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Identifying sources of pollution, sanitation strategy in the prevention of human brucellosis in a Northern population of Mexico

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Abstract: *Brucellosis is one of the most common zoonotic diseases in the world, and it represents a great threat to human and animal health. In Mexico, several years ago brucellosis occurs in both rural and urban population, both sexes, economically active. The aim of this study was to determine the sources of pollution and risk vehicles to apply a strategy of basic sanitation in the prevention of human brucellosis in the Comarca Lagunera Region, state of Durango and Coahuila, Mexico. Was performed, taking 300 samples of milk, cheese, cream, butter or any food that is made with unpasteurized milk or are of dubious origin. For each case, cultures were 5 milk, cheese, cream, water and foods made with dairy products and if necessary, could be carried out dog stool culture. Was isolated from the genus Brucella samples mainly dairy cheese and cream for human consumption, so that these remain a risk factor, of the 300 samples tested, 36 were positive corresponding to 12%. 34% of the 300 samples analyzed, representing 102 samples that were unable to determine the presence of Brucella due to the high concentration of contaminant bacterial flora, mainly Enterobacteriaceae, such as gender Klebsiella, Proteus and Escherichia, which are microorganisms own fecal matter to cause acute gastroenteritis mainly Escherichia coli. The origin of imported dairy products at higher risk for Laguna region of Durango and Coahuila. The promotion of health in this sense must see that the population is informed in writing and through the media. This will favor the producers of these products to achieve more efficient methods to develop its dairy products in a sanitary manner.*

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I. Introduction

Bacteria of the genus *Brucella* is the etiologic agent of brucellosis, is a bacterial zoonosis with worldwide distribution [1], a major zoonotic disease that affects humans and domestic animals [2]. It also leads to reproductive problems in large numbers of cattle [3], causing abortion and infertility [4], so that ruminants are highly susceptible to brucellosis. The genus *Brucella* contains fourteen different species have been characterized: six classical species *B. abortus* predominantly infects bovines, *B. melitensis* small ruminants, *B. suis* pigs, *B. canis* dogs, *B.ovis* rams, and *B. neotomae* from woodrats; of these, the first four can infect humans [5, 6]; an additional novel species from *B. maris*: *B. ceti* from cetaceans [7], and *B. pinnipedialis* from seals; *B. microti* from wild rodents and from mandibular lymph nodes of red foxes, *B. inopinata* a from breast implant infection in humans, *B. vulpis* from red foxes [8], and *B. papionis* from baboons. The genus *Brucella* consists of Gram (-) [3] and it behaves as a facultative intracellular parasite [4]. Facultative intracellular pathogens, a property that keeps them protected from the action of antibiotics and antibody-dependent effector mechanisms: this justifies the chronic nature of infection as they are able to adhere, penetrate and multiply in a variety of eukaryotic cells both phagocytic and non-phagocytic [5, 9]. *B. melitensis* is the most pathogenic to humans and is fewer specific species, infecting goats, sheep, cows, camels, and dogs [10]. *B. melitensis* remains the principal cause of human disease in the world, followed by *B. abortus*, and *B. suis*, while there are rare cases of *B. canis* and human infection with new diseases have emerged with new pathogens *Brucella* in marine mammals [11]. *B. melitensis* is the main agent of human brucellosis, which is related directly to infection in sheep and goats. Due to the absence of suitable vaccines for humans, prevention depends on the control/eradication of the disease in animals [12]. Due to continuous efforts to control and eradicate brucellosis in domestic animals, brucellosis levels have declined in many countries [13]. Since 1905, its existence is accepted in Mexico, though; the first isolation of a strain of *B. melitensis* is performed, by Placeres et al, in the State of Puebla, Mexico. Has caused and today cause great economic losses to livestock in the country and is one of the most important public health problems. The clinical manifestations are less apparent and as the bacteria are in the reproductive tract in females, abortion, premature births and retained placenta are the only visible signs in animals suffering. In contrast, human disease is manifested by a prolonged febrile course, disabling, with severe complications and may progress to a chronic disease, headache, profuse sweating, chills, joint pain, depression, weight loss and malaise. Occasionally localized suppurative infections arise in organs, including liver and spleen [14, 15]. The incubation period of brucellosis is between five days and several months (with an average of two weeks). The most typical symptoms, constipation, loss of appetite, weight loss and weakness. There is also an increase in the size of the spleen, liver, and lymph nodes [16, 17]. The disease is characterized in its initial phase and causes acute infection if not treated properly, can become a chronic and persist for the life of the host. The diagnosis of brucellosis in humans should consider clinical aspects, and have a detailed clinical history that includes some epidemiological data type. It is highly recommended to practice a bacteriological study, complemented by the search for antibodies. The modes of transmission may be direct or indirect [18]. The risk of this zoonosis is primarily a direct occupational being the farmers, veterinarians, slaughter workers most at risk. Brucellosis is transmitted clearly defined by two mechanisms: direct contact with infected material such as blood, urine, vaginal discharges, aborted fetuses and placentas of infected animals [14, 19]. The indirect way is through the ingestion of cow's milk, colostrum, sheep or goats or their products (butter, fresh cheese) containing viable microorganisms (10^9-10^{13} bacteria/ml), products that have been made from unpasteurized milk [16]. Control measures for the prevention of

