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BACTERIOLOGY (N BOREL, SECTION EDITOR)

New Scenarios for *Brucella suis* and *Brucella melitensis*

Luis Samartino[1,2] · **Matías Arregui**[1] · **Pablo Martino**[3]

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Abstract
Purpose of Review We pretend to highlight the most important advances reached in the last few years in the biology of *Brucella suis* and *Brucella melitensis* and focus attention on new tools for the comprehension, prevention and control of these zoonotic agents.
Recent Findings Important progress lately done in the field of pathogenesis is presented here. This involves current studies on proteins involved in the survival and antigenic structure of *Brucella*, as well as the findings of how *Brucella* has achieved modifications in order to adapt, replicate, survive and modulate the host´s immune system, improve the knowledge of the performance of these bacteria.
Summary Consequently, better approaches for vaccinology and diagnostic techniques are developed. Relevant epidemiological issues based on changes in the highly conserved genome of these bacteria will be valuable tools to describe outbreaks and disease status, along with risk factors that would otherwise be difficult to analyze.

Keywords *Brucella suis* · *Brucella melitensis* · Pathogenesis · Vaccines

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✉ Luis Samartino
samartino.luis@inta.gob.ar

Matías Arregui
arregui.matias@inta.gob.ar

Pablo Martino
pemartino@fcv.unlp.edu.ar

[1] National Institute of Agricultural Technology of Argentina (INTA), Veterinary and Agronomic Research Center (CICVyA), Institute of Pathobiology, De Los Reseros and Nicolas Repetto s/n, 1686 Hurlingham, Buenos Aires, Argentina

[2] School of Veterinary, University of Salvador, Champagnat St, 1629 Pilar, Buenos Aires, Argentina

[3] Provincial Research Council (CIC), 11 y 526, 1900 La Plata, Buenos Aires, Argentina

Introduction

Brucellosis is a worldwide zoonosis, affecting humans, domestic animals and wildlife. The disease is caused by a genus *Brucella* which is classified as α-Protobacteria and is composed of facultative intracellular bacteria. This organism has as a major characteristic to have a predilection for the reticuloendothelial system and the reproductive tract. We describe here some of the most recent relevant facts occurring due to *Brucella melitensis* and *Brucella suis* infection [1].

The Organism

Brucella suis is an intracellular pathogen that causes reproductive losses in swine and zoonotic infections in people worldwide. It is known that there is a certain restriction on hosts that is affected by the different species of *Brucella*; there is also a preference within the degree of biovars. For instance, *B. suis* has five biovars. These different biovars usually affect different animal hosts. *B. suis* biovars 1 and 3 infect pigs, and these biovars are almost worldwide; however, biovar 2 infects pigs and hare (*Lepus europeanus*), and it seems to be present mainly in Europe, being less pathogenic for people. Biovar 4 was isolated from reindeer (*Rangifer tarandus tarandus*) and caribou (*Rangifer tarandus*) in USA, Canada and Russia and seen as almost not pathogenic for pigs. Finally, biovar 5 has been isolated from rodents in Russia, and there is almost no

report of isolation since it was isolated for the first time [2]. In addition, this biovar was questioned as to if it belongs to *B. suis*; genome studies prove that biovar 5 is a member of this species of *Brucella* [3].

Brucella suis is commonly transmitted by ingestion or by contact with fluids from infected neonates, abortions or uterine discharges. Pigs usually eat remains of aborted fetuses and placentas. The clinical manifestations of swine brucellosis are associated with abortions in the middle of the gestation period, stillbirth, infertility, orchitis and epididymitis in boars. Abortions occur generally after 30 to 40 days post infection. It was also described that doses of 10^4 to 10^5 CFU/ml are enough to infect pigs experimentally by the conjunctival route. Another survey describes that boars can shed 10^4 to 10^5 CFU/ml. A synthetic review of *B. suis* epidemiology was described [2].

Brucella suis have particular characteristics differing from other *Brucella* species when it infects swine. This bacteria causes systemic infection with the consequence of reproductive failure, can infect pigs of all ages, and meanwhile the transmission of the disease can be venereal as well as the classical oral route [4].

In many countries, *B. suis* biovar 1 is usually isolated from cattle, originating an important inconvenience for bovine brucellosis programs due to false positive results in the serological test for bovines, taking into account that there is not a serological difference in cattle infected with *B. abortus*.

There are reports indicating that *B. suis* biovars 2 was isolated also from cattle in Europe [5]. Brucellosis infections have been reported also in wild (feral) swine. This condition brings an increasing complication in the United States where there is a high risk of infection in domestic pigs, as some farming practices could permit the mixing of both populations. Besides, feral swine can be infected by *B. abortus* creating conditions for "host jumping". This fact should be carefully controlled to avoid severe epidemiological consequences [5].

Brucella melitensis is one of the most important zoonotic agents worldwide [6].There are three known biovars of *B. melitensis*, 1, 2 and 3 [5]. Goats are the classic and natural hosts of *B. melitensis* and together with sheep are its preferred hosts. The most important transmission route in susceptible hosts is oral mucosa. *B. melitensis* infection in pregnant small ruminants affects the placenta and fetus inducing late gestation abortions in pregnant small ruminants. Goats infected with *Brucella* are the main source of transmission to susceptible animals in the flock and also to the animal handlers [1, 7]. Despite that abortion is the main clinical sign, it does not occur in all infected goats, but these animals still shed *Brucella* to the environment, becoming a major risk to other susceptible animal species that coexist in the same environment (e.g., cattle and sheep)[5]. The transmission of *Brucella* between different susceptible hosts can complicate the control of the disease. Keeping cattle together with other ruminants is a risk factor for the spread of brucellosis [8].

