ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE" TERAMO

INTERNATIONAL BRUCELLOSIS SOCIETY

VETERINARIA ITALIANA

Teramo Brucellosis 2022

INTERNATIONAL RESEARCH CONFERENCE

BRUCELLOSIS 2022 INTERNATIONAL RESEARCH CONFERENCE

Including the 74th *Brucellosis* Research Conference

Giulianova - Teramo, Italy

September 16 - 19 2022

Proceedings

edited by
Fabrizio De Massis, David O'Callaghan, Angela Arenas, Philip Elzer and Sue Hagius

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Proceedings of the Brucellosis 2022 International Conference, including the 74th Annual Brucellosis Research Conference, held in Giulianova, Teramo, Italy from September 16th to 19th, 2022.
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BRUCELLOSIS 2022 INTERNATIONAL RESEARCH CONFERENCE

including the 74th Brucellosis Research Conference

Giulianova – Teramo, Italy

September 16th – 19th 2022

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Opening Ceremony

Nicola D'alterio
Director General
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy

Dear Participants,

As Director General of the Istituto Zooprofilattico Sperimentale of Teramo, I am very proud to open this important international scientific conference, finally hosted in Italy thanks to the opportunity given us by the International Brucellosis Society.

Organising a residential event after such a long-lasting health emergency has been a true challenge for us, but we see it as an evidence of the reliability that is recognized to us by the International Scientific Community.

Challenging was also the choice to organise such an event, not in a big city, as usual, but in Giulianova, close to our headquarters in Teramo.

Located between the Apennine Mountains and the Adriatic coast, this is a territory with deep traditions of fishing and shepherding. A heritage we are proud of, for the history, the traditions and the delicious food. Definitely, we are strongly connected with this land. Therefore, this event represents an opportunity to let people know our beautiful region.

I thank all the women and men of the IZS engaged in the organisation of this Conference, in particular the Head of the World Organisation for Animal Health Reference Laboratory for Brucellosis, Dr Fabrizio De Massis, and the Head of the Italian national Reference Centre for Brucellosis, Dr Manuela Tittarelli.

During the next four days, 270 experts, scientists, researchers, policymakers and stakeholders will exchange knowledge and opinions on the factors influencing the emergence and spread of the different species of *Brucella*.

Although brucellosis is a zoonotic disease, well known for more than one century, it still remains a matter of concern for many countries, representing a serious threat to human health and hampering the improvement of animal production in several areas of the World.

Therefore, this Conference aims at making the point of the most recent knowledge about the disease and providing useful indications for its control and eradication.

For this reason, I would like to give our warm welcome to all of you, and to thank you for the effort you made to join this meeting. I wish you to enjoy this experience!

Opening Ceremony

Fabrizio De Massis

Conference Chair

Expert for the World Organization for Animal Health (WOAH), Reference Laboratory for Brucellosis (*Brucella abortus*, *Brucella melitensis*, *Brucella suis*); Reference Laboratory for Ovine Epididymitis (*Brucella ovis*)

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy

David O'Callaghan

Conference Vice-Chair

Bacterial Virulence and Chronic Infection (VBIC), INSERM U1047, and French National Reference Centre for Human Brucellosis

University of Montpellier, Medical School, Nîmes, France

Dear Authorities, Participants and colleagues,

It is a great honour and pleasure for us to welcome you to Giulianova, Teramo, for the Brucellosis 2022 International Research Conference. This meeting is including the 74th Annual Brucellosis Research Conference, the traditional meeting for brucellosis scientists, usually held in Chicago.

Over 170 years after the disease was first described in Malta, bovine and caprine Brucellosis are still of major public health concern, both in endemic and non-endemic countries, all over the world. The impact on animal and human health is still enormous, and control and eradication of this zoonotic disease remains a global and interdisciplinary challenge. A 'One Health' approach, with a dynamic and mutually complementary collaboration between all the interested partners is required: animal health, human health, livestock owners, the food industry and the regulatory bodies.

We believe that this conference will be an ideal forum to allow the exchange of information, ideas and discussion between the different actors in the field of brucellosis. It will bring together scientists working on fundamental aspects of *Brucella* biology with animal and human health experts working in the field with brucellosis. We are looking forward to the exciting exchanges between the different participants, describing their experience, their results and their visions of the future perspectives. We hope that the networking opportunity given by this conference will allow the establishment of new research collaborations. We strongly encourage you to make this Conference of benefit both to the brucellosis research community, and to decision-makers, with the final goal of a global eradication of brucellosis in animals and men, with a One-health perspective. We are here for this.

The *Brucella* genus is not just the two species that cause the bovine and caprine disease. Recent research is discovering new *Brucella* in non-conventional animal species, for which the zoonotic potential should be continuously assessed. These issues of animal health, human health, and One

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Health have a significant political, economic and societal dimension in the affected areas.

We are happy to see many oral and poster presentations on a broad spectrum of topics regarding brucellosis. We had wagered on this conference, most of you remember that the Conference was postponed due to the COVID-19 pandemic. We are very glad to have succeeded in meeting all of you in person rather than on line!

We thank the Italian Ministry of Health, the Regional government of Abruzzo and the Municipality of Giulianova, Teramo, who are working hard to ensure food security and food safety standards across all branches of veterinary medicine and public health, brucellosis included, and have shown strong political and financial support for this conference.

We thank the Scientific Committees, the Liaison Committee, and the staff of Veterinaria Italiana Journal for the hard preparatory work done. We thank the Session Chairpersons, the Keynote Speakers and the oral and poster presenters for having accepted to give their invaluable contributions to support to the Brucellosis 2022 international Research Conference. We also thank all the registrants for their participation and for willing to share their expertise with the audience. Thanks, finally, to all commercial companies for their contributions to sponsor the event.

That's it, so we wish you all a successful Conference and an enjoyable stay in Giulianova, Teramo.

Our very best wishes.

Opening Ceremony

Frank Boelaert

Senior Scientific Officer, Biological Hazards & Animal Health and Welfare Unit (BIOHAW)

Risk Assessment Production department (ASSESS)
European Food Safety Authority (EFSA), Parma, Italy

Update on brucellosis in EU

The European Union (EU) system for the monitoring and collection of information on zoonoses, including brucellosis, is based on Zoonoses Directive 2003/99/EC, which obliges EU Member States (MSs) to collect, for zoonoses, relevant and, when applicable, comparable data on zoonoses, zoonotic agents, and foodborne outbreaks. In addition, MSs shall assess the trends and sources of these agents, as well as outbreaks in their territory, submitting an annual report each year by the end of May to the European Commission (EC) covering the data collected. The EC should subsequently forward these reports to the European Food Safety Authority (EFSA). EFSA is assigned the tasks of examining these data and publishing the EU Annual Summary Report. In 2004, the EC entrusted EFSA with the task of setting up an electronic reporting system and database for monitoring zoonoses (EFSA mandate number 2004-0178, continued by 2015-0231). Data collection on human diseases from MSs is conducted in accordance with Decision 1082/2013/EU on serious cross-border threats to health. The case definitions to be followed when reporting data on infectious diseases to the European Centre for Disease Prevention and Control (ECDC) are described in Decision 2018/945/EU. ECDC has provided data on zoonotic infections in humans like brucellosis, as well as their analyses, for the EU Summary Reports since 2005. Since 2008, data on human cases have been received via The European Surveillance System (TESSy), maintained by ECDC. Since 2019, the EU Annual Summary Reports on zoonoses, zoonotic agents and foodborne outbreaks have been renamed the 'EU One Health Zoonoses Summary Report' (EUOHZ), which is co-authored by EFSA and ECDC.

The following key facts were made based on the EUOHZ 2020 report. In 2020, the number of confirmed cases of human brucellosis was 128 in the EU. The EU notification rate of 0.03 per 100,000 population was the lowest notification rate reported since the beginning of surveillance in the EU in 2007. From 2016 to 2020 there was a significantly declining trend of confirmed human cases of brucellosis in the EU. Three MS (Greece, Italy and Portugal) had significantly decreasing 5-year trends from 2016 to 2020. Forty-nine (38.3%) out of 128 human cases were reported with information on the Brucella species. *Brucella melitensis* was reported as the aetiological agent in 43 (87.8%) out of 49 cases. In 2020, one weak-evidence foodborne brucellosis outbreak was reported in the EU, due to *Brucella melitensis* in sheep meat and products thereof, affecting two persons from the same household, who contracted the infection abroad. In cattle, the trend is favourable in 20 officially brucellosis-free MSs and seven non-officially brucellosis-free MSs

(Bulgaria, Croatia, Greece, Hungary, Italy, Portugal and Spain). Overall, in the officially brucellosis-free regions of the EU there were six infected herds in 2020 resulting in an extreme low prevalence (< 0.001). In the non-officially brucellosis-free regions of the EU, bovine brucellosis remained a rare event with 603 positive herds (0.38%) out of 157,000 tested herds, which was the lowest annual count since 2012. In sheep and goats, a stable situation was reported for 19 officially Brucella melitensis-free MSs and eight non-officially *Brucella melitensis*-free MSs (Bulgaria, Croatia, France, Greece, Italy, Malta, Portugal and Spain). Overall in the non-officially *Brucella melitensis*-free regions of the EU, 349 (0.22%) sheep and goat flocks were reported brucellosis-positive out of 160,000 tested, which was the lowest count since 2012. The eradication of brucellosis in cattle and in sheep and goats is close to being achieved in Croatia and Spain, with almost no positive herds reported for these infections in recent years. Brucellosis in cattle, and in sheep and goats is still prevalent in Greece and in some regions of Italy and Portugal. In Italy and Portugal, the proportion of brucellosis-positive cattle herds, and sheep and goat flocks in non-officially free regions decreased in recent years. Brucellosis is still an animal health concern with public health relevance in southern European countries that are not officially free of brucellosis.

References

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Simona Forcella
SNE, Policy Officer
European Commission, DG SANTE, Unit G2 – Animal Health, Brussels, Belgium

The Animal Health law Focus on brucellosis

21 April 2021 marks an historical date for the veterinary legislation in the European Union. Starting from that day the Regulation (EU) 2016/429 on transmissible animal diseases ("Animal Health Law"), adopted in 2016, became applicable.

This robust, single legal framework for EU veterinary policies allowed the EU to streamline a huge number of legal acts (39 were repealed), and to simplify the rules. The EU veterinary policy focus on key priorities such as preventing (biosecurity and surveillance) and eradicating (regionalization and eradication programs) diseases and clarify the responsibilities of farmers, veterinarians and other operators on movements of animals.

One of the delegated act adopted by the Commission to make the new rules applicable includes the list of animal diseases relevant for the Union intervention, among them brucellosis, listed as "Infection with *Brucella abortus*, *B. melitensis*, *B. suis*". The renaming of the disease resulted from an European Food Safety Authorty Scientific Opinion on prioritization and categorization of listed diseases, and is aligned with the International Standards of the World Organisation for Animal Health (WOAH).

The EU legislation considers Brucellosis as a category B disease for kept bovine, ovine and caprine animals. This means that all Member States in the EU should have, for the whole country or for certain zones, the health status of free from infection with *Brucella abortus*, *B. melitensis* and *B. suis* as regards kept bovine animals and kept ovine and caprine animals, or approved eradication programmes.

Specific provisions apply to align the designation of the health status with the International Standards of the WOAH.

The eradication programme sets out the conditions for granting and mantaining of the status of free from infection, with or without vaccination, to an establishment.

For the granting of the health status at country or zone level, a Member States must fulfill certain conditions concerning absence of infection, active and passive surveillance, percentage of establishments and animal population with status free from infection, ban on vaccination and conditions on the introduction of animals and germinal products into the establishment.

Any suspicion of brucellosis in bovine, ovine or caprine animals has to be notified to the competent authority, and, in case of a confirmed outbreak, this needs to be followed up by specific disease control and eradication measures. The Animal Health Law also changed the approach to the testing methods switching from a prescriptive explicit description of testing methods used to confirm the diagnosis of brucellosis, to the modern and flexible "cascade" and the prominent role of the European Union reference laboratories.

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Prof. Dr. Romano Marabelli
Advisor to the Director General
World Organization for Animal health (WOAH), Paris, France

WOAH activities on brucellosis

The Mandate of the World Organization for Animal Health (WOAH, founded as OIE) is to improve animal health globally, thereby ensuring a better future for all. WOAH is the global authority on animal health, working across borders to improve animal health and welfare and to assist national Veterinary Services.

Since its foundation in 1924, WOAH has focused on setting International Standards to support the safe trade of animals and animal products and to improve the prevention and control of animal diseases. Additionally, WOAH continuously has collected data to improve the knowledge of animal health situations worldwide.

WOAH relies on organization's experience and expertise, with the support of its network of Reference Centers and Reference Laboratories, to foster the necessary changes and to provide leadership in global animal health governance, so that Veterinary Services are better equipped to anticipate and respond to new expectations.

Brucellosis is one of the most frequently reported zoonotic infections from WOAH Member Countries, causing a major socio-economic impact in affected areas. According to the official notification received, *Brucella abortus* is currently still present in 87 countries, *B. melitensis* in 67 countries and *B. suis* in 28 countries, while only 4 countries have self-declared them free from Brucellosis to date.

An effective control of brucellosis requires a strong collaboration between animal and human health sector, following a One Health approach. The concept of One Heath to address health risks in the human-animal-environmental interface is getting every day higher and higher importance in the political agenda.

WOAH is constantly committed to support the current 182 Member Countries in the control of Brucellosis in animals and humans, by strengthening the Veterinary Services and improving animal health and veterinary public health worldwide.

This goal is pursued through the setting of International standards, such as the Terrestrial Code, where the disease specific chapter on brucellosis (Chapter 8.4) was already present in the first edition of the Terrestrial Code, published in 1968. The latest modifications have been made in 2018, always with the purpose of mitigating the risk of brucellosis spreading in animals and humans.

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An even important International standard is the Terrestrial Manual, which provides recommendations for brucellosis diagnostic test and vaccine production in Chapter 3.1.4. First adopted in 1990, the chapter was last reviewed and adopted during the 2022 WOAH General Session.

Indeed, the expertise network constitutes the core of WOAH scientific excellence. In 2022, WOAH has a global network of 266 Reference Laboratories covering 108 diseases or topics in 38 countries, and 68 Collaborating Centers, covering 45 specialties in 31 countries. Reference laboratories ensure that the standards, guidelines and recommendations are scientifically sound and up to date. The brucellosis reference laboratory network is one of the largest networks: 11 labs for *Brucella abortus*, 10 labs for *Brucella melitensis*, 6 labs for *Brucella suis*, 1 lab for *Brucella canis*.

In this framework, the WOAH welcomes the initiative of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' of organizing the Brucellosis 2022 International Research Conference. We would like to express to Nicola D'Alterio, Director General of the Institute, and to Fabrizio De Massis, the WOAH Reference laboratory expert for *B. abortus*, *B. melitensis* and *B. suis*, the best wishes for a successful Conference, always in a perspective to increase and disseminate the knowledge about this disease, to improve animal health globally, thereby ensuring a better future for all.

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Summary

IZS TERAMO ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"
Teramo Brucellosis 2022 INTERNATIONAL RESEARCH CONFERENCE
VETERINARIA ITALIANA RIVISTA DI SANITÀ PUBBLICA VETERINARIA

K1 The ever expanding *Brucella* genus

David O'Callaghan [1]

Abstract

Papers on *Brucella* and brucellosis often begin with 'Brucellosis is one of the world's most common zoonotic diseases'. However, this just refers to *Brucella melitensis, B. abortus* and certain *B. suis* biovars. These are the most commonly isolated *Brucella* strains because they have been spread across the world by the livestock industry over past centuries. They are also the most common causes of human brucellosis mainly because they are the species that humans are most exposed to. The *Brucella* genus is much larger than these three species and, in the last 25 years, has rapidly expanded from 6 to 12 recognised species with several more strains not yet assigned officially. The new strains have been isolated from humans, diverse mammals and, more recently, fish, amphibians and reptiles. It is most likely that many more new strains will be identified in the future. Whole genome sequencing has shown that the genus can be divided into two distinct clusters; the 'classical' *Brucella*, including the major zoonotic strains and the 'atypical' *Brucella* containing many of the new isolates. Many of the 'atypical' strains are phenotypically distinct form the 'classical' strains showing rapid growth, motility, a chemically and antigenically distinct O-antigen and a unique metabolic capacities. These atypical strains highlight the evolutionary path of *Brucella* from a soil bacterium. Very recently, the taxonomy of the genus *Brucella* has been complicated by the reclassification of other members of the *Brucellaceae* to the genus. These bacteria are soil bacteria or endophytes associated with different plants that do not cause a brucellosis like disease. This change is greatly contested by the *Brucella* research community. Due to their importance to the livestock industry and to human health, most studies concerning the virulence and zoonotic potential of *Brucella* have been with on *B. melitensis, B. abortus* and *B. suis*. In this Keynote Lecture, I will give an overview of how the *Brucella* genus has expanded and discuss to what extent the new strains represent a threat for animal or human health. I will also comment on the problems associated with the inclusion of non-*Brucella* strains in the genus.
Presenting author: david.o-callaghan@umontpellier.fr

Keywords: *Brucella*, Evolution, Taxonomy, Virulence

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O1-1 The Global Spread of the Most Famous *Brucella* Species

Jeffrey Foster [1]

Abstract

The past decade has seen an explosion of genomic sequencing for *Brucella*, with over 1,110 genomes now available. However, the large majority of these genomes come from just two species, *Brucella abortus* and *B. melitensis*, which is no coincidence due to the ubiquity of these two *Brucella* and their pervasive impacts on livestock, wildlife, and humans worldwide. Phylogenomics can be used on contemporary isolates, as well as from ancient DNA from fossils, to understand the timing of spread and their evolution. The primary finding is that the current distribution of *B. abortus* and *B. melitensis* is intimately tied to human movement of livestock across the globe-as livestock get moved so too do their pathogens. Both *B. abortus* and *B. melitensis* have striking similarities to each other, linked to human colonization patterns but also have key, but unexplained differences. *Brucella abortus* has one successful lineage that is found throughout the world, but also contains considerable diversity of numerous and divergent lineages, especially in Africa and Asia. *Brucella melitensis* in contrast has three successful lineages but relatively little diversity outside of these three groups. This talk will compare and contrast *Brucella* genomes to help explain how human movement has shaped these two important *Brucella* species over the past few thousand years and continuing to today.
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Keywords: *Brucella abortus*, *Brucella melitensis*, Evolution, genomics

1 *Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, USA*

O1-2 East African *Brucella* phylogenomics: introducing OrthoPhylo, A turn-key tool for generating ortholog based bacterial whole genome phylogenetic trees

Earl Middlebrook[1], *Robab Katani*[2], *Jeanne Fair*[3]

Abstract

The number of publicly available bacterial genome sequences is staggering (1.1milion assemblies in NCBI alone) and the rate of deposition is only increasing, with *Brucella* sequences being no exception. This wealth of data is juxtaposed with the lack of phylogenetic methods to robustly place these sequences within an evolutionary context. Not only does a phylogenetic placement aid in taxonomic classification, but also informs the evolution of novel phenotypes (host switching), targets of selection (host immune evasion mechanisms), and horizontal gene transfer (AMR/virulence genes). Methods fo reconstructing trees include comparing 16S ribosomal or other single loci, multi locus, whole genome alignments and Kmer based SNP analysis. All of these methods suffer from narrow taxonomic resolution, with 16S working well for higher taxon divisions and whole genome alignments working well for closely related samples. Here I present OrthoPhylo, a phylogenetic pipeline that takes bacterial genomes, annotates them and identifies orthologs, converts protein to nucleotide alignments, then builds species trees with both concatenated alignments and gene tree to species tree methods. The workflow has been designed to accept large numbers of input genomes (>1000) by identifying samples that represent the diversity of the whole dataset, and using these genomes to build models and identify orthologs. This strategy allows the generation of trees for ~1000 bacterial assemblies in ~30hrs using 30 cpus, with the majority of this time being taken up by ML based tree generation. This pipeline is designed to be an easy to install, turn-key solution for generating high resolution bacterial trees from species that can differ by more than 30% nucleotide identity. Here I present findings on the state of publicly available *Brucella* assemblies and their phylogenetic placements with a focus on sequences from East Africa, an understudied region of endemic *Brucella* infections. This effort is a part of a recently initiated five-year cross-sectional survey to assess risk factors associated with brucellosis in the East African countries of Tanzania and Rwanda.

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Keywords: Phylogenetics, Africa, Genome, Assembly, Ortholog

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O1-3 Canine Brucellosis in France due to *Brucella canis*: an emerging disease?

Guillaume Girault [1], *Acacia Ferreira Vicente* [1], *Maëline Ribeiro* [1], *Ludivine Perrot* [1], *Fathia Petot-Bottin* [1], *Luca Freddi* [1], *Vitomir Djokic* [1], *Claire Ponsart* [1]

Abstract

Canine brucellosis due to *Brucella canis* is a neglected and underdiagnosed disease in many regions of the world. Currently, this disease is a main cause of abortions or infertility in dogs, or others symptoms like discospondilitys or subclinical forms. For the last two years, canine brucellosis reappeared in Europe and could become endemic. At least 8 cases have been confirmed in kennels from France, from dogs imported from Eastern Europe (Russia, Belarus and Romania) and the USA. Other outbreaks have been reported in United Kingdom, Germany, Italy, Portugal and the Netherlands or as sporadic cases in other countries. Serological, bacteriological and molecular biology approaches can be used and combined to identify or follow the infection. However, it is not possible to exclude the infection in case of negative results. The number of pets adopted in UK have largely increased during the COVID19 pandemic, especially new dogs breeds. This can be correlated with the emergence of canine brucellosis in European countries, as infected dogs have been mainly imported from foreign countries. Serological analyses are useful to identify and to follow the infection, due to the rough LPS avoiding the cross reaction with other smooth *Brucella* species. Bacteriological and molecular analyses have been also performed, as the isolation of strains allow genotyping and phylogenetic analyses. These analyses are important to track the source of infection and try to identify specific routes of transmission. This study presents an overview of canine brucellosis in France, with the combination of different approaches, allowing to hypothesize about introduction or emergence of the disease in the country.

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Keywords: Canine brucellosis, *Brucella canis*, France, European Union, Emerging disease

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O1-4 Evolution towards pathogenicity: phylogenomic insights into *Brucellaceae*

Maite Loperena-Barber[1], *Chantal Renau-Minguez*[2], *Carla Perpiñá-Clérigues*[2], *Paula Ruiz-Rodríguez*[2], *Mireia Coscollá*[2], *Ignacio Moriyón*[1], *Raquel Conde-Álvarez*[1]

Abstract

During evolution, some α-Proteobacteria like *Brucella* have become stealthy intracellular pathogens, not easily detected by the innate immune system. To gain knowledge into the emergence of this strategy and the virulence mechanisms of *Brucella*, we sought to compare phylogenetically close but biologically divergent bacteria. Accordingly, the aim of this work was to search for the genomic characteristics that might explain the biological differences between *Brucella*, a pathogen of livestock and humans, and the closely related genera *Ochrobactrum* and *Pseudochrobactrum*, which are mainly free-living bacteria and only occasionally opportunistic human pathogens. For this purpose, we focused the comparison on *P. algeriensis*, a recently described species isolated from lymph nodes of cattle. Genomic analyses suggest that *Pseudochrobactrum*, conserves the lipid A structure of *Brucella* LPS, a trait already observed in *O. intermedium* that could delay immunity activation and hamper a prompt clearance of the bacteria. In addition, *P. algeriensis* contains putative genes for free-lipid modifications, probably explaining its extreme resistance in vitro to cationic peptides that mimic the bactericidal peptides of innate immune system. Finally, *P. algeriensis*, unlike other *Pseudochrobactrum* species, presents the genomic capacity of producing a rhamnose based O-polysaccharide LPS, being in this regard similar to *B. inopinata* BO2, an early diverging Brucella strain. Likewise, *P. algeriensis* carries part of the operon coding for the Type IV secretion system (T4SS) that, while essential for intracellular multiplication of *Brucella*, has been described as involved in plasmid transfer in *Ochrobactrum*. This work supports the notion that lipid A and free-lipid modifications are conserved in *Brucellaceae* as mechanisms of resistance to cationic peptide antibiotics in soil or to their innate immunity counterparts, while the T4SS have diverged to different functions.

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Keywords: Cattle, *Brucellaceae*, *Pseudochrobactrum*, Evolution

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O1-5 Brucellosis on the Guiana Shield: emergence of a new *Brucella* species?

Anne Keriel[1], *Loic Epelboin*[2], *David O'Callaghan*[1]

Abstract

We report two cases of brucellosis in patients hospitalized in distinct towns of French Guiana, a french overseas territory localized on the Northeastern shore of South America. Both patients were clandestine Brazilian goldminers who had recently arrived in Guiana after passing through neighbouring Suriname and living in the deep in the Amazonian Forest. Both were men aged 39 and 45, respectively. The clinical pictures were febrile lumbar pain for the first, and deterioration of general condition and febrile low back pain evolving for 10 months, revealing L4-L5 spondylodiscitis with epiduritis and extension of the abscess to the psoas for the second. The diagnosis of brucellosis was made by blood culture or puncture of the psoas abscess, respectively. Both cases evolved favourably under rifampicin + doxycycline during their follow-up. Extensive phenotypical and genetic characterization of the bacterial isolates revealed that they represent a novel species of *Brucella* for which we propose the name *Brucella amazoniensis* sp. nov. These cases are very probably due to zoonotic transmission from a still unknown animal reservoir of the deep rainforest. Medical practitioners and professionals working in contact with the wildlife should thus be aware of the existence of such pathogens in this region of the world, which is recovered for more than 95% by the Amazonian Forest.
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Keywords: Amazonia, *Brucella* species, French Guiana, Goldmining

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O1-6 Atypical *Brucella* spp. are facultatively anaerobic in the presence of nitrates, correlating with increased expression of denitrification genes under anoxic conditions

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Abstract

Respiration is a fundamental process in living cells, resulting in ATP production following electron transfer from low-redox- potential electron donors to high-redox-potential electron acceptors such as O_2. In prokaryotes, enhanced respiratory flexibility allows the use of alternative electron acceptors, including nitrogen oxides, which contributes to their ability to colonize microaerobic or anaerobic environments. So far, *Brucella* spp. have been classified as strictly aerobic bacteria, defining O_2 as the sole electron acceptor. Use of nitrate as alternative electron acceptor has been suggested, as all genes involved in the denitrification pathway are present in *Brucella*, but data on possible growth under anaerobic conditions in the presence of nitrates have not yet been available. Comparative RNA-Seq analysis of zoonotic *Brucella suis* 1330 and the atypical species *B. microti* grown to mid-log in screw-cap tubes containing Tryptic Soy broth revealed specific upregulation of nor and nos denitrification genes in *B. microti*. Under anoxic conditions, both species grew in broth, but in the presence of $NaNO_3$ only, and in a NO_3-concentration-dependent manner for *B. microti.* Growth of *B. suis* was limited, as compared to classical conditions, whereas *B. microti*, as well as other atypical species such as *B. inopinata* BO1 and strains isolated from Australian rodents and African bullfrogs, were well-adapted to anaerobic conditions in the presence of $NaNO_3$ as electron acceptor. narG mutants of *B. microti* and *B. suis*, lacking nitrate reductase, lost growth capacities under anoxic conditions. Remarkably, denitrification kinetics in the absence of oxygen revealed significantly earlier and faster nitrite production and consumption in *B. microti*, correlating with RT-qPCR-monitored higher expression of nir, nor, nos genes encoding the corresponding reductases of the denitrification pathway. To our knowledge, this is the first experimental evidence of NO_3-dependent anoxic growth of *Brucella* spp., showing that, in contrast to classical *Brucellae*, atypical species and strains grow equally well under oxic and anoxic conditions. At least the novel *Brucella* spp. should therefore definitely be considered as facultative anaerobic bacteria. Presenting author: stephan.kohler@irim.cnrs.fr

Keywords: Atypical *Brucella*, Denitrification, Facultative anaerobic, RNA Seq

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P1-01 Genetic diversity of *Brucella* spp. isolates in Iran: A multilocus sequence typing analysis

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Abstract

Brucellosis is an endemic infection in Iran and causes important economic losses in livestock as well as a serious health problem in humans. The aim of the current study was to evaluate the genetic diversity of *Brucella* spp. isolated from livestock and human in Iran through the multi locus sequence analysis (MLST). A comprehensive phylogenetic analysis has been achieved to understand the relationship of *Brucella* genotypes identified in Iran to those originating from different regions across the globe. A total of 30 *Brucella* isolates were recovered from 8 milk samples collected from cows (n=7) and camels (n=1), human blood samples (n=12), cow lymph nodes (n=4), and 6 aborted fetuses from sheep (n=3), cows (n=2) and goat (n=1). MLST -9 analysis was used to characterize the genetic diversity among *B. abortus* and *B. melitensis* isolates. Classical biotyping and molecular identification through AMOS PCR and Bruce-ladder PCR showed that all *Brucella* isolates were either *B. abortus* or *B. melitensis. B. melitensis* was associated with ovine/caprine, camel, cow samples, and the majority of human blood samples (n=12). In contrast, *B. abortus* was related to bovine samples and a single human sample, indicating that both *B. melitensis* and *B. abortus* may contribute to the human brucellosis burden in Iran. *B. melitensis* isolates comprised 4 MLSA-9 genotypes, the common and globally distributed ST8, ST7, ST71, and ST102. However, four *B. melitensis* isolates represented a novel ST from human and cow sources. *B. abortus* isolates belonged to the common MLSA-9 genotypes ST1 and ST2 with relationships to biotype and other PCR-based typing methods. The current results represent the first molecular characterization of *Brucella* strains circulating in Iran and provide the basis for further studies examining the molecular epidemiology of *Brucella* spp. in Iran and their relationships to those circulating worldwide.

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Keywords: MLST, Brucellosis, Iran, *Brucella melitensis*, *Brucella abortus*

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P1-02 Identification of atypical *Brucella* spp. by MALDI ToF MS

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Abstract

Correct identification of atypical *Brucella* spp. and their discrimination against former *Ochrobactrum* species remains difficult with current routine diagnostic procedures. Matrix-assisted time-of-flight mass spectrometry (MALDI ToF MS) is a fast, reliable, cost-effective and high-throughput technique, increasingly used to identify microorganisms by their proteomic fingerprint. However, successful identification of a specific pathogen is only possible with an accurate reference database that covers the respective genus. Unfortunately, none of the major manufacturers of MALDI ToF MS systems offers databases comprising all currently known *Brucella* species. To overcome this drawback, various internal pipelines and databases have been established over the last years. Yet, the newly emerging species require an extension of these pipelines due to the lack of reference spectra and biomarkers. We describe the identification of several atypical *Brucella* strains, including former *Ochrobactrum anthropi* and *Ochrobactrum intermedium*, using their MALDI ToF MS spectra by taking advantage of unique, species-specific peak pattern profiles. These specific mass spectral peaks have been identified by comparing spectra of 110 different strains and roughly 2,600 spectra belonging to the six classical and two marine *Brucella* species, the novel species *B. microti*, *B. inopinata*, *B. papionis* and *B. vulpis* as well as various atypical *Brucella* isolates from frogs and rodents. The proteins corresponding to the species-specific peaks were identified using our in-house pipeline combined with data from NCBI. The identification of these species-specific spectral peaks and their subsequent assignment to *Brucella* biomarkers finally highlights the reliability of our method. In summary, classical and novel *Brucella* spp. can be quickly and easily identified using the here presented MALDI ToF MS pipeline, avoiding the commonly described misidentifications with former *O. anthropi*, *O. intermedium* and atypical *Brucella* strains. The ability of MALDI ToF MS to discriminate novel atypical *Brucella* and former *Ochrobactrum* species from classical *Brucella* and *B. inopinata*(-like) is especially important in the context of biological safety regulations.
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Keywords: Atypical *Brucella*, Diagnostics, Identification, MALDI ToF MS

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P1-03 A case report on a novel, non-core *Brucella* strain isolated from a White´s Tree frog in Germany

Christoph-Martin Ufermann[1], *Rainer Oehme*[2], *Dirk Hofreuter*[1], *Sascha Al Dahouk*[1]

Abstract

In the past 15 years many divers strains have been assigned to the genus *Brucella* that differ from the homogenous classical core *Brucella* species. These novel, atypical *Brucella* spp. are gaining increasing interest in the scientific community while major information about their epidemiology, hosts, reservoirs, pathogenicity, and zoonotic potential are still missing, and more and more isolates are being reported. In our case report, we describe brucellosis in a pair of White's Tree frogs (*Litoria caerulea*) kept in a German zoo. Differential phenotyping of bacterial cultures was done with classical microbiological methods. Illumina sequencing reads were used for genomic characterization and phylogenetic positioning of the *Brucella* strain within the genus. The seriously ill White'sTree frogs were submitted for pathologic examination to the responsible chemical and veterinary investigation office. Both frogs were in a poor nutritional status and were presented with additional abnormalities, *e.g.* skin ulcerations and edema. Bacterial cultures from several organs of both frogs were positive for *Brucella* and were confirmed by polymerase chain reaction. A *Brucella* sp. isolate was forwarded to the German Federal Institute for Risk Assessment for in-depth analysis. This isolate displayed non-fastidious and rapid growth and predominantly showed microbiological characteristics similar to other novel, atypical *Brucella* strains, such as *Brucella inopinata*-like BO2 or *Brucella* sp. UK8/14, the latter also isolated from a White'sTree frog. Genomic and phylogenetic characterization placed the isolate apart from the core clade within the novel, non-core clade together with various amphibian and human isolates (inopinata- clade). In summary, we here report another case of amphibian brucellosis caused by a novel, atypical and non-core *Brucella* strain that clusters together with and is alike to previously reported isolates from amphibian hosts and human brucellosis patients underlining that exotic frogs are potential reservoirs for *Brucella* spp., posing an underestimated zoonotic hazard for exposed individuals (*e.g.* exotic animal keepers, veterinarians).