human brucellosis are pasteurization of milk, livestock vaccination and elimination of infected animals [20]. Brucellosis is a disease of worldwide distribution anthropozoonotic been known for many years, however, remains a health and economic problem [1, 5, 21]. People become infected by inhaling contaminated dust or hair, for splashing in the conjunctiva, accidental ingestion, via skin abrasions or cuts or accidental self-inoculation of blood from infected animal or live vaccines [22]. *Brucella* spp can survive for long periods in the dust, manure, water, fetuses, soil, offal and dairy milk. In this context, the brucellae, being eliminated intermittently with milk, food becomes a source of infection for the population that consumes without any preliminary heat treatment. Both rural and urban population will be affected; the city with greater purchasing power will have a risk when buying milk products without sanitary control. The cheese, largely concentrated in the bacteria that can survive in these conditions a few months [1]. The same goes in the case of butter, cream or ice cream made with milk contaminated. Consuming raw or undercooked meat, from infected animals, represents a lower risk, because the muscle contains low amounts of *Brucella*. In contrast with viscera, udder and testicles contain significant amounts of bacteria. Fresh blood is potentially dangerous for those individuals who usually consume natural or mixed. Transmission from person to person is very rare, a reported case there is only circumstantial evidence suggests that transmission occurred through sexual contact. Of greater importance is the result of an infection such as blood transfusion or tissue transplantation, bone marrow is the most risk. Another form of transmission is from mother to child with acute brucellosis by breast milk or placenta causing abortion or brucellosis in the newborn. In Mexico, several years ago brucellosis occurs in both rural and urban population, both sexes, economically active, 20 to 45 years old with more cases in women. Children of about 10 years are a population at increased risk. The reported mortality is around 30 cases annually. The cultivation of the bacteria is the only strong evidence that this is a *Brucella* infection. Although it can be isolated from various sources, the blood is the material most often used for bacteriological culture. There are some recommendations that should be taken into account to achieve the successful cultivation in the first place the patient should not be under antibiotic therapy when taking the sample and should preferably be practicing during the acute phase of illness. Once you have the suspect colony growth, it is recommended that a presumptive identification. The aim of this study was to determine the sources of pollution and risk vehicles to apply a strategy of basic sanitation in the prevention of human brucellosis in the Comarca Lagunera Region, state of Durango and Coahuila, Mexico.

