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Animal Health

Review

Brucellosis: Aspects Limiting a Real Approximation to this Zoonosis. The Role of Goats

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ABSTRACT

Background: After being studied for 133 years, brucellosis is considered a re-emerging zoonosis. **Aim:** To analyze aspects limiting a real approximation to brucellosis, with emphasis on goats as reservoirs.

Development: A number of 126 specialized sources were reviewed (118 scientific papers and 8 books). A total of 50 references were chosen in order to meet the aim of this review and the journal's guidelines. Although *B. melitensis* is the most virulent species in humans, and goats are its natural reservoirs, the two national reports with a veterinary approach on the zoonosis are centered on *B. abortus*, in bovines. However, the outcome of international studies is different, with a predominance in developing countries. Dairies make a significant way of transmission of the disease. Five foreign articles consider the bacterial *biofilm* phenotype as a factor of virulence and the cause of chronicity; only two consider *Brucella*. Despite the existence of reliable diagnostic techniques, some of them Cuban, the visibility of brucellosis occurrence in the country is limited, especially in domestic animals.

Conclusions: A real approximation of Brucellosis as a zoonosis is biased due to mistaken underestimation; disregard of goats and their products, as sources of transmission; the exclusion of *biofilm* from related research; and poorly-publicized results of national screenings.

Key words: biofilm, *Brucella melitensis*, goats, disease reservoirs, zoonosis (Source: *MeSH*)

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INTRODUCTION

Several ancient zoonosis with a bacterial etiology have prevailed in the twenty-first century as re-emerging diseases, which among others, have been caused as a result of underestimation of the zoonosis during the second half of the twentieth century, when it was wrongly thought to be under control (Chatterjee, Bhaumik, Chauhan, and Kakkar, 2017; Barreto and Rodríguez, 2018a, 2019; Barreto, Rodríguez, García & Vázquez 2019a). Brucellosis is the best-documented example of this mistake.

In 1968, the World Health Organization (WHO) blamed this zoonosis for being "*... responsible of more diseases, miseries, and economic losses than any other animal-related disease known to affect humans"* (Álvarez-Hernández, Díaz Flores, and Ortiz Reynoso, 2015). Despite the severity of this imputation, 42 years later, Flores Castro (2010), warned about a breach favoring the persistence of the disease: "*...it exemplifies the lack of interaction between the public health and veterinary sectors, making it one of the most frequently occurring zoonosis in the world".* [1]

Five years later, in Cuba, Mendoza, Ramírez, Yera, Rosales, and Mora (2015): "*... since 1985, and especially during the 1990s, there were difficulties to develop control programs, and the compliance of regulations to control the disease, with a gradual worsening of prevention and diagnostic, instability, and a seeming trend to increased focalization".*

Interestingly, *Brucella melitensis*, the causal agent of brucellosis in goats, and the most virulent species to humans, drew the attention of more than one military power. Its low infecting dose (10-100 colony forming units) made it an excellent choice for possible spraying during bioterrorist warfare. After the attacks on the World Trade Center, in 2001, it regained the attention of the media (Chatterjee *et al.*, 2017).

The incomprehensible paradox is that on one end of the scale there are more than 10 million annual cases of a zoonosis which is endemic to a large portion of developing countries, as a result of faulty health care systems, and on the other end, the same etiological agent is used by military powers willing to invest their funds in the research of harmful products to people (Rossetti, Arenas-Gamboa, and Maurizio, 2017).

However, the goal of this brief review is far from warmongering. Instead, it will focus on certain factors that may have contributed to the prevalence of one of the oldest zoonosis, particularly among the ever-growing emerging diseases. Although *B. melitensis* is not the only species that causes human brucellosis, it is the most commonly found (Rossetti, Arenas-Gamboa, and Maurizio, 2017). Therefore, this review will tackle goats as one of the natural reservoirs.

[1]The quoted remarks were textually cited, and will be repeated throughout the article.

It is worth noting that 90% of the more than a billion goats today are located in the poorest countries, with shortages of resources for proper diagnostic to control this disease and its transmission (Geresu and Kassa, 2016). This element adds significant bias when assessing the global impact of this zoonosis, particularly in areas where management practices may reach over crowdedness between animals and people (Saminathan *et al*, 2016).