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Keywords: Atypical *Brucella*, Brucellosis, Case Report, Frog, non-core *Brucella*

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P1-04 The isolation of atypical *Brucella* species from captive Amazon milk frogs (*Trachycephalus resinifictrix*)

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Abstract

Brucellosis is a zoonosis found globally and is the result of infection by members of the genus *Brucella*. Twelve species of *Brucella* have been formally described, with the more recently described species significantly expanding the range of mammalian hosts. Furthermore, a growing number of phenotypically and genetically atypical strains have been reported from non-mammalian hosts, most notably amphibians. Reports of human infection with atypical *Brucella* sp. strains raise questions regarding the incidence and impact of emerging species. Initial bacterial identifications from the *post mortem* of three captive Amazon milk frogs (*Trachycephalus resinifictrix*) indicated *Ochrobactrum anthropi* or unspecified intralesional Gram negative bacteria. The tissues were sent to the UK Reference Laboratory for Brucellosis. The excised tissues were macerated prior to the inoculation of solid and liquid media. Supportive and selective (Farrell's) solid media and Brodie and Sinton broths were used for the isolation and propagation of potential colonies. The growth characteristics and morphology of target isolates from all three amphibians resembled members of the atypical *Brucella* group (*e.g. B. inopinata*). The isolates grew readily within 24 hours of inoculation at 37°C with 5-10% CO_2 on all media types. However, traditional methods of classical biotyping to characterise members of the *Brucella* genus did not identify a specific known species. Positive results of PCR targeting *Brucella* specific genes indicated that these isolates represent members of the emerging atypical *Brucella* group. Further analysis of the isolates was performed using whole genome sequencing and multi-locus sequence typing approaches, to characterise the isolates relative to other members of the atypical group. The primary isolation and characterisation of these isolates provides the opportunity to evaluate the potential clinical and diagnostic significance of emerging *Brucella* species. Accelerated growth characteristics coupled with negative monospecific sera reactions, used to identify the A and M epitopes of the O-polysaccharide, suggests that infection with these isolates may go undetected through the use of current serological and bacterial identification methods. These isolations emphasise the need for innovation in routine approaches to aid in the detection and identification of *Brucella*.

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Keywords: Brucellosis, Biotyping, Amphibians, Atypical

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K2 Next-generation proteomics in the fight against pathogens: a guide tour

Jean Armengaud [1]

Abstract

Since proteins are the workhorses of biological systems, their global study by proteomics provides a wealth of information. Next-generation proteomics is based on high-resolution tandem mass spectrometry which can record spectra on hundreds of thousands of different molecules, allowing the identification and quantitation of thousands of proteins. Highly complementary to genomics and transcriptomics, proteomics better explain the resulting phenotype and can be performed in a timely manner. Interestingly, it can be applied to any pathogen, delivering a better understanding of its specific characteristics as well as new insights into the host response to infection. Remarkably, the identification of virulence and antibiotic-resistance factors, as well as other key molecular players, became ultimately straightforward thanks to comparative proteomics. Pathogen detection by proteotyping is also a promising methodology that takes into account taxon-specific information that can be rapidly recorded by mass spectrometry and interpreted for rapid diagnosis. Indeed, tandem mass spectrometry is able to quickly distinguish closely related strains and helps in their taxonomical study. Finally, pathogens are often part of more complex microbial communities. Recent advances in metaproteomics make it possible to address pathogens in such a complex environment. Challenging questions in metaproteomics benefit from recent advances in bioinformatics and this methodology has gained maturity for envisioning now its large scale application in clinical settings. The different facets of proteomics and metaproteomics, including sample preparation, data acquisition, database construction, search strategy and dat interpretation, will be presented and discussed in light of several studies carried out on a variety of pathogens and biological questions.
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Keywords: Pathogens, Proteomics, Metaproteomics, Proteotyping, Virulence, Biomarkers

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O2-1 *Brucella* Databases at BioCyc.org: Pathways, Omics Tools, and Enhanced Genome Annotation

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Abstract

BioCyc.org is a web portal for >20,000 sequenced microbes, including 54 *Brucella* genomes. BioCyc couples high-quality curated data with a wide range of easy-to-use bioinformatics tools. Each of the *Brucella* databases in BioCyc was constructed using a similar methodology. A series of computational inferences were applied to the annotated genome from RefSeq, including prediction of metabolic reactions and metabolic pathways, transport reactions, operons, Pfam domains, and orthologs with other genomes in BioCyc. When available, additional data were imported from related databases, including protein features and Gene Ontology terms from UniProt, protein localization data from PSORTDB, and gene essentiality data from OGEE. Manual curation was performed on the databases for *B. abortus* 2308 and *B. ovis* ATCC 25840 to integrate information from the experimental literature. Mini-review summaries, literature references, and evidence codes were authored for selected proteins and pathways. The resulting *B. abortus* database contains 224 metabolic pathways and 3,874 protein features and the *B. ovis* database contains 236 pathways and 3,745 protein features. A particular focus was curation of virulence factors, such as the lipopolysaccharide and type IV secretion system. The BioCyc website provides extensive bioinformatics tools for searching and analyzing these databases and leveraging them for analysis of omics datasets. Genome-related tools include a genome browser, sequence search and alignment tools, and extraction of sequence regions. Pathway-related tools include pathway diagrams and navigation of zoomable organism-specific metabolic map diagrams. Operons, regulatory sites, and the full regulatory network can be displayed when such data are present. Comparative analysis tools enable comparisons of genome organization, of orthologs, and of pathway complements. Omics data analysis tools support enrichment analysis and painting of transcriptomics and metabolomics data onto individual pathways and the full metabolic map diagrams. The Omics Dashboard tool enables hierarchical exploration of omics datasets. A unique feature called SmartTables enables users to construct and store tables of genes, metabolites, or pathways, and to perform multiple analyses, such as converting a list of genes or metabolites into a list of all pathways in which those genes/metabolites participate.

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Keywords: Bioinformatics, Databases, Genomics, Metabolic pathways, Omics

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O2-2 Histidine metabolism and metal homeostasis in *Brucella abortus*

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Abstract

Copper is a heavy metal that plays an essential role as a co-factor for several proteins involved in biochemical processes. However, it can become toxic to bacteria when its concentration increases. To avoid damage, the bacteria have to put in place mechanisms to control the concentration of copper and to allow the homeostasis of the metal. Currently, the mechanisms used by *Brucella* abortus to maintain copper homeostasis are unknown. In our laboratory, different mutants deleted for several genes of the histidine biosynthesis pathway showed lower replication in two infection models: HeLa cells and RAW 264.7 macrophages. Histidine is an amino acid that has the ability to coordinate different metal ions. In the present work, we investigated the impact of the deletion of histidine synthesis genes on copper homeostasis in *B. abortus*. First of all, a bioinformatics analysis was carried out to identify histidine-enriched proteins in the bacterium, and confirmed the link between this amino acid and metals. Secondly, we demonstrated that his mutants showed lower growth when copper was present in the medium, illustrating a sensitivity to copper. In addition, we were able to isolate suppressors with various mutations in the opp operon, suggesting that this could be a way for the bacteria to escape the toxicity of copper. It was also found that *B. abortus* does not show this sensitivity when actors classically described as involved in copper resistance are deleted. In conclusion, our results suggest that *B. abortus* would use histidine as the first line of defense against copper toxicity rather than the systems usually described.
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Keywords: *Brucella*, Histidine, Homeostasis, Metals, Transporter

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O2-3 Metabolic Glycoengineering: A strategy to label *Brucella abortus* cell envelope with synthetic analogues of D-mannose

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Abstract

Metabolic Glycoengineering (MGE) is a powerful tool for the study of biomolecules in living systems. This technique consists in manipulating the metabolism of living cells, prokaryotic or eukaryotic, in order to modulate their glycosylation. MGE can be used to modify natural glycans, disrupt glycan biosynthesis and probe metabolic flux inside cells. An interesting approach is to install unnatural monosaccharides, with subtle modifications, into cellular glycans. We aim to chemically modify the bacterial cell surfaces by targeting the cell wall with unnatural clickable sugars that are metabolically transformed and incorporated. The metabolic incorporation into glycans pathway can be visualized by chemical probes, for instance fluorescent reporters, that could react with the unnatural carbohydrate via biorthogonal click reactions. In this project, the strategy is to explore the metabolic route of D-mannose because, to the best of our knowledge, clickable D-mannose derivatives have never been described for such an approach in the literature. Moreover, D-mannose is present only in some bacterial cell surface, which could lead to a method with a high specificity. We performed a multi-step stereoselective synthesis of clickable D-mannose derivative. In this study, we showed that D-mannose derivatives can be used to fluorescently label *Brucella abortus* cell envelope in a specific manner. Further investigations showed that the unnatural monosaccharide is incorporated in the lateral branch of the core of the lipopolysaccharide, since a wadC mutant is unable to incorporate the modified mannose, while a gmd mutant is still incorporating it. In contrast to what has been previously described, we here labelled the entire cell envelope and not only the growing pole of the bacterium, which suggests a putative remodeling of the bacterial surface of *Brucella abortus*.

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Keywords: Cell envelope, Click-chemistry, Lipopolysaccharide, Glycoengineering, Metabolism

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O2-4 Targeted methodology for the identification of protein- peptide vaccine candidates

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Abstract

Effective vaccines for infectious animal diseases, including zoonotic animal diseases of livestock and poultry, including brucellosis of ruminants, are often not available or not efficacious. This work presents a targeted approach and methodology to identify potential protein and peptide vaccine candidates. This methodology applies to all microbial organisms for which procedures are developed to study membrane proteins in particular. Certain microbial membrane proteins are important receptors of signal proteins of the host's immune response system for the initiation of a protective immune response or alternatively, some membrane proteins are activated to allow the organism to escape the host's immune surveillance system. Here we present an approach to directly target the membrane proteins which might afford effective protection. A membrane protein extraction procedure based on previously published procedures by investigators separates three fraction of membrane proteins using TX-100 differential protein extraction procedures. This method results in three types of protein fractions, detergent-soluble proteins, aqueous soluble proteins (including periplasmic proteins), and detergent-insoluble proteins. One and two-dimensional electrophoresis separates (and purifies) these proteins. Western Blots with antibodies from infected and naïve animals as controls are compared and appropriate protein bands or spots are excised, digested with a variety of proteolytic enzymes, and submitted for MALDI-TOF spectrometry. Analysis of the data with SwissProt database programs provides the protein ID and sequence. The identified sequences are then further analyzed by three-dimensional modeling using MolBio and protein external protein loops are identified. Sequences are further studied for loop/sequence homologies and function by the publicly available Resource Center of the Zhang Laboratory which identifies additional potential functionalities of the identified protein(s), providing a better understanding of the immune response regulation. Examples of proteins and peptides identified by this approach are presented.
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Keywords: Vaccine, Proteomics, Outer membrane vesicles, Brucellosis

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O2-5 Whole genome based tools in epidemiological surveillance of *Brucella abortus*, *Brucella melitensis* and *Brucella suis* bv2 in Italy in the last decade

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Abstract

Brucellosis is one of the most important zoonotic diseases in the Mediterranean basin. In Italy, *Brucella abortus* and *B. melitensis*, prevalent in the southern regions despite the ongoing eradication campaign, continue to pose public health risk and cause significant economic losses. In this work we use genomic tools to study the epidemiology of *B. abortus*, *B. melitensis* and *B. suis* bv2 in Italy over the last decade. We sequenced over 1,300 *Brucella* strains collected between 2011 and 2021. The sequences were typed using whole genome sequencing (WGS)-based methods, including single nucleotide polymorphism (SNP) and core genome multilocus sequence typing (cgMLST) and clusters of genetically related strains were identified. WGS typing results demonstrated the presence of three large clusters of *B. abortus*, corresponding to diverse sequence types. ST-30 was dominant in Sicily, while ST-1 and ST-2 were found mostly in mainland Italy. ST-1 strains, all assigned to biovar 1 were collected primarily from buffalo and largely restricted to Campania region. These strains formed a tight genomic cluster that has persisted in the region for the entire decade. *B. melitensis* was predominantly found in the south, with the highest prevalence in Sicily and Calabria. While in these regions we observed weak geographical segregation of the genotypes, the incursions of *B. melitensis* in the northern regions were generally caused by single clone introductions. Half of the cases of human brucellosis could be attributed to the locally circulating *B. melitensis* strains, while the others were mainly imported from North Africa. The population of *B. suis* bv2 from the wild boars and pigs in Italy was grouped in several lineages. Central European lineage, confined to the northwestern regions, and Eastern European lineage, found along the Apennine mountain ridge, both contained highly clonal strains suggesting that they arose from single introduction events, likely linked to the wild boar repopulation campaigns. Our study demonstrates that a large number of *Brucella* clones persisted over many years in Italy, escaping the control measures imposed in the eradication programmes. Integrating WGS analysis of *Brucella* strains with the current control strategies would be vital for more effective eradication campaign in Italy.
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Keywords: *Brucella*, Molecular epidemiology, Genomics, Whole genome sequencing - WGS

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O2-6 In silico pipeline for protein comparisons in *Brucella* genus

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Abstract

Although species of *Brucella genus* share more than 90% of their genomic content, their phenotypic characteristics may vary greatly among species and even strains. To explain some of those differences, *e.g.* zoonotic potential, pathogenicity or immune response specificity, we devised the supervised machine-learning algorithm that compares the 3D protein structure, molecular weight, hydrophobicity, iso-electric point and structure stability. Based on the available whole chromosome sequences of *B. abortus, B. melitensis, B. suis, B. ovis, B.canis* and *B. microti,* the database was created, and for the efficacy of the model, only annotated proteins were used to predict the 3D structure based on the Swiss model system. Further, using the Bio.SeqUtils the protein structure characteristics were calculated and used for the comparisons. Our in silico results showed two membrane (cyst, cysW), urease and one heat-chock chaperon proteins characteristic for *B. melitensis* strains, while in other species, they have 100% homology. In *B. ovis* strains two cytochrome oxidase subunits II and two sugar metabolic proteins were constantly truncated and their structure stability, iso-electric point and hydrophobicity varied greatly compared to other *Brucella* species. Also, *B. ovis* strains in 95% of the cases were missing and the remaining 5% had very shortened transcriptional regulator; iron-sulfur, NAD binding and ATP binding enzymes; dsbA family protein. Carbohydrate hydrolase is characteristically truncated in *B. microti*, while imidazolonepropionase has *B.canis* specific properties. All strains of *B. ovis* and *B. microti* have similar ATP-dependent helicase HrpB that differs in its' hydrophilic characteristics and iso-electric point compared to others, while these species completely miss lipids catabolismand cardiolipin sintase enzymes. Two proteins alcohol dehydrogenase AdhP and ornithine/lysine decarboxylase, were found to be present only in *B. abortus, B. suis* and *B.canis,* while only Cu(I)-responsive transcriptional regulator is 100% similar between three species. Differences in OMP 2a and OMP 31 among species were observed regarding molecular weights and protein stability. These in silico analyses are in concordance with published results, but further laboratory confirmations are necessary. However, our analysis used intra species differences and, for now, we have not included differences among biovars, nor comparisons of active sites.
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Keywords: Species protein comparison machine-learning, Protein structure, Protein functionality

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P2-01 Envelope stress response in *Brucella abortus*

Adélie Lannoy[1], *Xavier De Bolle*[1]

Abstract

Studies performed with *Escherichia coli* indicated that following an envelope stress, a signalling sensor is activated and induces a series of regulatory cascades which modify the expression of target genes allowing an adaptative response. While envelope integrity is obviously crucial for survival and growth, the envelope stress response in *B. abortus* is under-investigated. We therefore undertook a Tn-seq analysis in the presence of deoxycholate (DOC) in rich medium, compared to a control without DOC. DOC is a soft anionic detergent, used here to stress the envelope. We found that a two-component system (TCS) CenK-CenR and a homologous system to Mla pathway are required to grow in the presence of DOC. According to a Tn-seq performed with *B. abortus* in RAW 264.7 macrophages, CEnR, CEnK and MLA Mutants are attenuated, suggesting that mutants failing to adapt to envelope stress are indeed impaired for their ability to survive and/or grow inside these cells. A homologous system of CenK-CenR was first identified in Caulobacter crescentus, the model for alpha-proteobacteria, where it plays an essential role in maintaining cell envelope integrity. In *Brucella* this TCS is poorly characterized and previous studies focus only on the response regulator, that seems to play a role in responses to osmotic stress and acidic pH. We generated markerless ΔcenR and ΔcenRΔcenK mutants, and we show that they have a growth defect on DOC, confirming the phenotype suggested by the Tn-seq analysis on DOC. These results indicate that cenK and cenR genes are indeed involved in envelope stress sensing. In *E. coli* the Mla pathway maintains the lipid asymmetry and membrane integrity. This pathway allows a mainly retrograde transport of phospholipids between inner and outer membranes. The mla mutants in *E. coli* have a disruption of outer membrane permeability and integrity. We identified homologues of the Mla proteins in *B. abortus*. We generated markerless deletions strains for mlaE, mlaF, mlaD, mlaA and mlaEFDA and we found that each deletion strain has a growth defect on DOC, confirming the phenotype suggested by Tn-seq indicating that this system is indeed involved in envelope integrity.
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Keywords: *Brucella abortus*, Envelope, Stress Response, Two Component System

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P2-02 Comparison of the *Brucella abortus* population structure based in genotyping methods of distinct levels of resolution

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Abstract

Numerous techniques are currently available with different principles, costs, and levels of resolution to understand the transmission dynamics of brucellosis worldwide. We aimed to compare the *B. abortus* population structure of 53 Brazilian genomes using eight different genotyping methods [multiple-locus variable-number tandem-repeat analysis (MLVA8, MLVA11, MLVA16), multilocus sequence typing (MLST9, MLST21), core genome MLST (cgMLST) and two techniques based on single nucleotide polymorphisms (SNP) detection (parSNP and NASP)]. The strains were isolated from six different states between 1977 and 2008 and were previously analysed using MLVA8, MLVA11, and MLVA16. Then, their whole genomes were sequenced, assembled, and submitted to MSLT9 MLST21, cgMLST, and SNP analyses, using the mlst, chewbbaca, parSNP, and NASP programs, respectively. All the genotypes were compared in the R program (dendextend package). MLST9 and MLST21 were the two techniques with the lowest level of resolution, both presenting 4 genotypes. MLVA8, MLVA11, and MLVA16 showed a progressive level of resolution as more loci were analysed, with 6, 16, and 44 genotypes, respectively. The cgMLST showed the highest level of resolution, depicting 45 genotypes, followed by the SNPs methods, both with 44 genotypes. Hierarchical grouping by the mean distance among the correlation of the techniques showed clustering of MLST9 and MLST21, the second clustering of MLVA8, MLVA11, and MLVA 16 and a third clustering grouping parSNP, NASP, and cgMLST. In the assessed population, MLVA was more discriminatory than MLST and was more practical and cheaper to perform. Furthermore, SNP techniques and cgMLST provided the highest levels of resolution, showing high agreement with each other, which could be explained by the high clonality of *B. abortus* genomes (90% of its gene repertoire belongs to the core genome). In conclusion, it was observed that different techniques might be more indicated depending on available resources. It investigated epidemiological scenarios, with MLVA being a simpler and ideal technique for countries that are still in the disease control phase, and SNP and cgMLST techniques would be better choices for outbreak investigations and for surveillance in countries in the eradication or where brucellosis is already eradicated.

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Keywords: cgMLST, Epidemiology, MLST, MLVA, SNP

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P2-03 Study of outer membrane vesicles from *Brucella* species

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Abstract

Gram-negative bacteria release nanovesicles from their outer membrane, named outer membrane vesicles. The purpose of these vesicles in bacteria physiology is still unknow, and some models have been proposed to explain their production. In last decades, proteomics becomes an important tool to determine the proteins carried in the vesicles. Vesicles contain proteins able to elicit an immune response, so they have been proposed as a model to develop acellular vaccines. Previously, vesicles from *Brucella abortus* and *B. melitensis* have been studied and demonstrating to be protective in mice after the challenge with a virulent *Brucella* strain. In this study, OMVs of *Brucella suis, B. ovis, B.canis*, and *B. neotomae* were purified and analyzed by SDS-PAGE, transmission electron microscopy and liquid chromatography coupled to mass spectrometry to determine the pan-proteome. Additionally, antigenic proteins were detected by western blot using rabbit anti-*Brucella* specific sera. *Brucella suis* ATCC 23444 (1330), *B. ovis* ATCC 25840 (63/290), *B.canis* ATCC 23365 (RM6/66) and *B. neotomae* ATCC 23459 (5K33) were used in this study. OMVs of *B. melitensis*, *B. ovis, B.canis* and *B. neotomae* showed a spherical shape and bilayer lipid membrane, and sizes between 30-80 nm. The pan-proteome showed many homologous proteins, such as Omp16, Omp25, Omp31, SodC, Omp2a, and BhuA. Proteins contained in the vesicles from different *Brucella* species were detected by anti-*Brucella* sera. Also, the proteins found in the *Brucella* species tested here were also identified within the OMVs of *B. suis* and *B. abortus*. The presence of these proteins shared in vesicles of different *Brucella* species made them candidates to be evaluated as an acellular brucellosis vaccine or in diagnostic tests.

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Keywords: *Brucella*, Outer membrane vesicles, Proteomics

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P2-04 Whole Genome sequencing of *Brucella* strains isolated from water buffalo in southern Italy during 2019-2021

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Abstract

Brucellosis is the most widespread zoonosis in the world and is responsible for significant economic losses, particularly in bovine and water buffalo herds. The causative agent of the disease is *Brucella* spp. which can infect humans and numerous animal species, including cattle, water buffalo, sheep and pigs. In animals the main clinical manifestation of infection is abortion. The pathogen can be transmitted through direct contact with infected animals and parturition products or through consumption of contaminated raw milk and/or dairy products. The genus *Brucella* is characterized by a high level of homology of DNA. For this reason, advanced molecular methods are very useful tools for the characterization of field isolates and the identification of sources of infection. The aim of this study was to characterize by whole genome sequencing (WGS) *Brucella* strains isolated from water buffalo farms in the Campania Region in order to perform epidemiological trace-back studies. This study was carried out on 41 strains of *Brucella abortus* bv.1 isolated from lymph nodes of as many water buffaloes from 40 different farms in the Campania Region. All strains were sequenced for whole genome characterization. The obtained results showed very high homogeneity of the strains under study. Indeed they exhibited a unique MLST (ST1) genetic profile and 4 distinct MLVA genetic profiles. These data indicate a very low level of genetic variability of the analyzed *Brucella* strains and suggest an epidemiological situation characterized by the persistence of a few bacterial strains in close geographical areas, characterized by intense inter-human and commercial exchanges. This evidence suggests an inter-herd diffusion probably due to environmental factors associated with few sources of infection, likely common to most of the farms included in this study.

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Keywords: *Brucella*, Water buffalo, WGS, MLST, MLVA

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P2-05 Evaluation of DNA extraction methods for long-read whole genome sequencing of atypical *Brucella* spp. isolates

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Abstract

In recent decades the description of several new species of *Brucella* has expanded the known mammalian host diversity of the genus considerably. Of these more recently described species, *B. microti*, *B. inopinata* and *B. vulpis* have been described as 'atypical', exhibiting either atypical phenotypic traits (*B. microti*), or greater genetic diversity (*B. inopinata* and *B. vulpis*) than classically described *Brucella* species. There are also an increasing number of reports of the isolation of atypical *Brucella* sp. organisms from other vertebrate hosts, including fish, reptiles and particularly amphibians. Analyses based on whole genome sequencing (WGS) have indicated that, whilst such atypical isolates maintain a high degree of genetic homology with core *Brucella* species, they exhibit a degree of horizontal gene transfer, incorporating genomic regions with sequence identity to soil associated Alphaproteobacteria. The inclusion of genetic elements from outside the genus *Brucella* emphasises the need for robust and reliable WGS tools to accurately characterise the genomes of novel atypical strains. Long-read sequencing methods are particularly valuable for this, as they can be used to construct complete bacterial genomes more reliably than approaches based on the de novo assembly of short sequencing reads alone. However, the selection of suitable DNA extraction methods has been shown to be integral to the success of long-read sequencing approaches. Furthermore, as the pathogenic potential of atypical *Brucella* strains remains unclear, such DNA extraction methods should ideally be compatible with methods commonly used for release of inactivated bacteria from containment laboratories. Using inactivated cultures of three atypical *Brucella* species (*B. microti, B. inopinata* and *B. vulpis*) we evaluated DNA extraction methods for producing long-read sequencing data using Oxford Nanopore Technologies Minlon sequencing platform. We evaluated metrics of sequencing (*e.g.* read length N50 and read number) and compared de novo genome assemblies, in order to identify the most suitable method. The results of these analyses are presented here.

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Keywords: Amphibians, Atypical, *Brucella*, Whole genome sequencing

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P2-06 PORIFERA - Core-genome-based analytical pipeline for prediction of new markers to rapid identification of emerging *Brucella* species

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Abstract

Six new *Brucella* species isolated from humans, wild animals and environmental sources, were added to the six classical *Brucella* species potentiating an additional threat for humans. Other atypical *Brucella* strains isolated from wild rodents, frogs and fishes, will likely be proposed as new species in the future. This work aims to implement a core-genome-based analytical pipeline, PORIFERA, for prediction of new markers to rapid identification of emerging *Brucella* species. The comparison of core-genome at greater resolution of closely related *Brucella* spp. may provide information on specific genetic markers, such as single-nucleotide polymorphism (SNP). Twenty-three *Brucella* genomes (10 *B. melitensis*, seven *B. suis* and six *B. abortus*) were sequenced using Illumina technology. Genome assemblies were performed using de novo assembler SPAdes. The analysis involved the 23 newly sequenced genomes and 25 *Brucella* spp. complete genomes publicly available from eight of the 12 recognized species *B. suis, B. abortus, B.canis, B. ovis, B. microti, B. pinnipedialis* and *B. ceti*. Two *Brucella* sp. strains isolated from amphibians (09RB8910; 09RB8471) were used as outgroup. The alignment of core-genome was performed using Parsnp and the evolutionary history was inferred using Neighbor-Joining and Maximum Likelihood methods available in the Harvest suite (vs 1.1.2). Functional annotation had been accomplished to each SNP, as well as exclusion of intragenic SNPs in order to identify novel discriminatory and informative biomarkers. The evolutionary history was inferred from a total of 256 667 putative SNPs shared among the 46 more closely related genomes and the genomes of the amphibians isolates 09RB8910 and 09RB8471. From these, a total of 31034 SNPs were significantly associated with *B.canis* (n=2127), *B. ovis* (n=2018), *B. suis* (n=1782), *B. abortus* (n=1466), B. suis (n=1365), *B. ceti* (n=1463), *B. pinnipedialis* (n=891) and *B. microti* (n=786). These data will be further used for development of novel molecular methods to identify, genotype or direct assignment of *Brucella* species.
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Keywords: *Brucella* spp., Core-genome analysis, Genotyping, Molecular markers, Single nucleotide polymorphisms

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P2-07 Study of the genetic variability of *Brucella melitensis* isolated from goats and sheep of San Luis province in Argentina

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Abstract

The objective of this work was the analysis of the genetic variability of *Brucella melitensis* in the departments of Libertador General San Martín, Ayacucho and Belgrano that belong to the province of San Luis, Argentina. For this purpose, flocks with different prevalence values of brucellosis were identified by conventional serological techniques as Buffered Plate Antigen Test (BPAT), Fluorescence Polarization Assay (FPA) and Complement Fixation Test (CFT). Samples of spleen, lymph nodes, liver, testis, epididymis, semen, milk, and synovial fluid and abomasal contents of abortions from a total of 104 animals were processed, of which 98 were goats and 6 were sheep. 32 isolates of *B. melitensis* biovar 1 were obtained and identified by classical bacteriological methods. Also, the presence of the pathogen in tissues samples was evidenced by PCR. An animal was considered positive for PCR for *B. melitensis* when a specific band was obtained in at least one of the tissues from the same animal. After the molecular detection of *B. melitensis* in tissues with isolation, DNA samples from tissues from which it was not possible to isolate were analyzed and it was possible to demonstrate the presence of the agent in 62 animals (including 4 sheep). As genotyping method, the MLVA 16 scheme was used, which uses the variability of 16 molecular markers to generate an unequivocal individualization of the genomes of *B. melitensis* from different animals. The genotypes were analyzed to determine the phylogenetic relationship between them and their georeferenced data were added to have complete information on the presence and circulation of different genotypes in each department. This study allowed identifying 21 circulating genotypes in the goats from the different departments. The phylogenetic relationship model found some of the genotypes described as potential candidate founders of the lineage that is distributed in different areas of the province. The discriminatory ability of the technique in a real epidemiological situation was confirmed, including discriminating genotypes within the same herd. The genotypes from this work were no previously described in any international data base.
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Keywords: *Brucella melitensis*, Goats, Sheep

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P2-08 *Brucella melitensis* immunoproteomics reveal strain- specific antigens suitable for the unbiased diagnosis in a DIVA strategy

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Abstract

In the Mediterranean area, *Brucella melitensis* is among the most common species with sheeps and goats being the major reservoir of the pathogen. Diagnostic and prophylactic measures adopted for the control of brucellosis suffer from a lack of specificity and cross-reactivity, respectively. Also, live-attenuated *B. melitensis* Rev.1 administration in goat and sheep showed residual virulence and cross-reactivity with the current diagnostics, raising the need for studies to amend both the vaccinal and diagnostic strategies. Here, we employed an immunoproteomic approach to underline the specific immunogenic proteins of both *B. melitensis* Rev.1 and *B. melitensis* 16M strains, meant respectively as the reference of the vaccinal and field strains, and discriminate their antigens from each other and from those of the most cross-reactive specimens (*i.e. Escherichia coli* O157:H7 and *Yersinia enterocolitica* O:9). Following the optimized resolution of the *Brucellae proteomes*, independent incubation of the bacterial protein profiles with properly depleted sera from either Rev.1 or 16M infected animals revealed a distinct map of immunogenic proteins for the assayed bacteria. Differential immunogenic proteins have been identified both computationally and analytically via MS/MS, yielding a list of suitable candidates for the design of novel diagnostics and/or prophylactic strategies. Universal stress protein and related nucleotide-binding proteins (Uspa), nucleoside diphosphate kinase and putrescine utilization regulator were uniquely identified as predictive for the presence of the vaccinal strain. On the other hand, ABC transporter-substrate-binding protein (cluster4_ leucine/isoleucine/valine/benzoate, broad-specificity amino acid ABC transporter-substrate-binding protein) and ABC transporter-substrate-binding pro (cluster1maltose/g3p/polyamine/iron) were identified as putative biomarkers of *B. melitensis* 16M. The independent identification of the immunogenic proteins confirms previous in-silico bioinformatic prediction performed on the same bacterial strains. Sorting the major immunogenic proteins of both the vaccinal and field strains is pivotal for the design of unbiased serodiagnostic strategies crowning the conventional diagnostics for *Brucella*. Also, these may serve as the starting point for drawing optimized prophylactic strategies targeting other molecules than those addressed by the diagnostic tests; thus, differentiating infected from vaccinated animals. This improves monitoring campaigns which represents the keystone for the fair and efficient control and eradication of brucellosis in animals and humans.