II. Material and Methods

For the analysis took into account the population with higher incidence of brucellosis in which there is a higher prevalence of cases of human brucellosis. Once detected the communities, we conducted a survey of every sick person, to know the most likely vehicle by which people are becoming infected in the Laguna Region. Was performed, taking samples of milk, cheese, cream, butter or any food that is made with unpasteurized milk or are of dubious origin. Studies were also carried water and feces of domestic animals (dogs) to rule out the possibility that this is a risk vehicle together with others. To determine the number of crops to be held by community, also took into account the number of cases reported by the Sanitary Authority 2 and 6 of the Health Secretary. For each case, cultures were 5 milk, cheese, cream, water and foods made with dairy products and if necessary, could be carried out dog stool culture. For the cultivation of the different samples proceeded as follows, for milk sample was placed in the refrigerator at 4°C overnight and the next day cream was used as inoculum surface. Centrifuged at high speed in individual sterile tubes was measured an equal volume of sample from each tanning or nipple (at least 20 ml). The samples were centrifuged 15 min at 7.000 6.000 x g cream is used both as the sediment. Cream was inoculated with a handle, placing approximately 0.1 to 0.2 ml cream on the surface of two plates, one medium and one agar Farrell trypticase with serum. The cream was sown directly in the middle of Farrell plates using a swab. Cheese and other dairy milk were ground in peptone water or liquefied just before planting in the Farrell's medium. For samples of cheese and other dairy milk, weighed 100 g were placed in 500 ml of sterile peptone water sterile homogenizing with a blender or using a sterile jar with tight lid and the jar was placed at 4°C for one hour.

With a sterile swab sample was taken from the fat floating on the surface and seeded in duplicate in the middle of Farrell. For stool culture (Amoss and Poston), it was an emulsion of a gram of feces in saline solution, filtered through sterile gauze and the filtrate centrifuged at low speed to remove coarse particles. Supernatant was added to anti-*Brucella* serum and incubated 2 h at 37°C, subsequently was centrifuged again at moderate speed for 5 min, decanting the supernatant.

The pellet was resuspended and centrifuged again, sowing in Petri dishes containing sediment through Farrell. Once inoculated plates were taken in an atmosphere of CO_2, at a concentration of 5 to 10%, incubated at 37°C for 4 to 7 days. All suspect colonies on TSA reseeded with yeast extract or *Brucella* agar to observe the characteristic colonial morphology in the absence of antibiotics. Biochemical tests were made for, as well as agglutination with polyvalent anti-*Brucella* serum. We applied a statistical method to determine the number of cases to analyze and 5 products for each of them with 5% error and 95% confidence.

III. Results and discussion

We analyzed 300 samples of dairy products mainly found 36 samples corresponding to 12%. Identified the main sources of pollution and it was established that import dairy products from reaching the Laguna region of Durango and Coahuila most at risk are the States of Durango in the municipalities of Santa Clara and Cuencame and the State of Zacatecas. These products have in common; they are handmade products and lack of authorization. Was isolated from the genus *Brucella* samples mainly dairy cheese for human consumption, so that these remain a risk factor, of the 300 samples tested, 36 were positive corresponding to 12%. 34% of the 300 samples analyzed, representing 102 samples that were unable to determine the presence of *Brucella* due to the high concentration of contaminant bacterial flora, mainly Enterobacteriaceae, such as gender *Klebsiella*, *Proteus* and *Escherichia*, which are microorganisms own fecal matter to cause acute gastroenteritis mainly *Escherichia coli*. The highest proportion of samples positive for *Brucella* were cheeses and creams, this from the standpoint of food is more likely, since the material is a concentrated milk used for processing, another aspect is that the cream is the higher concentration of the agent, as the cream provides a favorable medium for the high protein and vegetable fats also provide protection from unfavorable external environment. 5 were used as controls trademark samples, which had label certifying its quality in terms of health, the results for the isolation of the genus *Brucella* were negative. The bacterial concentration was lower than that of craft shows. Control strategies available to prevent human brucellosis are