Another, perhaps not so influencing, but valid element, might be the assumption that these small ruminants have, by nature, a deflecting response to infectious diseases (Barreto and Rodríguez, 2018a, b). This was a wrong and spread criterion, which may be the cause of insufficient related publications.

For 133 years, almost all papers studied the etiological agent according to its planktonic phenotype, a bias limiting any approaching attempts to learn from this zoonosis (Almirón, Roset, and Sanjuán, 2013; Tang *et al.*, 2020).

Hence, the purpose of this review is to analyze the aspects limiting a real approximation to brucellosis, with special emphasis on goats as reservoirs.

DEVELOPMENT

In this study, 126 specific sources from 118 scientific articles published in reference journals (level I and II) and eight textbooks, were reviewed. The previously set goal, and the editorial guidelines of the journal chosen for publication, were the contrasting elements to select 50 references, which were classified into four levels depending to their present-day relevance: published this year (5), published during the last five-year period (33), and during this decade (12). Depending on their origin, 14 were published by national researchers, and 36 belong to foreign scientists. Then, based on abridged information, several critical aspects that limit real approximation to one of the oldest zoonosis were discussed; possibly, the same reasons that make it a reemerging disease.

Genus Brucella

Acknowledged species

Genus *Brucella* comprises these species: *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis*, *B. neotomae*, *B. ceti*, *B. pinnipedialis*, *B. microti*, and *B. inopinata*, the first four have proven their zoonotic character, especially *B. melitensis* (Alizadeh *et al.*, 2018). Recent isolates of *B.inopinata* from humans might expand this figure (Li *et al.*, 2020), which is still awaiting confirmation. This is the commonly used contrasting method, though DNA homology tests show that it is, in fact, a species (*B. melitensis*), and multiple biovars. Despite the general consensus in this regard, this choice is seldom used in related publications (Carroll, 2013).

According to a contrast analysis made to 10 species, there were preferential associations with animal species that hosted them, which have appeared as the result of gradual adaptative changes throughout time. Although these links are not strictly compelling, as evidenced when cattle transmits *B. melitensis* and *B. suis* to humans, along with the growing participation of multiple wild species as reservoirs. This adds to the limitations for the control of this zoonosis (Olsen and Palmer, 2014; Li *et al.*, 2020).

Morphological and physiological traits

These are facultative intracellular pathogenic, asporogen, non-toxigenic or fermenting, motionless, gram negative coccobacilli (alone, in pairs, or forming short chains). They do not have flagella and capsule, though compatible structures have been reported in some cases (Kaltungo, Saidu, Musa, and Baba, 2014). *B. melitensis* is the prototype and more virulent species to humans. Despite intracellular parasitism inherent to the pathogenesis of *Brucella* spp., they can survive in the environment for variable periods of time. This stage is remarkably favored during biofilm phenotype transit (Almirón, Roset, and Sanjuán, 2013; Tang *et al.*, 2020).

The previously described morphological and physiological qualities of *Brucella* spp., adjust to the evaluation made of the planktonic phenotype. These measurements have had slight fluctuations throughout the 133 years elapsed since the first official report (Kaltungo *et al.*, 2014). As occurs with the rest of the bacterium domain, planktonic or free-floating forms are one phase of the vital cycle that involves 0.1% of prokaryotes, whereas 99.9% involves *biofilm* bacteria (Samal and Das, 2018). This arrangement (sessile) ensures its persistence in adverse environments as responses (motility, communication through *quorum sensing*, formation of exopolysaccharide matrix, antimicrobial resistance, etc.), which are common to arrangements that are impossible for planktonic forms (Almirón, Roset, and Sanjuán, 2013).

Precisely, *biofilm* also justifies persistence and aging of such infections in the host (Samal and Das, 2018; Tang *et al.*, 2020). Accordingly, it is important to consider variable *biofilm* in research that tackle this zoonosis.