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Keywords: One Health, Brucellosis, DIVA strategy, Diagnosis, Biomarkers

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K3 Metabolic interaction between *Brucella abortus* and its host

Renee Tsolis [1]

Abstract

Brucella abortus has been shown to colonize many different cell types and tissues in the host. At sites such as spleen and lymph nodes, the bacterium persists intracellularly, eliciting only a mild granulomatous inflammatory response. In contrast, in the placenta *B. abortus* elicits a massive inflammatory response, and many of the bacteria are found to be growing in the extracellular space. This presentation will discuss our lab's modeling of *B. abortus* infection in mice and our approaches to understand the metabolic interactions between *B. abortus* and its replication niches within different types of macrophages and in the placenta. Ultimately, we aim to better understand how the pathogen exploits each environment to replicate and spread to the next host.
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Keywords: Metabolism, Innate immunity, Placenta, Virulence factors, Transmission

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O3-1 BNIP3L-dependent mitophagy induced by *Brucella abortus* in host cells is required for bacterial egress

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Abstract

Brucella species are Gram-negative facultative intracellular bacteria responsible for brucellosis, a world-wide zoonosis affecting various hosts including humans. Along the infection in most host cells, *Brucella* first interacts with the endosomal pathway and eventually reaches its replicative niche inside the endoplasmic reticulum (ER), in which bacteria multiply massively. Regarding the many physical and functional interactions between the ER and mitochondria, as well as the major role of mitochondria in inflammation and host-pathogen interactions, we aim to study the effect of *Brucella* on the mitochondrial population of infected cells and the crosstalk existing between them which is still poorly understood. In this work, we showed that *Brucella abortus* induces mitophagy through an increase in LC3/mitochondria colocalizing events, as well as an increase in the number of acidified FIS1 mitochondrial fragments at 48h post-infection in host cells. *Brucella abortus* -induced mitophagy is accompanied by a strong mitochondrial network fragmentation which is dependent on the mitophagy receptor BNIP3L as we demonstrated that a siRNA-mediated silencing of BNIP3L prevents the mitochondrial fragmentation and mitophagy in *Brucella abortus* infected cells. Our results also show that the expression of BNIP3L induced by *Brucella abortus* relies on the activation of the hypoxia-inducible factor HIF-1α in an oxygen-independent way. Instead, HIF-1α activation appears to be iron-dependent since FeCl2 supplementation prevents HIF-1α nuclear translocation and BNIP3L expression in *Brucella abortus* infected cells even if the origin of a putative iron starvation response in the host cells remains to be elucidated. In an attempt to better understand the functional role of the *Brucella abortus*-induced BNIP3L- mediated mitophagy, and what would be the resulting advantage for the host cell and/or the bacteria, our results show that BNIP3L silencing drastically reduced the number of reinfection events at 72 h post-infection, suggesting that bacterial egress from the host cell could be impaired. Future research will be needed to decipher the dynamics and the role of mitophagy and mitochondrial membranes in the last steps of *Brucella abortus* intracellular cycle. Altogether, those results should highlight new molecular mechanisms and critical steps involved in *Brucella* trafficking during infection.
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Keywords: BNIP3L, Egress, HIF-1 alpha, Mitophagy, Trafficking

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O3-2 RNA-immunoprecipitation/miRNA-Seq reveals miRNA- like, small noncoding RNAs of *Brucella suis*, translocated into the cytoplasm of infected murine macrophages

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Abstract

Intravacuolar bacterial pathogens have evolved a variety of strategies to subvert host cell functions during infection. In the past two decades, cellular microbiology has focused on the interplay between bacterial proteins with host cell proteins, lipids and DNA. However, over the same period, small noncoding bacterial RNAs have emerged as central players in gene expression regulation and pathogenesis. Moreover, the translocation of microRNA-like sRNAs (miRNAs) from intracellular bacteria into the host cell cytoplasm to manipulate cellular functions has been recently reported. Using the izMiR miRNA detection algorithm, we bioinformatically identified sequences encoding candidate pre-miRNAs, and which were similar to human pre-miRNAs, in the genomes of several intravacuolar bacterial pathogens. To validate bioinformatics predictions, we immuno-precipitated the RNA-induced silencing complex (RISC) from RAW 264.7 murine macrophages infected or not by *Brucella suis* 1330. Following RNA extractions, samples from infected and non-infected cells were differentially analyzed by small RNA-Seq. This approach led to the identification of six RISC-associated transcripts specifically aligning with the *B. suis* genome, matching the izMiR-based predictions, and showing the characteristics expected for authentic miRNA. Among these, *B. suis* MIR6325 and MIR11484 are of particular interest, since bioinformatics analysis using the MR-microT algorithm led to the identification of highly-ranked candidate eukaryotic targets. Ongoing work is aiming at the experimental validation of host cell target mRNAs for miRNAs MIR6325 and MIR11484, using a RISC-Trap approach. In parallel, we are generating *B. suis* deletion mutants for several validated pre-miRNAs to evaluate their phenotypes and possible impact in the context of host cell infections by using next-generation sequencing, cellular microbiology, high-resolution microscopy and screening approaches. Our study will shed light on an emerging, yet poorly explored domain of host/pathogen interactions, and may help to identify targets for the development of new biomarkers and antimicrobials.

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Keywords: *Brucella suis*, izMiR miRNA prediction, miRNA, RISC, RNA-Seq

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O3-3 Lipopolysaccharide synthesis and traffic in the envelope of the pathogen *Brucella abortus*

Caroline Servais,[1] *Victoria Vassen*,[1] *Audrey Verhaeghe*,[1] *Xavier De Bolle*[1]

Abstract

The lipopolysaccharide (LPS) is essential for most Gram-negative bacteria as it is a main component of the outer membrane (OM). In the pathogen *Brucella abortus*, smooth LPS (S-LPS) is crucial for virulence. Being part of the Rhizobiales, *Brucella* displays unipolar growth and its LPS was shown to be incorporated at the growth sites, *i.e.* the new pole and the constriction site. When synthesized, the rough LPS (R-LPS) is anchored to the cytoplasmic leaflet of the inner membrane (IM) and is then flipped to the periplasmic leaflet by MsbA, an essential ABC transporter. The O-antigen is independently flipped to the periplasmic leaflet of the IM and is then ligated by a periplasmic ligase to a fraction of the R-LPS, giving S-LPS. S-LPS and R- LPS are translocated by the essential LPS translocation machinery (Lpt). LptDE and LptB2CFG form the OM and IM complexes, respectively. Nothing is known about the localization of MsbA or the Lpt complex, and the O-antigen ligase remains unidentified in *Brucella*. Here, we showed that the IM complex (LptC and LptF) is localized at the growth sites, while LptD is dispersed all over the OM. We hypothesize that LPS could be translocated only when the entire complex is formed at the growth sites and that a fraction of LptD would be inactive. Surprisingly, MsbA was mainly localized at the old pole and time-lapse microscopy suggested that MsbA was able to move during growth. Based on this, we proposed that MsbA would be mobile in the IM, actively flipping LPS. Then, LPS would diffuse in the IM until it encounters the Lpt pathway. In addition, we identified WadA as the main O-antigen ligase in *Brucella* spp. WadA presents a N-terminal glycosyltransferase domain, that was shown to add the last glucose onto the R-LPS core, and a C-terminal predicted to be an O-antigen ligase domain. We showed that deleting the biggest periplasmic loop of the O-antigen ligase domain generates a rough phenotype, that could be restored to smooth by adding a copy of the O-antigen ligase domain only. This work highlights a new class of bifunctional O- antigen ligase.
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Keywords: Lipopolysaccharide, Lpt pathway, O-antigen ligase, Unipolar growth

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O3-4 A *Brucella* effector that modulates host retrograde transport to promote intravacuolar replication

Jean Celli,[1] *Elizabeth Borghesan*,[2] *Erin P. Smith*,[2] *Sebenzile Myeni*,[3] *Chloe Dugelay*,[4] *Laurent Terradot*[4]

Abstract

Brucella spp. remodel host cellular membrane transport pathways via delivery of Type IV secretion (T4SS) effector proteins to generate a replication-permissive vacuole (rBCV) that supports their intracellular proliferation. Whether Type IV effectors also mediates intracellular growth within the replication vacuole is mostly unknown. Here we show that the T4SS effector BspF is not required for rBCV biogenesis but specifically promotes *Brucella* replication within rBCVs. Ectopically expressed and bacterially delivered BspF interfered with vesicular transport between the trans-Golgi network (TGN) and recycling endocytic compartment. BspF targeted the recycling endosome, inhibited retrograde traffic to the TGN and interacted with the Arf6 GTPase-activating Protein (GAP) ACAP1 to dysregulate Arf6-/Rab8a-dependent transport within the recycling endosome, which resulted in accretion of TGN-associated vesicles by rBCVs and enhanced bacterial growth. The predicted structure of BspF and interaction with ACAP1 identified residues within its Gcn5-related acetyltransferase (GNAT) domain required for BspF's modulation of Arf6 activity through ACAP1, the resulting interference with retrograde transport and BspF's role in bacterial intracellular growth. Altogether, these findings provide mechanistic insight into *Brucella* modulation of membrane transport that promotes their own proliferation within intracellular vacuoles.

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Keywords: *Brucella*, Type IV secretion, BspF, Pathogenesis, Retrograde membrane transport

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O3-5 Novel H-NS-like Protein MucR Coordinates Virulence Gene Expression During Host-Association in *Brucella* spp. Through Silencer/Counter-Silencer Interactions

Ian Barton,[1] *Krishna Patel*,[1] *Joshua Pitzer*,[1] *Brandon Garcia*,[1] *Daniel Martin*,[1] *Ilaria Baglivo*,[2] *Zhongqing Ren*,[3] *Xindan Wang*,[3] *R. Martin Roop II*[1]

Abstract

Correct timing of virulence gene expression is critical for successful disease outcomes and the persistence of pathogens within the host environment. The global transcriptional silencer H-NS is a nucleoid-associated protein (NAP) that is important for coordination of virulence in many bacteria including *Escherichia coli, Shigella, Salmonella,* and *Vibrio*. In these bacteria, H-NS-mediated silencing is overcome through direct antagonization via transcriptional counter-silencers that bind to gene promoter regions, displace H-NS, and permit transcriptional activation. *Brucella* spp. and related members of α-proteobacteria lack functional H-NS homologs, so it is unclear whether other proteins are involved in performing analogous functions during host-association and pathogenesis. We have identified the Zn finger protein MucR as a novel H-NS-like protein that is critical for virulence in *Brucella* spp. by binding to and directly repressing virulence gene promoters in an H- NS-like manner. We show that MucR specifically interacts with AT-rich DNA regions containing multiple TA steps. Building on previous work, we show that oligomerization is required for proper MucR activity. Further, we demonstrate the stress- responsive regulator and SlyA-homolog, MdrA, acts as a direct counter-silencer to MucR through competition on virulence gene promoters. Consistent with the role of MucR as an H-NS-like protein, hns from *E. coli* is able to functionally complement mucR mutants in *Brucella* spp. Together these data demonstrate the role of MucR as a novel H-NS-like protein and highlight the importance of silencer/counter-silencer interactions in the pathogenesis of *Brucella* spp. and related bacteria. Presenting author: bartoni19@ecu.edu

Keywords: Virulence, Gene regulation, Transcriptional silencing, Host-association, Pathogenesis

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O3-6 Structural insights into the NyxA/B effector family

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Abstract

Brucella spp. survival and replication inside host cells requires a type IV secretion system (T4SS) named VirB. The T4SS is a molecular nanomachine that translocates effector proteins into the host cell to subvert host cell function, metabolism and defenses. Although many VirB substrates have been identified in *Brucella*, few of them have been characterized functionally and structurally. NyxA and NyxB are homologous and conserved proteins found to be translocated by *Brucella* into the host during infection 1. The proteins were found to interact with the human Sentrin specific protein SENP3 and modify its localization in the cell. In this study we have investigated the structural properties of NyxA/B. We purified NyxA and NyxB recombinantly and solved the crystal structure of NyxB at a resolution of 2.5 Å. The protein reveals a novel dimeric fold that was confirmed by Multi-angle light scattering (MALS) and Small-Angle X-ray Scattering (SAXS) experiments. Structural analysis of the NyxB surface revealed an acidic pocket delineated by several residues strictly conserved in NyxA. In the context of the dimer, these surfaces are juxtaposed to form an extended concave negatively charged The structure enabled the design of specific mutants at a groove formed by the dimer. A structure-function study will be presented, using site- directed mutagenesis, *in vitro* and in cellulo interaction assays, providing insights into NyxA/B interaction with SENP3.
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Keywords: Effector, X-ray crystallography, Type IV secretion system, *Brucella*, Structure

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P3-01 Manipulating *Brucella abortus* two-component regulatory system BvrR/BvrS promoter activity through environmental stimuli

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Abstract

The two-component regulatory system BvrR/BvrS is required for *Brucella abortus* transition from an extracellular to an intracellular lifestyle. BvrS is a sensor histidine kinase transducing unknown external stimuli to the BvrR transcriptional regulator through phosphorylation. Active BvrR then binds to gene regulatory regions, affecting their transcription. The system is a master regulator controlling the expression of genes related to cell envelope homeostasis, carbon and nitrogen metabolism, the virulence factor VirB and its regulator VjbR. *B. abortus* bvrR/bvrS mutants are avirulent in mice models. Low concentration of nutrients and pH were recently described as environmental cues affecting the BvrR transcriptional regulator activation through phosphorylation. Here, we describe the environmental cues that affect BvrR/BvrS promoter activity. Using a pbvrR::luxA/luxB transcriptional fusion, the promoter activity was determined during in vitro growth and ex vivo in a cellular infection model. Conditions and chemical compounds simulating the environment found during intracellular trafficking were evaluated: nutrient restrictions, different pH, presence of metals, carbon, and nitrogen sources, at different concentrations and time. The results obtained for each condition tested, individually or in combination will be presented in this report. Some of the assessed conditions affected bvrRp activity and demonstrated a similar effect on BvrR phosphorylation. A combination of conditions tested was found to repress bvrRp activity. Altogether, these results show that conditions that modulate bvrRp activity are not necessarily the same as those sensed by the BvrR/BvrS two-component system but might influence BvrR activation. This work provides a first attempt toin vitro manipulate the bvrRp activity and hence contribute to the understanding of the type of environmental signals need it for regulating bvrR/bvrS transcription.

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Keywords: Two-component regulatory system, Gene-regulation

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P3-02 How does Gefitinib inhibits *Brucella* infections?

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Abstract

Brucellosis is a zoonotic disease caused by *Brucella*, with extensive implications in both economic and public health. To date, no human vaccine has been developed and the current treatment with antibiotics can cause severe side effects and relapses. Due to the need for new treatments to fight brucellosis, one possibility is to target host cells mechanisms to block entry and/or intracellular replication of bacteria. In addition to such host-directed therapy (HDT), we used a drug repositioning strategy, which consists in evaluating drugs already on the market. Previous findings from our lab showed that Gefitinib, a drug currently used for the treatment of a type of lung cancer, strongly inhibits the proliferation of *B. melitensis, B. abortus* and *B. suis* inside macrophages and trophoblasts in vitro, even proving a curative effect. In vivo evaluation of the drug is currently ongoing in a murine model of brucellosis in collaboration with the group of Prof. Renee Tsolis (UC Davis, CA, USA). The aim of this study is to understand the molecular mechanisms by which Gefitinib achieves the control of *Brucella* infection. BeWo cells (human cytotrophoblasts) were treated with Gefitinib. The activation of several intracellular signaling pathways was analysed using Western Blot (WB). In parallel, cells were infected with fluorescent *B. melitensis* using gentamicin protection assays and cells were analyzed using confocal microcopy. WB analyses revealed a decreased phosphorylation of EGFR upon stimulation with Gefitinib and alteration of downstream signaling pathways was detected, including FAK and MAPKs. Gefitinib treatment increased both the number and size of Lysotracker-positive vesicles (LPV). Upon infection, the number of bacteria per infected cell is significantly decreased and *Brucella* highly colocalized with LPV in Gefitinib treated cells. Gefitinib modifies intracellular molecular pathways and bacteria are rapidly eliminated from infected cells by an apparent increase in lysosomal activity. These results suggest Gefitinib as a promising therapeutic alternative for the treatment of human brucellosis.
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Keywords: *Brucella,* Gefitinib, Mechanism, Treatment, Trophoblasts

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P3-03 Characterization of IalB family protein in *Brucella abortus*

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Abstract

Invasion associated locus B (IalB) was originally described as a protein required for intra-erythrocytic parasitism in *Bartonella* spp.. *Brucella* spp. and *Bartonella* spp. are closely related alfa-proteobacterial pathogens that cause chronic infections in mammals. *Brucella* spp. genomes encode orthologous IalB proteins. One of them, encoded by locus BAB1_0368 in *B. abortus*, has been described as immunogenic in *B. abortus* and it was identified as a component of outer membrane vesicles in *B. melitensis* 16M. However, the contribution of IalB proteins to *Brucella* lifestyle is still not characterized and their biological roles remain unknown. This study is focused on characterizing and understanding the contribution of the members of IalB family protein (PF06776 in Pfam database) to *B. abortus* physiology and pathogenesis. To achieve this, single and multiple mutants were obtained by unmarked gene deletion in *B. abortus.* To evaluate the impact of these deletions in bacterial physiology, we first evaluated growth rates of these strains in rich medium Tryptic Soy Broth by using an automated growth curve analyzer. In addition, a fluorescent D-amino acid derivative was used to detect differences in bacterial size/morphology and in peptidoglycan synthesis by fluorescence microscopy of fixed cells. Finally, to address the role of IalB proteins in interaction with host cells, entry and intracellular replication of the mutant strains in non-professional phagocytic cells (HeLa) was evaluated. Single mutants in some ialB genes showed statistically significant differences to the parental strain 2308 in generation time, intracellular replication and in cell size and morphology. These preliminary results suggest a role of IalB proteins in *B. abortus* cell shape, as well as in vegetative and intracellular multiplication.
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Keywords: *Brucella abortus*, Growth rate, IalB, Intracellular replication

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P3-04 A new model for the synthesis of the homopolymeric O-antigen of *Brucella*

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Abstract

Lipopolysaccharide (LPS) is the mayor outer membrane antigen in gram-negative bacteria and, in pathogens, is a central macromolecule in the pathogenic process. In *Brucella* the LPS has been shown to be a virulence factor required for the successful establishment of a persistent infection and that it affects the efficient intracellular replication of the bacterium, a hallmark of the infectious cycle. To date the accepted model for the synthesis of the O-antigen of *Brucella* fits the canonical homopolymeric pathway: the synthesis of the complete structure is completed on one poly-prenol-phosphate that is, afterwards, translocated to the periplasmic side of the inner membrane and ligated to the lipid-A-core completing the synthesis of the LPS. In this presentation we will present evidence that the synthesis of the O-antigen in *Brucella* occurs through a different mechanism as the one previously proposed and that it does not adjust to the canonical homopolymeric model. We found that *Brucella* uses two decaprenol-phosphate intermediaries: one onto which the perosamines and formyl- perosamines are polymerized and a second one, primed with the trisaccharide NAc-Qui-Man-Man that serves as an acceptor for the polymerized perosamines. We have additionally identified a protein with no known function to date, that controls the length of the O-antigen linked to the LPS modulating the number of perosamines polymerized on the first lipid intermediary. This mechanism of synthesis constitutes a new model for homopolymeric O-antigens.
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Keywords: LPS, O-antigen, Homopolymer

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P3-05 Regulatory patterns influenced by the Two-Component System BvrR/BvrS in *B. abortus*

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Abstract

An essential element required for *B. abortus* intracellular replication is the two-component system BvrR/BvrS. Intracellularly, the regulatory protein BvrR is activated by phosphorylation in response to cues found in early and late compartments. We generated two-point mutations in the BvrR putative phosphorylated aspartate 58 to understand the BvrR-regulated transcriptional response. Aspartate 58 was substituted with either an alanine (BvrRD58A-dominant negative) or a glutamic acid (BvrRD58E-dominant positive), and the sequences were inserted in plasmids. We introduced these plasmids expressing bvrR mutants and the wild-type version into a *B. abortus* bvrR- and analyzed the strains' phenotypes. The wild type and the BvrRD58E mutant restored the phenotypes affected in *B. abortus* bvrR- according to polymyxin assays, ex vivo replication, and expression of proteins such as Omp25, VirB8, and VjbR. In contrast to *B. abortus* bvrR-, we observed that the BvrRD58A mutant is not as attenuated in several phenotypes tested. For example, VjbR expression was recovered in the BvrRD58A mutant compared to *B. abortus* bvrR- confirming that unphosphorylated BvrR exerts transcriptional control. Interestingly, although BvrRD58A binds to the virb promoter, VirB8 expression was not restored. Based on these results, we propose three regulatory patterns defined by BvrR phosphorylation, (i) unphosphorylated BvrR binds and regulates the expression of genes directly, (ii) phosphorylated BvrR binds and regulates the expression of genes directly, and (iii) unphosphorylated BvrR binds to gene promoters, but additional regulators are needed to promote gene expression. These findings serve as a working model for understanding how the response regulators of two-component systems control gene expression.

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Keywords: Two component system, Regulatory protein, Phosphorylation, Regulatory patterns

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P3-06 Is CuxR the missing link between CdG signaling and *Brucella* virulence?

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Abstract

Bis-(3',5')-cyclic diguanylic acid, also known as cyclic-di-GMP (CdG) is a secondary messenger that plays important roles in the basic physiology and virulence of many bacteria. CdG functions in three ways, by binding to effector proteins, binding to transcriptional regulators, or binding to riboswitches and modulating their activity. The latter two effect gene transcription while the former modulates protein function. Diguanylate cyclases (DGCs) produce CdG from two GTP molecules, while phosphodiesterases (PDE) degrade it. Their regulation affects the level and activity of CdG with in the cell. Previous work demonstrated a link between CdG signaling and *Brucella* virulence, but the genetic regulators that mediate this link have not been identified. The recent discovery and characterization of the CdG- responsive UDP-xylose regulator (CuxR) in Sinorhizobium meliloti, a close phylogenetic relative of *Brucella*, has provided a possible clue in this regard. The DNA-binding activity of CuxR, an AraC-type transcriptional activator, is dependent on the presence of CdG. CuxR controls the expression of genes encoding an arabinose-based exopolysaccharide known as APS in S. meliloti in a CdG-responsive manner. The genes encoding CuxR and the aps operon reside in a single genetic locus inS. meliloti that is conserved in *Brucella*. Derivatives of virulent strain *B. abortus* 2308 with null mutations in the cuxR and aps genes are currently being constructed to determine if - a) CuxR mediates CdG signaling in *Brucella*; b) if CuxR regulates expression of the *Brucella* aps genes, and c) if the cuxR and/or aps genes are required for the wild-type virulence of *B. abortus* 2308 in mice and cultured mammalian cells. In addition to providing important insight into the specific role that CdG signaling plays in *Brucella* virulence, these studies also have the potential to define a link between exopolysaccharide (EPS) production and virulence in these bacteria. This is an exciting possibility because although *Brucella* strains have been shown to have the genetic capacity to produce EPSs, it is unknown if they play a role in the pathogenesis of brucellae in their mammalian hosts.
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Keywords: Cyclic-di-GMP, Exopolysaccharides, Virulence

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P3-07 Structural study of type IV secretion system protein VirJ

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Abstract

Brucella spp. survival and replication inside host cells requires a type IV secretion system (T4SS). The T4SS translocates effectors proteins into the host cell to modulate the intracellular fate of the bacterium in order to establish a secure niche were it actively replicates. Although many VirB substrates have been identified in *Brucella*, we still know very little about the secretion mechanism that mediate their translocation across the two membranes and the periplasmic space. In Gram negative bacteria, T4SSs are composed of 12 proteins, VirB1 through VirB11 and VirD4 according to the nomenclature used for the prototypal Agrobacterium tumefaciens VirB/D T4SS. VirD4 serves as cytoplasmic receptor for substrate binding to facilitate entry into the translocation channel. The T4SS of *Brucella* is homologous to the VirB/D system, but lacks the virD4 gene raising questions about effector translocation in this system. VirJ has been identified as a periplasmic protein essential for the translocation of two *Brucella* effectors BPE123 and SepA. These two effectors are initially secreted in the periplasm before being translocated to the host cell in a VirJ-dependent manner by the T4SS. Indeed, VirJ also interacts with T4SS components VirB5 and VirB8 and thus could facilitate delivery of selected effectors to the T4SS machinery. In this study we have investigated the structural properties of VirJ by biochemistry and modelling. We first purified the VirJ protein to investigate its biochemical properties. Multi-angle light scattering (MALS) experiments show that VirJ is monomeric in solution domain. We then predicted the structure of monomeric VirJ using alfafold 2. The structure reveals that VirJ contain 2 domains separated by a short linker. Domain 2 adopts a hydrolase fold with eight b-sheet surrounded by six α helices that appears to be conserved in *A. tumefaciens* VirJ. Analysis of structure-sequence conservation points towards a potential active site composed of one serine, one histidine and one aspartate. We then present the results of VirJ-effectors interactions and discuss potential mechanism for VirJ-guided translocation.
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Keywords: *Brucella*, Type IV secretion, VirJ, Biochemistry, Structural model analysis

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P3-08 Suppression of *B. melitensis* Rev.1 erythritol catabolism as a strategy to avoid genital tropism to develop a safe brucellosis vaccine

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Abstract

Small ruminant brucellosis by *Brucella melitensis* is manifested mostly as abortion and infertility, symptoms that result from the marked tropism and intense multiplication of these bacteria within the cells of the genital organs and placenta. Animal mass vaccination is critical to control brucellosis and lessen human infection in countries with high prevalence. However, Rev.1, the only available vaccine for small ruminants, keeps genital tropism and thus induces abortion in pregnant females. An obvious strategy for minimizing the abortifacient effects is the suppression of the tropism for the placenta, a phenomenon that has been postulated to be connected to the abundance of erythritol in this organ and the preferential use of this polyol by *B. melitensis*. Based on this hypothesis, we obtained non-polar deletion mutants in ery1 and ery2, genes of the recently unraveled erythritol catabolic pathway. Rev.1Δery1 was unable to metabolize erythritol and its growth was not affected by the presence of this polyol in rich medium. Rev.1Δery2 was also unable to use erythritol but its growth was inhibited by this polyol. Studies in THP-1 monocyte-derived-macrophages and BeWo trophoblasts showed that while both mutants multiplied in macrophages like Rev.1, Rev.1Δery2 multiplication in trophoblasts was significantly lower. In our pregnant mouse model, the deletion of both genes resulted in a decrease in abortions and reduced bacterial replication in the placenta and vertical transmission to the fetuses. The virulence (splenic multiplication curves) and protection assays in mice confirmed the attenuated profile of Rev.1Δery2. In contrast, Rev.1Δery1 showed a multiplication profile similar to Rev.1 and optimum protection against the *B. melitensis* H38 challenge. These results led us to assess the safety of mutant Rev.1Δery1 in pregnant sheep (work O5-2 presented by Muñoz, P. M. et al in this Conference). Presenting author: azuniga@unav.es

Keywords: Rev.1, Erythritol, Metabolism, Vaccine, Safety

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P3-09 Development and evaluation of the *Galleria mellonella* (greater wax moth) model to study *Brucella* host pathogen interaction

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Abstract

Brucellosis is a zoonosis caused by bacteria of the genus *Brucella*. These gram-negative bacteria cause long-lasting infections, a process in which *Brucella* PAMP-modifications (*e.g.* lipopolysaccharide [LPS]) hamper a prompt and effective activation of innate immunity. Laboratory models are essential to investigate *Brucella* virulence and although mice and, less frequently, guinea pigs have been used, ethical and practical considerations impede their use in high-throughput screening studies. Although lacking the complexity of mammalian immune system, the insect *Galleria mellonella* (greater wax moth) is increasingly being used as infection model as it conserves key aspects of innate immunity with mammals and has been useful in virulence analyses of relevant pathogens such as *Klebsiella, Legionella, Mycobacteria* and *Listeria*. To assess the potential of this model for the study of *Brucella* virulence, we evaluated *G. mellonella* larva survival upon infection with *B. abortus* 2308W wild-type and attenuated mutants (*i.e.* defective in the VirB Type-IV Secretion System [T4SS] or in the LPS-O- polysaccharide [O-PS]), and *B. microti* CCM4915. Then, we explored whether the survival profiles were related with a differential replication of brucellae by CFU-counting of whole-larva homogenates and fluorescence microscopy of primary- phagocyte isolates. Finally, we evaluated the ability of *G. mellonella* immunity to efficiently recognise *Brucella* by quantification of the pro-phenoloxidase system and melanisation activation after infection or LPS inoculation. As compared to *K. pneumoniae* 52145, *B. abortus* and *B. microti* induced a delayed and less severe mortality profile. Moreover, typical- brucellae did not trigger an early-melanisation response, consistent with the low immunostimulatory properties of *Brucella* LPS. Finally, we observed that *Brucella* virulence in *Galleria* is influenced by VirB and O-PS, since the corresponding mutants displayed even more marked delayed and less severe mortality profiles than the parental strain. Intriguingly, bacterial replication within larvae was affected by the lack of O-PS, but not of a functional T4SS. In light of these results, the *G. mellonella* model may represent an alternative tool for the study of *Brucella* interaction with innate immune, although the suitability of this model for the study of other aspects of *Brucella* pathogenesis, such as *Brucella* intracellular life, remains to be elucidated.

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Keywords: *Brucella*, *Galleria mellonella*, Survival, Phagocytosis, Melanisation

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P3-10 Control of the subcellular localization of host nuclear proteins by *Brucella* effectors

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Abstract

Bacterial targeting of the nucleus is a major virulence strategy shared by a number of plant and animal pathogens. In order to manipulate nuclear functions and eukaryotic gene expression, bacteria inject into host cells nuclear-targeting effector proteins, known as nucleomodulins. We have recently identified two such effectors from *Brucella abortus* that we named NyxA and NyxB. We have found that these two effectors modulate subcellular spatial dynamics of nuclear proteins during infection. We show that NyxA and NyxB target the host de-SUMOylase SENP3, extensively impacting nuclear dynamics. SENP3 is required for efficient intracellular replication within host cells. Furthermore, we have found that key components of ribosomal biogenesis machinery accumulate in the host cytosol of infected cells, suggesting a novel host response to infection. The consequences of these interactions will be further discussed in the context of *Brucella* pathogenesis.

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Keywords: Nucleomodulins, Effectors, SENP3

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P3-11 In-vitro model of macrophages bovine cell for the characterization of pathogenic mechanisms of bovine brucellosis

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Abstract

Brucella infection, affecting several animal species, constitutes one of the main zoonosis worldwide with a high economic impact. Disease pathogenicity is mainly associated with the ability of *Brucella* to evade the host immune system by surviving inside both phagocytic and non-phagocytic cells, especially macrophages, dendritic cells and granulocytes. This leads to a chronic persistent infection, in a mechanism not yet completely characterized. The macrophages, among cells of the innate immune system, constitute the first defense line against pathogens and an important immuno-modulating determinant. For this reason, the investigation on *Brucella* strains behavior inside macrophages could be essential to better understand and define the pathogenic mechanisms in humans and host species. In this context, we investigated the virulence of *B. melitensis* and *B. abortus*, the *Brucella* strains with the highest zoonotic potential, on BoMac cells, anin vitro cellular model of bovine peripheral macrophages. This study has the purpose of characterizing the bovine-pathogen interaction and developing an *in vitro* system serving as a reference model of bovine brucellosis infection. We observed the internalization of both *B. melitensis* and *B. abortus* in BoMac macrophages, their survival and proliferation inside host cells up to 72h from infection. Further investigations are ongoing on host gene expression and production of pro- and anti-inflammatory cytokines to characterize the bovine macrophages immuno-modulating response in the in vitro infection model. The characterization of pathogen virulence factors and the host cells molecular response, constitute the basis for understanding infectious disease mechanisms and development of more effective tools for their control. Presenting author: k.zilli@izs.it

Keywords: Brucellosis, Macrophages, Pathogenic mechanisms

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K4 Internal affairs: defining how cytosolic receptors sense *Brucella* and contributes to host defense

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Abstract

Brucella has developed a stealth strategy through pathogen-associated molecular patterns reduction, modification, and hiding to ensure low stimulatory activity. This strategy allows *Brucella* to reach its replication niche before activating antimicrobial mechanisms by host immune responses. However, inside the host cells, *Brucella* releases vital molecules for the bacteria that trigger the activation of host cytosolic receptors. First, we defined that *Brucella* LPS is the ligand for the receptor caspase-11. Additionally, we determined that *B. abortus* is able to trigger pyroptosis leading to pore formation and cell death, and this process is dependent on caspase-11 and gasdermin-D (GSDMD). Mice lacking either caspase-11 or GSDMD were significantly more susceptible to infection with *B. abortus* than wild-type animals. Our findings suggest that caspase-11/GSDMD-dependent pyroptosis triggered by *B. abortus* is important to infection restriction in vivo and contributes to immune cell recruitment and activation. Besides LPS, DNA is another important bacterial ligand. Then, we determined that the cGAS/STING pathway is able to recognize bacterial genomic DNA and cyclic dinucleotides. Further, we have demonstrated that STING but not cGAS is critical for host protection against *Brucella* infection in macrophages and *in vivo*. Additionally, we revealed that STING contributes to an inflammatory M1-like macrophage profile upon *Brucella abortus* infection. This metabolic reprogramming is induced by STING-dependent stabilization of hypoxia-inducible factor-1 alpha (HIF-1a). HIF-1a stabilization reduces oxidative phosphorylation and increases glycolysis during infection with *B. abortus* and enhances nitric oxide production, inflammasome activation and IL-1b release in macrophages that are involved in reduced bacterial replication. In summary, identifying innate immune receptors and their ligands is critical to the development of new vaccines and control measures against *Brucella* infection. In addition, we speculate on the prospect of targeting immunometabolism in the effort to develop novel therapeutics to treat brucellosis and other bacterial infections.
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Keywords: *Brucella abortus*, Innate immunity, Inflammasome, STING, Immunometabolism

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O4-1 Antibody response elicited by *Brucella abortus* strain RB51 vaccine in young water buffaloes (*Bubalus bubalis*) using a triple dose and the WOAH vaccination schedule. A long term trial

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Abstract

Brucella RB51 is a live modified vaccine, obtained from a rough phase mutant of the virulent strain *B. abortus* 2308. The RB51 strain lacks the side chain O of the LPS thus RB51 vaccinated and non-infected animals do not react to official serological tests (RBT and CFT). This supports the use of the vaccine for a DIVA strategy. RB51 vaccine has been shown to be safe and effective in cattle and bison. Its use in water buffalo has been proposed using a different vaccination protocol but knowledge of long term effects of RB51 vaccination in this species remain incomplete. Aim of the study was to evaluate the kinetics of RB51- antibodies in water buffaloes vaccinated according to the protocol described for the bovine species in the WOAH Manual, modified with the use of a triple dose. Thirty buffaloes 5-9 months old were vaccinated with the commercial vaccine RB51 (CZ Veterinaria, Spain) using a triple dose. Six buffaloes were included as controls. A booster vaccination was administered at 12 month of age. When turning 19-21 months, female animals (both vaccinated and controls) were induced to pregnancy. Blood samples were collected all along the trial, covering vaccination, pregnancy and lactation time. RB51 - specific antibodies were detected and quantified using a specific CFT based on RB51 antigen. All vaccinated animals showed a positive serological reaction following each vaccine injection, but titres and duration of the humoral response differed among animals. For 259 days after booster vaccination, comparison of CFT values between vaccinated and control groups remained significant, despite some of the vaccinated animals turned seronegative. Subsequently, a decrease of antibody titres was recorded with a fluctuating trend observed in several animals, alternating periods of negativity with periods of CFT positivity with titres close to the test cut-off (1:4 serum dilution). Positive animals were still recorded after 2,5 years post vaccination. No relevant changes in antibody titres were recorded during pregnancy or lactation. In conclusion, with the vaccination schedule applied, CFT-RB51 clearly discriminates between vaccinated and unvaccinated controls up to 8 months after the booster. Afterwards, results interpretation becomes less clear-cut considering CFT-RB51 only.