pasteurization of milk, livestock vaccination and elimination of infected animals [20]. Animal vaccination significantly influences the incidence of disease in humans, by consumption of dairy products and animal-sharing practices. The respective roles of vaccination and health education are a cause of future research, since there has been substantial reduction in annual incidence between two periods (1997-2002) of study among individuals whose herds had been vaccinated yet, this reduction was due to an effort to provide health education, pasteurization of dairy products, masks and gloves, especially during animal handling, etc. [20], as well as conducting animal vaccination, the incidence in 1997 was 13.2 per 1000 individuals in 2002 was 0.7 per 1000 individuals, the application of these sanitary measures reduce brucellosis in animals and in humans. Another study showed that an important exposure to smooth strains *Brucella* spp is detected in the people that work and live on cattle farms with animals that tested positive for *Brucella abortus*, due to the execution of risky cultural practices. The quantitative difference between the cases reported to the epidemiological surveillance system of the Ministry of Health, Alajuela, Costa Rica, and the results of that study indicated that this disease should be included as a differential diagnosis for cases of acute febrile illness and that an effective and efficient epidemiological surveillance system should be implemented [22]. Furthermore, Martinez et al (2017) reported that the conditions that have the greatest influence on the health status of the population are the lack of basic sanitation, lack of training or technical advice for goat farmers who live off the product of their animals, low income, little state budget, as well as deficiencies in quantity and quality in infrastructure and basic sanitation at each critical point of production. The consumption of dairy products made with unpasteurized milk is a health problem in the Lagunera region due to its availability and low cost. This is an arid and semi-arid zone, endemic to brucellosis and of great importance for the exploitation of livestock, favored by climatic conditions characterized by high temperatures and low humidity. Faced with this situation, the need to carry out the present investigation was raised, the objective of which was to identify contamination by *Brucella* sp and the lack of compliance with basic sanitation measures in dairy production. Ullah et al (2020) published that although vaccination is recommended and treatment is possible for brucellosis, they are not considered safe for human health, hence regular screening and culling of the reactor animals remain the only choice to monitor and eradicate brucellosis. Introduction of the new stock at these farms should be carried out only after screening and quarantine. Furthermore, farm workers should be advised to adopt protection measures as a routine. Abortion at these farms should not go unnoticed and must be investigated to confirm its cause to adopt recommended control measures. If abortions occur, disinfection of the area should be ensured along with strict biosecurity measures to restrict chances of dissemination.

IV. Conclusion

Through this study, the origin of imported dairy products at higher risk for Laguna region of Durango and Coahuila, which were dairy products from Zacatecas and Durango (Santa Clara and Cuencame) States arriving on the market to the Shire Laguna, these products are handmade and pose no health certification labels. The promotion of health in this sense must see that the population is informed in writing and through the media (TV and radio). This will favor the producers of these products to achieve more efficient methods to develop its dairy products in a sanitary manner.

Referencias

[1]. Ullah Q, Jamil T, Melzer F, Saqib M, Hussain MH, Aslam MA, Jamil H, Iqbal MA, Tahir U, Ullah S, Qureshi ZI, Neubauer H. 2020. Epidemiology and Associated Risk Factors for Brucellosis in Small Ruminants Kept at Institutional Livestock Farms in Punjab, Pakistan. Front Vet Sci 7

[2]. Ocampo-Sosa AA, García-Lobo JM. 2008. Demonstration of IS711 transposition in *Brucella ovis* and *Brucella pinnipedialis*. *BMC Microbiol* 8: 17

[3]. Gopaul KK, Koylass MS, Smith CJ, Whatmore AM. 2008. Rapid identification of *Brucella* isolates to the species level by real time PCR based single nucleotide polymorphism (SNP) analysis. BMC Microbiol 8: 86

[4]. González D, Grillo MJ, De Miguel MJ, Ali T, Arce-Gorvel V, Delrue R-M, Conde-Álvarez R, Muñoz P, López-Goñi I, Iriarte M, Marín C-M, Weintraub A, Widmalm G, Zygmunt M, Letesson J-J, Gorvel J-P, Blasco JM, Moriyon I. 2008. Brucellosis vaccines: assessment of *Brucella melitensis* lipopolysaccharide rough mutants defective in core and O-polysaccharide synthesis and export. PLoS ONE 3: e2760

[5]. Castro HA, Sofia Raquel Gonzalez y María Inés Prat. 2005. Brucelosis: una revisión práctica. Acta Bioquímica Clínica Latinoamericana 39: 203

[6]. Chain PS, Comerci DJ, Tolmasky ME, Larimer FW, Malfatti SA, et al. 2005. Whole-genome analyses of speciation events in pathogenic Brucellae. Infect Immun 73: 8353

[7]. Foster JT, Okinaka RT, Svensson R, Shaw K, De BK, et al. 2008. Real-time PCR assays of single-nucleotide polymorphisms defining the major *Brucella* clades. J Clin Microbiol 46: 296