Pathogenicity and pathogenesis mechanisms

For many years, there existed consensus in relation to the alleged "absence of virulence factors" in the particular case of *Brucella*. This term was coined in view of failed efforts to identify genes codifying for capsules, plasmids, fimbriae, and exotoxins, as part of target studies done in the late twentieth century. They dealt with attributes that conditioned the pathogenicity of other bacterial species and genus. The pathogenesis of some even involved intracellular parasitism (Döhmer, Valguarnera, Czibener, and Ugalde, 2014). In spite of those absences, *B. melitensis* has several variants that allow it to fool the defensive mechanisms of the hosts (Gourley, Petersen, Harms, and Splitter, 2015; Głowacka, Żakowska, Naylor, Niemcewicz, and Bielawska-Drózd, 2018). Some examples are given below.

Lipopolysaccharides, which make the outermost envelope of bacteria, and have transcended as parietal antigens (O antigens), play a key role in the pathogenesis of this agent. The polysaccharide ends block the binding of C1q to the bacterial surface, thus hindering the incorporation of the other components of the complement, using the traditional ways, as well as its lytic effects (Conde-Álvarez *et al.*, 2012). Lipid A is lethal to neutrophils. The lipid and polysaccharide fractions, together, protect from antibacterial peptides, and their mediation to activate its complement in the *so called* lectin pathway (Alizadeh *et al.*, 2018).

Perhaps, the last idea is related to the expressed resistance to a broad diversity of peptides (lactoferrins, defensins, and lysosomal compounds produced by nuclear polymorphic leukocytes). Furthemore, by regulating of adenine-mono-phosphate and guanine-mono-phosphate systems (AMP and GMP, respectively), these bacteria can inhibit the integration of phagolysosomes, with an ensued release of myeloperoxidase and tumorous necrosis factor (TNF). Their survival as facultative parasites in macrophages is linked to the interruption of apoptosis in these phagocytic cells. This strategy allows them to avoid multiple immune responses of the host, such as macrophage activation and cytotoxic T lymphocyte production, particularly due to a halt in the expression of TNF (Alizadeh *et al.*, 2018).

In terms of invariants, *Brucella spp.* can be considered to follow a pattern in its pathogenesis: adhesion to mucous membranes, as a previous step to transit through the epithelial barrier. Its placement at this level has a key goal: to unleash the response of macrophage monocyte system, phagocytic cells where it settles despite the above described neutralization. As intracellular parasites, they travel through the endothelial reticulum to the local lymphatic nodes (retropharyngeal, inguinal, iliac): liver, spleen, and bone marrow. After 7-30 days, they make it to the blood torrent, causing intermittent bacteremia. It is an agent bearing a chemotropism to steroid-dependent reproductive tissues (prostate, testicles, epididymis, gravid uterus, and placenta). The non-gravid uterus may act as a reservoir (de Figueiredo, Ficht, Rice-Ficht, Rossetti, and Adams, 2015; Samal and Das, 2018).

Recently, an arsenal of virulent genes has been confirmed to exist in *B. melitensis* strains isolated from different mammal species in Egypt. The high percentage of strains carrying *virB* (98.1%), *bvfA* (92.3%), and *ure* (96.2%) genes is high, which is associated to the success in replication, survival (both intracellular), and resistance to low pH, respectively (Hamdy and Zaki, 2018). Other related topics (Li *et al.*, 2017), and the reemerging of this zoonosis, are discussed in the proposal of Barnwal, Kaur, Heckert, Gartia, and Varani (2020).

The discussion made in this part corresponds to research done in relation to the planktonic phenotype of *Brucella.* A study of *B. abortus* genomics and proteomics that compared the planktonic and biofilm phenotypes, demonstrated the existence of numerous virulence genes, which were regulated by the latter. Their participation may be critical in terms of invasiveness, colonization, and virulence of the agent, as well as in its avoidance strategies of defensive mechanisms of the host (Tang *et al.*, 2020).

Brucellosis

It is a zoonotic infectious disease produced by species of genus *Brucella*. Its synonymity is spread, due to the number of years of studies and ailments. Some of the most commonly terminologies are Malta fever, Mediterranean fever, undulating fever, and Bang's disease (Al-Arnoot, Abdullah, Alkhyat, Almahbashi, and Al-Nowihi, 2017). It affects a lot of mammal species; humans are not the exception, causing unspecific manifestations of fever. When it becomes chronic, it leads to peripheral arthritis, sacrolitis, orchitis, endocarditis, and neurobrucellosis (Abdelhady *et al.*, 2017).