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Keywords: Brucellosis, Water buffalo, RB51, Immune response

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O4-2 Antibody response elicited by *Brucella abortus* strain RB51 vaccine in young water buffaloes (*Bubalus bubalis*) using a triple dose and the vaccination schedule authorised in Caserta province, Italy

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Abstract

Eradication of animal brucellosis is still a priority in Italy and the disease has a relevant impact on water buffaloes in the province of Caserta, Campania Region, Italy. In order to reduce the prevalence of infection, vaccination with the live attenuated vaccine *B. abortus* strain RB51 has been authorized by Central Veterinary Authority (Ministry of Health) in water buffalo herds in endemic territories, under controlled conditions. Namely, vaccination of young animal only (6-8 months old females, before sexual maturity), using a dose 3 times higher than the one used for cattle, followed by a second injection of same dose, 1 month after the first administration. In fact, when used on adult animals RB51 can be excreted in milk or induce abortion in pregnant animals. The aim of the study was to acquire information on development and persistence of RB51 specific antibodies induced by the vaccination schedule authorized from the time of vaccination (6-8 months old) to the stage of adult (26-28 months old). 22 buffaloes 7 - 8 months old were vaccinated with the commercial vaccine RB51 CZ Veterinaria, S.A. (Spain) using a triple dose. Four buffaloes were included as controls, inoculated with placebo and immediately mixed with vaccinated animals. A booster vaccination was administered after 30 days. Blood samples were collected regularly after vaccination. Sera were tested using a specific Complement Fixation test performed using RB51 antigen (RB51-CFT). RB51- CFT identified as positive 80% of the animals 9 days after first vaccination. Following booster vaccination, all vaccinated animals tested positive for approximately 2 months. Compared to our previous studies, results suggest that age of vaccination influences antibody response and RB51-CFT results. Two months after booster vaccination, antibody titers and numbers of vaccinated animals testing positive for RB51-CFT was variable and decreased in a non-progressive trend. Actually, we observed that several animal alternated positive and negative results to RB51-CFT up to 18 months after booster vaccination. This information represent a relevant element to develop diagnostic protocols to survey and detect the possible unauthorised application of RB51 vaccination on adult animals.

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Keywords: Brucellosis, Water buffalo, RB51, Immune response, Complement Fixation Test

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O4-3 Use of brucellin skin test to identify water buffaloes (*Bubalus bubalis*) vaccinated with the live attenuated vaccine *B. abortus* strain RB51

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Abstract

The use of the live attenuated *Brucella abortus* RB51 vaccine in water buffaloes was exceptionally authorised in Italy in 2007 as an additional measure to reduce the impact of Brucellosis in some endemic areas. Vaccination was restricted to prepubescent animals only, to avoid the known risks of excretion with milk or abortion when administered to adult females. To survey non-authorised vaccinations, a diagnostic protocol was developed combining RB51 complement fixation (RB51-CFT) and brucellin skin tests (BST). The aim of the study has been to assess performances (Se and Sp) of BST when applied to a known population of buffaloes vaccinated with RB51, and controls. Data from two different RB51 vaccine trials were combined, and included 49 vaccinated animals and 9 controls. Both trials considered two separate injections of a triple dose of RB51 vaccine to young animals, but age and timing of vaccination and timing of booster dose differed slightly. BST was performed using commercial brucellergene OCB. In the first (long term) trial, BST was performed 3 months after delivery, *i.e.* 23-25 months after booster vaccination. In the second trial, BST was executed 18 months after the booster dose. Different cut-off for skin thickness increase were considered to evaluate BST performance. In both trials most vaccinated animals showed a BST positive reaction, that was more evident at 72hrs compared to 48hrs, this independently of the vaccination protocol applied. BST showed best value of Se (83,7%, 70,9-91,4 C.I.) and Sp (100%, 71,7-100 C.I.) when considering a skin thickness increase of 1,5 mm as cut-off. As expected, some vaccinated animals (8/49, 16,3%) tested negative to BST. Taken together, both study results indicate that BST, when applied at herd level, is a suitable test to identify RB51 vaccinated animals for long time after vaccination. Conversely, due to the low sensitivity, application of BST on individual animals is questionable.

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Keywords: Brucellosis, Water buffalo, Immune response, Brucellin skin test

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O4-4 Effects of age on immune response induced by *Brucella abortus* S19 or RB51 vaccination in heifers

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Abstract

The purpose of this study was to compare the immunological response induced by bovine brucellosis vaccination (S19 and RB51) in calves vaccinated in different ages between 3 and 8 months, to determine the ideal age for vaccination of calves. One hundred and five female calves aged 3 to 8 months were randomly divided in nine groups (n=12), in a 3×3 (3 vaccination regimens x 3 ages) experimental design, as follows: three groups were vaccinated with S19 at 3-4 (G1), 5-6 (G2) or 7-8 (G3) months of age, three groups were vaccinated with RB51 at 3-4 (G4), 5-6 (G5) or 7-8 (G6) months of age and three groups were inoculated with saline (controls) at 3-4 (G7), 5-6 (G8) or 7-8 (G9) months of age. Vaccination of all groups occurred at day 0 and assays to evaluate the induced immune response were performed at days 0, 28 and 56 after vaccination, from peripheral blood from all heifers at each time point. Peripheral blood mononuclear cells (PBMC) were isolated and cultured in the presence of γ irradiated *Brucella abortus* 2308 antigen to quantify CD4+, CD8+, CD4+CD45RO+ and CD8+CD45RO+ T lymphocytes. Culture supernatants were also tested for IFN-γ by ELISA. For each response variable, we fitted a mixed model with a fixed effect for the interaction age (3-4, 5-6 and 7-8) × treatment (S19, RB51 and control) × time (0, 28 and 56), using the packages lme4 and emmeans in the software R. The model for IFN-ɣ production by the PBMC following vaccination exhibited a significant interaction only treatment and time, showing no effect of the age on vaccination. There was a significant higher expression of IFN-ɣ after vaccination with S19 or RB51 at 28- and 56-days post-vaccination in comparison with control animals regardless the age of the calves, however, non-significant difference was observed between S19 and RB51 at any time or age. In conclusion, our results showed no effect of the age (3 to 8 months) on the immune response induced by vaccination of heifers with either S19 and RB51, as well as that both vaccines elicited a strong IFN-ɣ response after vaccination.
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Keywords: Bovine brucellosis, Bovine vaccines, IFN-γ, TH1 immune response

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O4-5 TIRAP exon 5 polymorphisms influence the outcome of *B. melitensis* infection in goats

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Abstract

TIRAP is an adaptor protein necessary for TLR4/2 inflammatory signaling in response to bacterial infection. The *Brucella* effector protein TcpB induces TIRAP degradation, down-regulating the immune response and enhancing *Brucella* intracellular replication. Two single nucleotide polymorphisms (SNPs, S180L y D96N) in TIRAP exon 5 (TIRAP-E5) were associated with an enhanced resistance to *M. tuberculosis, P. falciparum* and other infections in humans. The aim of this work was to evaluate if SNPs within caprine TIRAP-E5 influence the susceptibility to *B. melitensis* infection in goats. A candidate-gene association study was conducted using 100 DNA samples from *Brucella*-seropositive (cases) and seronegative (controls) female creole goats. Animals were unvaccinated against *Brucella* spp. and belonged to ten flocks with prevalence of brucellosis (10 -72%). Genotyping of TIRAP-E5 was carried out by PCR and DNA sequencing. Genetic associations were evaluated by the Fisher's exact test. Monocyte derived macrophages (MDMs) cultures were obtained from peripheral blood of seronegative goats, and infected with *B. melitensis* 16 M (MOI 1:10) for 30 min. At different times post-infection, intracellular *Brucella* counts and cytokine mRNA expression were determined by a gentamicine protection assay and qRT-PCR after RNA isolation, respectively. Sequence alignment of caprine and human TIRAP-E5 showed an identity of 76%. We detected three polymorphic SNPs (rs914(T/C), rs459(C/G) and S189N(G/A)) at TIRAP-E5 in the studied goat population. The rs914-C allele was significantly more frequent in controls than cases (p=0.002). Moreover, the genotypes rs914-CC+CT and the haplotype C-C-G (rs914- rs459-S189N) were associated with absence of *Brucella*-specific antibodies (p= 0.001), while rs459-CG and T-C-A were associated with presence of *Brucella*-specific antibodies (p=0.043; p=0.045). Bioinformatic analyzes suggested that the associated polymorphisms could affect RNA folding and/or protein function. Besides, MDMs with the rs914-CT genotype controlled more efficiently *Brucella* intracellular growth at 24 h post-infection (p.i.) (p=0.02), and expressed higher levels of pro-inflammatory cytokines IL-12 e IL-18 at 4 h p.i. than rs914-TT MDMs (p < 0.05). Consistent with previous reports in humans, the results presented here suggest that genetic variability in TIRAP exon 5 would influence the outcome oBf. melitensis exposure in goats.

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Keywords: Caprine-brucellosis, Genetic-Resistance, SNP, TIRAP

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O4-6 PMNs supports *Brucella* dispersal with reduced immune recognition

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Abstract

Neutrophils (PMNs) are the first line of defense against bacteria entering the body. However, it has been shown that *Brucella* induce low PMN activation and survive within these leukocytes by resisting their microbicidal mechanisms. *Brucella* also induce the premature cell death of PMNs, which release chemokines and express 'eat me' signals. These PMNs are then phagocyted by mononuclear cells where *Brucella* replicate. This evidence suggests that PMNs may behave as vehicles protecting Brucella from immune recognition, favoring their dispersion to the target organs. To test this hypothesis, we analyzed the course of infection in mice intraperitoneally infected with *B. abortus* alone (Ba) or with *Brucella*-infected PMNs (Ba-PMN). We evaluated bacterial loads, histopathological analyzes in selected tissues, cytokine production in serum, anti-*Brucella* antibody titers, and hematological parameters. We observed that mice infected with Ba-PMN had lower bacterial loads in the spleen and bone marrow at seven days of post-infection compared to Ba. The bacterial load then became equivalent at 30 days post-infection. The pathological index demonstrated a similar trend to the bacterial load. Ba-PMN infected mice showed less granulomatous inflammation in the spleen and bone marrow at seven days post-infection than the Ba group but with similar indexes at day 30. Comparably, Ba-PMN infected mice showed less IFN-gamma and IL-6 at the beginning of the infection than the Ba group but with similar concentrations at the end of the experiment. Ba-PMN infected mice also showed fewer anti-*Brucella* antibody titers at day 30 than the Ba group. No significant differences were observed between infected groups in the hematological values at 7 or 30 days. Despite both groups reaching similar bacterial loads by day 30, the bacterial loads and the immunological parameter were lower at the beginning of the infection in the Ba-PMN group. We conclude that the course of infection in the Ba-PMN group was stealthier than in the Ba group. Despite the slower course of *Brucella* infection in the Ba-PMN, PMNs supported their dispersion to a similar extent but with reduced immune recognition.
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Keywords: *Brucella*, Neutrophils, Pathogenesis, Mice

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K5 The bumpy road to a *Brucella* vaccine: the near hits and good catches

Angela Arenas-Gamboa[1]

Abstract

Exactly ninety-nine years ago, Matilda's milk and Dr. John Buck's laboratory accident became one of the most important strides in the history of *Brucella* vaccines and is considered a success story where 'chance favored the prepared mind'. Despite that a century has passed, to this date, there have been no significant advances in the development of superior vaccines, and S19 is still considered to be the best choice in terms of protective efficacy against *B. abortus*. Here, we discuss lessons learned as well as new approaches to better understand the mechanisms behind enhancing protective efficacy and safety, and their importance in the development of next generation vaccines.
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Keywords: Vaccines, Vaccination, Animal Models, Pathology, Efficacy

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O5-1 Registered Influenza Viral Vector Based *Brucella abortus* Vaccine for Cattle in Kazakhstan: Age-Wise Safety and Efficacy Studies

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Abstract

Vaccination is an effective tool for controlling brucellosis in livestock. A novel influenza viral vector based *Brucella abortus* vaccine (Flu-BA) was introduced for use in cattle in Kazakhstan in 2019. In this study, the safety and efficacy of the vaccine was evaluated in male and female cattle at different ages, and during pregnancy as a part of its registration process. Our data demonstrated that the Flu-BA vaccine was safe after prime or booster vaccination in calves (5-7 months old male and female), heifers (15-17 months old) and cows (6-7 years old) and was not abortogenic in pregnant animals. A mild, localized granuloma was observed at the Flu-BA injection site. Vaccinated animals did not show signs of influenza infection or reduced milk production in dairy cows, and the influenza viral vector (IVV) was not recovered from nasal swabs or milk. Vaccinated animals in all age groups demonstrated increased IgG antibody responses against *Brucella* Omp16 and L7/L12 proteins with calves demonstrating the greatest increase in humoral responses. It does not elicit antibodies that respond positively to routine serologic brucellosis diagnostic tests, and therefore meets the DIVA criterion. Following experimental challenge with *B. abortus* 544, vaccinates demonstrated greater protection and no signs of clinical disease, including abortion, were observed. The vaccine effectiveness against *B. abortus* 544 infection was 75, 60 and 60%, respectively, in calves, heifers and adult cows.*Brucella* were not isolated from calves of vaccinated cattle that were experimentally challenged during pregnancy. Our data suggests that the Flu-BA vaccine is safe and efficacious in cattle, including pregnant animals; and can therefore be administered to cattle of any age.

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Keywords: Bovine brucellosis, Calves, Cows, Heifers, Protective efficacy, Registration trials, Vaccine

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O5-2 Improving Rev.1 vaccine safety in pregnant ewes

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Abstract

Lack of safety in pregnant animals hinders Rev.1 mass vaccination campaigns, the most cost-effective strategy to control *B. melitensis* in endemic and low-income countries. In addition to its abortifacient effect, Rev.1 is virulent to humans and streptomycin (Strp)-resistant, an antibiotic of choice for treating human brucellosis. Our goal was to develop a Rev.1 vaccine of improved safety for pregnant sheep. Three Rev.1 mutants were studied: Rev.1ΔwadC, lacking the lipopolysaccharide core lateral branch, that enhances immune system recognition; Rev.1Δery1, unable to catabolize erythritol, involved in *Brucella* genital tropism; and Rev.1StrpS, carrying the *B. melitensis* 16M rsmG instead of Rev.1 rsmG (Rev.1ΔrsmG::Tn7BmersmG) and dispaying increased Strp sensitivity. In mice, the mutants protected similarly than Rev.1 against *B. melitensis* showing the same (Rev.1Δery1) orless (Rev.1ΔwadC and Rev.1StrpS) residual virulence. Two similar but independently conducted safety experiments were carried out in sheep (one for Rev.1ΔwadC and one for Rev.1Δery1 and Rev.1StrpS). Coetaneous 17-month- old *Brucella*-free ewes were synchronized reproductively and mated. Pregnant ewes were randomly allotted into groups of 12-13 animals. At the middle of pregnancy (73-78 days), each group was vaccinated conjunctively with 1-2 × 109 CFU of the corresponding mutant while a control group received the same conjunctival dose of the commercial Rev.1 vaccine (Ocurev®, CZV, Spain). Fever, anorexia, apathy, abortion, perinatal death or lesions were recorded daily. Also, bacterial excretion in vaginal fluids and milk or presence in fetuses was monitored weekly and immediately after abortion/delivery by culturing samples on duplicate plates of both CITA and Farrell selective media. After deliveries, ewes were necropsied and main target organs (spleen, uterus, and mammary, crural, prescapular, iliac and cranial lymph nodes) submitted to bacteriological assessment. The antibody response was assessed weekly by Rose Bengal, Complement Fixation, Agar Gel Immunodiffusion and iELISA tests. Similarly to Rev.1, all mutants induced *Brucella* antibodies 2 weeks after vaccination. However, whereas 61-75% of Rev.1 controls excreted the vaccine and 46-58% suffered undesirable reproductive symptoms, Rev.1ΔwadC, Rev.1Δery1 and Rev.1StrpS did not induce excretion, abortion or perinatal death. Thus, these three safe Rev.1 mutants are suitable candidates for further efficacy experiments in sheep.

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Keywords: Vaccine, Ovine, Rev.1, Safety, Pregnant ewes

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O5-3 Screening for vaccinal candidates in *Brucella canis*: A genomic based strategy for selection of potential DNA target regions

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Abstract

Brucella canis is transmitted among dogs and from dogs to humans mainly through contact with vaginal secretions, fetus and placenta from infected animals. In Costa Rica, a diversity of outbreaks related to the disease have occurred in commercial kennels, causing significant economic losses as well as awareness from health and animal authorities. To date, no commercial vaccines are available against canine brucellosis. In this work, we used successive passages in different in vitro culture media as a strategy to induce spontaneous mutations in a virulent strain of *B.canis*. Subsequently, a genomic-based approach was performed to screen for DNA targets that could be potentially selected to assess attenuation. Distinct strains with different number of passages in in vitro culture media were selected and genetically characterized through whole genome sequencing. Genomic analysis revealed no major genetic or structural rearrangements among passages in comparison to the original strain. In addition, we found 8 different Single Nucleotide Polymorphisms (SNPs) between the original strain and strains with different number of passages as potential targets, located in different genes and in intergenic regions. Non reverting deletion mutants of selected target SNPs were derived from virulent *B.canis* strain and were tested for attenuation in a murine model. Significant differences in virulence profiles were observed between the deletion mutants and the virulent *B.canis* parental strain. These results contribute on the searching for potential DNA targets for the development of vaccine candidates for canine brucellosis.

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Keywords: Attenuation, Canine brucellosis, Genomic analysis, Spontaneous mutations

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O5-4 BM Delta-pgm, a superior vaccine for the control of brucellosis in small ruminants

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Abstract

As part of GalvMed's 'Brucellosis Vaccine Prize' initiative, our lab took on the challenge to develop a new vaccine for the control of small ruminant brucellosis that is superior to the one that has existed for more than 50 years. Based on our previous knowledge and experience in the development of vaccines for bovine and porcine brucellosis, we developed the vaccine strain *B. melitensis* Delta-pgm (BM Delta-pgm) by "clean" deletion of the phosphoglucomutase (pgm) coding gene. BM Delta-pgm has a general defect in sugar metabolism that prevents it from polymerizing polysaccharides containing glucose and/or galactose; therefore, it is unable to synthesize cyclic-beta-glucans and assemble the O-polysaccharide (O-PS) to the lipid A- core and, as a consequence, has a "rough" phenotype. These characteristics make BM Delta-pgm avirulent as we demonstrated extensively in the murine infection model. Immunological and vaccination/challenge studies in the murine model indicate that BM Delta-pgm is immunogenic, generates a good IFN-gamma-mediated Th1 response, does not generate antibodies against O-PS and provides protection against challenge, not only by *B. melitensis*, but also by *B. abortus* and *B. suis*. These characteristics prompted us to evaluate the safety and protective efficacy of BM Delta-pgm in sheep. Between 2018 and 2019, we conducted four safety evaluations in ewes at early and late gestation. Abortigenicity, colonization and dissemination of the vaccine strain in milk and vaginal secretions were evaluated. The results indicate that BM Delta-pgm is safe and generates less than 4% abortions when applied to pregnant ewes. During 2020, an evaluation of the protective efficacy of the vaccine was performed in comparison with a non-vaccinated and a Rev.1 vaccinated control groups, all challenged with *B. melitensis* 16M at mid-gestation. Humoral and cellular immune response, lambing status, milk shedding, postpartum vaginal discharge and colonization of dams and lambs were evaluated. All parameters evaluated indicate that BM Delta-pgm is a safe vaccine strain, prevents abortion and colonization of dams and lambs conferring more than 90% protection against challenge with virulent strains. We propose BM Delta-pgm as a new vaccine with superior characteristics to the existing one for the control of brucellosis in small ruminants.

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Keywords: Small ruminants, Vaccine, Rough LPS, DIVA strategy

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O5-5 Pathogenesis of *Brucella ovis* in pregnant mice and protection induced by the candidate vaccine strain *B. ovis* ΔabcBA

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Abstract

Ovine brucellosis caused by *Brucella* ovis is a major cause of reproductive failure in sheep. This study aimed to evaluate transplacental infection and pathogenicity of *B.ovis* wild type strain ATCC 25840 (WT *B.ovis*) and the candidate vaccine strain *Brucella ovis* ΔabcBA in pregnant mice. A total of 40 BALB/c mice were equally divided into 4 groups: (i) non immunized and uninfected control mice (3/10 mice became pregnant); (ii) non immunized and challenged with WT *Brucella ovis* (5/10 pregnant); (iii) inoculated only with *Brucella ovis* ΔabcBA (6/10 pregnant); (iv) immunized with *Brucella ovis* ΔabcBA and challenged with WT *Brucella ovis* (5/10 pregnant). Female mice bred, and five days after visualization of the vaginal plug, they were inoculated intraperitoneally (ip) with 100 µL of sterile PBS, 100 µL of 1×106 CFU of *Brucella ovis* ΔabcBA, or 100 µL of 1×106 CFU of *Brucella ovis* WT, according to each group. At the 17th day of gestation, samples of spleen, liver, uterus, placenta, fetus and mammary gland were obtained for bacteriology, histopathology and immunohistochemistry. Non immunized mice challenged with *B.ovis* WT developed necrotizing placentitis as well as microgranulomas in the liver and spleen. These findings support the notion that B.ovis infection in pregnant mice induces lesions that are similar to those caused by *B.abortus* in the same animal model. *Brucella ovis* ΔabcBA was not recovered from any of the sampled organs, and it did not cause any gross or microscopic lesions, indicating that it is a safe and attenuated strain in this experimental model. In addition, *Brucella ovis* ΔabcBA was induced protective immunity as demonstrated by decreased numbers of *B.ovis* WT in the liver, uterus and fetuses of immunized mice after the challenge with *B.ovis* WT.
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Keywords: Transplacental Pathogencity, Vaccine, *Brucella ovis*, Pregnant, Nectotizing Placentitis

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O5-6 Identification by Transposon-sequencing of essential genes for chronic infection by *Brucella* in mice

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Abstract

Because of the importance for public health and damages induced by *Brucella* (B.) infection in livestock farming, scientists and field workers try to control and eradicate the disease in animals. However, existing vaccines do not meet all the safety criteria for animals. Currently, the only vaccines able to induce a protection to natural domestic animal hosts against brucellosis are live attenuated vaccines (LAVs), as *B. abortus* S19 and *B. melitensis* Rev.1. Unfortunately, they have some risks of human contamination, interfere with diagnostic tests and importantly, they can induce abortions in animals. Developing safer vaccines for animals would help to reduce the impact of brucellosis. First generation LAVs relied on empirical and somewhat unpredictable attenuation. Recent advances in genetics make it possible to envisage the rational construction of LAVs. Our objective is to achieve by Transposon Sequencing (Tn-seq) a functional map of the *Brucella melitensis* genome in order to construct a candidate LAV capable of persisting long enough to induce protective immunity but incapable of persisting in tissues or invading the placenta in pregnant animals. For this purpose, Tn-seq analyses were performed under different conditions in the mouse model. To generate the Tn-seq, we have generated a library of about 106 transpositional mutants. We have identified in mice genes required for the persistence of *B. melitensis* in the lung after an intranasal inoculation and in the spleen after an intraperitoneal injection. By using these protocols, we avoided the bottleneck which is a limit of the method. Our results show that essential genes for the persistence of *Brucella* vary according to the analyzed tissues. Many of these genes are involved in bacterial metabolism, suggesting that *Brucella* has different nutritional requirements depending on the colonized tissues. Tn-seq predictions were validated by constructing markerless deletion mutants and testing them in mice. On this basis, several candidate vaccines have been selected and are in the validation phase in mice.
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Keywords: Tn-seq, Functional map, Mice, Vaccination, Candidate

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O5-7 *Brucella abortus* RB51 vaccine strain and raw milk consumption: an emerging public health risk

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Abstract

The RB51 vaccine is an attenuated live strain of *B. abortus* and has been used in the United States since 1996 as part of the State-Federal Brucellosis Eradication Program. It is estimated that 4,000,000 calves are vaccinated annually with RB51 and an unknown proportion of these vaccinated animals may become chronic shedders of RB51. Since August 2017, the U.S. Centers for Disease Control and Prevention (CDC) has reported three confirmed human cases of brucellosis in Texas, New Jersey and New York due to *B. abortus* RB51. These cases were each associated with consumption of domestically acquired unpasteurized (raw) dairy products. The epidemiological investigation of a human RB51 case from New York led to a dairy farm in Pennsylvania (PA) as the potential source of infection. Laboratory testing by USDA's National Veterinary Services Laboratory (NVSL) on animal samples collected from the PA farm identified one cow to be shedding RB51 in milk. Whole- genome sequencing (WGS) analysis of the RB51 strains cultured from the PA cow identified two distinct RB51 strain types that match the genetic profiles of RB51 strains isolated from two human RB51 brucellosis cases- one reported in October 2017 from New Jersey and another reported from New York in November 2018. CDC acquired the PA cow under an approved research protocol to investigate currently unknown questions related to the molecular diversity of RB51 in a host, patterns of shedding, host factors associated with chronic infection, and exposure risk to humans. From March to September 2019, we collected samples of quarter milk, blood, urine, rectal and vaginal swabs for culture, bacterial quantification and sequencing as well as antibiotic susceptibilty tesitng. Findings from this testing show intermittent shedding patterns of RB51 in the milk, genetic and phenotypic variations of the RB51 vaccine strain isolated in the mammary system and host immune response to infection with the RB51 vaccine strain.

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Keywords: RB51, raw milk, human brucellosis

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P5-01 Evaluation of different adjuvants associated with vaccination candidate *Brucella ovis* ΔabcBA in a murine model of infection by *Brucella ovis*

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Abstract

Brucellosis is an infectious disease caused by facultative intracellular Gram-negative bacteria, of great importance in animal and human health. Among the known species, *Brucella ovis* is capable of infecting sheep, mainly causing epididymitis and reproductive failure in ram, leading to important economic losses, thus highlighting the need to develop vaccines to help control this disease. Previous studies have shown good levels of protection againstB. ovis infection both in a murine model and in the natural host using the *B. ovis* ΔabcBA strain abcBA encapsulated by alginate. Therefore, this study aimed to evaluate the increase in the vaccine potential of the candidate *B. ovis* ΔabcBA associated with different adjuvants, polymeric matrix or alginate microcapsule and Chitosan, in mice challenged with *B. ovis*. We observed that *B. ovis* ΔabcBA encapsulated by alginate with chitosan, but not associted to polimeric matrix favored lower bacterial recovery in both the spleen and liver of challenged animals, demonstrating better efficacy in controlling the infection. It should also be noted the ability of this vaccine formulation to lead to a greater synthesis of immunoglobulin production, especially immunoglobulin G, where this increase in total levels is correlated with the increase in certain subclasses, with a significant emphasis on the higher levels of the IgG2a subclass, involved in the favoring the control of infections by intracellular agents. Addicionally, use of this vaccine formulation resulted in lower score of histopathological lesions in target organs. These results demonstrate the vaccine potential of the *B. ovis* ΔabcBA strain encapsulated by alginate with chitosan, leading to both a higher protection index, as well as inducing the significant production of antibodies related to a protective immune response, making possible its future evaluation in a natural host.
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Keywords: Attenuated vaccine, Biopolymer, Immune response, Ovine brucellosis

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P5-02 Exploring new candidates to develop a *B. ovis* vaccine based on S-LPS devoid mutant H38ΔwbkF and core defective derivates

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Abstract

Brucella ovis, though non-zoonotic, is a serious cause of reproductive failure in sheep. No country has been declared free from this infection and eradication by test-and-slaughter has been claimed very seldom. Rev.1, the only available vaccine for small ruminants, is effective against *B. melitensis* (zoonotic) and *B. ovis*. However, Rev.1 is banned in those regions where *B. melitensis* is eradicated to avoid interferences in *B. melitensis* serosurveillance. Consequently, *B. ovis* is re-emerging in *B. melitensis*-free countries and a *B. ovis* specific vaccine not interfering in the smooth lipopolysaccharide (LPS) based tests used to diagnose *B. melitensis* is needed. In a recent work[1], we demonstrated that subcutaneous vaccination of rams with the rough (R) O-polysaccharide (O-PS) mutant H38ΔwbkF confers protection similar to Rev.1 against *B. ovis*, while not interfering in the Rose Bengal and Complement Fixation tests used for *B. melitensis* diagnosis. However, since H38ΔwbkF interferes in *B. ovis* serodiagnosis, we also tested a *B. ovis* mutant (Bov::CAΔwadB) defective in the LPS-core lateral branch. While Bov::CAΔwadB did not provide protection, it caused low if any interference in the *B. ovis* agar gel immunodiffusion (AGID) test recommended by WOAH, an observation related to its modified core epitopes. The aim of this work was to explore whether similar LPS-core defects in H38ΔwbkF reduces the interference in *B. ovis* diagnosis while maintaining its efficacy against *B. ovis*. We constructed two mutants (H38ΔwbkFΔwadB and H38ΔwbkFΔwadC) carrying the expected core and O-PS defects, as shown by SDS-PAGE and Western-blot. In mice, both mutants showed marked attenuation with respect to H38ΔwbkF and did not protect against *B. ovis*. Thus, both were discarded for further research in sheep. These results confirmed the important role of the core for *Brucella* virulence and that over attenuation leads to ineffective vaccines. Therefore, we are exploring the use of the conjunctival route with H38ΔwbkF as a strategy to reduce the persistence of vaccinal antibodies and assessing protective efficacy of H38ΔwbkF by this route in rams.