[8]. Scholz HC, Revilla-Fernández S, Dahouk SA, Hammerl JA, Zygmunt MS, Cloeckaert A, Koylass M, Whatmore AM, Blom J, Vergnaud G, Witte A, Aistleitner K, Hofer E. 2016. *Brucella vulpis* sp. nov., isolated from mandibular lymph nodes of red foxes (Vulpes vulpes). Int J Syst Evol Microbiol 66: 2090-2098

[9]. Solis Garcia del Pozo J, Solera J. 2012. Systematic review and meta-analysis of randomized clinical trials in the treatment of human brucellosis. PLoS One 7: e32090

[10]. Cassataro J, Velikovsky CA, de la Barrera S, Estein SM, Bruno L, et al. 2005. A DNA vaccine coding for the *Brucella* outer membrane protein 31 confers protection against *B. melitensis* and *B. ovis* infection by eliciting a specific cytotoxic response. Infect Immun 73: 6537

[11]. Ariza J, Bosilkovski M, Cascio A, Colmenero JD, Corbel MJ, Falagas ME, Memish ZA, Roushan MRH, Rubinstein E, Sipsas NV, Solera J, Young EJ, Pappas G. 2007. Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. PloS Medicine 4(12): e317

[12]. Zabalza-Barangua A, San-Roman B, Chacon-Diaz C, de Miguel MJ, Munoz PM, Iriarte M, Blasco JM, Grillo MJ. 2018. GFP tagging of *Brucella melitensis* Rev1 allows the identification of vaccinated sheep. Transbound Emerg Dis 66(1):1-33

[13]. Bogdanovich T, Skurnik M, Lubeck PS, Ahrens P, Hoorfar J. 2004. Validated 5' nuclease PCR assay for rapid identification of the genus *Brucella*. J Clin Microbiol 42: 2261

[14]. Chanto G, Rojas N, Ching A, Zuniga R, Castro E, et al. 2007. [Prevalence of serum antibodies for the bacterium *Brucella* sp. in a tropical human population]. Rev Biol Trop 55: 385

[15]. Mantur BG, S.K. Amaruath y R.S. Shindle. 2007. Review of clinical and laboratory features of human brucellosis. Indian J Med Microbiol 25

[16]. Dellamea AB. 2006. Nueva vacuna contra la brucelosis. Acta Bioquímica Clínica Latinoamericana 40: 83

[17]. Kunda J, Fitzpatrick J, Kazwala R, French NP, Shirima G, et al. 2007. Health-seeking behaviour of human brucellosis cases in rural Tanzania. BMC Public Health 7: 315

[18]. Minas M, Minas A, Gourgulianis K, Stournara A. 2007. Epidemiological and clinical aspects of human brucellosis in Central Greece. J Infect Dis 60: 362

[19]. Ortega CA, José Paredes A. y Alfredo Guillén O. 2007. Prevalencia de anticuerpos contra *Brucella* sp en donantes del banco de sangre de un hospital de Lima. Rev Peru Med Exp Salud publica 24: 431

[20]. Jelastopulu E, Bikas C, Petropoulos C, Leotsinidis M. 2008. Incidence of human brucellosis in a rural area in Western Greece after the implementation of a vaccination programme against animal brucellosis. BMC Public Health 8: 241
[21]. Falagas ME, Bliziotis IA. 2006. Quinolones for treatment of human brucellosis: critical review of the evidence from microbiological and clinical studies. Antimicrob Agents Chemother 50: 22
[22]. Rosales-Galeano C, Puentes-Mojica C, Arias-Rojas O, Romero-Zúñiga JJ. 2020. Aspectos epidemiológicos de la brucelosis en humanos en las Áreas Rectoras Aguas Zarcas y Los Chiles, Costa Rica, 2015-2017. Ciencias Veterinarias 38(1): 1-16
[23]. Martínez RA, Ortega SJL, Cervantes FM, Urtiz EN, Hernández GSI, Alba RJJ. 2017. Contaminación por *Brucella* sp e incumplimiento en medidas de saneamiento básico en la producción de lácteos. Enf Inf Microbiol 37(4): 128-132

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