Camels ,which are not known to be primary hosts of *Brucella*, are, however, highly susceptible to *B. melitensis*, playing an importance role in the epidemiology of brucellosis [9]. Alpine ibex (*Capra ibex*) and Chamois (*Rupicapra rupicapra*) can be also infected and can trigger an outbreak of brucellosis [10].

Brucella melitensis as well *B. suis* can be transmitted to humans mostly as a food borne disease or, particularly, to farmers engaged on agricultural practices. Brucellosis in humans is a major systemic disease in which bacteria are located within phagocytes of the reticuloendothelial system (RES). The liver is one of the main organs of the reticuloendothelial system, and it is involved in all cases of brucellosis in both humans and animals [11]. Human brucellosis has many clinical signs, but fever and musculoskeletal symptoms are the most common. The mechanisms predisposing *Brucella* to an osteoarticular site are not clear but these sites represent an important niche for bacterial persistence [12]. In addition, neutrophil recruitment is related to *Brucella*-induced pathologies in both ruminants and in humans [13].

The Standard Immunological Diagnosis

The most widely implemented serological diagnostic techniques are those based on the detection of antibodies against smooth LPS, which are considered *Brucella* immunodominant surface antigens.

Screening tests like Rose Bengal are broadly used worldwide; however, the buffer plate antigen test is also used as screening for porcine and caprine brucellosis. Another excellent test is complement fixation, but it is too cumbersome and requires very well-trained personnel. Progressively, this test has been replaced by enzyme-linked immunosorbent assays which brought important advantages improving sensibility, specificity and automation. Lately, another diagnostic method was introduced for brucellosis diagnostics: the fluorescence polarization assay (FPA) which was demonstrated to be feasible for the diagnosis of brucellosis in pigs as well as goats. Thus, there are enough serological methods for the diagnosis of brucellosis but they also have some limitations [14]. As an example, none of these tests has the ability to identify the type of *Brucella* species, onset of infection, course of the disease, or even if the animal has active disease at the time of sampling, because antibody titers may remain high for a long period of time after the onset of infection. In addition, REV 1 vaccinated animals are very difficult to distinguish from truly infected ones. Lately, the lateral flow test (LFA) was also evaluated in several species, including ruminants; those studies claim that LFA is more sensitive and specific than traditional methods previously mentioned in this review and suggest

using it as confirmatory tests. However, although it is promissory, we understand that more work should be done in order to evaluate and standardize the reagents of the test which are allowed to be prescribed by the World Organisation for Animal Health (OIE).

We also understand that the major challenge with these diagnostic tests is to increase the diagnostic specificity, mainly in places where Rev 1 vaccine, Suis 2, and/or Strain 19 are implemented, thus, false positives results are common. To solve this difficulty, different approaches have been applied using different protein antigens with possibly diagnostic activities. For example, cooper/zinc superoxide dismutase, lumazine synthase, or BP26, are the most well-known candidates; however, still none of these proteins can be standardized to be an "ideal" antigen [15].

Recently, a couple of studies were done, trying to improve the diagnosis of porcine brucellosis. An experiment demonstrates that a skin test using a genetically defined mutant (*B. abortus* ΔmanBcore) results in a 100% specificity in pigs infected with *B. suis* biovar 2 and also in a free herd, while they were false positives with standard diagnostic tests [16]. In a related study in pigs, the performance of an Indirect ELISA with R-LPS, gel immunodiffusion, counterimmuno-electrophoresis, latex agglutination and indirect ELISA with OPS free proteins in comparison with current serological tests based in S-LPS was evaluated. Protein tests show 100% specificity, but the sensitivity was lower than 63%. However, among S-LPS tests, gel immunodiffusion shows 68% sensitivity and 100% specificity. The authors suggest that gel immunodiffusion could be a practical alternative to intradermal testing [17]. Knowing that these tests need to be validated, and probably used in determined regions, we understand that for regions where productive animals are raised extensively and/or the number of animals is quite large, neither the skin test nor the gel diffusion test will be able to replace neither standard immunoassays nor fluorescent polarization.

The Advanced Diagnostic Tests

Serological tests based on antibody detection of smooth *Brucella* are the basis of ruminants as well as porcine brucellosis diagnosis. The high sensitivity and specificity of these tests are the reasons for which they are chosen [18–20]. But, *Brucella spp*. proteins play an important role triggering cell mediated immune (CMI) response in the host, which is the most relevant protection against *Brucella* infection. Measurement of IFN-γ could be useful to differentiate vaccinated animals from truly infected ones and can be used to monitor evolution of infected animals predicting any reproductive problems [21•].

During stages of the disease, IFN-γ may shift. The variation in the presence of this cytokine in the pregnant sheep can be correlated to the stage of the disease and the dissemination of *B. melitensis*. After a first bacteremia, colonization of placenta in pregnant ewe, plus the cessation of circulation in peripheral blood, can be determinants of a decline in the production of specific IFN-γ by blood cells. If an abortion occurs, a second circulation of *B. melitensis* takes place in the infected sheep, inducing an increase in the IFN-γ response. Moreover, detection of IFN-γ shows infection earlier than tests based on LPS antibodies, but it is not able to detect all infected animals, in contrast with the classical serological tests [21•].

There are advances to investigating the properties of specific OPS epitopes in serodiagnosis related to the M epitope. Recent structural evidence shows the existence of an M-type of each smooth *Brucella* [22].