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Keywords: *Brucella ovis*, Lipopolysaccharide, Serodiagnostics, Vaccine

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P5-03 Development of a *B. melitensis* Rev.1 mutant lacking streptomycin resistance

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Abstract

Brucella melitensis is the etiological agent of ovine and caprine brucellosis. Since this species is the major cause of human brucellosis, vaccination of small ruminants is essential for its control and eradication avoiding brucellosis in humans. However, *B. melitensis* Rev.1 (the only vaccine available) is abortifacient when applied to pregnant sheep and goats, virulent to humans and resistant to streptomycin (Strp), one of the antibiotics of choice for treating human brucellosis. The goals of this work were to study the streptomycin resistance mechanisms in Rev.1 strain and to correct genetically this resistance to develop a potentially safer vaccine. In bacteria, Strp resistance is associated with mutations in the 16SrRNA, in proteins of the 30S ribosomal subunit and in enzymes modifying the 16SrRNA. Thus, we compared the corresponding genes in Rev.1 and *B. melitensis* 16M (reference strain of the biovar 1 and Strp-sensitive [Strps]). Whereas the 16SrRNA sequences were identical, we identified point mutations in Rev.1 rpsL (encoding the 30S S12 protein) and rsmG (encoding a methyltransferase acting on N7 of G527 of 16SrRNA) leading to P91L (mutation previously described) and P81R changes, respectively. Consistently with rpsL essentiality, we could not obtain Rev.1rpsL mutants. However, we demonstrate the involvement of rpsL in Rev.1 Strp resistance by introducing the 16M rpsL gene in Rev.1. The rsmG deletion in 16M (BmeΔrsmG mutant) conferred Strp resistance, while rsmG deletion in Rev.1 (Rev.1ΔrsmG) did not affect Strp resistance. Introduction of 16M rsmG in Rev.1ΔrsmG (Rev.1ΔrsmG::Tn7BmersmG -abbreviated as Rev.1StrpS-) resulted in increased Strp sensitivity. Rev.1StrpS showed an attenuated profile both in BeWo trophoblasts and THP-1 monocyte-derived-macrophages. In mice, Rev.1StrpS conferred similar protection against *B. suis* and resulted in lower residual virulence and abortifacient effects than Rev.1. The mutant was proven safe in pregnant sheep (see the results of the work presented by, P. M. Muñoz.). Protective efficacy experiments are in progress in sheep.
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Keywords: Rev.1, Streptomycin, Vaccine, Ovine, Safety

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P5-04 Production and immunization of sheep using Irradiated *Brucella* Vaccine

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Abstract

Brucellosis is a highly contagious disease during animal abortions due to the created aerosols which may infect animals and humans in the surrounding area. Treatment of animals is not economically feasible, while the drugs to treat human disease are expensive and take a long time. The control of disease is mainly by animal vaccination. Irradiated *Brucella melitensis* Rev.1 vaccine was produced. Three groups of four animals (total 12) between the ages of 2 and 5 months were vaccinated. One group of ewes received the irradiated vaccine; a second group was vaccinated with live *B. melitensis* Rev.1, and the control group remained unvaccinated. Samples were collected twice a week and checked for humoral and cellular immunity using RBPT, cElisa, iElisa, cytokine Elisa, flow cytometry and RT-qPCR. The animals were challenged 18 months post- vaccination by being housed with infected sheep and goats (contact transmission). In addition, samples were collected during the challenge trial and checked for humoral immunity using RBPT, SAT and cElisa and iElisa. Bacterial isolation was also conducted using PCR to identify genus and species. Cytokine analysis is ongoing. *B. melitensis* Rev.1 irradiated vaccine was produced and was showed no growth and good metabolic activity. All animals vaccinated using live *B. melitensis* Rev.1 vaccine were serologically positive for brucellosis, while the other two groups remained negative. Furthermore qPCR and flow cytometry investigation indicated elevation of cytokines in the two groups that received either the live or irradiated vaccines, whereas unvaccinated animals indicated stable cytokine levels. The challenge trial of the vaccinated animals caused gradual appearance of brucellosis antibodies in the two vaccinated animal groups. The animal experiment was implemented using three groups of ewes; one unvaccinated as the negative control; one vaccinated using live *B. melitensis* Rev.1, and one vaccinated using irradiated *B. melitensis* Rev.1. All immunity monitoring results of the tested animal groups were compatible with the expected response when vaccinated with an efficient vaccine.
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Keywords: Brucellosis, Irradiated vaccine, Metabolic activity, Cellular immunity, Flow cytometry

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P5-05 Effectiveness of *Brucella abortus* S19 and RB51 vaccine strains: a systematic review

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Abstract

Brucella abortus S19 and RB51 are the most used vaccines to control bovine brucellosis worldwide; therefore, this study aimed to perform a systematic review on the effectiveness of these two vaccine strains (field trials to evaluate bovine brucellosis vaccines efficacy). The literature review was conducted on April 03rd, 2021 on five databases (CABI, Cochrane, PubMed, Scielo, Scopus and Web of Science) and included papers published between 1976 and 2014. The search strategy recovered a total of 6027 papers, which after selection based on title, abstract, full-text and considering the eligibility criteria ended up with 17 papers and 33 trials included. The strain most used in the trials was S19 (25/33, 75.75%), at the dose of 109 colony forming units (CFU) (18/25, 72%), by subcutaneous route (21/25, 84%), in a single dose (20/25, 80%), and in adult animals (23/25, 92%). RB51 was used in 8 (24.24%) trials, %), at the dose of 109 colony forming units (CFU) (5/8, 62.5%), mainly by subcutaneous route (8/8, 100%), in a single dose (4/8, 50%) and animals younger than 12 months (6/8, 75%). The higher field challenge (non-experimental) observed was 39% and the lower, 0.64% (mean 13.36% ± 12.89%). The incidence of brucellosis and/or abortion were the outcomes assessed in 12 (36.36%) trials, whereas reduction of abortion rate and/or reduction in brucellosis prevalence were the outcomes assessed in 21 trials (63.63%). The most used serology test to evaluate brucellosis infection was Complement Fixation test (26/33, 78.78%), followed by Rivanol test (24/33, 72.72%). The great heterogeneity in the initial prevalence of the disease before vaccination (natural challenge), in the vaccination protocols and doses used, and in the adoption or not of other control policies associated with vaccination, prevented a meta-analysis of the studies included in the systematic review. In conclusion, the systematic review results suggest that the S19 at the dose of 109 CFU is effective to reduce brucellosis prevalence in the herds when used in adult animals.
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Keywords: Bovine brucellosis, Vaccination, Field assay, Field efficacy

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P5-06 Development of inactivated *Brucella abortus* vaccines from *Brucella abortus* biovar 3

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Abstract

Bovine brucellosis caused by *B. abortus* is endemic in cattle in Bangladesh. It is a top priority zoonotic disease in Bangladesh. There is an urgent need to control brucellosis in livestock of Bangladesh. Although vaccination is employed in many countries to control bovine brucellosis but *Brucella* vaccines are not available to use in livestock in Bangladesh. The objective of this study was to develop inactivated *B. abortus* vaccines using *B. abortus* biovar 3 isolated from cattle in Bangladesh. Two inactivated *B. abortus* vaccine: *B. abortus* vaccine was prepared. Preclinical efficacy trail of the vaccines were conducted in mice. BALB/c mice at 6-8 weeks of age were immunized subcutaneously with *B. abortus* alum and oil adjuvant vaccines. Booster vaccine was administered at 28 days post vaccination (DPV). Challenge infection of vaccinated and control mice was given at 42 DPV with virulent *B. abortus* biovar 3. Sera were collected from five randomly selected mice at 7, 14, 21, 28, 35 and 42 DPV for detection of antibody response by rose Bengal plate test (RBPT), Indirect ELISA. Bacterial load in the spleen of mice were determined at 7 days post challenge. Cell mediated immune response (CMI) in vaccinated mice was measured by delayed type hypersensitivity (DTH) reaction. *B. abortus* specific antibody response was detected in 80% vaccinated mice by RBPT. The ELISA OD value of sera of alum and oil adjuvant vaccinated mice were (0.152±0.06 and 0.244±0.02),(0.155±0.02 and 0.252±0.003),(0.45±0.02 and 0.546±0.030), (0.65±0.01 and 0.575±0.009),(0.69±0.11 and0.696±0.005), (0.626±0.08 and 0.702±0.061) and (0.616±0.08) and 0.782±0.083) at 0, 7, 14, 21, 28, 35 and 42 DPV, respectively ($p<0.05$). Bacterial load of alum and oil adjuvant vaccinated mice (log105.64±0.43 cfu/g and and log10 5.35±0.50 cfu/g) was significantly reduced as compared to unvaccinated control (log106.45±0.78 cfu/g) ($p<0.05$). Swelling of footpad of mice was observed (1.16±0.13 mm, 1.14±0.16 mm and 0.96± 0.10 mm at 24hr, 48hr and 72hr, respectively). Data of this study indicates that inactivated *B. abortus* vaccine induces both humoral and CMI response and confer protection in mice against virulent challenge infection. Field trail of the inactivated vaccines in dairy cattle is under progress.

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Keywords: Inactivated *B. abortus* vaccine, BALB/c mice, Immune response, protective efficacy

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K6 The diagnosis of brucellosis

John McGiven [1]

Abstract

The diagnosis of brucellosis encompasses multiple scenarios, methodologies, requirements, objectives, hosts and pathogen species. Definitive diagnosis is only possible via isolation and identification of *Brucella* but this presents challenges due to imperfect sensitivity, biorisk, capability and cost. Other approaches are needed to navigate these issues including measurement of the host immune response and detection of DNA. Alongside epidemiological information the results from such tests can inform a suitable risk-based diagnostic interpretation and response. Despite the challenges of brucellosis control the diagnostic element is in many cases strong. For example, the *Brucella* sLPS is a gift to the diagnostician. It is abundant on the cell surface, is robust, multivalent, amphiphilic, T-independent and subject to the adaptive immune response and a single type may be universally applied for detection of infection with [nearly] all smooth stains. It underpins all primary serological assays, the appropriate implementation and interpretation of which should be a primary objective of any testing system. Diagnostic weaknesses include the non-universal attainment of good quality diagnostic antigen, an issue compounded by costs and the biological risks. Induction of false positive serological reactions due to vaccination or infection with cross reactive organisms occur - although their occurrence and significance varies considerably by circumstance. Measurement of the cellular immune response may assist diagnosis but access to the required reagents is poor. At the molecular level, recombinant and synthetic approaches offer some basis for insight and solution. Recombinant skin test antigen is being developed for bovine tuberculosis. Data using synthetic OPS based oligosaccharides suggests that antigen presentation impacts upon antibody induction - information which may provide new options for resolution of FPSRs. An alternative strategy is the application of other abundant cell surface antigens. The atypical properties of the rLPS core make this an attractive target. Select excipients appear to improve its diagnostic potential with respect to infection with smooth and with rough strains. At the global level the prevalence of disease in humans and animals remains poorly understood. Are limitations in existing diagnostic tools contributory to this knowledge gap? If so, what more can be done and how can this be achieved?
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Keywords: Diagnosis, Culture, PCR, Serology, Antigen

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O6-1 Investigation into efficacy of rLPS based serodiagnostic antigens

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Abstract

Antigens produced from *Brucella* rough lipopolysaccharide (rLPS) antigens have the potential to aid in the serodiagnosis not only of infection due to rough species and strains of *Brucella* but also infection due to smooth strains. In the latter case this would assist in resolving samples where a false positive result against smooth antigens (*i.e.* those containing O polysaccharide) may be suspected. A 'Rough Antigen' extract prepared from *B. abortus* RB51 using phenol/chloroform/petroleum ether (PCP) has been evaluated for the serodiagnosis of porcine (*B. suis*) and canine brucellosis (*B.canis*) and found to be highly effective. Analysis of the PCP fraction revealed it to be a protein free mixture of rLPS (~60%) and outer membrane phospholipids (~40%). We investigated if further purification of the rLPS may yield a diagnostically superior antigen and produced an antigen that was higher in rLPS purity (>95%). However, this antigen was diagnostically inferior. Furthermore, the depleted material (~5% rLPS) remained diagnostically similar to the original PCP extract. Fragmentation Mass Spectrometry of the depleted antigen suggested the predominance of phospholipids (including phosphatidyl choline) and ornithine lipids. Thin Layer Chromatography (TLC) of the depleted antigen confirmed this predominance and elution of the TLC fractions enabled the specific components to be tested by ELISA. This evaluation confirmed that the major antibody binding material in the extract was rLPS rather than any of the co-extractants. This finding raised the possibility that the presence of surfactants such as phospholipids is enabling and enhancing the diagnostic efficacy of the rLPS in the ELISA. To investigate this we evaluated a range of surfactants by using them to dilute the purified rLPS. The ELISA result from these dilutions showed no increase in the response to a positive serum in the case of dilution with octyl glucoside and CHAPS, but an increase of tenfold or more in the titre of the response after dilution with phospholipids or dodecylmaltoside. We believe that this observation is related to the critical micellar concentration of these compounds and demonstrates that for some antigens, the inclusion of non-antibody binding components can enhance diagnostic performance.
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Keywords: Canine, Diagnosis, Elisa, Lipopolysaccharide, Porcine

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O6-2 Cross-reaction comparative evaluation of five enzyme-linked immunosorbent assay and Golden Standard for porcine brucellosis diagnosis

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Abstract

Porcine brucellosis is listed as categories D and E disease in the regulation (EU) 2018/1882, Animal Health Law (AHL). The AHL (prescribes measures in the EU member states for trade conditions and surveillance/notification. Moreover, according to the regulation, the diagnostic methods should be validated in accordance with international standards for this purpose. Rose Bengal and Complement fixation tests are mainly used for *Brucella abortus*, *B. melitensis* and *B. suis* serological diagnosis. Although performed for brucellosis diagnosis in domestic animals, these tests were initially validated only for bovines. Furthermore, in pigs, it seems to have a high level of false positive serological reactions (FPSRs) caused primarily by *Yersinia enterocolitica* O:9. To date, no method for porcine brucellosis diagnosis has been validated for the intended purpose according to current World Organisation for Animal Health (founded as WOAH) principles. This study aims to compare the FPSRs performances of five ELISA kits for porcine brucellosis diagnosis [1ID Screen *Brucella suis*, 2ID Screen Brucellosis serum indirect multi-species (Innovative Diagnostics, France), 3Ingezim *Brucella* Compac, 4Ingezim *Brucella* Porcina (Ingenasa, Spain), and 5*Brucella abortus/melitensis/suis* DIVA (VMRD, USA)] comparing to Rose Bengal (RBT) and Complement Fixation test (CFT). A panel of 88 brucellosis-free pig sera from artificial insemination centers6 in France, that presented a serological positive reaction in at least one method (RBT, CFT or i-ELISA), were examined for analytical specificity (cross-reaction activity). The false positive reactions were observed in all tests: RBT (80/88), CFT (56/85) and 3 with anti-complementary reaction, Kit7 (6/88), Kit8 (55/88), Kit9 (81/88), Kit10 (44/88), and Kit11 (60/88). The RB and CF methods for brucellosis diagnosis showed a high level of cross-reaction comparing with ELISA kits for porcine brucellosis. ELISA is a robust and simple test to perform, however each ELISA kit presents different performances. Therefore, validation of this method for porcine species is needed for surveillance and trade.

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Keywords: Porcine brucellosis, ELISA, Serological cross-reaction

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O6-3 Machine Learning for MALDI-TOF MS identification of *Brucella*

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Abstract

MALDI-TOF mass spectrometry (MS) is a fast and reliable method for bacterial identification widely used. Most common databases used for this purpose lack reference profiles for *Brucella* species, hampering the correct species identification and reliable sub-species characterization. Here, we report the creation of peptide mass reference spectra, which were used to train a machine learning (ML) algorithm for predicting *Brucella* species. We selected two datasets composed of 107 *Brucella* strains for the ML alghoritm training, and 160 *Brucella* strains for validation. The strains have been isolated from our diagnostic activities and included: *B. melitensis* bv 3 and bv1, *B. abortus* bv 1 and bv3; *B. suis* bv 2, *B. ceti* and *B. ovis*. All strains were typed by means of molecular and biochemical analyses. Strains were heat inactivated, and protein extraction was performed using ethanol-formic acid protocol. The samples were spotted on a 96-spot steel plate target and covered with alpha-cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution (Bruker Daltonics) before MALDI-TOF MS analysis. The ML algorithm XGBoost was trained with features engineered from mass spectra observations related to the corresponding *Brucella* species. We used a 5-fold cross-validation, repeated 10 times, to evaluate 50 models whose hyper-parameters were selected according to the random search procedure for determining the greatest accuracy model. Subsequently, the selected model was validated by testing 480 samples from the validation dataset. The *Brucella* species identification had 99.4% accuracy with 100% diagnostic sensitivity (dSe) and specificity (dSp) for *B. abortus, B. ceti* and *B. ovis.* However we observed a small decrease of performance for *B. melitensis* and *B. suis* bv2 which showed 97.2% dSe and 99.2% dSp, respectively. Overall, the ML algorithm misidentified 3 *B. melitensis* with *B. suis* bv2. Our results showed that MALDI-TOF MS is reliable for *Brucella* identification to the species level from culture plates. The trained ML algorithm revealed to be specific and highly sensitive and appears to be an efficient and reproducible method for the rapid detection of the genus *Brucella*. Considering the presence of at least 12 *Brucella* species, the preliminary dataset needs to be enlarged for comprehensive representation of the entire genus.

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Keywords: *Brucella*, Machine Learning, MALDI-TOF MS

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O6-4 Swab types and storage conditions affect *Brucella* recovery and DNA detection

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Abstract

Brucellosis is a worldwide zoonosis that once established, is very difficult to eradicate with persistence in animals, environment and humans. The classical *Brucella* species, *B. abortus*, *B. melitensis* and *B. suis* affect mainly cattle, small ruminants, and pigs, respectively, causing abortions and infertility. Detection of bacteria in live animal can be very challenging, since infected animals do not show systematically clinical symptoms and excretion of bacteria in bodily fluids can be rare and/or intermitted. Sampling with swabs (genitalia, tissue lesions and contaminated environments) from seropositive animals with or without clinical symptoms, can be very useful for bacterial detection and isolation in bacteriological and/or molecular diagnostics. However, no comparison of swab types has been performed in order to ensure successful detection of *Brucella*. The aim of this study was to compare main commercially used types of swabs for sampling and diagnostics of *Brucella* spp., and determine the optimal storage conditions for testing. To achieve this, we tested bacterial and molecular methods for detection of classical *Brucella* species using nine swab types, all with different tip materials, treated immediately after spiking, after 72 h at +4°C, and after 72 h at -20°C. Our results show how storage conditions, freezing (-20°C) vs refrigeration (+4°C), negatively affect *Brucella* survival even on swabs compared to direct treatment, while no impact on qPCR detection was observed. We demonstrate that *Brucella* recovery and detection capacity strictly depend on swab tips type, independent of the storage conditions. In particular, bacterial survival is highly variable regarding the type of swabs used for isolation and storage conditions, while molecular detection is uniform. Interestingly, a new type of flocked swab, which includes a conservation medium, showed the highest levels of recovery in both bacterial culture and qPCR, irrespective of the storage conditions (20% higher with than without protective medium). The use of this swab provide increased *Brucella* viability at refrigeration and freezing temperatures, which significantly increases the diagnostic sensitivity compared to the other tested swabs. To further evaluate performances of flocked swabs under field condition, a multi-centric study targeting infected animals will be conducted within the European national Laboratory Network.

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Keywords: Swab, Storage condition, Recovery, qPCR, Bacteriology

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O6-5 Proteomics-based identification of immunodominant *Brucella canis* proteins as candidates for serodiagnosis and vaccine development

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Abstract

Canine brucellosis is an emerging disease and *Brucella canis* can be a potential health threat for dog holders. Diagnosing canine brucellosis has pitfalls since serological methods that recognize *B. abortus* and *B. melitensis* infections fail to detect anti-*B. canis* antibodies. Therefore, we used an immunoproteomics approach to identify immunodominant proteins as antigen candidates to improve serological testing. We performed two-dimensional gel electrophoresis with *B. canis* cell extracts followed by MALDI-ToF MS analysis and could correlate 182 protein spots with 82 *B. canis* proteins. Western Blot analysis of 2D SDS-PAGE gels using the sera from *B. canis*-infected dogs, verified by culture, PCR or serological tests, and sera from uninfected dogs, detected 50 immunoreactive *B. canis* proteins. Hence, 32 out of 82 identified proteins did not react with any sera, although seven of these proteins have been identified in previous studies as immunodominant in *B. abortus* and/or *B. melitensis*. A total of 14 proteins, like GroEL, DnaK or KatA, were false-positive hits since they reacted with sera of *B. canis* - free dogs. In contrast, 36 immunogenic proteins were detected by serum from bacteremic dogs and 16 out of these proteins also reacted with serum from infected but non-bacteremic dogs. Some of these immunoreactive proteins, *e.g.* three periplasmic substrate-binding proteins, seem to be specific for *B. canis* and have not been described before as immunodominant in *Brucella.* A subset of immunodominant proteins were also found in *B. canis* outer membrane vesicles that might serve as vaccination platform. In summary, we identified several immunoreactive proteins for the detection of acute and chronic canine brucellosis.

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Keywords: *Brucella canis*, Proteomics, Immunodominant proteins, Serodiagnostics, Vaccine

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O6-6 Preliminary study of interferon gamma test used for diagnosis of brucellosis in the water buffalo

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Abstract

Brucellosis (BRC) is a worldwide zoonosis with a strong economic and public health impact. In water buffalo, BRC is sustained by *Brucella abortus* and *B. melitensis*. Currently, diagnosis in ruminants is based on indirect serological assays required by actual legislation, the Rapid Rose Bengal Serum Agglutination (RBT) and the Complement Fixation (CFT) tests, that mainly detected antibodies against lipopolysaccharide (S-LPS) and thus, reveal the humoral immune response. In contrast, little is known about the cell-mediated (CM) response during brucellosis infection in buffalo. The aim of this study is to develop a diagnostic test based on the assessment of CM immunity. To this aim we are evaluating the use of the interferon-gamma test (IFN-γ), recently introduced for the diagnosis of bovine tuberculosis also in the buffalo species. Preliminary results of a field study are herein described. Serum and heparinised blood samples, collected from buffaloes, in 11 BRC-free herds (261 samples) and in 15 brucellosis-infected herds (158 samples), that were confirmed by bacteriological test, were analyzed. RBT and CFT assays were carried out according to the WOAH official procedures. Blood samples (1 mL) for the IFN-γ test were stimulated by two different concentrations of Brucellergene OCB (Zoetis, France) (40 U/well and 100 U/well) introducing phosphate-buffered saline (PBS), as negative control for the basal value of IFN-γ. The IFN-γ production was detected by the BOVIGAM TB kit (Thermo Fisher Scientific) according to the manufacturer's instructions. To evaluate the relationship between nonparametric values of CFT and IFN-γ assays, Chi-square statistic test was performed. Statistical analysis highlighted a higher association rate between CFT and IFN-γ tests positivity using the antigenic stimulus at 100 U/well (phi=0.32, P<0.0001) in respect to 40 U/well (phi=0.26, P<0.001). Although previous studies in bovine have been carried out at 40 U/well, our results suggest that in buffalo the antigen stimulation should be done at 100 U/well. These preliminary results suggest that the IFN-γ test could be used in buffalo as an ancillary test as its performance are similar to CFT test. Further studies are needed to evaluate the accuracy of the test.

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Keywords: Brucellosis, CM immunity, Diagnosis, Interferon-gamma test, Water buffalo

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P6-01 Diagnostic characterization of histopathological findings and bacterial isolation in canine brucellosis

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Abstract

Canine brucellosis is a critical problem in dogs leading to reproductive illnesses including abortion and infertility. *Brucella canis* significantly influences the reproductive organs targeting local lymph nodes (LN) and causing a fluctuating bacteremia. It may also have systemic effects in chronic cases. The aim of this study was to gain insight into the diagnostic characterization of *B. canis*-infected dogs using histopathological findings and bacterial isolation from different organs to further understand the pathogenic changes in canine brucellosis. We collected different specimens from 24 asymptomatic seropositive dogs, including 10 tissues (superficial inguinal LN, uterus, etc.), blood and urine. We performed an in-depth histopathological analysis and bacterial isolation to detect the pathogenic changes and presence of *B. canis* in the collected specimens. Of the 24 seropositive dogs, 20 were confirmed as true positives with *B. canis* cultured from more than one specimen. Within the bacteria-positive dogs, 15 (75%) showed remarkable histopathological findings in six kinds of tissues (submandibular LN, lung, liver, kidney, uterus, testis, epididymis), including granulomatous and suppurative lesions. The histopathological findings correlated with bacterial presence in a majority of the cases (66.7%). Dogs with histopathological lesions in the liver exhibited multifocal granulomas around the hepatic vein. Seven dogs had *B. canis* isolated from the uterus; one dog showing progressive endometritis with severe necrosis and brownish exudate in gross findings and a severe granulomatous pyoendometritis with inflammatory infiltration of neutrophils, lymphocytes, plasma cells. This study further defined the pathogenic characteristics of canine brucellosis, revealing bacteriological and histopathological findings. Moreover, it suggests that dogs infected with *B. canis* demonstrate significant systemic pathology as well as reproductive organ effects. Work supported by APQA B-1543081-2021-22-02.
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Keywords: *Brucella canis*, Pathological characteristics, Histopathological finding, Bacterial isolation

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P6-02 Use of *Brucella Ovis* antigen for canine brucellosis diagnostic by Agar Gel Immunodiffusion Assay

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Abstract

Canine brucellosis caused by *Brucella canis* is a zoonotic infectious disease that is common worldwide. This infection is not registered in Ukraine, due to the lack of diagnostic tools. One of the available laboratory diagnostic methods is the agar gel immunodiffusion test (AGID), which is recommended for testing of dogs with suspected brucellosis. The objective of the work was to identify an antigen for AGID to detect antibodies against *B. canis*. The study was conducted using thermo-extract R- antigen with 4×109 CFU of *Brucella ovis* 67/B strain. Reference anti-*B. canis* serum was used as a reference positive sample that was kindly provided by Claire Ponsart (ANSES). Antigen tests were performed by AGID according to George and Carmichael (1978) using field sera (n=547) taken from stray dogs from five regions of Ukraine. For comparison purposes, these samples were also tested using commercial C. *Brucella* Ab Test Kit / Rapid Test Kit (Bionote, Republic of Korea). The antigen was made by thermo-extraction of *Brucella ovis* 67/B. The determination of the resulting antigen activity was studied by AGID using the reference anti-*B. canis* serum in dilutions from 1:5 to 1:40. It was found that the minimum dilution at which a precipitation line was detected in agar is 1:20 in all repetitions. Furthermore, 54 (9.87%) samples of blood serum from dogs were positive, which indicated the circulation of *B. canis* in stray animals. All AGID positive samples were also confirmed using commercial C. *Brucella* Ab Test Kit / Rapid Test Kit. Our studies have shown the suitability of the obtained thermo-extracted R- antigen from *B. ovis* 67/B strain for its use in AGID to detect antibodies against *B. canis.* We also found a high seroprevalence of canine brucellosis among stray animals in Ukraine that may require the introduction of wide monitoring among domestic dogs.
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Keywords: *Brucella canis*, *Brucella ovis*, Canine Brucellosis, Diagnostics, Serological tests

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P6-03 The cloning, expression and immunological evaluation of the Omp31 protein from *Brucella melitensis* and evaluation of its possible use for diagnosis in bovine brucellosis

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Abstract

This work describes molecular methodologies for the production of OMP31r for the serological diagnosis of bovine brucellosis, which can be caused by biovars of *Brucella abortus*. Brucellosis is easily transmitted to man and causes an acute febrile illness, undulating fever, which can become chronic and cause serious multisystem complications. Unequivocal diagnosis of *Brucella* infection can only be made by isolation and identification of *Brucella* but in situations where bacteriological analysis is not possible, diagnosis can be based on serological methods. Furthermore, false positives can be expected in vaccinated animals, because the antibodies cross-react with infection by the wild-type strain. Serological monitoring tests typically use total antigen such as: *Brucella* buffered, *i.e.* rose bengal test (RBT) or buffered plate agglutination test (BPAT), or lipopolysaccharides in various ELISA formats. At this point, selecting one or more antigens and using molecular biology to obtain it in its recombinant form would provide a fast and efficient way to innovate in serological diagnostic methods. The Omp31 antigen has been reported for the diagnosis of brucellosis in sheep and goats. In this work, the Omp31 gene from *B. melitensis* was isolated to be cloned and expressed in a *Escherichia coli*, where the protein is obtained safely and in large quantities. In this research work, a wide variety of techniques such as bioinformatics, molecular biology, biochemistry and immunology were applied. Omp31r showed a sensitivity and specificity of 77 and 90% and was not recognized by sera from vaccinated animals and by a hyperimmune serum against *B. abortus* RB51, which is the strain used as a vaccine, which is important since it does not produce false positives, that is to say, it does not produce cross-reaction, and the consequent economic cost for the farmer of slaughtering an animal that is not really sick. The Omp31r has optimal performance and quality for use in practical laboratory work and research projects that require the serological diagnosis of Brucellosis sp. This achievement will undoubtedly allow commercial independence in relation to antigen production, taking a big step at the institutional level on the path towards the production of diagnostic kits against *Brucella*.

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Keywords: *Brucella abortus, Brucella melitensis*, Brucellosis, Serology, Bioinformatics, Serodiagnostics

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P6-04 Livestock brucellosis monitoring using bacteriological method in Ukraine for the period 2017-2021

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Abstract

The epidemiological and epizootic brucellosis situation remains tense in many countries around the world. The territory of Ukraine is free of brucellosis in farm and domestic animals. However, there is a risk of contracting the disease from Russia, Georgia, Turkey, and other countries during export and import operations. The aim of this study was to survey for brucellosis in livestock farms and wild faun, and to assess the epizootic situation of brucellosis in Ukraine. The study was based on biological material from seropositive animals, aborted fetuses, stillbirths, and game meat samples. Brucellosis was monitored using bacteriological methods in accordance with the WOAH Terrestrial Manual 2009. Chapter 2.7.2. Caprine and ovine brucellosis (excluding *Brucella* dependent), WOAH Terrestrial Manual Chapter 2.7.8 and "Guidelines for brucellosis diagnosis in animals", approved by the Ministry of Agro-Industrial Complex of Ukraine, State Department of Veterinary Medicine in 1998. For the period 2017-2021, the regional state laboratories of the State Service of Ukraine on Food Safety and Consumers Protection and the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise tested 1,327 samples of biological material (aborted fetuses, stillbirths, game meat samples to rule out brucellosis). In 2017, 542 samples [(cattle - 416 samples (76.8%), pigs - 109 samples (20%), small cattle - 17 samples (3%)]. In 2018 - 160 samples [(cattle - 116 samples (72.5%), pigs - 39 samples (24.4%), small cattle - 5 samples (3%)]. In 2019, 210 samples [(15.8%) (cattle - 178 samples) (84.8%), pigs - 29 samples (13.8%), small cattle - 3 samples (1.4%)]. In 2020, 240 samples [(cattle - 175 samples (72.9%), pigs - 57 samples (23.8%), small cattle - 8 samples (3.3%)]. During 2021 - 175 samples [(cattle - 122 samples (69.7%), pigs - 49 samples (28%), small cattle - 4 samples (2.3%)]. No brucellosis pathogens were detected using bacteriological testing or guinea pig bioassays. Given the danger of brucellosis to human health, through transmission of the pathogen through unpasteurized milk, cheese, butter, uncooked meat and offal, monitoring of livestock for brucellosis is mandatory.
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Keywords: Brucellosis, Bacteriological research, Ukraine, Biological material

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P6-05 Evaluating the OPS linkage composition for all biovar type strains of *B. abortus*, *B. melitensis* and *B. suis* and strains described by the WOAH for use in the production of vaccines and diagnostics

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Abstract

The O-polysaccharide (OPS) of smooth classical *Brucella* species is a critical virulence determinant, essential serodiagnostic antigen and a crucial component in two of the three vaccines described by the WOAH. Yet, despite its significance, the OPS structure has only been determined for a few (n=7) strains. For other strains the structure and approximate proportion of α1-2 or α1-3 glycosidic linkages is inferred from monoclonal antibody binding profiles; this determines the A or M dominance of strains. The genetic basis for linkage type and proportion has not been identified so cannot be inferred from sequence data or PCR. It is possible that linkage type may have a functional role for *Brucella*. The ratio and positioning of α1-2 or α1-3 may also play an increasing role in diagnosis and vaccine design given the advent of synthetic oligosaccharide antigens. To better characterise the OPS structure and define relative abundance of the α1-3 glycosidic linkages, we evaluated smooth type strains of *B. abortus* (n=10), *B. melitensis* (n=4) and *B. suis* (n=5) (including vaccine strains *B. abortus* S19 and *B. melitensis* Rev.1) and *Y. enterocolitica* O:9, using 1H Nuclear Magnetic Resonance (NMR). Assignments of the anomeric proton were made at chemical shifts of 5.05 ppm for α1-3 and 5.17 ppm for α1-2 linkage types. Relative abundance of linkage types was determined as a ratio of peak heights at these chemical shifts. We compared this ratio with monoclonal antibody (Mab) binding data (determined by iELISA). Structural analysis confirmed that α1-3 glycosidic linkages are present in all evaluated *Brucella* stains (other than *B. suis* biovar 2), in line with the abundance suggested by MAb binding profiles. A finding from this work that may have a more immediate application is that the OPS from the attenuated *B. abortus* vaccine strain S19 showed the same glycosidic linkage composition profile as the OPS from diagnostic antigen strains *B. abortus* S99 and S1119-3. Strain S19 may be a less virulent and safer alternative for use as a diagnostic antigen. SDS-PAGE demonstrated that S19 has similar length OPS to S19 and S1119-3, and this may also be an important diagnostic property.
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Keywords: Monoclonal antibody, NMR, O-polysaccharide, Structure, Antigen

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P6-06 The seroprevalence and geographic distribution of camel brucellosis in Kordofan States, Western Sudan

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Abstract

This work has been formulated to determine the special geographical distribution of brucellosis in camels in Sudan's Kordofan States (North, West and South) and to compare different diagnostic tests for their sensitivity, specificity and agreement. In this study we used competitive enzyme-linked immunosorbent assay (cELISA) as the gold standard as compared with the modified Rose Bengal test (mRBPT), Buffer Acidified Plate Antigen (BAPA), and Serum Agglutination Test (SAT). The study area was chosen according to movement of camels and high densities of camels and ruminants. A total of 388 apparently healthy camels were sampled during the period from May to December 2018 to determine the seroprevalence of brucellosis. Within the collected samples, 43 (11.08%), 41 (10.56%) and 30 (7,73%) were found positive by mRBPT, BAPA, and SAT respectively. The 43 mRBPT positive samples were tested by cELISA where 38/43 (88.4%) were confirmed positive. Using cELISA as the gold standard, a sensitivity of 52.4% and a specificity of 100% were recorded for BAPA and a sensitivity of 58.8% and a specificity of 83.3% for SAT. KAPPA coefficient agreement between cELISA and BAPA (16%) and between cELISA and SAT (31%) mean there is poor agreement between cELISA as a golden standard test and the other two tests (≤40 consider poor). The results of this study revealed that brucellosis occurs in camels from the Western States, which confirms and extends earlier findings regarding the widespread infection in camels in the Western Sudan. Accordingly with a map of camel brucellosis distribution, we acknowledge that control measures can be more effectively targeted in high prevalence areas like the Skiekan locality. *Brucella* organisms that infect camels are contagious for humans, and the disease is considered to have an economic impact as it affects reproduction, production, and camel trade; and it needs much more attention to attain successful control.

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Keywords: Camel brucellosis, Distribution, BAPA and cELISA

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P6-07 The comparison of a fourplex HRM and Taqman qPCR assays for the detection of *Brucella* spp. and *Coxiella burnetii* in ruminants abortion cases from Botswana

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Abstract

Brucellosis in livestock significantly impacts many countries' economies, primarily affecting animal health and productivity. It causes direct losses for the animal industry and is a barrier to the international trade of live animals. It is a zoonosis as well, hence affecting public health. A newly developed multiplex high-resolution melt (HRM)real-time (q) polymerase chain reaction (PCR) for simultaneous detection of four abortive zoonotic agents in domestic ruminants was compared with probe-based qPCR assaysto detect *Brucella* spp. and *Coxiella burnetii*. Ninety-seven (97) clinical samples from cattle, sheep, and goat abortion cases submitted to Botswana National Veterinary Laboratory from 2010 to 2021 for disease diagnosis, and 34 milk samples submitted to the same laboratory for routine quality control were subjected to fourplex HRM and Taqman assays. The fourplex assay detected *Brucella* spp. in 13 samples and co-infection of *Brucella* spp. with *Coxiella burnetii* in 45 samples.There was a perfect agreement between the HRM assay and each of the two qPCRs (kappa =1). Similarly, the Bland-Altman analysis showed a mean Cq difference of -0.7 for HRM versus *Brucella* qPCR and 0.63 for HRM versus *Coxiella* qPCR, suggesting a good agreement. In conclusion, the HRM fourplex assay is a valuable diagnostic tool to detect *Brucella* species with reliable Cq values comparable with the Taqman assays.