This is one of the diseases humans have dealt with for centuries. However, as late as 1887, David Bruce (1855-1931) isolated the etiological agent of the then called Malta fever. He named it *Micrococcus melitensis*, perhaps because it really is a coccobacillus, whose morphology may be misleading. This is an insignificant detail, considering that the application of Koch's postulates set the agent-disease relationship categorically. Hence, years later, the scientific community agreed to name the disease brucellosis and the causal microorganism *Brucella melitensis* (Banai and Corbel, 2010).

Some specialists have deemed it as the most widely spread disease in the contemporary world (Yasmin and Lone, 2015). This criterion competes with the assertions made about leptospirosis (Barreto and Rodríguez, 2018a). However, this is not about competition and rivalry; rather, it has to do with encouraging awareness and the need to update the current knowledge and the respect of entities (unfortunately not the only ones), which affect a wide scope of animals species that provide food for humans, whose profitability is remarkably decreasing (Abedi *et al.*, 2020). Although this issue is relevant, it must be dependent from the effects of these products as sources of transmission of such zoonosis (Barreto and Rodríguez, 2018b).

In the world, today, there are some highly involved areas. In Europe, for instance, the Mediterranean region is worth mentioning, as well as in southeast and middle Asia (including India and China), and the Middle East countries. In Africa, the area south of the Sahara desert, known as sub-Saharan Africa, and in Latin America, the zoonosis is present in Mexico, Venezuela, Colombia, Argentina, Chile, and many others, though the reports are few. This figure comprises different geographical and cultural locations: every day 3.5, 10^{12} people are exposed to brucellosis (Álvarez-Hernández *et al.*, 2015; Obregón Fuentes *et al.*, 2015; Yasmin and Lone, 2015; 2016; Rossetti, Arenas-Gamboa, and Maurizio, 2017).

Although to some, this is ailment has been controlled in industrial countries, this appreciation is both optimistic and mistaken, which was similar to the one withstood last century. The truth is that, globally, it is an ever-growing re-emerging zoonosis (Olsen and Pamer, 2014). This controversial topic is influenced by several factors, of which socio-economic issues are critical (El-Sayed and Awad, 2018; Abedi *et al.*, 2020).

Diagnostic

The diagnostic of *Brucella* spp. has been broad and diverse since 1887. At the beginning, it was based on its microscopic and cultural traits. In addition to it, though they are gram negative, they generally are of irregular coloring. They only grow in enriched culture media, forming small, curved, and non-hemolytic colonies after 2-5 days of incubation at 37ºC. *B. abortus* only acts like that in 5-10% CO_2 environments in primary isolates. It is a painstaking, high-risk choice whose implementation is authorized in highly-safe biological facilities only (Wong, Ng, and Tan, 2018). It is also important to consider that it is a category B biological agent (Carroll, 2013).

The logical limitations of the microbiological diagnostic gave way to serological techniques, such as agglutination tests (quick on plate, and slow in 2-mercaptoethanol tubes); complement fixation reaction (CFR); ELISA-like immunoenzymatic assays (to detect IgA, IgG, and IgM) (Araj, 2010; Genç, Bûyûktanirm, and Yurdusev, 2011; Al Dahouk, Sprague, and Neubauer, 2013; Geresu and Kassa, 2016). These variants made the study of this zoonosis possible in the last century, and some even became official diagnostic methods of the World Health Organization.

In Cuba, the commercial Italian serological kit *Febrile Antigen Brucella* (FAB) was introduced in 2011 for human screenings. In 2015, it was replaced by Brucellacapt™, a single step immunocapture agglutination technique that enables the detection of agglutinating (IgA, IgG, and IgM), and non-agglutinating antibodies (IgA e IgG) against *Brucella* spp (Echevarría Pérez, Fuentes, Rodríguez Olivera, and Lugo Suárez, 2019).