Constructions of an alternative M epitope [23, 24] with a unique structures that does not exist in nature has been in order to develop a diagnostic platform based on ELISA [25].

The combination of these proposed diagnostic antigens with a vaccine that has A epitopes can potentially create a diagnostic technique that is not adversely affected by the application of a vaccine. The only smooth *Brucella* biovar that does not possess this M epitope is *B. suis* biovar 2 [5].

Closely related *Brucella* isolates are difficult to distinguish; meanwhile, it is not possible to distinguish them by using a serological tests or classical bacteriology; even with PCR, typing is not possible. The genome sequence amplification method based on PCR is an alternative tool. The identification of similar *Brucella spp*. is achievable with multiple gene sequence analyses, such as multi-locus sequencing (MLS) and multi-locus variable-number tandem repeat (MLVA). These techniques use a limited number of candidate genes and the intraspecies relationships of *B. melitensis* may be difficult to resolve. The whole genome single nucleotide polymorphisms (SNP) based on phylogenetic analysis can help with this trouble, providing higher resolution power and resolving genetic relationships between the *B. melitensis* species [26••].

Whole genome SNP-based phylogenetic analysis can identify isolates, and demonstrate the phylogenetic links in *B. melitensis* and *B. suis* in relation to the geographical origins. Some mutations or acquisition of genome changes may be related to bacterial adaptation to different environments or even to a specific host [26••, 27].

MLVA has been confirmed as a useful technique to assess *B. melitensis* genetic relations between isolates and the traceability to enhance prevention, control and eradication of brucellosis. Using similar techniques with *B. suis*, isolates can be differentiated into clades despite slight differences at the genome level. *B. suis* contains, like *B. melitensis,* sufficient polymorphisms for genetic analyses, such as genotypes distribution, tracing swine outbreaks, or the identification of potential feral or domestic sources [20].

To use a large database of MLVA profiles of *Brucella* isolates is crucial to the usefulness of MLVA as an

epidemiological tool. MLVA has been shown to be useful in revealing the most prevalent genotypes, distribution, circulation and relatedness of *Brucella* isolates, mainly in endemic areas [28–30].

The quantitative real-time PCR (qPCR) assay can be also used as a sensitive diagnostic tool for detection of *B. suis*. It is remarkable that the high sensitivity of this assay outperforms previous PCR assays for the specific detection of *B. Suis*. The specificity of this qPCR is 100% for *B. suis* and negative for another *Brucella spp.* or another bacterium. It also can be used as a screening method, because it is a low cost, fast and reliable assay for large sample pools of *B. suis* 1 to 4 [31, 32].

New diagnostic techniques must be evaluated to improve the performance of the standard ones. Therefore, sensibility and specificity, simplicity, the ability to differentiate vaccinated from infected animals, and costs are some variables to be assessed before they can be widely implemented.

The Pathogenicity

Knowledge of genomics has led to the identification of variations in the genome of different species and strains of *Brucella*, allowing understanding virulence factors and survival of bacteria in specific hosts. With techniques such as cDNA microarrays, it is feasible to determine genome transcript changes of *B. melitensis* genes. It is predictable that the majority of upregulated genes in the log phase cultures are associated with growth.

Recent information describes that environmental stress resistance and adaptation to intracellular life are related to the cell envelope's associated genes of *the B. melitensis* [33, 34].

There is a quorum sensing (QS) communication system that controls the expression of certain genes, showing a reaction to population density and regulating virulence determinants as well [35]. There is a described inducer called acylhomoserinelactone that works as a signal synthesized by *luxI,* affecting the operation of the transcriptional regulator LuxR. Deletion of *Brucella vjbR*, a LuxR-like transcriptional regulator, decreases the ability to survive in the intracellular environment of *B. melitensis* (e.g., potential vaccine candidates) [36].

We know that the pathogenic potential of *B. melitensis* and *B. suis* mostly depends on its ability to survive within host cells. There are proteins present in the cell envelope, which are a virulence factor of *B. melitensis* that promotes survival of bacteria in host cells such as macrophages [34, 37]. Interaction between pathogens and hosts determines a change in gene expression patterns in the cell envelope proteins that starts from a cascade of events , leading either to the colonization or the elimination of pathogens in the host [38].

When *Brucella spp*. makes its way through the membrane of a host's cells, it faces extreme environmental conditions designed to get rid of bacteria, and it has developed strategies to avoid the host's immune system. *Brucella* containing vacuoles (BCVs) avoid the linkage with lysosomes and can resist in an acidified environment. They also have the ability to interact with components of the early endosome after being entered by the host cell [39].

There are some acquisitions that *Brucella* has achieved in order to adapt, replicate, survive and modulate the host´s immune system. Some of these important achievements are the well-known VirB T4SS, a perosamine-based O antigen (associated with intracellular replication), the ability to sequester metal ions and the skill of interfering with the immune system via TIR domain proteins [3].

The type IV secretion system (T4SS) is essential for the BCV biogenesis. T4SS translocates effector proteins, which modulate traffic through the membrane throughout the secretory and endocytic pathways [40]. *Brucella* operates systems to move DNA or proteins into the susceptible cells, having these as an important role in bacterial virulence. In *Brucella spp*., the VirB T4SS allows development of pathogenesis in the generation of a niche inside the host cell and is involved in protein translocation across the cell envelope [41, 42].