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Keywords: Abortive agents, *Brucella* spp., Real-time PCR, Ruminants, Zoonosis

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P6-08 Clinical and laboratory surveillance of suspected infections in kennel workers in an outbreak of *Brucella canis*

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Abstract

Humans and dogs commonly share the same domestic environment. Zoonotic pathogens may be harboured by dogs, and potentially be transmitted to humans and owners that often consider their dogs as family members. *Brucella canis* is one of those pathogens at risk of transmission to humans. The first evidence of *B. canis* in a dog in Italy was reported in 2010, based on serological and PCR findings. More recently, *B. canis* was isolated for the first time in Italy, during a large outbreak occurred in a commercial breeding kennel. Although canine brucellosis is considered a zoonosis, confirmed human cases are relatively uncommon, with roughly 50 cases identified in the US since 1973. Lab employees, voluntaries, veterinarians, owners and people having close and frequent contacts with infected dogs are considered at higher risk of infection. However, human brucellosis caused by *B. canis* is underestimated due to the general lack of specific serological tests and misconceptions concerning its prevalence. Uncontrolled spread of infection in dogs may have important public health implications so that actions are required to acquire data on distribution of this underhanded zoonosis of pets, as well as to control its spread in high-risk environments. Following detection of canine brucellosis in the Italian breeding kennel, more than one person among veterinarians and kennel's operators reported symptoms compatible with *Brucella* infection. Since all these people had an active and long lasting role in the management and handling of the dogs in the brucellosis outbreak, *B. canis* was not excluded as the possible cause of their illness. Diagnosis of human brucellosis relies on bacterial culture, molecular or mass spectrometry identification, and serological tests. Unfortunately, sensitivity of culture is extremely low and serological tests available for classical *Brucella*, are not effective to detect *B. canis* antibodies. Thus, the same tests available for dogs where applied to investigate suspect clinical cases arose in workers involved in the outbreak. In the view of a public health approach controlling for canine brucellosis, it would be important and urgent to develop validated laboratory protocols to support diagnosis of infection in humans.

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Keywords: Brucellosis, *Brucella canis*, Dogs, Zoonosis, Surveillance

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P6-09 Brucellosis in Buffaloes: Serologic Diagnostic

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Abstract

Brucellosis is an endemic disease in many regions of the world. Buffalo herds have been increasing, and it is praised for the elaboration of premium products. Buffaloes are frequently infected by brucellosis and several serological tests have been employed for the detection of this disease. Here we show comparative performance among these serological tests for diagnosis brucellosis in buffaloes. Blood samples were taken from a herd of 50 adult unvaccinated animals, where abortions were reported. Sera were frozen at -20° C until processing. Buffer Plate Antigen (BPAT), Flourescent polarization (FPA) and complement fixation (CFT) were simultaneously performed according to the Manual of Standards for Diagnostics Test and Vaccines (WOAH). FPA was carried out in a SENTRY 100® (Diachemix) fluorescence polarization instrument using a 1/100 serum dilution. CFT was done through micro method, titrating the complement to 50% of hemolysis. The same cut-offs (FPA: 105 mP; CFT: 41 ICFTU (International complement fixation test units) were used as in cattle for the determination of positive and negative animals. 22 samples were positive to BPAT, FPA and CFT. All sera that were positive for BPAT showed strong agglutination. FPA results obtained in the positive sera were in the range between 170 and 250 mP while the average measurement of the negative control was 75 mP. CFT was positive at very high serum dilutions in all confirmed samples. No false positive results for BPAT were obtained in any sample. FPA and CFT confirmed exactly the same results that BPAT anticipated. Serological diagnosis for brucellosis in buffaloes is sometimes controversially. These results suggested that FPA might be used as a confirmatory test for brucellosis in buffalo, due to the same performance relative to CFT, its adjustable cut- off useful in different epidemiological situations, its reliability and ease of performance. CFT is a very useful test, as long as proper equipment and trained laboratory professionals are available. We show here a very precise methodology using BPAT as screening test and FPA as confirmatory one. A larger study is needed in order to obtain a cut-off that responds to the current epidemiological situation of brucellosis in buffaloes.

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Keywords: Brucellosis, Buffaloes, Serological, Diagnosis

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P6-10 Development and validation of a real-time PCR assay for detection of *Brucella* spp. in samples of animal origin

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Abstract

Brucellosis is a zoonosis with serious implications for human and animal health. Direct diagnosis in domestic ruminants depends on the isolation of *Brucella* from abortion material, vaginal secretions, milk or from tissues taken after slaughtering. Even if serological methods are sufficient to determine the sanitary status of a herd, bacteriological isolation is still the gold standard to establish a definitive diagnosis of Brucellosis in a single animal. The culture-based method to detect *Brucella* spp. can include the use of an enrichment medium that could be incubated for up to 6 weeks. It presents several inconveniences such as: managing laboratory spaces, intensive manipulation of potentially infectious material with increased risk of infection for the laboratory personnel. The aim of this study was to develop and validate a robust and reliable real-time PCR designed to amplify a portion of IS711 genetic element which is unique to *Brucella* species. It includes a chimeric DNA as internal amplification control that is coamplified with the target sequence to monitor the presence of PCR-inhibitory substances in clinical samples. It allows a higher predictivity in identifying the positive samples, or in case of a negative result, it avoids the long-term culture based-methods. To validate the method a total of 335 samples from domestic ruminants and marine mammals were examined and the results were compared with those obtained using the culture method. The Receiver Operating Characteristic (R.O.C.) curve was calculated from the resulting data which allowed us to identify the optimal threshold value (best cut-off) with results of sensitivity, specificity, accuracy of 91.9%, 94.5%, 94.3%, respectively, which places the diagnostic method in the range of values attributable to highly accurate testing. This developed real-time PCR could represent a valid and timely tool for the diagnosis of brucellosis and, therefore, a support to the eradication program of this zoonosis.
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Keywords: *Brucella*, Real-time PCR, Diagnosis, Method validation

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P6-11 The Brucellosis conundrum: antibody vs microbial detection

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Abstract

Brucella is a highly monomorphic genus and consist of gram-negative pathogenic species. The disease is a systemic infection that can affect numerous organs and tissues and is shared between animals in a herd. Small ruminants and cattle are typically infected with *Brucella melitensis and Brucella abortus*, respectively. The presence o *Brucella* in livestock contributes to massive economic losses as well as veterinary and public health distress in developing countries. Due to its unspecific signs and symptoms that are similar to those of other febrile diseases, its slow growth rate on culture, and the complexity of its serodiagnosis, brucellosis remains difficult to diagnose. In the present study, samples including blood (serum), liver, kidney, spleen and lymph node tissues were collected from 275 animals (180 cattle, 60 sheep and 35 pigs) from 5 abattoirs in the Eastern Cape province, South Africa. A random sampling method was used for this study. Serum samples were tested using Rose Bengal test (RBT) and confirmation was done using Complement Fixation Test (CFT). Tissue samples were screened using the PCR for the detection of *Brucella* species and positive samples were subjected for culture. The PCR assay amplified *Brucella* directly from tissues in 77 of 180 cattle, 28 of 60 sheep and no *Brucella* was detected from the pigs. The direct culture technique detected *B. abortus* from 23 cattle and 1 sheep whereas mixed infections of *B. abortus* and *B. melitensis* were detected from 4 cattle and 3 sheep using AMOS-PCR assay. RBT revealed a seroprevalence of 2.9% (1 of 35), 6.2% (4 0f 60) and 6.1% (11 of 180) from pigs, sheep and cattle respectively. Only 1 cattle sample was confirmed positive on CFT. The result of this study emphasises the importance of using more than one diagnostic technique for the detection of *Brucella* species in livestock. The sensitivity and time rate of PCR technique suggest that the assay could be used for *Brucella* diagnosis in livestock from abattoirs. The study further shows again the low sensitivity and specificity of brucellosis serological tests for diagnosis.
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Keywords: Abattoirs, Brucellosis, Livestock, Seroprevalence

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P6-12 Evaluation of a new double-antigen indirect ELISA using a rough-LPS extract from *B. suis* for the diagnosis of porcine brucellosis

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Abstract

Current serological tools (complement fixation test -CFT- or Rose-Bengal test -RBT-) do not offer satisfactory results for swine brucellosis diagnosis. A recently developed indirect ELISA (iELISA) for the detection of anti- *B. suis* antibodies, based on a combination of two antigens derived from both rough (r-) and smooth (s-)*Brucella* LPS (McGiven et al., 2012), was shown to limit false positive serological reactions (FPSR). Based on the same concept, the ID Screen® *Brucella* suis Indirect is a double- well iELISA using smooth and rough extracts as antigens. Test performance was assessed by testing 3 groups of swine sera (origin: France): i) positive reactors from infected herds (n=286; positive in CFT and RBT) ii) FPRS from free herds (n=202; positive in RBT) iii) negative reactors from free herds (n=720, RBT negative). Results were expressed as S/P values and interpreted as per manufacturer's instructions. Animals are considered to be infected with *B. suis* when positive with both the s- and r-LPS antigens. FPRS should only react on the s-LPS, and are considered as negative. A Receiver Operating Characteristic (ROC) curve analysis was performed. Measured sensitivity was 72.7% (IC95% 67.5-77.9%, n=286); specificity was 99.9% (IC95% 99.6-100%, n=720). Out of 202 FPRS tested, 197 were found negative with the new ELISA, drastically reducing the false positive reactors rate: measured specificity on FPRS was 97.5% (IC95% 95.3-99.7%, n=202). Different cut- off values were tested; it is possible to increase theB. suis sensitivity without significantly affecting specificity. Increasing sensitivity could be of interest when testing herds where the presence *B. suis* has been already confirmed. To conclude, this new iELISA could improve surveillance and control programs of porcine brucellosis by reducing the impact of FPRS in herds, historically negative for *Brucella* infections.
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Keywords: Diagnostics, *Brucella suis*, ELISA, FPRs reduction

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K7 Challenges in human brucellosis

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Abstract

Brucellosis remains one of the most common zoonotic human infections, over a century after core methods of prevention were first implemented in Malta. Despite successful eradication in animals and humans from some countries, there has been expanded recognition of human brucellosis throughout southeast and east Asia. Infection has re-emerged in zones of conflict and it is a common cause of fever in North and East Africa. In Western Europe and North America, increasing canine infections with *B. canis* pose an uncertain threat to humans. The protean clinical features of human disease are well described but diagnostic awareness among clinicians is variable, especially outside endemic areas. Differentiation from tuberculosis is critical when deciding on therapy, particularly in resource poor settings. Diagnostic needs include: implementation of standardized serological tests; development of serological tests for organisms such as *B. canis*; consensus on the role of PCR and interpretation of significance of prolonged PCR positivity after treatment. Reference databases for matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and similar diagnostic tools need enhancement to differentiate *Brucella* spp. from each other and from newly incorporated species such as *Brucella* (*Ochrobactrum) intermedium*, which are rarely pathogenic. Therapy requires at least two antimicrobial agents to reduce the risk of clinical relapse, but there have been no well-designed prospective trials for decades to determine: the optimum dose, duration and safety of modern aminoglycosides instead of streptomycin; the role of fluoroquinolones; optimum duration of treatment for focal skeletal disease; the best therapeutic combinations and duration for children. Staff exposure to live organisms is still a problem in human and veterinary diagnostic laboratories. The need for dual therapy regimens for post exposure prophylaxis (PEP) is debatable and could be reduced to a single drug, with simpler post exposure monitoring programmes to improve PEP adherence. If safe vaccines become available, their use is likely to be limited to selected groups. Mortality should not exceed 1% but human morbidity is substantial, yet clinical research is difficult to fund and poses logistic challenges. This might improve if human brucellosis was formally recognized as a neglected tropical disease.
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Keywords: Brucellosis, Diagnosis, Epidemiology, Human, Therapy

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O7-1 Brucellosis in a farming community in central South Africa: A longitudinal cohort study (2015-2018)

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Abstract

Brucellosis is a widespread zoonotic disease of public health importance that is often associated with occupational exposure. Despite brucellosis being considered a priority zoonotic disease in South Africa, the true incidence and prevalence of the disease in animals and humans is largely unknown. To enhance the current understanding of human brucellosis in South Africa, the persistence of *Brucella* antibodies and the number of new infections in a farming community were investigated. A longitudinal study was conducted in a farming community within a 40 000 km^2 area in the Free State and Northern Cape provinces, South Africa. Participants (n=232) were selected from three cross-sectional studies conducted over a 36-month period from 2015-2018. Participants enrolled in at least the first and last surveys were included in the cohort. Seroprevalence was determined using an anti-*Brucella* IgG enzyme-linked immunosorbent assay (ELISA). Seropositive samples were tested further using an anti-*Brucella* IgM ELISA and immunocapture agglutination test to assess the antibody profiles. Statistical analysis was conducted using STATA v14. Of 232 participants, 87.9% (204/232) were from a farming population (people living and working on 102 domestic and game farms) and 12.1% (28/232) were (para)veterinary professionals. The median age was 39 years (interquartile range: 31-51 years) at first enrolment and 84.9% (197/232) were male. The IgG seropositivity was 13.4% (31/232), 14.2% (19/134) and 12.9% (30/232), respectively for the three surveys. Of the reactors, 83.9% (26/31) remained IgG seropositive and at least 6.5% (2/31) also had persistent IgM antibodies. Five of the participants seroconverted over the study period. This is the first longitudinal cohort study investigating human brucellosis in South Africa. A large proportion of reactors had persistent specific antibodies over the 36-month study period. Because unrecognize seroconversion was detected in the farming community, it is possible that more individuals were antibody-positive than would otherwise be reported, and that they could have ongoing infection if not treated appropriately. The findings from this study once again highlight the need to interpret serological results in light of the clinical context for the diagnosis of active brucellosis in high-risk populations especially in endemic areas.

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Keywords: Farming community, Human brucellosis , Longitudinal, Seropositivity, South Africa

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O7-2 First analysis of antimicrobial resistance in *Brucella* strains isolated in Italy

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Abstract

Brucellosis is one of the world's most widespread zoonosis. The disease affects livestock causing significant economic losses and can be acquired by humans through close contact with infected animal material or consumption of raw dairy products. While the treatment in animal brucellosis is not indicated, human brucellosis is generally treated with doxycycline combined with rifampicin or aminoglycosides. The levels of antimicrobial resistance (AMR) in *Brucella* strains circulating in European countries have not been estimated. The aim of this study is to evaluate antimicrobial susceptibility of *Brucella* strains isolated from livestock and humans in Italy from 2019 to 2022. A total of 165 strains were analyzed: 96 *B. abortus*, 61 *B. melitensis*, two *B. canis* and four *B. suis*. Additionally, two *Ochrobactrum anthropi* strains, isolated from a cattle and a human, were included in the study. The susceptibility to nine antimicrobials: gentamicin, streptomycin, doxycycline, tetracyciline, chloramphenicol, rifampicin, ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole were assessed using broth microdiluition method. Minimum inhibitory concentrations (MIC) values were interpreted according to CLSI guidelines for potential bacterial agents of bioterrorism and for slow-growing bacteria. More than a half of the analyzed *Brucella* strains (86/163) were susceptible to all tested antibiotics. The other strains were shown to be resistant/non-susceptible to two antimicrobials, rifampicin and doxycycline, only. Four strains of *B. melitensis* were full resistant to rifampicin (with a MIC of 4ug/ml). Intermediate resistant to rifampicin was detected in 73/163 (44.8%) *Brucella* strains and six of these, identified as *B. abortus*, were also non-susceptible to doxycycline (with a MIC of 2 ug/ml). The two *O. anthropi* isolates were resistant to rifampicin and chloramphenicol and non-susceptible to streptomycin. The *O. anthropi* strain isolated from cattle was additionally non-susceptible to trimethoprim/sulfamethoxazole. *Brucella* strains isolated from wild animals show higher susceptibility to some of the tested antibiotics than strains isolated from farmed animals. The data obtained indicate that a considerate part of *Brucella* strains circulating in Italy show a reduced susceptibility to rifampicin, one of the commonly used antimicrobials for human treatment. This suggest that monitoring of *Brucella* spp. AMR in animal populations could provide indications for most effective empirical treatment regime for locally-acquired human brucellosis.
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Keywords: Antimicrobial Resistance (AMR), Brucellosis, Minimum Inhibitory Concentrations (MIC)

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O7-3 Whole genome sequencing for tracing geographical origin of imported cases of human brucellosis in Sweden

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Abstract

Human infections with *Brucella melitensis* are occasionally reported in Sweden, despite the fact that the national flocks of sheep and goats are officially free from brucellosis. The aim of our study was to analyze 103 isolates of *B. melitensis* collected from patients in Sweden between 1994 and 2016 and determine their putative geographic origin using whole genome sequencing (WGS)-based tools. The majority of the strains were assigned to East Mediterranean and African lineages. Both in silico Multiple Loci VNTR (Variable Number of Tandem Repeats) Analysis (MLVA) and core genome Multilocus Sequence Typing (cgMLST) analyses identified countries of the Middle East as the most probable source of origin of the majority of the strains. Isolates collected from patients with travel history to Iraq or Syria were often associated with genotypes from Turkey, as the cgMLST profiles from these countries clustered together. Sixty strains were located within a distance of 20 core genes to related genotypes from the publicly available database, and for eighteen isolates, the closest genotype was different by more than 50 loci. Our study showed that WGS based tools are effective in tracing back the geographic origin of infection of patients with unknown travel status, provided that public sequences from the location of the source are available.
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Keywords: *Brucella melitensis*, MLVA, MLST, WGS, cgMLST, Brucellosis

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O7-4 Screening of FDA approved drugs to treat Brucellosis

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Abstract

Treatment of Brucellosis in humans for six to eight weeks with a combination of doxycycline plus an aminoglycoside and/or rifampicin has been shown to be effective in the majority of cases. But they're a small percentage (2 to 5%) of such treated cases has been shown to relapse; relapse rates were higher with two-week regimen irrespective of combination of therapeutics. Therefore, there is a need for novel therapeutics that can effectively treat brucellosis with no cases of relapse. There is no cost-effective treatment of Brucellosis in food-producing animals. Drug repurposing is an efficient way of generating novel clinical opportunities for already existing drugs with the advantage of an economical accelerated drug development timeline with reduced costs. In the current study, we screened the MedChemExpress library consisting of FDA- approved drugs and additional clinical molecules against *Brucella abortus*. Out of the 2,591 drugs, 87 drugs were identified that did inhibit *B. abortus* at a concentration of 8 µM or less. The minimum inhibitory concentration (MIC) of the eleven drugs identified in the initial screening were tested against *B. abortus* using commercially certified concentrations of drug set. We found that Auranofin, Cinacalcet and Setraplatin are the most potent non-antibiotic drugs, identified in the MIC assay. They were further evaluated against two clinically important species *i.e.*, *B. suis* and *B. melitensis*. The MIC values were similar and in the range of 0.06-4 µg/ml. Synergistic activity with approved antibiotics doxycycline and gentamicin against *Brucella*.
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Keywords: Drug screening, FDA approved drugs, Minimal Inhibitory Concentration, *Brucella*

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O7-5 Human brucellosis exposure in confirmed cutaneous anthrax cases, Dien Bien, Vietnam with an update on human prevalence regionally

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Abstract

Anthrax and brucellosis are important zoonoses worldwide. Often, they are reported separately with separate control strategies in livestock; routine vaccination for both accompanied by culling for brucellosis. The status of both diseases is poorly understood in Vietnam. In Vietnam, anthrax has been identified as a priority zoonotic disease for control in a One Health Circular (#16, 2013). Vietnam has a likely substantial, but poorly understood, brucellosis risk. There were no data available for Vietnam in the 2006 global assessment. Brucellosis rates are unknown, but the disease has been confirmed for humans and livestock, including recent genotyping in southern provinces. Here, we implemented the fluorescence polarization assay (FPA) using the Sentry 200 handheld FPA reader (Ellie Labs) and the B1002 test kits (based on an O-polysaccharide for *Brucella abortus*, which also reacts with *B. suis* and *B. melitensis*. We performed tests on human and animal samples. Human serum samples (collected 2011-2016) were randomly selected from the NIHE serum bank and livestock samples were provided from NCVD and NIHE (2015 - 2018); additional swine samples were provided by ILRI. We are currently running active hospital surveillance across six provinces in northern Vietnam, with 1,018 samples collected to date and 312 tested. We confirmed 1.39% human exposure to brucellosis (5/359; 95% CI: 0.045% - 3.22%). Four of those exposures were detected in Dien Bien province and two of those were confirmed cutaneous anthrax cases. The two co-infections were detected in family members from a single household in 2011 with the remaining two cases independent of each other in 2015. The fifth case was reported from Ha Nam province in 2016. These results suggest brucellosis may be widespread underappreciated/underreported in Vietnam. Preliminary results from ongoing surveillance suggest similar prevalence rates. We tested 1107 animals. While human samples were limited to northern Vietnam, animal samples were widely distributed. Domestic swine from southern Vietnam were 9.4% (17/180) seropositive. A second group of swine samples from ILRI had 2.2% seroprevalence (11/500). The remaining samples represented domestic cattle/buffaloes; all sero-negative. Our results suggest brucellosis surveillance for Vietnam is warranted and future policy might list brucellosis as reportable.

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Keywords: Brucellosis, Brucellosis human, Vietnam, Anthrax, serology, Surveillance

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O7-6 Going chronic: about a complicated case of pediatric brucellosis being the first 'endemic' incident of human Brucellosis in Belgium (2021-2022)

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Abstract

Brucellosis is a major worldwide bacterial zoonotic infection caused by *Brucella* spp. In Belgium, the disease incidence is low, and the few yearly notified cases are of imported nature. Here, we report the case of a 4-year-old Belgian boy initially diagnosed and treated for uncomplicated brucellosis, which nine months later relapsed to neurobrucellosis. Confirmatory diagnosis of the acute disease occurred end of July 2021 through a combined assessment of serology and positive blood culture. The isolated strain was identified as *Brucella melitensis* bv3. Antimicrobial susceptibility testing did not highlight resistant phenotypes and combination therapy of trimethoprim/sulfamethoxazole and rifampicin was administered for 6 weeks. The epidemiological investigation pointed to 'endemic' contamination due to regular consumption of raw-milk-derived cheeses sold within a participative Belgian market. Multiple Locus Variable-Number Tandem Repeat Analysis (MLVA) and core-genome multilocus sequence typing (cgMLST) traced back the genetic background to a clade of Sicilian strains. In June 2022, the child was re-admitted to the pediadric intensive care unit (PICU) for fever and seizures. Less than 24h after admission, when neurobrucellosis was highly suspected, he was treated with IV ceftriaxone, rifampicin, trimethoprim- sulfamethoxasone and dexamethasone with the aim to give 6 weeks of tritherapy followed by a bitherapy for a total duration of 1 year. The clinical and neurological progress was excellent and the patient was discharged on home at day 12. Our case is a call of attention to both public health authorities and clinicians. Raw milk and its products have become increasingly desirable for many European families as a natural and better nutrition food. Awareness about the hazards associated with the consumption of raw milk and its products should be promoted among end-users. On the other hand, patient management in the context of pediatric brucellosis remains challenging, given the paucity of reported data. Diagnosis of chronic cases is yet difficult and specific laboratory tests/biomarkers to confirm chronic brucellosis are needed. The surveillance of brucellosis in the food chain could be upgraded considering the growing evolution of the social economy in Belgium and the European Union.

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Keywords: Antimicrobial susceptibility testing, Cheese, MLVA, Neurobrucellosis, Pediatric, Whole genome sequencing

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P7-01 Brucellosis of veterinary health care workers engaged in *Brucella* vaccination program in West Bengal, India

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Abstract

Recently the National Animal Disease Control Program in India targeted a massive *Brucella* vaccination program. In the state of West Bengal about 6000 Veterinary healthcare assistants participated in the program in the month of September, 2021. Among them more than 400 Veterinary healthcare assistants developed symptoms and signs suggestive of brucellosis. Laboratory tests based on STAT, RBT, and ELISA indicated 13% confirmed Brucellosis cases. However, there were also many asymptomatic cases which were not tested. Most of them stated history of needle prick injury during the vaccination program, which was mainly due to disturbed animals. Manifestations started after about a month of the prick injury. Few of them also complained regarding their improper training and supply of proper personal protective equipments. All of them treated in referral hospitals and a standard protocol of Government Health Department was followed. During follow up, it was found that some of them showed gross psychiatric problems. Thus proper training of the Veterinary healthcare assistants is necessary and care should be taken during vaccination of animals. A prophylactic treatment in healthcare assistants with needle prick injuries should be given, and supply of personal protective equipments should be monitored.
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Keywords: *Brucella* vaccination, Needle prick injury, Occupational brucellosis

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P7-02 Human brucellosis, an important however a neglected zoonotic disease: status in occupational and non-occupational groups in Punjab (India)

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Abstract

Brucellosis is one of the major neglected zoonotic diseases in India, and has been reported in animals and humans across almost all states of the country. Several health agencies have been working on this pathogen all over India. The Centre for One Health (COH), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) has been established with the aim to strengthen the One Health Framework to work on one health issues in India. The Centre is very actively engaged in the surveillance of antimicrobial resistance, food safety and several endemic zoonotic diseases including brucellosis. Brucellosis not only affects the health of animals but also has impact on human health especially of occupational risk groups, for example veterinarians and dairy farmers. The tertiary care hospitals are now including human brucellosis in differential diagnosis in cases of fever of unknown origin. In the present study, where in COH is rendering brucellosis diagnostic facility to the various stakeholders in Punjab state of India, a total of 417 human blood samples were tested for *Brucella* agglutinins using Rose Bengal plate test and serum tube agglutination (STA) tests during the period 2020-22. The blood samples were of the veterinary personnel, dairy farmers who approached COH for brucellosis testing and of the patients with history of fever of unknown origin referred by the multispecialty hospital. Out of 417 samples, 85 (20.3%) were found positive for antibodies to *Brucella* with STA titre ranged from160-10240 IU. Forty-one out of 161 patients referred by multi-speciality hospital for brucellosis were seroprevalence; majority of them belong to non-occupationally exposed population. The findings revealed the endemic status of brucellosis in the state, thus reflecting the continuous exposure of human to this zoonotic disease, and indicates the presence of infection in both occupational as well as non-occupational groups. Veterinarians and farmers most of the time due to nature of their work have the awareness about this pathogen, but non-occupational groups need to be educated about the transmission, prevention and control of this disease.
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Keywords: Brucellosis, Punjab, India, Occupational exposure, Non-occupational group

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P7-03 The epidemiology of human brucellosis in the British Isles 2000-2020: an ongoing travel-related threat in a non-endemic region

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Abstract

Although successfully eradicated in some parts of the world, brucellosis remains one of the most significant zoonotic diseases. Here, we describe human cases of brucellosis in the British Isles, where bovine brucellosis, caused by *B. abortus*, has been successfully eradicated, and ovine/caprine brucellosis, caused by *B. melitensis*, has never been reported. In total 188 cases of brucellosis were confirmed by biotyping of isolated cultures submitted by hospitals to the WOAH an FAO Refernce Laboratory for Brucellosis (APHA, UK), largely isolated from blood, between 2000 and 2020. A significant number of bacterial isolates submitted were excluded as *Brucella* and a subset of these isolates were identified by 16S rRNA sequencing to be organisms potentially confounding hospital automated or semi-automated bacterial identification systems. The vast majority of confirmed *Brucella* cases were caused by *B. melitensis* (91%) and, where data were available, all appeared associated with travel to endemic regions - most commonly Somalia, Turkey, India, and Saudi Arabia. These findings confirm that *B. melitensis* appears by far the most significant *Brucella* species associated with human brucellosis globally. There were a smaller number of cases of *B. abortus* (6.4%), none of which were associated with international travel outside of the British Isles (UK and Republic of Ireland). They appear to reflect more recent eradication of bovine brucellosis from Ireland, or - in one case - possible reactivation of latent brucellosis acquired prior to eradication in Great Britain. Although known to be a potentially significant human pathogen, only two cases (1.1%) were caused by *B. suis*. Molecular typing of isolates by multi-locus variable number tandem repeat analysis (MLVA) and multi-locus sequence typing (MLST) revealed clustering consistent with the likely geographical origin of cases and, where no travel history was available, could be useful in indicating the potential source of infection.
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Keywords: Brucellosis, Human, Epidemiology, Molecular epidemiology

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P7-04 Evaluation of a Real-Time PCR for the diagnosis of Brucellosis in humans

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Abstract

Diagnosis of brucellosis is made by culture and serological tests. Microbiological isolation is considered the gold standard; however, it requires a minimum of viable bacteria in the sample and an incubation period of up to 4 weeks. The time limitation led to the evaluation of a new molecular biology method based on real-time PCR for rapid and more sensitive detection of *Brucella*. To evaluate a molecular biology test for the detection of *Brucella* spp. in humans. The design of primers and probes was carried out using the Beacon Designer v8.1 (BD8) program, using the sequence of the 31 kDa outer membrane protein (BSCP31) for genus, and for the detection of specie the alkB gene regions and BMI1162 for *B. abortus* and *B. melitensis*, respectively. An internal reaction control (IPC) was added, with the purpose of positive validation of samples. The PCR test was evaluated using 64 samples of blood from individuals with suspected brucellosis. These samples were also processed by serology with Rose Bengal, standard agglutination and agglutination tests in the presence of 2-mercaptoethanol. Statistical analysis was performed with a 2×2 contingency table using EPIDAT 3.0 software. The designed oligonucleotides were successfully tested, a detection limit of 5 fg was obtained, which is equivalent to 1 CFU in the serum sample. The diagnostic sensitivity and specificity were 97.2 and 96% respectively. In this work the identification of *Brucella* spp. at the level of genus and species was performed by a highly sensitive real-time PCR test. This test considerably is reducing the identification time for *Brucella* species whereby we can recommend it as a support tool for the timely diagnosis of human brucellosis.

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Keywords: Brucellosis, Diagnostics, Human Brucellosis, Diagnosis, Molecular biology

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P7-05 The seroprevalence of Brucellosis and molecular characterization of *Brucella* species circulating among preslaughtered animals and workers in slaughterhouses in Amman, Mafraq & Karak Governorates

Ekhlas Hailat,[1] *Tareq Al Sanouri*,[1] *Sereen Iweir*,[1] *Mohannad Al Nsour*[1]

Abstract

Brucellosis is a zoonotic disease of occupational nature, and it is endemic in Eastern Mediterranean countries including Jordan. In 2022, we conducted a seroprevalence and molecular study in Jordan among the high-risk occupation of slaughterhouse workers in the central slaughterhouses of three governorates, Amman, Karak and Mafraq, to study brucellosis risk factors and estimate *Brucella* seropositivity among pre-slaughtered animals and workers. A questionnaire was administered, and blood samples were collected from all workers (356) in the slaughterhouses (Amman n=300, Karak n=22 and Mafraq n=34). Job categories represented in the study included administrative staff, veterinarians, slaughterers and animal skinners, viscera handlers, loaders of live animals, meat sellers, carcass handlers, cleaners, and drivers. The collected samples were tested for *Brucella*-specific IgM and IgG antibodies using the enzyme-linked immunosorbent assay (ELISA). The positive samples were then tested using polymerase chain reaction (PCR). Anti-*Brucella* antibodies were detected in 11.8% of the samples. The seroprevalence rate was highest among viscera handlers (15.7%) followed by cleaners (15.6%), and slaughterers (11.7%). About 35.4% reported eating while working, and 19.9% don't wear any personal protective equipment (PPEs). Moreover, 140 blood samples were collected from animals in each slaughterhouse (Amman n=88, Karak n=20 and Mafraq n=32), and serologically tested. Close to 16% were positive by Rose Bengal, and 37% were positive by Fluorescent Polarization Assay. All human and animal samples were negative by PCR. In conclusion, brucellosis is an infection of occupational health importance. The use of PPEs and safer working practices in slaughterhouses should be advocated. This can be done by conducting awareness sessions in endemic countries like Jordan. Regular laboratory testing of brucellosis in animals arriving at slaughterhouses should be conducted.
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Keywords: Brucellosis, Zoonosis, Epidemiology, Slaughterhouses, Jordan

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P7-06 Prevalence and risk factors of Brucellosis in lactating cow and its public health importance in military dairy farms of Bangladesh

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Abstract

Brucellosis is a great concern for dairy farming globally including in Bangladesh. It is also an important zoonosis having public health significance. The seroprevalence, risk factors, and species of *Brucella* prevalent in Bangladesh are known to large extent but the military dairy farms are totally unknown. The objective of the current work was to determine the Sero-molecular prevalence, identify risk factors, detect *Brucella* species, and assess workers' knowledge, awareness, and practices relevant to the transmission of brucellosis and its public health importance in Military Dairy Farms of Bangladesh. Serum and milk samples from 1003 lactating dairy cows of eight military dairy farms and 715 serum samples of dairy farm workers and hospital patients were collected during the 36 months from 2017 to 2020. Five different commercial diagnostic test kits were used to detect the prevalence of *Brucella* infection for testing all the collected sera and milk samples. Multivariable logistic regressions were used to identify important risk factors for brucellosis. The overall 2.39% seroprevalence of brucellosis was recorded with all the CFT, SAT, and ELISA assays and 3.09% with RBT, whereas only 0.20% of tested milk samples showed positive with MRT in the lactating dairy cows. The result also showed that Out of 715 tested human sera, only 4 (0.5%) milker's sera were positive for RBT with a 95% confidence interval of 0.2 to 1.5. Brucellosis was also significantly higher in cows that calved more than three times odds ratio [OR] =3.7; (95% confidence interval [CI]: 1.5-9.1) than those calved one to two times. The odds of brucellosis were about 43 times higher (OR = 42.9; (95% confidence interval [CI]: 10.7- 100.107) in aborted cows than in non-aborted cows. This study showed that 55.4% had adequate knowledge about brucellosis as a human disease. Results suggest that *B. abortus* was endemic and first time identified as human brucellosis in occupationally exposed military dairy farm workers in Bangladesh. The risk factors identified in this study would help to prevent, control, and eradication of brucellosis not only in eight military dairy farms but also in other dairy industries in Bangladesh.

