superior diagnostic capabilities, both in sensitivity and specificity, compared to the agglutination test for identifying cases of brucellosis.

In the Ecuadorian context, given the high prevalence of bovine brucellosis in dairy herds, the prevalence of uncontrolled livestock practices, and the consumption of unpasteurized milk in rural areas, the incorporation of ELISA into human seroprevalence studies would provide more precise evidence regarding the magnitude of zoonotic risk. The documented use of indirect ELISA in cattle in Ecuador, with good indicators of sensitivity, specificity, and reproducibility, supports its applicability in *Brucella* infection diagnosis, whether due to past or active exposure (Garrido et al., 2025).

Several international studies have consistently demonstrated that ELISA surpasses conventional agglutination testing in sensitivity and specificity in endemic contexts. For example, research conducted in China concluded that ELISA (IgG and IgM) achieves sensitivities of up to 98.7% and specificities exceeding 98%, while agglutination only reaches 63.8% sensitivity, highlighting a significant risk of false negatives in chronically exposed populations (Xu et al., 2020).

Techniques such as PCR, qPCR, LAMP, WGS, FISH, ELISA, NGS, microarray analysis, dPCR, and biosensor-based assays allow for the rapid, accurate, and sensitive identification of *Brucella* in various biological samples, including milk, blood, tissues, and other fluids. These advances are crucial not only for diagnosis in domestic animals but also for the surveillance and control of the disease in wild species, which can act as continuous reservoirs of infection (Islam et al., 2025).

The results obtained using commercial plate agglutination tests cannot be considered a

reliable representation of the true brucellosis infection status. The percentage of individuals who test positive using commercial tests is implausibly high, especially when these data are compared with other diagnostic methods and with the information available in the literature on the prevalence of brucellosis in different populations (Lukambagire et al., 2021).

Taken together, the evidence from recent research in both human and animal contexts confirms that the ELISA assay is currently one of the most reliable tools for detecting *Brucella*, outperforming traditional agglutination tests in diagnostic performance (Garrido et al., 2025). This underscores the importance of incorporating the ELISA assay into human seroprevalence studies in rural communities like Olmedo, allowing for a precise assessment of zoonotic risk and contributing to the design of disease prevention and control strategies.

CONCLUSIONS

Although a small proportion of the evaluated population showed reactivity in the febrile antigen agglutination test, in contrast to the total absence of antibodies detected by the ELISA assay, there is no serological evidence of active or past *Brucella* infection, despite the high consumption of unpasteurized milk in this rural area of Olmedo. These results suggest that unpasteurized milk continues to be consumed within the evaluated population, thus maintaining various risk factors. These practices highlight the need to establish ongoing epidemiological surveillance, health education programs, and the promotion of safe dairy consumption as part of strategies aimed at preventing this infection in the population. Furthermore, the importance of using reliable diagnostic methods such as the ELISA assay to promptly identify potential cases of brucellosis and reduce risks in rural communities is emphasized.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Andrea N. Mero, Sanddy L. Mieles, and Nicole A. Lagos. **Data curation:** Andrea N. Mero, Sanddy L. Mieles, and Nicole A. Lagos. **Formal analysis:** Andrea N. Mero, Sanddy L. Mieles, and Nicole A. Lagos. **Investigation:** Andrea N. Mero, Sanddy L. Mieles, and Nicole A. Lagos. **Methodology:** Andrea N. Mero, Sanddy L. Mieles, and Nicole A. Lagos. **Supervision:** Andrea N. Mero, Sanddy L. Mieles, and Nicole A. Lagos. **Visualization:**

Andrea N. Mero, Sanddy L. Mieles, and Nicole A. Lagos. **Writing – original draft:** Andrea N. Mero, Sanddy L. Mieles, and Nicole A. Lagos. **Writing – review & editing:** Andrea N. Mero, Sanddy L. Mieles, and Nicole A. Lagos.