The transcriptional regulator MucR plays a key role in the expression of T4SS, in the QS system, and it is also involved in responses to different environmental hazards. Analysis of the *mucR* mutant using RNA-seq and RT-PCR identified the genes affected by the transcriptional regulator MucR. If the *mucR* gene was deleted, the tolerance to acid is affected [43, 44].

Through RNA-seq, a genome-wide transcriptome analysis can be performed; this helps to improve the knowledge in how certain pathways play a key role in regulation of intracellular adaptation.

In *Brucella*, T4SS transports effector molecules into the infected cells leading to control signaling pathways, and aiming to ensure survival for the bacteria. VirB (which contains 12 open reading frames) is responsible of encoding T4SS of *Brucella*, termed virB1 [44].

Almost all genes homologous to *mucR* are involved in the endurance, survival and adaptation of bacteria to the host cells [43]. The virB operon for intracellular invasion and multiplication is also required by *B. suis*. Survival and multiplication in macrophages or epithelial cells is affected in T4SS mutants. We must bring up that single nucleotide polymorphism (SNP) performed in the attenuated vaccine strain *B. suis* S2 shows a mutation occurring in almost all the genes of the *virB* operon, coding for a type IV secretion system (T4SS), compared with virulent isolates [27].

In *B. melitensis*, activity of MucR It has been shown to modulate lipid A core modification, and resistance to environmental hardness, cationic peptides, oxidative stress and detergents [45].

There is a characteristic weak immune response in *Brucella* infection, possibly because of the implementation of a strategy

to avoid recognition and control the host cells [46]. This last strategy rises due to the pro-inflammatory responses induced by the TIR-containing protein TcpB (TIR domain containing protein in *Brucella*)/Btp1 (*Brucella* TIR domain containing protein) and another characterized protein, BtpB. It was shown that TcpB and BtpB proteins were translocated into host cells via a T4SS-dependent pathway [47].

An important assumption of the *Brucella* pathogenesis can be attributed to the number of 'hidden' proteins that might allow the pathogen to elicit inflammatory responses further suppressing host immune response [48].

An uncommon and recently description behavior of *B. melitensis* is the location of the bacteria inside murine erythrocytes after intraperitoneal inoculation. The circulation of antibodies induced by immunization drastically reduces blood persistence of *Brucella* following challenge. This invasion of erythrocytes may constitute an unexpected step that helps *Brucella* to avoid the host defenses. It is important to emphasize that these red cells are not capable of endocytosis [49].

This last paragraph is intended to highlight strategies or abilities than are unknown about the pathogenicity of *Brucella.* Much effort should be made to resolve these unanswered questions.

The Vaccinology

B. melitensis Rev.1 is the only vaccine that is being used for the prevention of brucellosis in specific hosts. This vaccine has proved to be the best candidate for prophylaxis of brucellosis in sheep and goats [50–53]. Unfortunately, there are some side effects related to the use of this vaccine, like the potential reversion of virulence, the capacity of this strain of causing infection in humans and the streptomycin resistance encoding information. For these reasons, vaccination with this strain is restricted to animals [52]. To reduce these limitations, subunit vaccines, recombinant vaccines, and new attenuated live bacteria are being designed.

The clearance of intracellular pathogens depends on Th1-type responses and IFN-γ. This cytokine activates macrophages and promotes immune-responses turning antibody responses toward protective IgG2a [54, 55]. Protection against *Brucella ssp.* largely relies upon the CMI responses, which induce microbicide actions to eradicate bacteria from intracellular niche [56].

Subunits vaccines depend on the identification of antigens that raise a protective immunity, but it is not fully known which antigens are responsible for triggering humoral and cellular immune responses [57].

Recombinant protein-based subunit vaccines are alternative candidates for human immunization against brucellosis [52, 58]. The presentation of different antigens in the same vaccine can promote a better protection than that induced with a single one, especially choosing antigens expressed at different moments of the life cycle of *Brucella* [59].

The combination of rOmp31 with rTF, for example, triggers strong immune responses. The immunization of mice with rOmp31 + rTF caused a considerable decrease of *B. melitensis* colonization in mouse spleen [60].

Recombinant proteins rOmp19 and rP39 were tested as protective immunogens when administered simultaneously (rO + rP). The cocktail protein was found to be immunogenic and demonstrates that the multivalent proteins are more immunogenic than the individual ones [51]. The recombinant rO + rP cocktail established a Th1 p redominant immune responses both for in vitro cell culture and for in vivo mouse models [61].

The use of analytical methods based on the availability of genomic sequences are tools that can potentially propose subunit antigens more efficiently as vaccine candidates [62]. Reverse vaccinology is based on in silico genome analysis and the identification of *Brucella* antigens with characteristics such as immunogenicity or structure, requirements that are necessary if they are to be proposed as vaccines [63].

The molecular chaperone DnaK is involved in protein folding, interaction by binding to unfolded polypeptide domains when *Brucella* is under adverse conditions. This protein is able to elicit a Th1 cellular response and confers significant protection against *B. melitensis*. It is also confirmed that combination of antigens generates a more important protection compared to the immunization with a single protein, as it is exemplified with DnaK in a cocktail with Omp31. Despite the promising results, the protection with this candidate is lower than with other potential subunit vaccines [64].

Another chaperon protein, RHspA, is a potential candidate vaccine for *Brucella* because it elicits a specific cellular response, produces a remarkable amount of antigen-specific IFN- and IL-12 and gives adequate protection. This candidate appeared to work successfully for immunization to prevent *Brucella* infections, but this protective immunity is inferior to protection in mice vaccinated with Rev-1 [65].