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Keywords: Prevalence, Risk factors, Brucellosis, Lactating cow, Public health

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K8 Control of Brucellosis in domestic ruminants. Yes we can!

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Abstract

The incidence of human brucellosis is persistently high and even increasing. However, the situation of the main reservoir of this zoonosis with major health and economic consequences, namely the domestic ruminant population, remains largely unknown. Indeed, apart from reports on the situation in few regions where sound and effective programmes are in place, and a few targeted studies on limited areas in various countries, there is currently no clear picture of the epidemiological situation and the control programmes in place around the world. It is surprising, in this respect, that presentations on this subject at international congresses are rare, as this congress again demonstrates. Clearly, some knowledge or tools still need to be developed or refined. However, the experience gained in several regions, notably Europe and North America, and the research carried out over many decades show that effective tools are available and the appropriate methodology sufficiently advanced to make substantial progress in the control or even eradication of this reservoir. Resource constraints, both human and financial, but also and above all a lack of political will to conduct long-term control programmes in close association with the concerned stakeholders, are obviously to blame. This presentation, which opens the session on 'Epidemiology, control and eradication', aims to reiterate the main elements of a diagnostic, health decision and control strategy based on the knowledge acquired in the epidemiology of the disease and on the basic principles of infectious disease diagnosis in general. It is hoped that, at a time when the One Health concept is on everyone's lips, the animal and public health authorities will become more actively engaged in the fight against this infection, which is so detrimental to both human and animal populations.
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Keywords: Brucellosis, Control, Epidemiology, Domestic ruminans, key features

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O8-1 T-Racing: a contact tracing tool for supporting epidemiological investigation during livestock disease outbreaks

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Abstract

A user-friendly web application (T-Racing) was developed in an R environment to support epidemiological investigations through interacting maps, network graphs, and tables. The application relies on Temporal Network Analysis techniques and accounts for the dynamic nature of animal trade, to perform a quick and efficient back and forward tracing activity. T-Racing makes use of web services to retrieve data from diverse Official National Databases, through the plumber package and has been distributed using Shinyproxy. Movement data underlying the spatial-temporal tracing pattern can be explored according to time frame, animal species, movement to slaughterhouses, and movements in and out of the selected farm. The forward/backward search can be performed from one or more farms, selected among those which had moved animals or notified outbreak of disease within a selected time frame and or falling in a user-defined spatial buffer. Considering search results may often involve thousands of farms/movements (which would not be possible to visualize or use, when many), the T- Racing application is able to provide tools that may interactively and iteratively handle huge networks. Users can add or remove links/nodes according to epidemiological information and/or spatial location, node's property, and network deepness,to get the most relevant results, including paths among outbreaks caused by genetically similar pathogens. Moreover, the result can be restricted to paths followed by single identified animals linked to the seeds. This tool may strongly support animal disease control measures since it can rapidly organize essential contact information from large datasets, and identify connections in a multi-area outbreak investigation thus supporting the epidemiological investigation.

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Keywords: Animal Health, Epidemiology, Tracing, Temporal Network Analysis, Shiny

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O8-2 Follow up investigations on non-infected dogs adopted from the *B. canis* outbreak

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Abstract

Canine brucellosis due to *Brucella canis* is a contagious disease characterized by abortions in females and epididymitis, testicular atrophy, prostatitis and infertility in males. In April 2020 a *B. canis* outbreak was notified for the first time in Italy. The infection occurred in a breeding kennel in central Italy that was hosting mostly chihuahua but also other toy breed and involved more than 600 animals. After confirmation of infection, a ban for animal movement and selling was applied. A no-kill strategy was implemented for outbreak management and a procedure was developed to identify *B. canis* non-infected dogs subsequently given for adoption, this in order to reduce kennel population. This study describes the procedure applied to select *B. canis* non-infected animals eligible for adoption and report results of follow-up laboratory investigation carried out on adopted animals finalised to exclude any late occurrence of infection. To reduce the dog population hosted in the infected kennel a protocol was developed that combined neutering and *B. canis* serological testing. Negative animals were transferred to a buffer kennel for a second round serological and bacteriological testing. Negative animals were considered eligible for adoption. Following adoption, a procedure for tracing and monitoring rehomed dogs was developed and is unde implementation. More than 300 animals were identified as *B. canis* non-infected and rehomed. To date, over 12 months since first adoption, none of the rehomed animals developed infection. Our results suggests that whenever applicable, rehoming of *B. canis* negative animals should be considered and included in the toolkit for management of *B. canis* outbreaks.

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Keywords: *Brucella canis*, Outbreak management, No-kill strategy, Rehoming

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O8-3 Risk factors for the persistence and spread of Brucellosis in buffaloes in the province of Caserta (2015-2020)

Maria Ottaiano,[1] *Roberta Brunetti*,[1] *Paolo Calistri*,[2] *Alessio Di Lorenzo*,[2] *Daria Di Sabatino*,[2] *Francesca Cito*,[2] *Paolo Chiodini*,[3] *Loredana Baldi*,[1] *Esterina De Carlo*[1]

Abstract

Despite the fact that brucellosis has been eradicated in many countries, it is still present in various geographical areas and causes considerable economic and health damages. In Campania, the infection is present in the provinces of Salerno and Caserta, where in the latter province a drastic increase in the prevalence and incidence of infection in buffalo has been observed in the last years. Campania is the area with the highest concentration of buffalo farms in Italy. About 80% of Campania's buffalo population is present in Caserta. This zootechnical activity is characterized by the presence of over 1400 farms and about 300000 heads, occupying a prominent role in the agricultural production system and representing an important component of the regional economy as part of buffalo mozzarella from Campania DOP. The results of official controls carried out from 2015 to 2020 in the buffalo farms of the Caserta Province were analysed. The data was extracted from the National Veterinary Information Systems and from the Laboratory Management System of the Experimental Zooprophylactic Institute of Southern Italy. The statistical analysis was carried out through the software R version 4.1.0. The dataset consists of 4,583 serologically controls in buffalo farms, of which 353 were positive over the six years considered. The association (Chi Square and Wilcoxon Tests) between a series of covariates was examined and the response variable "presence / absence" of the disease in the company was evaluated. A mixed effects logistic regression model was carried out to evaluate the contribution of possible factors over time and quantify the risks for the development of the disease. The infection is concentrated in the geographic areas with the highest density of animals and farms for Km2. More than 50% of the positive farms were repeatedly positive over time. From the analysis of the model, it emerged that the presence of abortions, a higher number of animal movements and herds density are significantly associated with the detection of infection in herds. The presence of infection's geographic clusters suggests the presence of environmental factors that may have facilitated the spread of brucellosis between neighbouring farms.

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Keywords: Brucellosis epidemiology, Buffalo, Risk analysis, Caserta

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O8-4 Outbreak of bovine brucellosis in the Bargy mountain: special feature of *B. melitensis* infection in cattle

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Abstract

Since France is officially free of bovine brucellosis, a single autochthonous outbreak of cattle brucellosis occurred in 2012 in the Bargy, linked to a wild reservoir of *B. melitensis* in Alpine ibex. On October 12, 2021, a positive ELISA result on pooled milk sample was confirmed during the monthly serological monitoring in a farm of 240 dairy cattle. Two cows presented a positive reaction after brucellin testing and one of them was seropositive. Post mortem investigations of both cows led to the isolation of three *Brucella melitensis* isolates from the lymph nodes and milk of a primiparous cow that calved on September 14, 2021 and grazed in 2020 in the Bargy mountain pasture. Sequencing of the 3 isolated strains demonstrated the great genomic proximity with the strains of the 2012 cattle household and those isolated from ibex since 2012. The slaughter of the herd in January 2022 mobilized 12 people on site for 2 days for the collection of 1516 samples (1462 samples from 214 cattle; 54 samples from 18 euthanized calves). Bacteriological cultures were performed from blood, three pairs of lymphnodes, spleen, genital swab and genital tract. Samples were processed by the entire network of approved local laboratories with negative results. Three females presented non-negative serological results at slaughter (1 Rose Bengal (RB) positive, 1 RB and Complement fixation (CF) positive, 1 doubtful ELISA). Additional analyses are underway (molecular analyses, enriched cultures), in order to investigate animals that have presented particular risk factors (abortion, FC or ELISA result close to the threshold, taking colostrum from the infected cow, transhumance). Current results are in favor with a recent infection, together with a limited intra-herd dissemination. The authors would like to thank the French authorities, the laboratory network (LDA 73, LDA 01, LDA 13, EVA (LDA 31), LABOCEA, PUBLIC LABOS (LDA TARN), TERANA), the slaughterhouse and staff who contributed to the investigations. Presenting author: claire.ponsart@anses.fr

Keywords: Outbreak, *Brucella melitensis*, Ibex, Cattle, Whole genome sequencing - WGS

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O8-5 Human brucellosis: First calculated estimate of global annual incidence

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Abstract

Brucellosis presents a major concern worldwide, especially for people living under resource-limited settings. Until this date, a reliable estimate of the global annual incidence has been elusive for human disease. In order to truly comprehend its burden, it is vital for the international community to recognize its distribution and frequency. To address this gap, we employ a novel approach in which available data from the World Organization of Animal Health (WOAH), World Bank, and the United Nations (UN) is used to estimate the annual number of newly infected people worldwide and regionally, utilizing risk metrics and at- risk populations. Estimates are developed based on three different models that utilize Bayesian inference, bootstrap resampling, and weighted average interpolation that take into consideration missing information, underreporting, inadequate diagnostic capacity, and diagnostic misclassification. Alarmingly, a conservative estimate of the annual global incidence is many times higher than the historical speculation. Incorporating diagnostic misclassification into the models expands the estimate to an alarming order of magnitude. As expected, the vast majority of the estimated cases are predominantly occurring within Africa and Asia. Nevertheless, although the magnitude of the burden is significantly lower in areas within the Americas and Europe, some of these regions are still a concern and should be closely monitored for potential disease reemergence. As human brucellosis is evidently neglected in the regions where the risk of disease is most prominent, its reinstatement as a priority Neglected Zoonotic Disease by the World Health Organization (WHO) should become urgent to curb the disease spread.

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Keywords: *Brucella*, Brucellosis, Brucellosis epidemiology, Brucellosis incidence, Human brucellosis

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O8-6 Insights into the seroprevalence of *Brucella canis* infection in dogs in Portugal

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Abstract

In Portugal canine brucellosis due to *Brucella canis* has historically not been regarded as endemic, but little is known about the real epidemiologic situation. Laboratory testing is only done in animals traveling from Portugal to endemic areas, or after veterinarian request following clinical symptoms. Serological positive reactions are rarely reported, and *B. canis* has never been isolated in Portugal. As it is not considered a notifiable disease, no official prevalence data are available, representing a challenge for studying its epidemiology. The purpose of this retrospective study was to get insights into the occurrence of *B. canis* in dogs in Portugal. We screened a collection of serum samples received between 2014 and 2021 at the National animal health reference laboratory (INIAV) for serological control. The collection includes 642 samples from different regions in Portugal, including Azores and Madeira. All samples were tested by complement fixation test (based on *B. ovis* antigen, CFT-*B. ovis*); from these, 438 samples were also tested by rapid slide agglutination test (RSAT and ME-RSAT) and/or immunochromatographic test (ICT). In addition, 252 samples from dogs showing clinical symptoms (including blood samples, vaginal swabs, aborted fetus) were submitted for detection of *Brucella* spp. with polymerase chain reaction (PCR). There was no positive serology for smooth *Brucella* spp. The frequency of positive serologic results was 9,7% (62 in 642 dogs) using CFT-*B. ovis*, but 7,3% of the tested samples showed anti-complementary reaction. From the 62 positive dogs, 21 tested positive in RSAT, ME-RSAT and/or ICT. Regarding the samples submitted to PCR, 19% (48/252) resulted positive. Although the results obtained belong to pre-selected samples (not reflecting the national occurrence) collected from a heterogeneous group of dogs, this first preliminary results suggest a low seroprevalence of *B. canis* infection in Portugal. The study still ongoing in order to increase the number of samples, the geographic coverage and the evaluation of breeding kennels across Portugal.
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Keywords: Canine brucellosis, *Brucella canis*, Serological tests, Seroprevalence

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P8-01 Develop a series of maps to predict the spatial distribution of Brucellosis in South Caucasus

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Abstract

Zoonotic diseases are an important cause of human morbidity and mortality; around 75% of recently emerging human infectious diseases are zoonoses. The movement of people, goods, and animals, globally, as facilitated by free trade and tourism may allow emerging diseases to spread quickly. Animal movement caused by natural migration, trading and marketing livestock represents an especially critical aspect in dissemination of zoonotic diseases. In order to predict the spatial distribution for selected pathogen - *Brucella* spp., we developed models linking geo-referenced covariate information on geographical, ecological, social factors with geo-referenced samples of the selected pathogen. The proposed work will modernize national surveillance and identify areas at risk for Brucellosis in the South Caucasus. Quantitative disease ecology provides useful tools to analyze spatially referenced data on distributions of animal hosts and infectious agents, and their spatial concordance with environmental and social parameters. Animal movement caused by trading and marketing livestock represents an especially critical aspect in dissemination of zoonotic diseases. The Atlas will identify areas where brucellosis diseases might occur, but human cases have not been recognized, but where further surveillance should be targeted. Effective surveillance systems rely on local and national participants' ability and willingness to accurately report disease outbreaks, and their capability to implement local and national responses. Early identification of zoonotic disease emergence is essential to rapidly contain outbreaks, yet many local and national authorities lack the human and technical capability, capacity, and supporting financial resources to do so. By developing the Atlas, we will acquire information on the specifics of where and how the pathogen emerged, in what populations, in the past and, by extension, identify other similar regions where their circulation has gone undetected.
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Keywords: Atlas, Brucellosis, Caucasus, Surveillance, Zoonoses

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P8-02 Knowledge, attitude and practices associated with Brucellosis among livestock owners in Baure Local Government Area (LGA) of Katsina State-Nigeria

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Abstract

A questionnaire was adopted and modified to assess the knowledge, attitude, and practices regarding brucellosis among livestock owners in the Baure local government area (LGA) of Katsina State, Nigeria. Of the 80 participants, the majority had heard about animal brucellosis (89%); but few (11%) had heard about human brucellosis. Based on this survey, 59% of the respondents were unaware about the zoonotic potential of brucellosis and have poor knowledge of the hazards associated with milk consumption, as 57% drink soured or fermented milk, 37% drink raw unpasteurized milk, and only 6% drink pasteurized milk. Fever is believed to be a sign of brucellosis by 51% of the respondents and is frequently misdiagnosed as malaria. The questionnaire revealed 95% practice natural mating for herd reproduction; and 58% and 29%, respectively, of the respondents purchase animals based on experience and trust. A majority of the owners had 1, 2, 3, or >3 abortions in their herd within the study period; and 81% have never screened their animals for brucellosis. Eighty-five percent of those surveyed are unaware of the existence of *Brucella* vaccines, while 15% know about vaccines against brucellosis; however, 94% never vaccinate their animals against brucellosis. There is little understanding about brucellosis screening as 79% of the participants are not familiar with that practice. Categorical data analysis based on the 4-point scale grade 75% of the respondents as having poor knowledge of brucellosis, 13% have an average knowledge, and only 12.5% have good knowledge of brucellosis. There is a lack of positive attitude and practice based on a 2-point scale with 90% has poor practices. The current study revealed that the knowledge and understanding about brucellosis among livestock owners in Baure LGA is very limited.

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Keywords: Brucellosis, Surveillance, Zoonoses, Katsina State-Nigeria

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P8-03 16S Microbiome characterization of *Brucella* positive and negative raw milk samples collected from three regions in Tanzania

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Abstract

Brucellosis is endemic in many low- and middle-income countries, including Tanzania, where the disease is often transmitted via the consumption of raw milk or direct contact with infected animals/products. *Brucella* species can localize in the mammary lymph nodes and glands of infected dairy animals that may shed the pathogen in milk for extended periods of time and present a significant health risk to consumers of unpasteurized dairy products and individuals in direct contact with infected animals. Since the sale and consumption of raw and unprocessed milk is common amongst pastoral and agropastoral communities in Tanzania, large proportions of the population are susceptible to milk-borne and zoonotic diseases, including brucellosis. We have recently initiated a five-year cross-sectional survey to assess risk factors associated with brucellosis, including raw milk consumption in Tanzania. To determine the total microbial diversity present in raw milk and to identify potential associations with other pathogens or microbial signatures of milk from infected animals, characterization of *Brucella*-positive and negative samples using next-generation sequencing is being performed. In brief, we have collected 176 bovine milk samples from dairy cattle (76), and Boran crossbred cattle (100) from seven herds in three regions of Tanzania, Dodoma (Central), Morogoro (East), and Tanga (Northeast). Milk samples were collected aseptically, and Fluorescence Polarization Assay (FPA) was used to screen the milk samples as an initial screen to ascertain *Brucella*'s status. DNA extraction from the FPA+ and select FPA- milk samples were performed by applying a magnetic-based extraction method, followed by real-time PCR-based detection of the IS711 insertion sequence to confirm the *Brucella* status. Studies to assess 16S rRNA-based microbiome diversity will next be performed, and genomic DNA will undergo PCR with primers 515F-806R to amplify the V4 region of the 16S gene for microbiome profiling following the Earth Microbiome Project protocol Next-generation sequencing of the 16S RNA and Illumina short-read sequencing and are in progress.

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Keywords: 16S Microbiome, Tanzania, Raw milk, *Brucella* Positive and Negative, Cattle

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P8-04 Molecular phylogenetic correlations of *Brucella abortus* strains isolated from specific regions in South Korea

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Abstract

Bovine brucellosis is a re-emerging disease in South Korea with a recent increase in incidence on cattle farms in specific regions. Given this, a molecular phylogenetic investigation is a practical approach to tracking the infection source to reveal the reliable etiological evidence of the incidence. We analyzed phylogenetic characteristics using multi-locus sequence type analysis (MLSA) to prove the genetic correlation of Brucella abortus strains isolated from two specific regions, Jeonnam (JN) and Gyeongnam (GN). We analyzed 81 *B. abortus* strains from domestic cattle in nine provinces within these two regions in the last three years using MLSA with 18 specific single nucleotide polymorphisms (SNPs) which could display phylogenetic characteristics from genome sequences verified by draft whole genome sequencing (WGS). Fifty-eight strains isolated from eight JN provinces were divided into two sequence types (STs), either ST3 or ST5. All 23 strains from the one province in the GN region belonged in only ST3. In comparison with 172 internal reference strains isolated from other regions, 49 strains from four (4/8) provinces of JN region and all strains from the GN province were classified as ST3, which accounts for 31.2% of the internal reference strains. Formation of this ST3 cluster in five specific provinces over the three year period indicated a considerable molecular epidemiological relationship among these areas. Accordingly, we found *B. abortus* strains having the same genotypes distributed in some specific regions that could circulate across certain areas by indigenous strains. Furthermore, we propose that this investigation will be an integral part of providing reliable genetic evidence to support an effective action plan to control disseminating the disease. Work supported by APQA B-1543081-2021-22-02.
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Keywords: *Brucella abortus*, Phylogenetic correlation, MLSA, Molecular epidemiological relations

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P8-05 STOP Mr. BruBug: an educational tool to aware people about brucellosis

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Abstract

Brucellosis, an important zoonotic disease across the globe especially in thedeveloping nations, leads to economic as well as public health loss. Several measures, for example vaccination, surveillance, test and-cull, biosecurity measures etc. can be used as control strategies to combat this problem. In addition, health education about brucellosis could also encourage the occupational risk populations to adopt and implement control measures. Health education has been considered as a significant part of disease control, and several educational tools have been developed for educating people about the transmission and control of path-ogens having impact on human health, for example, *Taenia solium, Taenia saginata, Schistosoma* spp. soil transmitted helminths etc., however to the best of our knowledge, no such tool on *Brucella* spp. has been developed. We developed 'STOP Mr. BruBug', a game to educate both occupational and non-occupational risk groups. The educational tool has three levels; each level would help to improve knowledge regarding the transmission, prevention and control of brucellosis among several stakeholders, for example, veterinarians, medical professionals, livestock farmers, policy makers and laypersons. Further, to see the impact of this educational tool on the knowledge level of the stakeholders, evaluation will be performed, and we believe that this tool would help to enhance the knowledge regarding prevention and control of brucellosis among stakeholders.

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Keywords: *Brucella abortus*, Phylogenetic correlation, MLSA, Molecular epidemiological relations

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P8-06 *Brucella* infection in stray cattle reared in cow shelters (gaushalas) in Punjab, India: lessons learned from an observational study

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Abstract

Bovine brucellosis is an important zoonosis across several states of India.,and has been reported in domestic animals and people. Data on the prevalence of brucellosis in stray cattle in India is limited, however this information would be helpful to provide epidemiological information to develop control policies to combat this infection and its further transmission to humans and animals. Study was conducted in stray cattle reared in animal shelters (also called gaushalas in local language) in Punjab, India; of 23 gaushalas visited (one from each district of Punjab), 587 blood samples were collected and tested to assess the exposure to *Brucella* spp. Of 587 sera, 62 [10.56% (95% CI: 8.33%, 13.31%)], 63 [10.73% (95% CI: 8.48%, 13.50%)] and 68 [11.58% (95% CI: 9.24%, 14.43%)] were positive for the presence of antibodies to *Brucella* using Rose Bengal Plate Agglutination Test (RBPT), Standard Tube Agglutination Test (STAT) and Indirect Enzyme Linked Immuno Sorbent Assay (I- ELISA), respectively. The estimated true prevalence of 11.48 % (95% CI: 8.9%, 14.64%), 10.69 % (95% CI: 8.27%, 13.67%), and 13.28 % (95% CI: 10.50%, 16.66%) was observed using RBPT, STAT and I-ELISA, respectively. Exposure to *Brucella* spp. in animals was detected in 22 out of 23 cow shelters (gaushalas) and shelter/herd level prevalence was determined using combination of tests in series, *i.e.* if it was positive in RBPT or STAT result, and a positive indirect ELISA result. The shelter/herd level prevalence was estimated to be 96 % (95% CI: 79%, 99%). After adjusting for other variables in the final model, history of abortion was associated with very large odds of having a positive test (adjusted odds ratio 7.43, 95% confidence interval: 3.25-16.99, p = < 0.001). History of ROP was also associated with greater odds of having a positive *Brucella* infection test (adjusted odds ratio 5.25, 95% confidence interval: 1.38-19.88, p = 0.01). Infection with *Brucella* spp. in stray cattle indicates a potential risk to the farm workers and dairy farms. Further investigations are required to generate more epidemiological data on status of bovine brucellosis in stray cattle as well as shelter workers in Punjab and India.

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Keywords: Brucellosis, Cow-shelters, Gaushalas, India, Prevalence, Punjab

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P8-07 *Brucella canis* in Great Britain: Cases, case definitions, management and control

Anna Haughey,[1] *Andrew Taylor*,[1] *Roland Ashford*,[1] *Stephen Wyllie*,[2] *Andrew Frost*,[2] *Katherine Russell*,[3] *Zoe Gibney*,[3] *Nicholas Beeching*,[4] *Alessandro Gerada*,[5] *Adrian Whatmore*,[1] *John McGiven*[1]

Abstract

Canine brucellosis is commonly characterised by reproductive disturbances and discospondylitis in dogs. It is mainly caused by *Brucella canis* (*B. canis*), a zoonotic bacteria which is primarily transmitted through sexual contact between dogs and contact with infectious abortion material. Contact with other fluids have also been reported to transmit the bacteria, however, these are less infectious. Prior to 2017 no cases of canine brucellosis had been bacteriologically confirmed in Great Britain (GB) and serological evidence of infection was also exceptionally rare. In 2017 *B. canis* was isolated from two dogs (separate cases) imported from Eastern Europe, followed by a third bacteriologically confirmed case in 2018 and then a fourth larger case in 2020, also bacteriologically confirmed. Between January 2020-April 2022 there have been 58 cases in GB as determined primarily based on serology and epidemiology. Investigations have resulted in the testing of 171 dogs and 100 of these were serologically positive and, in combination with the epidemiological information, considered infected. Twelve were confirmed positive by bacteriology. Cases have been associated with dogs that have originated from Romania, Macedonia, Bosnia, Hungary, Afghanistan, South Africa, and Greece. GB have adopted a risk-based approach to determine the qualitative probability that a dog is infected and the likelihood of onward transmission of infection. The assigned probability alongside individual factors and wider implications informs the level of investigation required and the approach to controlling the spread of disease. To aid detection of cases in GB, from February 2021 positive tests for *B. canis* were made officially Reportable. So far, no domestic onward transmission of *B. canis* has been observed in GB dogs apart from one case in 2020 and one case in 2022. There is one confirmed case of human brucellosis due to infection with *B. canis* (at the time of writing). Teams at the Animal and Plant Health Agency continue to work closely with colleagues from GB Public Health Agencies and specialist clinicians from the *Brucella* Reference Unit at Royal Liverpool & Broadgreen Hospital to control the disease and protect human and animal health.

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Keywords: *Brucella canis*, Serology, Culture, Epidemiology, Risk

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P8-08 Seroprevalence of Brucellosis, isolation and characterization of *Brucella* and identification of the associated Risk Factors in small ruminants with history of abortion at two districts of South Omo Zone, Ethiopia

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Abstract

Brucellosis is one of the contagious neglected bacterial diseases of domestic and wild animals, caused by bacteria of the Genus *Brucella*, and distributed worldwide including Ethiopia. However, there was scarcity of epidemiological data on its occurrence in pastoral areas. A cross-sectional study was conducted from September 2018 to June 2019, to estimate the seroprevalence of brucellosis and isolate *Brucella* from small ruminants in two randomly selected pastoral districts, out of eight districts in South Omo Zone, Ethiopia. A pre-tested questionnaire was used to clarify the purpose. Blood samples were collected from a total of 124 small ruminants with history of abortion for serological test. Subsequently, 30 vaginal swabs were investigated from seropositive animals for *Brucella* isolation. All serum samples collected were screened serologically using the modified Rose Bengal Plate Test (mRBPT) and sera positive with mRBPT were confirmed with Complement Fixation Test (CFT). An overall seroprevalence in small ruminants with history of abortion was 21% (26/124; 95% CI: 0.14 - 0.28) using combined mRBPT and CFT. A multivariable logistic regression analysis revealed that risk factors considered in the study districts: species, history of abortion (OR: 0.28, 95% CI: 0.18 - 0.43), and parity numbers (OR: 0.20, 95% CI: 0.059 - 0.72) were significantly associated with *Brucella* infection. *Brucella* was isolated from 5(16.7%) of the 30 samples cultured on *Brucella* Selective Agar. All isolates, 5(16.7%) were from vaginal swabs. The isolates were *B. melitensis* based on biochemical, and bacteriological culture test result, though further test is required at biovariant level. In conclusion, the present serological test showed that brucellosis is highly prevalent among aborted small ruminants in the study area. Moreover, the isolation of *B. melitensis* from aborted goats' vaginal swabs may be considered one of the confirmatory for the *Brucella* infection. Therefore, strategic control measures should be implemented, such as regular testing of breeding animals to reduce brucellosis is required to reduce its economic impact and risk of zoonotic infection in the area.

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Keywords: Abortion, South Omo Zone, *Brucella*, Isolation, Seroprevalence, Small ruminants

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P8-09 An epidemiological study of Brucellosis in camels (*Camelus dromedarius*) in Khartoum State, Sudan

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Abstract

A study was conducted from April to September 2012, to determine the seroprevalence and risk factors for brucellosis infection in camels (*Camelus dromedarius*) in Khartoum State, Sudan. A total of 415 camels in 39 herds were included in the study from four localities and blood samples were collected and screened by RBPT. Twenty four samples tested positive giving an individual animal prevalence rate of 5.8%. All RBPT positive reactors were further tested by cELISA which confirmed 21 seropositive cases out of 24 RBPT reactors (87.5%). Eighteen herds were found seropositive among the 39 herds included in the study giving a herd prevalence of 46%. In the univariate analysis there was a significant increase in seropositivity of brucellosis in camel with respect to age and herd size (P≤ 0.05). Conversely, governorate, locality, sex, feeding, type of management, type of production, contact with other camels, source of new camels, source of water, housing, contact with other ruminants and contact with dogs were not found significantly associated with brucellosis (P≥ 0.05).Multivariate analysis showed that large herd size comprising more than 20camels was significantly associated with seroprevalence of camel brucellosis (Exp B=5.660; 95% CI: 1.258 -25.463; P≤ 0.05). The results of the present study indicate that *Brucella* exists within the camel herds in Khartoum State. The disease is widely distributed among large camel herds in the State. Further studies need to be done on *Brucella* infection in the other ruminants to determine which measures should be followed for control of brucellosis.

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Keywords: Camel brucellosis, Risk factors, Rose Bengala Test, cELISA Prevalence

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P8-10 The One Health Integrated investigation for brucellosis suspected cases-Shargelneel locality, Khartoum State. Sudan, February 2016

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Abstract

On 2nd February, 2016, cases of human brucellosis were reported from Sharg elneel locality, they suspected milk bought from nearby dairy farms to be the source of their infection. An outbreak investigation was done jointly to verify the existence of an outbreak, identify the source of infection and to raise awareness of health cadres. It was decided to study patients who fulfill criteria of case definition of brucellosis, from the 1st of January 2015 to 2nd February, 2016. Five patients were investigated; simultaneously random blood samples were collected from two dairy farms and awareness posters were distributed. Medical records of nine health facilities were reviewed. 27 health cadres were interviewed. Two awareness workshops were conducted. The effect of this intervention was assessed. All five patients investigated were positive for brucellosis. Three of five had a history of unpasteurized milk consumption, and one had direct contact to animals. 20 blood samples were randomly selected from two dairy farms, six were positive for Rose Bengal Test and the results was confirmed by ELISA. Nine physicians were interviewed two of them considered brucellosis priority in diagnosis of fevers and diagnosed cases, one of nine is not aware about treatment protocol. Nine lab technicians were interviewed for six of them the diagnostic kids is available, two registered positive cases, all of them are well aware and trained. All nine statistician were didn't register cases of brucellosis. Consumption of milk was the main cause of the outbreak. The burden of brucellosis is not clearly defined. Brucellosis is neglected in clinical diagnosis. Awareness of community is weak.We recommended assessing the effect of intervention within six months period, raising awareness of medical cadres and study prevalence of human brucellosis in Sharg elneel locality. Integration with veterinarians is important in prevention and control.

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Keywords: One health

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P8-11 *Brucella canis* serological survey in kennel dogs in North of Italy: case reports and preliminary data

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Abstract

Canine brucellosis is a worldwide infection and zoonotic disease caused by *Brucella canis* (Bc), which major public health concern is due to close contact between dogs and humans. In dogs, brucellosis most commonly manifests with reproductive symptoms, lymphadenopathy, osteoarticular and neurological disorders, although the occurrence of asymptomatic and chronic infections are frequently reported. The present study aims to assess the seroprevalence in 227 kennel/free-ranging dogs, 30% female and 70% male dogs, average age 5 years old (range 1-16 Y/o), enrolled within the Ricerca Corrente IZSVE 12/19. The dogs were screened for Bc specific antibodies through a microplate serum agglutination tests (mSAT). Dog's sera were tested using two-fold dilutions (from 1:20 to 1:640) and incubated for 48 hours at 37°C. In case of serologic titers ≥1:20, samples were submitted to the National and WOAH Reference Laboratory for Brucellosis, where complement fixation test (CFT), immunofluorescence test (IF) and bacterial isolation from blood and urine were attempted. Moreover, PCR targeting *Brucella* spp. genes was performed with DNA extracted from the clinical samples. Results reported an overall serologic prevalence of 1.77 % (4/227). Among the positive animals, one dog (entire male, 7 Y/o, mixed breed) with a concurrent parasitic disease (Trichuris spp.) was found positive for *B. canis* antibodies (mSAT 1:40) and negative for the bacterial isolation: this dog was found seronegative one month later. The second significant case was a dog (entire male, 5 Y/o, Rottweiler) with testicles decreased in volume, reporting a positive serology (mSAT 1:20, CTF 1:10, IF 1:80) and negative bacterial isolation. The dog was re-tested after one month: mSAT 1:20, CTF negative, IF 1:40, negative bacterial culture (blood and urine), and borderline positivity for real time PCR *Brucella* spp. This dog was consequently neutered and further investigations are ongoing. Currently, prevention and control of canine brucellosis are not easily achieved, especially due to the difficulty in identifying infected dogs: the development of novel diagnostic methods and hopefully of specific regulations will represent a crucial point for surveillance of canine brucellosis, and raising awareness among human health in a One-Health perspective.