REFERENCES

Chamorro, A. F. (2022). *Sero-epidemiología e identificación de Brucella SPP. con técnicas moleculares en ganado bovino en la provincia del Carchi* [Master's thesis, Universidad Internacional SEK]. https://repositorio.uisek.edu.ec/handle/123456789/4588

Garrido, A. D., Barrionuevo, M. Y., Moreno-Caballeros, P., Burbano-Enríquez, A., Salas, V. A., Galante, M. C., Wielick, C., Ron-Román, J., & Saegerman, C. (2025). Bayesian Estimation of the True Bovine Brucellosis Prevalence in Vaccinated and Non-Vaccinated Ecuadorian Cattle Populations, and the Sensitivity and Specificity of a Competitive and Indirect ELISA Using a New Synthetic Antigen. *Microorganisms*, *13*(1), 69. https://doi.org/10.3390/microorganisms13010069

Garrido-Haro, A., Barrionuevo-Samaniego, M., Moreno-Caballeros, P., Burbano-Enríquez, A., Sánchez-Vázquez, MJ, Pompei, J., Humblet, M.-F., Ron-Román, J., & Saegerman, C. (2023). Seroprevalencia y factores de riesgo relacionados con la brucelosis bovina en el Ecuador continental. *Pathogens*, *12*(9), 1134. https://doi.org/10.3390/pathogens12091134

Hurtado, J. A. (2017). La brucelosis: un problema de salud en la sombra. *Enfermedades Infecciosas y Microbiología*, *37*(4), 115. https://www.medigraphic.com/cgi-bin/new/resumen.cgi?IDARTICULO=86243

Ibarra, E., López, E., López, F., & Silva, J. (2021). “Una sola salud”, (One Health): Estudio de caso brucelosis en Carchi – Ecuador. *Horizontes de Enfermería*, (11), 70-80. https://doi.org/10.32645/13906984.1085

Islam, M. S., Habib, M. A., Tonu, N. S., Haque, M. S., & Rahman, M. M. (2025). Beyond serology: A meta-analysis of advancements in molecular detection of Brucella spp. in seronegative animals and biological samples. *Veterinary Medicine and Science*, *11*(1), e70200. https://doi.org/10.1002/vms3.70200

Loubet, P., Magnan, C., Salipante, F., Pastre, T., Keriel, A., O’Callaghan, D., Sotto, A., & Lavigne, J.-P. (2024). Diagnosis of brucellosis: Combining tests to improve performance. *PLoS*

Neglected Tropical Diseases, *18*(9), e0012442. https://doi.org/10.1371/journal.pntd.0012442

Lukambagire, A. S., Mendes, Â. J., Bodenham, R. F., McGiven, J. A., Mkenda, N. A., Mathew, C., Rubach, M. P., Sakasaka, P., Shayo, D. D., Maro, V. P., Shirima, G. M., Thomas, K. M., Kasanga, C. J., Kazwala, R. R., Halliday, J. E. B., & Mmbaga, B. T. (2021). Performance characteristics and costs of serological tests for brucellosis in a pastoralist community of northern Tanzania. *Scientific Reports*, *11*(1), 5480. https://doi.org/10.1038/s41598-021-82906-w

Mondragón-Lenis, I. M., Vélez-Londoño, J. D., Call, D., Sánchez-Jiménez, M., & Cardona-Castro, N. (2020). Primer caso confirmado de brucelosis humana por Brucella melitensis, una zoonosis presente en Colombia. *Infectio*, *24*(4), 259–261. https://doi.org/10.22354/in.v24i4.886

Ormazábal, M. C., Hidalgo, M., Salas, M., Benito, S. M., Pérez, A., & del Amo, C. (2022). Brucelosis humana. *Revista Sanitaria de Investigación*, *3*(1). https://revistasanitariadeinvestigacion.com/brucelosis-humana/

Rivadeneira, P. N. (2022). *Determinación de la prevalencia y factores de riesgo de brucelosis en explotaciones* [Undergraduate thesis, Universidad de las Fuerzas Armadas]. https://repositoriobe.espe.edu.ec/server/api/core/bitstreams/28c48bd9-c601-47b8-be5c-2321b8287d92/content

Xu, N., Wang, W., Chen, F., Li, W., & Wang, G. (2020). ELISA is superior to bacterial culture and agglutination test in the diagnosis of brucellosis in an endemic area in China. *BMC Infectious Diseases*, *20*(1), 11. https://doi.org/10.1186/s12879-019-4729-1