Brucella melitensis B115 strain, a natural rough stable strain, has demonstrated its usefulness for immunization against *B. melitensis* in the mouse model. Unfortunately, vaccinated animals had abortions, in a similar proportion to that observed using Rev-1, and it was documented as a possible occurrence of *in vivo* phenotype reversion phenomena (from rough to smooth) [66].

Modified *B. melitensis* 16 M has been used as a vaccine candidate with the deletion of *wzt*, a gene encoding the O-PS export system ATP binding protein, becoming a *Brucella* of the rough phenotype. *B. melitensis* 16MΔ*wzt* is able to confer a certain degree of protection against challenges with *B. melitensis* 16M (lower than the protection generated with Rev-1). It is noteworthy that 16MΔ*wzt* does not induce O-PS specific antibodies [67].

Brucella melitensis vaccine strain M5-90, similar to *B. melitensis* vaccine strain Rev.1, is mostly implemented for immunization of sheep and goats in China. A modified M5-90 has been used as a candidate, deleting genes associated with virulence factors. *B. melitensis* strain M5-90Δ*wboA* induced slightly higher levels of IFN- than M5-90. The *wboA* gene is responsible for encoding an essential glycosyltransferase for the biosynthesis of the *Brucella* O antigen [68].

Another approach on trying to arrive to a potential vaccine candidate against *B. melitensis* that has being used is the recombination of viruses carrying *Brucella*´s DNA. Recombinant pseudorabies virus vaccine strain expressing bp26 (an outer membrane protein, that is conserved and immunogenic in all *Brucella* species) of *B. melitensis* was generated. The spleens of mice vaccinated with recombinant virus had strong lymphocyte proliferative response and high levels of IFN-γ [69].

A novel influenza viral vector *B. abortus* (Flu-BA) has also being tested. The *Brucella* proteins L7/L12 and Omp16 are present in most species of *Brucella*. The Flu-BA vaccine should be able to provide cross-protection against other *Brucella* species, including *B. melitensis* [70].

The use of bioinformatics to predict B and T cell epitopes and construct a recombinant multi-epitope protein (rMEP), is a promising *Brucella* vaccination strategy.

In vivo tests using BALB/C mice showed a strong humoral immune response, indicated by specific IgG levels and with adequate induction of Th1 and Th2 response. This vaccine does not confer better bacterial clearance against *B. melitensis* 16 M infection and generated a lower level of protection than the commercial live vaccine [71].

Many strategies have been proposed to arrive at the ideal vaccine against *B. melitensis*; none have generated protection in similar levels as *B. melitensis* strain Rev-1. More studies have to be done to achieve the replacement of the current vaccine, despite the disadvantage of Rev-1 vaccination.

It is well known that there is lack of vaccines against infections with *B. suis* in most parts of the world. However, in China there is a live attenuated *B. suis* strain 2 vaccine (S2) that is used to immunize pigs and also ruminants. This fact was considered controversial for different investigators. Recently, this vaccine was evaluated in BALB/c mice challenged with pathogenic reference *Brucella* strains. The spleen of immunized mice showed an increase on TNF-α and a relatively good protection against infection to *B. suis* but also to *B. abortus* and *B. melitensis*. This candidate showed low virulence as there were no bacteria recovered in spleen post vaccination. *Brucella* specific IgG was detected in vaccinated mice, and produced IFN-γ and TNF-α conferring protection in mice from a virulent challenge by *B. melitensis* M28, *B. abortus* 2308 and *B. suis* S1330. Adverse effects, such as clinical signs or tissue damage were not described in vaccinated animals [72].

Recently, a new study described the construction of a novel immunogen candidate against porcine brucellosis. A highly attenuated *B. suis* Delta-*pgm* (Δ*pgm*) mutant strain was described, which confers a robust protective immune response.

B. suis Δ *pgm* strain provides a strong pro-inflammatory immune response with high levels of IFN- and TNF-α, consistent with strains that have shown protective properties without interfering with classical serological diagnostic tests. These results clearly demonstrate that this strain was able to trigger a robust cellular immune response that raised a remarkable protection against the virulent *B. suis* suggesting that it could be a potential candidate for the prevention of brucellosis in swine [73•].

Contemporary literature shows tremendous efforts that have been done employing the most current technology available, to get a better or newer vaccines to prevent brucellosis, however still the old fashion immunogens are the only ones which are helping the world to control this extended zoonosis.

Conclusion

A major effort to understand the biology of *Brucella melitensis* and *B. suis* has been done in the last few years. The chief progress toward a comprehensive understanding of the mechanism of surviving from these fantastic bacteria evidenced from the standard agglutination tests to the sequencing of the bacterial genome evidence. Modern technology helps to understand *Brucella* behavior, thus, research on traffic within cells, gene activation, precise diagnostic tools, remarkable advances in T and B cell induced factors, and new vaccine candidates, are continuously developed. However, we wonder why these organisms are still causing severe disease worldwide to animals and people. Maybe the scientific work is not enough so far, maybe control of eradication programs are not strong enough, but undoubtedly, scientists will continue looking for and reaching more precise diagnostic tests, safe and efficient vaccines, and overall discover those secrets that still these bacteria have kept hidden that allow them to provoke our intellect. Very much research has been done; however, and, at this moment, brucellosis research is in progress *Brucella* is waiting for us!!