Despite the undeniable advantages of the above mentioned techniques, the minimum required working conditions can only be found in diagnostic laboratories. This limitation, along with the complexity of some others, encouraged the development of other, simpler variants, with compatible sensitivity levels to the previous techniques, and easy to use in *in situ* screening comprising large numbers of animals. This group includes the rapid lateral flow immunochromatographic assays (Geresu and Kassa, 2016). Kits like these are being developed at the AIDS diagnostic laboratories (DAVIH), Mayabeque province, Cuba. They use protein A-colloidal gold (LFIA-PA) (Díaz *et al.*, 2015), and protein G-colloidal gold (Fragas Quintero *et al.*, 2018) as conjugates, which are appropriate for this type of diagnostic, and have an ELISA-like performance (Echevarría Pérez *et al.*, 2019).

In spite of the impressive development reached by a whole range of rapid and accurate assays for the detection of *Brucella* spp., including Cuban assays, the publication of scientific papers documenting the behavior of this zoonosis in the country is limited, especially in the field of veterinary, as opposed to Mendoza *et al.* (2015).

Exceptionally, besides the previously mentioned works, Obregón Fuentes *et al.* (2015a) said, *"...since 2010, the Cuban authorities of MINSAP (Ministry of Public Health decided to rescue the serological diagnostic type in humans suspected of having brucellosis, and appointed the National Laboratory of Defense against Brucellas* (*LNRB*), from the Deputy Office of Microbiology at IPK, responsible for this". In the paper, these authors demonstrated the effectiveness of *Febrille Antigen Brucella* for screening in Cuba. A few months later, they suggested an immunochromatographic variant to diagnose *Brucella from* environmental samples (Obregón Fuentes *et al.*, 2015b). IPK has followed the same line of work in order to achieve a more realistic diagnostic of the zoonosis in humans (Echevarría Pérez *et al.*, 2019). Nevertheless, when compared to other countries, the number of published scientific papers is low and polarized to a particular part of the island.

Regarding the veterinary side, the existing collaboration between the National Center of Animal Health (CENSA), and the AIDS Research Laboratory (LISIDA), in Mayabeque, is worth mentioning. Their goal was to suggest two immunochromatographic methods for the diagnostic of brucellosis in cattle (Fragas Quintero *et al.*, 2018). ¿But, how about goats? Just four references (Barreto, Rodríguez, Delgado, and Bidot, 2017a; Barreto, Bidot, Rodríguez, and Delgado, 2017b; Barreto and Rodríguez, 2018a,b) have included this topic among other diseases affecting small ruminants

Role of goats as reservoirs

Although cattle and camels, like certain species of wild animals end up as intermediate hosts of *B. melitensis* in Central Asia, and the Middle East (Olsen and Palmer, 2014), the role of goats should not be overlooked. They have been excellent hosts to this entity for centuries, as evidenced in most retrospective research (Kaltungo *et al.*, 2014; Rossetti, Arenas-Gamboa, and Maurizio, 2017). This supremacy dates back to early stages of the history of humans, just when these small ruminants were being first domesticated. Since, the production and disease developed together, producing endemism (Kaltungo *et al.*, 2014). Hence, efforts have been made to develop effective vaccines for sheep and goats (Salmon-Divon and Kornspan, 2020).

A large part of the planet comprising the developing countries also possesses the largest number of goats. The grounds are evident: it is the most suitable species to confront the harsh rearing conditions existing today, nutritional shortages, and specialized veterinary care by other domesticated animals. Contrary to that adversity, this species provide the largest amounts of protein (meat, milk, and derivatives), to the people (Tosser-Klopp *et al.*, 2014; Rossetti, Arenas-Gamboa, and Maurizio, 2017). These resources also are multiplying carriers of zoonosis, particularly cheese and other dairies made from non-pasteurized milk (Barreto *et al.*, 2017a,b; Barreto and Rodríguez, 2018a; Abedi *et al.*, 2020).