Compliance with Ethical Standards

Conflict of Interest Luis Samartino, Matías Arregui and Pablo Martino declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Poester FP, Samartino LE, Santos RL. Pathogenesis and pathobiology of brucellosis in livestock. Revue scientifique et technique (International Office of Epizootics). 2013;32(1):105–15.
2. Díaz AE. Epidemiology of brucellosis in domestic animals caused by *Brucella melitensis*, *Brucella suis* and *Brucella abortus*. Revue scientifique et technique (International Office of Epizootics). 2013;32(1):43–51. **3-60**.
3. Wattam AR, Foster JT, Mane SP, et al. Comparative phylogenomics and evolution of the brucellae reveal a path to virulence. J Bacteriol. 2014;196(5):920–30.
4. Meirelles-Bartoli RB, Mathias LA, Samartino LE. Brucellosis due to *Brucella suis* in a swine herd associated with a human clinical case in the State of Sao Paulo, Brazil. Trop Anim Health Prod. 2012;44(7):1575–9.
5. Godfroid J, DeBolle X, Roop RM, et al. The quest for a true One Health perspective of brucellosis. Revue scientifique et technique (International Office of Epizootics). 2014;33(2):521–38.
6. Welburn SC, Beange I, Ducrotoy MJ, et al. The neglected zoonoses–the case for integrated control and advocacy. Clin Microbiol Infect : Off Publ Europ Soc Clin Microbiol Infect Dis. 2015;21(5):433–43.
7. Baldi PC, Giambartolomei GH. Pathogenesis and pathobiology of zoonotic brucellosis in humans. Revue scientifique et technique (International Office of Epizootics). 2013;32(1):117–25.
8. Muendo EN, Mbatha PM, Macharia J, et al. Infection of cattle in Kenya with Brucella abortus biovar 3 and Brucella melitensis biovar 1 genotypes. Trop Anim Health Prod. 2012;44(1):17–20.
9. Gwida M, El-Gohary A, Melzer F, et al. Brucellosis in camels. Res Vet Sci. 2012;92(3):351–5.
10. Mick V, Carrou GL, Corde Y, et al. Brucella melitensis in France: persistence in wildlife and probable spillover from alpine ibex to domestic animals. PLoS One. 2014;9(4):1–9.
11. Young EJ, Hasanjani Roushan MR, Shafae S, et al. Liver histology of acute brucellosis caused by *Brucella melitensis*. Hum Pathol. 2014;45(10):2023–8.
12. Magnani D, Lyons E, Forde T, et al. Osteoarticular tissue infection and development of skeletal pathology in murine brucellosis. Dis Model Mech. 2013;6(3):811–8.
13. Lacey CA, Keleher LL, Mitchell WJ, et al. CXCR2 mediates *Brucella*-induced arthritis in interferon gamma-deficient mice. J Infect Dis. 2016;214(1):151–60.
14. Smirnova EA, Vasin AV, Sandybaev NT, et al. Current methods of human and animal brucellosis diagnostics. Adv Infect Dis. 2013;03(03):177–84.
15. Mcgiven JA. New developments in the immunodiagnosis of brucellosis in livestock and wildlife. Rev Sci Tech Off Int Epiz. 2013;32(1):163–76.
16. Dieste-Perez L, Blasco JM, De Miguel MJ, et al. Performance of skin tests with allergens from B. melitensis B115 and rough B. abortus mutants for diagnosing swine brucellosis. Vet Microbiol. 2014;168(1):161–8.
17. Dieste-Perez L, Blasco JM, de Miguel MJ, et al. Diagnostic performance of serological tests for swine brucellosis in the presence of false positive serological reactions. J Microbiol Methods. 2015;111:57–63.
18. Garcia-Bocanegra I, Allepuz A, Perez JJ, et al. Evaluation of different enzyme-linked immunosorbent assays for the diagnosis of brucellosis due to *Brucella melitensis* in sheep. Vet J. 2014;199(3):439–45.
19. Gupta VK, Shivasharanappa N, Kumar V, et al. Diagnostic evaluation of serological assays and different gene based PCR for detection of *Brucella melitensis* in goat. Small Rumin Res. 2014;117(1):94–102.
20. Leiser OP, Corn JL, Schmit BS, et al. Feral swine brucellosis in the United States and prospective genomic techniques for disease epidemiology. Vet Microbiol. 2013;166(1-2):1–10.
21. • Perez-Sancho M, Duran-Ferrer M, Garcia-Seco T, et al. Interferon-gamma responses in sheep exposed to virulent and attenuated *Brucella melitensis* strains. Vet Immunol Immunopathol. 2014;160(1-2):123–8. **This publication provides a sustainable basis for experiments related to the diagnosis of B. melitensis in sheep as a function of the variation of INF- , and how this Interleukin varies according to the stage of the disease and pregnancy.**
22. Kubler-Kielb J, Vinogradov E. Reinvestigation of the structure of Brucella O-antigens. Carbohydr Res. 2013;378:144–7.
23. Ganesh NV, Sadowska JM, Sarkar S, et al. Molecular recognition of Brucella A and M antigens dissected by synthetic oligosaccharide glycoconjugates leads to a disaccharide diagnostic for brucellosis. J Am Chem Soc. 2014;136(46):16260–9.
24. Guiard J, Paszkiewicz E, Sadowska J, et al. Design and synthesis of a universal antigen to detect brucellosis. Angewandte Chemie (International ed in English). 2013;52(28):7181–5.
25. McGiven J, Howells L, Duncombe L, et al. Improved serodiagnosis of bovine brucellosis by novel synthetic oligosaccharide antigens representing the capping M epitope elements of *Brucella* O-Polysaccharide. J Clin Microbiol. 2015;53(4):1204–10.
26. •• Tan KK, Tan YC, Chang LY, et al. Full genome SNP-based phylogenetic analysis reveals the origin and global spread of *Brucella melitensis*. BMC Genomics. 2015;16:93. **Impressive research in order to understand the pathogenesis of Brucella melitensis.**
27. Sankarasubramanian J, Vishnu US, Gunasekaran P, et al. A genome-wide SNP-based phylogenetic analysis distinguishes different biovars of Brucella suis. Infect, Genet Evol : J Molec Epidemiol Evol Genet Infect Dis. 2016;41:213–7.
28. De Massis F, Ancora M, Atzeni M, et al. MLVA as an epidemiological tool to trace back *Brucella melitensis* Biovar 1 re-emergence in Italy. Transbound Emerg Dis. 2015;62(5):463–9.
29. Garofolo G, Di Giannatale E, De Massis F, et al. Investigating genetic diversity of *Brucella abortus* and *Brucella melitensis* in Italy with MLVA-16. Infect, Genet Evol : J Molec Epidemiol Evol Genet Infect Dis. 2013;19:59–70.
30. Shevtsov A, Ramanculov E, Shevtsova E, et al. Genetic diversity of *Brucella abortus* and *Brucella melitensis* in Kazakhstan using MLVA-16. Infect, Genet Evol : J Molec Epidemiol Evol Genet Infect Dis. 2015;34:173–80.
31. Hänsel C, Mertens K, ME et al. Novel real-time PCR detection assay for *Brucella suis*. Vete Rec Open. 2015;2.
32. Lopez-Goni I, Garcia-Yoldi D, Marin CM, et al. New Bruce-ladder multiplex PCR assay for the biovar typing of *Brucella suis* and the discrimination of *Brucella suis* and *Brucella canis*. Vet Microbiol. 2011;154(1-2):152–5.
33. Scholz HC, Vergnaud G. Molecular characterisation of *Brucella* species. Revue scientifique et technique (International Office of Epizootics). 2013;32(1):149–62.
34. Wang Y, Chen Z, Qiao F, et al. The type IV secretion system affects the expression of Omp25/Omp31 and the outer membrane properties of *Brucella melitensis*. FEMS Microbiol Lett. 2010;303(1):92–100.
35. Uzureau S, Lemaire J, Delaive E, et al. Global analysis of quorum sensing targets in the intracellular pathogen *Brucella melitensis* 16 M. J Proteome Res. 2010;9(6):3200–17.

36. He Y. Analyses of *Brucella* pathogenesis, host immunity, and vaccine targets using systems biology and bioinformatics. Front Cell Infect Microbiol. 2012;2:2.
37. Brambila-Tapia AJ, Armenta-Medina D, Rivera-Gomez N, et al. Main functions and taxonomic distribution of virulence genes in *Brucella melitensis* 16 M. PLoS One. 2014;9(6):e100349.
38. Awwad E, Adwan K, Farraj M, et al. Cell envelope virulence genes among field strains of *Brucella melitensis* isolated in West Bank part of Palestine. Agricult Agricult Sci Proc. 2015;6:281–6.
39. Liu Q, Liu X, Yan F, et al. Comparative transcriptome analysis of *Brucella melitensis* in an acidic environment: identification of the two-component response regulator involved in the acid resistance and virulence of *Brucella*. Microb Pathog. 2016;91:92–8.
40. Myeni S, Child R, Ng TW, et al. *Brucella* modulates secretory trafficking via multiple type IV secretion effector proteins. PLoS Pathog. 2013;9(8):e1003556.
41. Lacerda TLS, Salcedo SP, Gorvel J-P. *Brucella* T4SS: the VIP pass inside host cells. Curr Opin Microbiol. 2013;16(1):45–51.
42. Gomez G, Adams LG, Rice-Ficht A, et al. Host-*Brucella* interactions and the *Brucella* genome as tools for subunit antigen discovery and immunization against brucellosis. Front Cell Infect Microbiol. 2013;3:17.
43. Dong H, Liu W, Peng X, et al. The effects of MucR on expression of type IV secretion system, quorum sensing system and stress responses in *Brucella melitensis*. Vet Microbiol. 2013;166(3-4): 535–42.
44. Walldén K, Williams R, Yan J, et al. Structure of the VirB4 ATPase, alone and bound to the core complex of a type IV secretion system. Proc Natl Acad Sci U S A. 2012;109(28):11348–53.
45. Mirabella A, Terwagne M, Zygmunt MS, et al. *Brucella melitensis* MucR, an orthologue of Sinorhizobium meliloti MucR, is involved in resistance to oxidative, detergent, and saline stresses and cell envelope modifications. J Bacteriol. 2013;195(3):453–65.
46. von Bargen K, Gorvel JP, Salcedo SP. Internal affairs: investigating the *Brucella* intracellular lifestyle. FEMS Microbiol Rev. 2012;36(3):533–62.
47. Li W, Ke Y, Wang Y, et al. *Brucella* TIR-like protein TcpB/Btp1 specifically targets the host adaptor protein MAL/TIRAP to promote infection. Biochem Biophys Res Commun. 2016;477(3):509–14.
48. Chiliveru S, Appari M, Suravajhala P. On *Brucella* pathogenesis: looking for the unified challenge in systems and synthetic biology. Syst Synth Biol. 2015;9(1-2):73–5.
49. Vitry MA, Hanot Mambres D, Deghelt M, et al. *Brucella melitensis* invades murine erythrocytes during infection. Infect Immun. 2014;82(9):3927–38.
50. Wang W, Wu J, Qiao J, et al. Evaluation of humoral and cellular immune responses to BP26 and OMP31 epitopes in the attenuated *Brucella melitensis* vaccinated sheep. Vaccine. 2014;32(7):825–33.
51. Ghasemi A, Salari MH, Zarnani AH, et al. Immune reactivity of *Brucella melitensis*-vaccinated rabbit serum with recombinant Omp31 and DnaK proteins. Iran J Microbiol. 2013;5(1):19–23.
52. Yang X, Skyberg JA, Cao L, et al. Progress in *Brucella* vaccine development. Front Biol. 2013;8(1):60–77.
53. Coelho AM, Pinto ML, GarcÍA DÍEz J. Impact of B. melitensis Rev-1 vaccination on brucellosis prevalence. Turk J Vet Anim Sci. 2015;39:261–70.
54. Vitry MA, De Trez C, Goriely S, et al. Crucial role of gamma interferon-producing CD4+ Th1 cells but dispensable function of CD8+ T cell, B cell, Th2, and Th17 responses in the control of *Brucella melitensis* infection in mice. Infect Immun. 2012;80(12): 4271–80.
55. Vitry MA, Hanot Mambres D, De Trez C, et al. Humoral immunity and CD4+ Th1 cells are both necessary for a fully protective immune response upon secondary infection with *Brucella melitensis*. J Immunol. 2014;192(8):3740–52.