In Cuba, throughout this millennium, goat breeding has been encouraged, as a source of milk for the elder in nursing homes, and lactose-intolerant children (Barreto *et al.*, 2017b; Bidot and

Moriche, 2018). Between January and May 2015, a total of 215 production systems (private and government-owned) were identified in the province of Ciego de Ávila, totaling 25 735 heads (86% of the animals in the province). Among others, this research identified “deficient control of parasites, poor residual treatment before use, and limited orientation and technical training by the Small Livestock Company, the Cuban Association of Animal Production, and other local entities” (Delgado Fernández, 2016).

Later, a random survey was applied to private farmers in the province, which focused on the identification of the most commonly found diseases observed in their herds. All reported affectations by parasitosis, podal disorders, and in a lower proportion, spontaneous miscarriages. Despite the last one, farmers did not refer to the existence of brucellosis, leptospirosis or tuberculosis. Moreover, no samples were sent to the local laboratory of Animal Health, or specialized veterinary assistance was offered (Barreto *et al.*, 2017a).

What kind of controls are implemented to the milk regarded as “ready”, which can guarantee it is free from agents like *Brucella* and *Mycobacterium*, just to mention a couple of bacterial genus? *Fasciola hepatica* could be added as one of the expanding zoonosis, and its relation to the causal agent of tuberculosis

Contrary to the remarkable role of *B. melitensis* in human brucellosis, having goats as reservoirs, the few papers published recently emphasized on cattle and *B. abortus* (Rosales, Castillo, Reyna, Serrano, and Fernández, 2018; Fragas Quintero *et al.*, 2018). This approach is the result of underestimating goats and sheep as part of maintenance and transmission of the etiological agent (Rossetti, Arenas-Gamboa, and Maurizio, 2017), limiting a real contact with the disease, and the adoption of control measures.

Clinical manifestations of the disease in goats

It is a chronic infectious disease, which is characterized by recurrent or persistent bacteria, with an extensive negative impact on herd productivity. The range of signs in goats is broad; in females, it causes miscarriages (65%), placentitis, and birth of inviably animals. It causes changes in the genital system of males where orchitis is frequent. Although mortality is centered on fetuses and newborn, it generally causes enormous economic losses, especially in countries regarded as endemic, with limited diagnostic and control, particularly considering that the disease can occur without external signs (Olsen and Palmer, 2014). During miscarriage and delivery, the infected animals excrete large amounts of *Brucella* spp. (10^{12} ufc/g), which is one of the transmission ways to the rest of the herd and surrounding humans (Rossetti, Arenas-Gamboa, and Maurizio, 2017).

Animal transmission occurs by licking the fetal membranes, miscarried fetuses, newborn litters, and the genital organs of other infected females. Pasture and pens are often contaminated, so the etiological agent can access the host by ingestion, inhalation or through the conjunctive tissue, skin, and udder, particularly on milking farms. The mammary glands are a frequent site of infection in goats, so mastitis is a common characteristic of caprine brucellosis. The sexual transmission of *B. melitensis* in small ruminants is outstanding (Olsen and Palmer, 2014).

Final considerations

There are multiple interacting factors that contribute to the existence of brucellosis among today's re-emerging diseases. One of the contributing reasons is underestimation of the zoonosis that was at its highest in the late twentieth century. Although spread throughout the world, it is predominant and endemic in the poorest countries, the ones with few resources for diagnostic and control. *Brucella melitensis*, apart from other reservoirs, keeps a ruling association with goats. Susceptible animals, etiological agents, and humans are equally predominant in any context. The first are a subsistence way of these populations. Derivatives, particularly dairies, are a significant way of transmitting the zoonosis. Although there are simple diagnostic systems for *in situ* animal screening, these are not applied to goat populations. Assuming the causal agent is the planktonic phenotype, not biofilm, introduces a significant bias similar to the unjustified underestimation of the ailment, and limits any further studies to approach brucellosis.

CONCLUSIONS

Any approximation to brucellosis is limited by a group of overlapping factors: mistaken assessment as zoonosis, the same as in goats and their products as sources of transmission, and the exclusion of variable biofilm in related research. Nationally, there is little dissemination of screening results, particularly in the field of veterinary medicine.

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AUTHOR CONTRIBUTION

Conception and design of research: GBA, HRT, HBR; data analysis and interpretation: GBA, HRT, HBR; redaction of the manuscript: GBA, HRT, HBR.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.