56. Golshani M, Rafati S, Dashti A, et al. Vaccination with recombinant L7/L12-truncated Omp31 protein induces protection against *Brucella* infection in BALB/c mice. Mol Immunol. 2015;65(2): 287–92.
57. Olsen SC. Recent developments in livestock and wildlife brucellosis vaccination. Revue scientifique et technique (International Office of Epizootics). 2013;32(1):207–17.
58. Hop HT, Simborio HL, Reyes AW et al. Immunogenicity and protective effect of recombinant *Brucella abortus* Ndk (rNdk) against a virulent strain *B. abortus* 544 infection in BALB/c mice. FEMS Microbiol Lett. 2015;362(4).
59. Pasquevich KA, Estein SM, Garcia Samartino C, et al. Immunization with recombinant *Brucella* species outer membrane protein Omp16 or Omp19 in adjuvant induces specific CD4+ and CD8+ T cells as well as systemic and oral protection against *Brucella abortus* infection. Infect Immun. 2009;77(1):436–45.
60. Ghasemi A, Jeddi-Tehrani M, Mautner J, et al. Simultaneous immunization of mice with Omp31 and TF provides protection against *Brucella melitensis* infection. Vaccine. 2015;33(42):5532–8.
61. Tadepalli G, Singh AK, Balakrishna K, et al. Immunogenicity and protective efficacy of *Brucella abortus* recombinant protein cocktail (rOmp19+rP39) against *B. abortus* 544 and *B. melitensis* 16M infection in murine model. Mol Immunol. 2016;71:34–41.
62. Sette A, Rappuoli R. Reverse vaccinology: developing vaccines in the era of genomics. Immunity. 2010;33(4):530–41.
63. Gomez G, Pei J, Mwangi W, et al. Immunogenic and invasive properties of *Brucella melitensis* 16M outer membrane protein vaccine candidates identified via a reverse vaccinology approach. PLoS One. 2013;8(3):e59751.
64. Ghasemi A, Jeddi-Tehrani M, Mautner J, et al. Immunization of mice with a novel recombinant molecular chaperon confers protection against *Brucella melitensis* infection. Vaccine. 2014;32(49): 6659–66.
65. Ghasemi A, Zarnani AH, Ghoodjani A, et al. Identification of a new immunogenic candidate conferring protection against *Brucella melitensis* infection in mice. Mol Immunol. 2014;62(1):142–9.
66. Perez-Sancho M, Adone R, Garcia-Seco T, et al. Evaluation of the immunogenicity and safety of *Brucella melitensis* B115 vaccination in pregnant sheep. Vaccine. 2014;32(16):1877–81.
67. Wang Z, Niu JR, Wang XL, et al. Evaluation of a *Brucella melitensis* mutant deficient in O-polysaccharide export system ATP-binding protein as a rough vaccine candidate. Microbes Infect / Institut Pasteur. 2014;16(8):633–9.
68. Li ZQ, Shi JX, Fu WD, et al. A *Brucella melitensis* M5-90 *wboA* deletion strain is attenuated and enhances vaccine efficacy. Mol Immunol. 2015;66(2):276–83.
69. Yao L, Wu CX, Zheng K, et al. Immunogenic response to a recombinant pseudorabies virus carrying bp26 gene of *Brucella melitensis* in mice. Res Vet Sci. 2015;100:61–7.
70. Tabynov K, Ryskeldinova S, Sansyzbay A. An influenza viral vector *Brucella abortus* vaccine induces good cross-protection against *Brucella melitensis* infection in pregnant heifers. Vaccine. 2015;33(31):3619–23.
71. Yin D, Li L, Song D, et al. A novel recombinant multi-epitope protein against *Brucella melitensis* infection. Immunol Lett. 2016;175:1–7.
72. Zhu L, Feng Y, Zhang G, et al. Brucella suis strain 2 vaccine is safe and protective against heterologous Brucella spp. infections. Vaccine. 2016;34(3):395–400.
73.• Czibener C, Del Giudice MG, Spera JM, et al. Delta-pgm, a new live-attenuated vaccine against *Brucella suis*. Vaccine. 2016;34(13):1524–30. **This new research shows the advantages of a vaccine candidate to prevent porcine brucellosis.**