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Keywords: *Brucella canis*, Serology, Epidemiology, Diagnostics Methods

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P8-12 First confirmed domestic transmission of *Brucella canis* between dogs in the UK: outbreak investigation and public health risk assessment

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Abstract

Brucella canis (B. canis) causes a zoonotic infection of dogs. It is primarily transmitted to humans via exposure to reproductive fluids, particularly from parturition and abortions. Human infections are rarely reported world-wide but could pose a serious risk for vulnerable individuals. Historically, UK *B. canis* cases have only been reported in imported dogs. In June 2020, a canine infected with *B. canis* was reported to Public Health England and an Incident Management Team convened. We aim to describe the outbreak and implications for the public health risk assessment. Self-reported information from the index and linked households, microchip database searches and site visits were used to identify humans and dogs potentially exposed to *B. canis* and assess ongoing risks. Humans and dogs were tested for *B. canis* serologically and cultures were performed on tissue samples from two dogs. Dogs were categorised as positive, non-infected or suspected for *B. canis* according to the long-term management plan. Nine households were potentially exposed to *B. canis*. There were nine canine abortions or stillbirths in the households in the preceding 18 months. Three humans had a single positive Rapid Slide Agglutination (RSA) test at 6 weeks, but negative results for subsequent samples. The positive results were determined to be of uncertain significance. The three patients were asymptomatic and did not receive treatment. 75% (27/36) of dogs tested were seropositive and both cultures grew *B. canis*. All seropositive dogs originated from the index household. Puppies born to seropositive parents were sold to a further six households and are being followed up. This outbreak of *B. canis* identified the first documented transmission of *B. canis* between dogs in the UK. Despite high-risk exposures over a prolonged period no humans developed symptoms of brucellosis during the investigation. Increased awareness is needed among dog owners, veterinarians and health professionals about the risks and prevention of transmission of *B. canis*.

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Keywords: *Brucella canis*, Field Epidemiology, Outbreak

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P8-13 Changes of laboratory findings in two dogs infected with *Brucella canis* following antibiotic treatment and orchiectomy: a case report

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Abstract

Canine brucellosis caused by *Brucella canis* (*B. canis*) is an emerging infection affecting dogs worldwide and potentially transmissible to humans. The disease is frequently observed in stray dogs or in breeding kennels where is responsible for important economic losses. Disease control measures consider treatment or euthanasia of infected animals, as no vaccines are available. Use of antibiotics is not encouraged due to the uncertain success of the cure and the high risk of disease relapse. Still this represents the only alternative to euthanasia, usually combined to orchiectomy and ovary-hysterectomy to reduce the risk of disease transmission. Data on the combined effect of castration and antibiotic treatments of *B. canis* infected dogs are limited and very often follow up information are missing. The aim of the study was to describe from a diagnostic laboratory perspective the effect of antibiotic therapy and castration on male infected dogs. Two male dogs of 8 months and 6 years old were identified as *B. canis* infected during trace back activities related to the *B. canis* outbreak occurred in Italy in 2020. One animal derived from the infected breeding kennel (patient 1) and was showing cryptorchidism while the second animal (patient 2) was exposed to direct contact with the infected dog, sharing the same environment. Laboratory investigations for *B. canis* were carried out before and after antibiotic treatment and orchiectomy, on sera, EDTA blood, urine and testicles. Animals were treated with two different therapeutic protocols. Before treatment, both animals showed high level of antibodies to microplate agglutination test and *B. canis* was also isolated from blood and urine. One month after antibiotic treatment, we observed a decrease of antibody titers and just for patient 1, *B. canis* was detected only by PCR from blood and urine. Orchiectomy was executed after one moths of antibiotic therapy and and no bacteria were detected in the testicles. Animal tested negative to both serology and bacteriology at follow up analyses carried out 1 year later. Despite the encouraging results, periodic follow up remain mandatory to exclude possible relapses of infection. Data also demonstrated that antimicrobial treatment influences laboratory test outcome.

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Keywords: *Brucella canis*, Canine brucellosis, Antibiotic treatment, Diagnosis

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P8-14 Brucellosis in Cameroon: Isolation, characterization, and prevalence of a zoonotic biothreat

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Abstract

Brucellosis negatively affects agricultural economies as well as animal and human health throughout Africa. Historically, the presence of *Brucella* in Cameroon has not been confirmed, and the disease situation not fully understood, as previous studies were based solely on serological tests. In this study, we attempt to better understand the disease extent in the major animal species of agricultural and public health concern including cattle, sheep, goats, and pigs within the most important regions of Cameroon in terms of trade, movement, and animal numbers (Far North, North, and West). Currently, blood, milk, urine, vaginal swab, hygroma fluid, placenta, and lymph node samples are being collected from each of the livestock species at slaughterhouses across each region. Serum and milk are subject to the Rose Bengal Test (RBT) for serology. All samples are subject to culture, as well as real-time PCR (RT-PCR). Countrywide, approximately 88% of sample collection, 82% RBT, 75% culture, and 9% RT-PCR has been completed. So far, RBT suggests a countrywide seropositivity of 6.79% for cattle, sheep (1.10%), goats (2.24%), and pigs (2.81%). Culture has resulted in 0.65% positivity for cattle and 0% in the other species. Although RT-PCR has only been conducted on 393 total animals, 10 cattle (5 North, 3 West, and 2 Far North) and 1 goat (West) have tested positive for *Brucella abortus*. To this point in our study, it appears that *B. abortus* is endemic across the country. Interestingly, despite the initial assumption that *B. melitensis* and *B. suis* are present, we have not yet identified the presence of these species. Furthermore, generalization that *B. melitensis* is a significant problem throughout Africa should not be assumed. Therefore, this study reveals that control strategies in Cameroon should likely be directed at cattle farming and the associated supply chain countrywide. Forthcoming, we will complete sampling and diagnostic assays, sequence DNA using whole genome sequencing, and conduct a region-specific epidemiologic risk investigation. Our overall findings within this project will serve to enhance the capabilities of the Cameroonian government, as well as human and animal health services to prevent, detect, and respond to the apparent brucellosis biothreat.

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Keywords: Brucellosis, *Brucella*, Cameroon, Brucellosis Prevalence, Brucellosis Control

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P8-15 Sustainable brucellosis control programme

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Abstract

Brucellosis is endemic in the Republic of Azerbaijan. There was a testing and slaughter program in the country; but with about 740,000 small farms with livestock scattered in about 4,300 villages, it was very difficult to enforce. Since 2015, within the framework of the Agricultural Competitiveness Improvement Project, the strategy has been changed to universal vaccination of small ruminants and cattle. We carried out a series of cross-sectional epidemiologic studies of the disease in livestock. The series started in 2009 with a pilot study in four regions, and its efficacy was evaluated in 2015 when a seroprevalence study was conducted in 51 regions which revealed a reduction in small ruminant seroprevalences from 3.3% to 0.14% in pilot regions. Another seroprevalence study was conducted in early 2020 in 25 randomly selected regions. The pilot study program of vaccination with conjunctival Rev.1 of all non-pregnant female small ruminants of breeding age and all females between 3 and 8 months was expanded to the whole country in 2015 and was again associated with a reduction in small ruminant seroprevalences when assessed in 2020. The overall RR for all species in a comparison of the 2015 and 2020 surveys in 25 regions was 0.8% (0.7, 0.9). Differences between overall prevalence in individual regions in both surveys were small in most cases. This may have been due to sampling variability in selection of livestock owners at different locations and variability in prevalence and proportions of species of livestock. Small ruminant seroprevalences were lower in 2020 than in 2015. The pilot study program of annual vaccination with conjunctival administered Rev.1 to all female small ruminants between 3 and 8 months old and all non-pregnant females of breeding age in the first two years conducted between 2009 and 2015 resulted in lower brucellosis seroprevalence leading to the adoption of a national control program using the same vaccination strategy. Vaccination of cattle began in 2017 with vaccination of female calves 3 to 8 months of age and was expanded in 2020 to include vaccination of adult non-pregnant females.

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Keywords: Epidemiology, Control, Vaccination, Rev.1, Prevalence

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K9 Brucellosis in wildlife and livestock

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Abstract

Brucellosis is a highly contagious disease caused by bacteria in the genus *Brucella*. This disease causes devastating losses to commercial and small-scale farmers in endemic regions. However, the control of this disease is challenging as it is mainly focused on livestock but also occurs in wildlife. This presentation highlights the multidisciplinary approach to detecting and characterising brucellosis as the biology of *Brucella* infection and detection is essential. Accurate diagnosis of brucellosis using serological tests is an existing shortcoming complicating brucellosis control, which is further complicated by brucellosis in wildlife using tests developed for livestock. The isolations of *Brucella* species from cattle and wildlife that could be due to spillover in livestock or between wildlife and livestock were further investigated. Multiple transmission patterns were observed such as spillover in livestock, spread within a herd to the introduction of infected animals. This research emphasised vaccination and testing as basic control measures which are more challenging in wildlife than livestock. Despite the large body of research on brucellosis, breaking the chain of transmission and controlling this disease remains challenging and underscores the interdisciplinary and collaborative approach required to reduce the impact of this disease on livestock and wildlife.
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Keywords: Brucellosis, wildlife, livestock, *B. abortus, B. melitensis*

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O9-1 Isolation of *Brucella suis* biovar 2 in a male roe deer (*Capreolus capreolus*), in central Italy

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Abstract

In May 2022, a moribund male roe deer (*Capreolus capreolus*) was rescued near Spoleto (Umbria region, Italy) and admitted to a Wildlife Rescue Center. On clinical examination, the subject, in older age, showed severe cachexia, disorientation, motor difficulties and marked increase in volume of the scrotal sac. The animal died few hours after admission. Anatomopathological examination and subsequent diagnostic tests were performed by IZSUM. Post mortem external examination showed ticks infestation, poor body condition score and a dorsal abscess of approximately 10 cm diameter. The animal showed reddened lungs and severe renal congestion, suggesting a stress myopathy as cause of death. The left scrotum appeared significantly increased in volume (10×6 cm) and, on cut surface, it showed areas of fibrosis with caseous-purulent orchitis in a well- organized abscess. The right one was slightly smaller in size (6×4 cm) and showed purulent epididymitis and orchitis. Serum was subjected to Rose Bengal test (RBT) and complement fixation test (CFT) for Brucellosis according to WOAH procedure. Spleen, kidney, liver, testes and testicular lymph nodes were taken for molecular (Fast qPCR) and bacteriologica investigations, while histology was performed also on epididymis and ascellar lymph nodes. RBT and CFT were both positive. CFT title was ≥ 1702,4 IU/ml. PCR resulted positive for *Brucella* spp.. WOAH procedures for *Brucella* isolation were performed and after one week of incubation, *Brucella* spp. strain was isolated from testis samples. The National Reference Laboratory for Brucellosis, at the IZS in Teramo, confirmed the presence of *Brucella* spp. and typing the isolated strains as *Brucella suis* serovar 2. *Brucella suis* can infect domestic and wild species. *Brucella suis* biovar 2 is the main strain responsible for brucellosis in wild boars in Italy and in Europe. This is the first case of *Brucella suis* isolation from a roe deer in Italy. Brucellosis in this roe deer might have been a consequence of transmission between the wild boar population and wild ruminants. In-depth epidemiological investigations of the affected territory will be necessary to understand the dynamics of the infection in wildlife and its potential spread to extensively farmed domestic species.

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Keywords: Roe deer, *Brucella suis*, Central Italy

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O9-2 *Brucella ceti* infection in cetaceans from Italian Seas: associated lesions and epidemiological data

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Abstract

Brucella ceti infections have been increasingly reported in cetaceans, although a limited characterization of Mediterranean *Brucella* spp. isolates has been previously reported. We focused on 23 cases of *B. ceti* infection occurred in striped dolphins stranded along the Italian coastline from 2012 to 2021, investigated by the network of Istituti Zooprofilattici Sperimentali laboratories, coordinated by the National Reference Centre for Diagnostic Investigations on Stranded Marine Mammals (C.Re.Di.Ma.). We assessed the gross and microscopic findings, the results of microbiological, biomolecular and serological investigations, as well as the detection of other relevant pathogens, and the results of whole genomic sequencing and comparative genomic analysis. Pathological changes consistent with *B. ceti* infection were detected in the central nervous system of 19 animals, showing non-suppurative meningoencephalitis; 5 of which showed ***B. ceti***-associated pathological findings also in other tissues. In 6 cases severe coinfections were detected, mostly involving Dolphin Morbillivirus (DMV). We classified the 23 isolates into two sequence types, the ST26, prevalent, and the ST49. Whole genome SNP analysis showed that strains from Italy clustered into five genetically distinct clades. Plotting these clades onto the geographic map suggests a link between their phylogeny and topographical distribution (Adriatic Sea, Ionian Sea, Ligurian Sea, Sardinian Sea, Tyrrhenian Sea) alongside a potential indication of separation of the circulating striped dolphins. These results represent an exhaustive characterization of *B. ceti* isolated from Italian waters and show the usefulness of WGS for understanding of the evolution of this emerging pathogen. The severity of *B. ceti*-associated lesions reported herein confirms the role of this microbial agent as a primary neurotropic pathogen in striped dolphins, as well as a probable cause of stranding events and death, as previously described. Moreover, our findings highlight the importance of a multidisciplinary approach in the monitoring of stranded cetaceans, with epidemiological data and laboratory informations truly shared across sectors in a One Health-based perspective.

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Keywords: *Brucella ceti*, Marine mammals, Neuropathology, Cetaceans, Italy

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O9-3 Brucellosis in vampire bats of Costa Rica: *B. nosferati* nov.

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Abstract

Forty-two *Brucella* strains were isolated from the salivary glands, mammary glands, milk, placenta, fetus, uterus, brain, lung liver, kidney, intestinal content, from seventeen out of 71 vampire bats (*Desmodus rotundus*) and six fetuses, as described before for other mammals[1]. Rose Bengal Test and cELISA performed in 55 vampires sera resulted in 22 seropositive animals. Immunohistopathology revealed that the bacterium extensively replicates in vampire bat tissues, including the placenta, and causes placentitis. Whole genome sequencing (WGS) and phenotypical characterization demonstrated that these isolates represent a new classical *Brucella* species with clear-cut genetic markers, radiating into a distinct branch from all other species. The proposed name is *B. nosferati* nov. Phylogenetic analysis showed that all *B. nosferati* strains were closely related and clustered together with *Brucella* sp. BCNN84.3 strain, previously isolated from an orchiepididymitis case of dog in Costa Rica. These bacteria are pathogenic, showed M type S-LPS, and, similar to other classical smooth strains, had all the virulent arsenal. The natural behaviour of *D. rotundus* inhabiting tropical and subtropical areas of North, Central, and South America makes this vampire bat a potential vector of brucellosis, as it is the case of rabies and bartonellosis.

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Keywords: Vampire, *Desmodus rotundus*, Placentitis, Costa Rica

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O9-4 Pathogenesis of *Brucella suis* biovar 1 in the armadillo (*Chaetophractus villosus*)

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Abstract

Brucella suis biovar 1 has the broadest animal host spectrum. Affects domestic animals and wildlife species. The aim of our study was to investigate the pathogenesis of *B. suis* biovar 1 infection in the armadillo (*Chaetophractus villosus*) under experimental conditions. One gravid female and three adult males were inoculated with a suspension containing 1×106 CFU/mL (colony-forming units) of *B. suis* biovar 1 by oral route. In addition, the gravid female and one male received the same suspension by the conjunctival route. A young male and two females not inoculated were kept in contact with the animals inoculated. The serum samples were analysed using the Buffered Plate Antigen test and Fluorescence Polarization Assay for the detection of *Brucella* antibodies. The inoculated armadillos showed positive antibody titres 2 weeks post-inoculation. After 55 days, the animals that tested positive for *Brucella* (5 animals) were euthanized under anaesthesia (tiletamine and zolazepam, 5.0 mg/kg/I.M). Of the three uninoculated animals, one female was seropositive for Brucella infection. *Brucella* was isolated from the spleen, liver, mesenteric lymph nodes, uterus, testes, and urine. Characteristic histologic lesions were found in the epididymis. These results suggest that armadillos can be a reservoir for the spread of *B. suis* infection, and the persistence of *Brucella* in armadillo tissues constitutes a risk for humans, because of the cultural practice of armadillo meat consumption in rural communities. The animals used in this study were handled by trained personnel, according to the standards and conditions approved by the Animal Ethics Committee of INTA (National Institute of Agricultural Technology) and the International Guiding Principles for Biomedical Research Involving Animals (Council for International Organization of Medical Sciences and the International Council for Laboratory Animal Science). It should be noted that armadillo is not an endangered species.
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Keywords: *Brucella suis*, Pathogenesis, Armadilo

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P9-01 Results of serological studies on brucellosis in wild boars and hares in Ukraine in 2020

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Abstract

Brucellosis poses a significant danger for the health of humans and animals. Wild mammals, especially wild boars and hares, are the main reservoir of infection in nature. Ukraine is free of brucellosis in farm animals, but this disease still unknown in wild fauna. The aim of our work was to conduct serological research on brucellosis among wild boars and hares in different regions of Ukraine and analyze the results of the investigation. Authors present the results of serological screening performed by complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA, ID.vet) over 2020. In total, 411 blood serum samples from forestries in 13 regions of Ukraine and 220 samples - from 24 regions, respectively, were tested and analyzed from wild boars and hares. In addition, the differential diagnosis on yersiniosis performed by the ELISA (INDICAL Bioscience). Positive reactions on brucellosis in wild boars were registered in 11 investigated oblasts. In summary, 21 (5.1%) sera samples tested positive to *Brucella* spp. by CFT. By ELISA, positive reactions detected much more - 64 (15.6%) due to the significant number of hemolyzed samples (109 samples tested only by ELISA). In hares, brucellosis antibodies were detected in animals from two oblasts. Positive reactions were registered in 1.8% of samples by CFT and in 1.4% by ELISA, respectively. However, because of possible cross-reactivity between antibodies for brucellosis and yersiniosis in brucellosis serological tests, we cannot exclude that some of the animals were infected by *Yersinia enterocolitica* O:9 and not *Brucella* spp. Antibodies were not detected in hare samples. The perspectives of further research regarding brucellosis in Ukraine should improve the differential diagnosis between brucellosis and yersiniosis. Future studies may include the use of molecular methods and expansion of epizootic monitoring in wild fauna of Ukraine. Presenting author: serolog@i.ua

Keywords: Brucellosis, Serological studies, Ukraine, Wild fauna

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P9-02 Antibody detection in heifers challenged with local strains of *Brucella suis* biovar 1 previously isolated from armadillo (*Chaetophractus villosus*) and hares (*Lepus europaeus*)

Luis Ernesto Samartino,[1] *Marcelo Fort*,[2] *Marta Kin*,[3] *Hugo Gimenez*[2]

Abstract

Bovine brucellosis is an infectious disease caused mainly by *Brucella abortus*; however, *B. melitensis* and occasionally *B. suis* may infect cattle. In Argentina brucellosis has been controlled in most of the cattle; however, routine serological surveillance detects animals which are positive on standard serological tests with low titers that are persistent or in many cases become negative later. In the geographic zone known as "La pampa" where most of the Argentinian cattle are concentrated, bovines share the habitat with wildlife present in the region. Previous documents indicate that in animals like armadillo and hares, the prevalence of *Brucella* antibodies is 16 and 6 % respectively. Interestingly, only *B. suis* biovar 1 has been isolated from those species. The objective of this research was to evaluate the serological pattern of heifers challenged with 1.5×107 IC of three different strains of *B. suis* biovar 1. Twelve pregnant cows, 2 years old, were utilized. Three of them (group A) were inoculated with *B. suis* isolated from armadillo; 3 heifers were challenged with *B. suis* isolated from hare; 3 were challenged with *B. suis* 1330; and 3 animals (group 4) were not challenged as a control. Blood was collected prior to challenge and every 20 days until the end of the study 150-day post challenge. For serological analysis, the Buffer plate antigen test (BPAT) was used as a screening test and fluorescent polarization (FPA) as the confirmatory test. All animals were negative 20 days before and the same day of challenge. All animals in group A and C, as well as two animals from group B were positive on BPA and FPA after challenge and remained so until day 60. Two heifers, one from group A and another from group B, persisted as positive until the end of the experiment (day 150) with high titers on the FPA test. All animals delivered their calves normally. We concluded that in this experiment *B. suis* inoculated into pregnant animals does not cause abortions but does induce serological titers which interfere the with test results and may cause confusion with the status of this animals.

Presenting author: luersa00@yahoo.com.ar

Keywords: Brucellosis, Cattle, *B. suis*, Armadilo, Hares

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3 *Chordate Biology, Department of Natural and Exact Science UNLPampa, Santa Rosa La Pampa, Argentina*

P9-03 Brucellosis in terrestrial and marine wildlife species from the European perspective

Tariq Jamil,[1] *Kadir Akar,*[2] *Sevil Erdenlig,*[3] *Jayaseelan Murugaiyan,*[4] *Vassilios Sandalakis,*[5] *Evridiki Boukouvala,*[6] *Anna Psaroulaki,*[5] *Falk Melzer,*[1] *Heinrich Neubauer,*[1] ***Gamal Wareth***[1]

Abstract

Brucellosis is a major zoonosis worldwide. The disease infects humans and a wide range of domestic animal and wildlife species resulting in substantial economic losses in the livestock industry and a significant public health impact. The disease is endemic in the Mediterranean basin, including the European parts. Brucellosis has been extensively investigated in humans and domestic animals in several European Union (EU) countries. The disease has been eradicated in livestock, and brucellosis remains a rare event in domestic animals in many EU countries. However, the situation in wildlife is still not obvious. Therefore, the current study aims to clarify the situation of brucellosis in terrestrial and marine wildlife species in Europe. All published articles on wildlife brucellosis in EU countries in the last twenty years have been analyzed. In the last two decades, brucellosis was reported in terrestrial and marine wildlife species in 20 and 10 European countries, respectively. Wild boars and brown hares were the most studied terrestrial wildlife species. Poland, Croatia, and Belgium showed the highest seroprevalences among wild boar caused by *B. suis* biovar 2. The most investigated marine wildlife species were seals and porpoises. Most samples were collected from dead carcasses, and brucellosis was mainly due to *B. ceti* and *B. pinnipedialis*. The highly pathogenic *B. melitensis* and *B. abortus* have been reported from terrestrial and marine wild animals, pointing to a zoonotic threat to wild animal handlers and hunters. Countries reporting brucellosis in both terrestrial and marine wildlife species are Germany, Croatia, Norway, Sweden, Italy, and the Netherlands. Most reports detected anti-*Brucella* antibodies by serology, and *B. suis* biovar 2 was the main isolates found in wild pigs. Currently, no vaccine is available for wild animals. Culling infected wildlife and developing specific diagnostic criteria for wildlife brucellosis remains a challenge.

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Keywords: Brucellosis, Distribution, European Union, Wildlife species

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3 *Faculty of Veterinary Medicine, Harran University, Eyyübiye Sanliurfa, Turkey*
4 *Department of Biological Sciences, SRM University AP, Andhra Pradesh, India*
5 *School of Medicine, University of Crete, Crete, Greece*
6 *Hellenic Agricultural Organization - DIMITRA-VRI, Thermi, Thessaloniki, Greece*

K10 Brucellosis and OneHealth: inherited and future challenges

Ignacio Moriyón[1]

Abstract

OneHealth is the collaborative efforts of multiple disciplines to attain optimal health for people, animals and the environment. While few studies have quantified the benefits, it is accepted that OneHealth strategies are necessary to combat brucellosis, a consensus supported by the fact that historically the concept owes much to observations made on brucellosis by Schwabe and Steele, and by experiences in the eradication of cattle and small ruminant brucellosis. Furthermore, the analysis of brucellosis in conflicting scenarios supports the inclusion of political and ethical considerations in the OneHealth concept. In brucellosis, One-Health actors include Public Health and Veterinary Services (with emphasis on notification systems), microbiologists, medical and veterinary practitioners and breeders, and brucellosis awareness plus a correct use of diagnostic, epidemiological and prophylactic tools are essential. Nevertheless, although the concept is clear, a series of inherited and new challenges pose significant obstacles, some aggravated by global warming and breeding intensification to meet food demands. Studies in endemic scenarios show that disease awareness, stakeholder sensitization/engagement and breeder trust are unresolved issues, all particularly difficult in brucellosis because of the protean characteristics of this zoonosis. Of paramount importance are infrastructural weaknesses, often accentuated by geography and climate. Capacity building faces misconceptions derived from an uncritical adoption of diagnostic and eradication strategies that were successful in countries with suitable means, and is hampered by technical matters requiring reference laboratories in endemic areas, problems not solved by new tools whose value in the real scenario is unknown. Although improving diagnostics and vaccines is challenging, urgent needs are research in semi-domestic or domestic species other than cattle and small ruminants and in wildlife and to develop a safer small ruminant vaccine. Results of this research, lessening the infrastructure requirements, realistic capacity building, creating reference laboratories in critical areas and a stepwise implementation of measures not directly transposed from the so-called developed countries are prerequisites for OneHealth implementation in the combat against brucellosis.

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Keywords: Brucellosis, OneHealth

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AGENDA

15 - 16 - 17 - 18 - 19

September 2022

Teramo Brucellosis 2022

INTERNATIONAL RESEARCH CONFERENCE

Introduction

Although described in the late 1850s and its causative agent isolated in 1886, brucellosis remains a matter of concern worldwide. The disease has been eradicated in a number of countries but continues to threaten human and animal health in many areas of the world, as well as animal productions. Moreover, new *Brucella* strains or species have emerged; and "classical" *Brucella* species are confirming their capacity to influence social, cultural, economic and agricultural environments. The need to enhance current knowledge for the scientific community and to share the most updated information with policy makers remains a priority of paramount importance in the context of the control and eradication of the disease in endemic areas.

On behalf of the International Brucellosis Society, the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZS - Teramo) is organizing the "Brucellosis 2022 International Conference," including the 74th Annual Brucellosis Research Conference, which will be held in Giulianova, Teramo, Italy from September 16th to 19th, 2022. The IZSAM is the WOAH Reference Laboratory for Brucellosis and National Reference Centre for Brucellosis.

During the conference, scientists, researchers, veterinarians, physicians, policymakers, national competent authorities, representatives of the International Organisations, and other stakeholders will exchange knowledge and opinions on the determinants influencing the emergence and spread of the different species of *Brucella*. This will include recent advances in understanding the biology, and evolution of the genus *Brucella*. The most recent developments in laboratory diagnostic procedures, as well as the most modern tools for surveillance and control, as well as disease impact on economics and world trade will be discussed.

Researchers from all over the world will take this opportunity to share their insights about the strategies, methods and tools to control the impact of brucellosis on diverse social, cultural and environmental conditions. The congress will strengthen the network of existing intersectorial and multidisciplinary collaborations, essential to fight this continuously changing and adapting disease.

Aims of the conference

- To provide an environment for discussion about recent progress made by the international scientific community on the knowledge about all aspects of brucellosis.
- To support a high-qualified international forum for scientists, researchers, veterinarians, medical clinicians, and public health professionals to exchange experiences and views on the most up-to date understanding on brucellosis issues.
- To enhance the knowledge about the main risk factors and critical points influencing the occurrence and transmission of brucellosis in man and animals with a particular focus in developing countries and Africa.
- To foster collaboration among participants in identifying common goals, projects, tools, and solutions to address the challenges associated with the control and eradication of brucellosis in endemic countries, as well as with the prevention of disease re-emergence in free areas.

Conference topics

The Conference programme includes 9 sessions. Each session, chaired by a recognised International expert, will host keynote lectures followed by oral presentations. Poster sessions will offer further opportunities to present and discuss scientific findings. All conference sessions will be run in plenary.
The Brucellosis 2022 International Research Conference will cover the following thematic areas:

1	Taxonomy, Evolution, Emerging Species
2	Genomics and Proteomics
3	Pathogenesis and Host-Pathogen Interaction
4	Immunology
5	Vaccination
6	Diagnostic
7	Human Brucellosis
8	Epidemiology, control and eradication
9	Wildlife Brucellosis

AGENDA

15 - 16 - 17 - 18 - 19
September 2022

Thursday, 15 September 2022

17:00 Welcome Reception and participants' registration

18:30 Welcome Cocktail

20:30 End of the day

Friday, 16 September 2022

08:00 Registration

09:00 Opening Ceremony

NICOLA D'ALTERIO
Director General, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

JWAN COSTANTINI
Major of Giulianova, Teramo, Italy

MARCO MARSILIO
President of the Abruzzo Region, Italy

PIERDAVIDE LECCHINI
Director General, Animal health and veterinary drugs, CVO, Ministry of health, Rome, Italy

SIMONA FORCELLA
Policy officer, European Commission – Directorate-General For Health And Food Safety - Unit G2 - Animal Health, Brussels, Belgium

FRANK BOELAERT (vdc)
Senior scientific officer, Biological Hazards & Animal Health and Welfare unit (BIOHAW), European Food Safety Authority (EFSA), Parma , Italy

KEITH SUMPTION
Chief Veterinary Officer/ Leader Animal Health Programme (NSAH)
Chief, Joint Zoonotic Diseases and AMR Centre (CJWZ),
Food and Agriculture Organization of the United Nations (FAO), Rome. Italy

ROMANO MARABELLI
Advisor to the Director General, World Organization for Animal Health (WOAH), Paris, France

FABRIZIO DE MASSIS (conference chair)
WOAH and National Reference Laboratory for Brucellosis Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

11:00 **Coffee Break**

AGENDA

Friday, 16 September 2022

SESSION 1
Taxonomy, Evolution, Emerging Species

Chair: **ADRIAN WHATMORE**
Animal and Plant Health Agency, Addlestone, Surrey, UK

11:30 **Keynote Lecture:** The ever expanding *Brucella* genus

David O'Callaghan
VBIC, INSERM U1047, University of Montpellier, National Reference Center for Brucella, Microbiology laboratory, Nimes, France

12:00 The global spread of the most famous *Brucella* species

Jeffrey Foster
Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, USA

12:15 East African *Brucella* phylogenomics: introducing OrthoPhylo, A turn-key tool for generating ortholog based bacterial whole genome phylogenetic trees

Earl Middlebrook
Los Alamos National Labs, Los Alamos, USA

12:30 Canine Brucellosis in France due to *Brucella canis*: an emerging disease?

Guillaume Girault
EU, WOAH & National Reference Laboratory for Animal Brucellosis, Animal Health Laboratory, Paris-Est University/ANSES, Maisons-Alfort, France

12:45 Evolution towards pathogenicity: phylogenomic insights into *Brucellaceae*

Maite Loperena-Barber
Institute of Tropical Health (ISTUN), University of Navarra, Spain

13:00 Brucellosis on the Guiana Shield: emergence of a new *Brucella* species?

Anne Keriel
University of Montpellier, INSERM / Hospital Center of Nîmes, France

13:15 Atypical *Brucella* spp. are facultatively anaerobic in the presence of nitrates, correlating with increased expression of denitrification genes under anoxic conditions

Stephan Köhler
Infectious Diseases Research Institute (IRIM), CNRS, University of Montpellier, France

13:30 **Lunch Break / Poster Session (Session 1)**

AGENDA

Friday, 16 September 2022

SESSION 2
Genomics and Proteomics

Chair: **GIULIANO GAROFOLO**

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy

15:30 **Keynote Lecture:** Next-generation proteomics in the fight against pathogens: a guide tour

Jean Armengaud
National Institute of Agronomic Research (INRA), Bagnols sur Cèze, France

16:00 *Brucella* Databases at BioCyc.org: Pathways, Omics Tools, and Enhanced Genome Annotation

Ron Caspi
Bioinformatics Research Group, SRI International, Menlo Park , California, USA

16:15 Histidine metabolism and metal homeostasis in *Brucella abortus*

Charline Focant
Research Unit in Microorganisms Biology (URBM), University of Namur, Belgium

16:30 Metabolic Glycoengineering: A strategy to label *Brucella abortus* cell envelope with synthetic analogues of D-mannose

Angeline Reboul
Research Unit in Microorganisms Biology (URBM), University of Namur, Belgium

16:45 Targeted methodology for the identification of protein-peptide vaccine candidates

Louisa B. Tabatabai (vdc)
Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames Iowa, USA

17:00 Whole genome based tools in epidemiological surveillance of *Brucella abortus*, *Brucella melitensis* and *Brucella suis* bv2 in Italy in the last decade.

Anna Janowicz
WOAH and National Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

17:15 In silico pipeline for protein comparisons in *Brucella* genus

Vitomir Djokic
EU, WOAH & National Reference Laboratory for Animal Brucellosis, Animal Health Laboratory, Paris-Est University/ANSES, Maisons-Alfort, France

17:30 **Coffee Break / Poster Session (Session 2)**

19:00 End of the first day

AGENDA

Saturday, 17 September 2022

SESSION 3
Pathogenesis and Host-Pathogen-Interaction

Chair: **MARTY ROOP**
Brody School of Medicine, East Carolina University, Greenville, North Carolina, USA

09:00 **Keynote Lecture:** Metabolic interaction between *Brucella abortus* and its host
Renée Tsolis
University of California, Davis, California, USA

09:30 BNIP3L-dependent mitophagy induced by *Brucella abortus* in host cells is required for bacterial egress
Jeremy Verbeke
Research Unit in Cell Biology – Namur Research Institute for Life Sciences (NARILIS), University of Namur, Belgium

09:45 RNA-immunoprecipitation/miRNA-Seq reveals miRNA-like, small noncoding RNAs of *Brucella suis*, translocated into the cytoplasm of infected murine macrophages
Stephan Köhler
Infectious Diseases Research Institute (IRIM), CNRS, University of Montpellier, France

10:00 Lipopolysaccharide synthesis and traffic in the envelope of the pathogen *Brucella abortus*
Caroline Servais
Research Unit in Microorganisms Biology (URBM), University of Namur, Belgium

10:15 A *Brucella* effector that modulates host retrograde transport to promote intravacuolar replication
Jean Celli
Paul G. Allen School for Global Animal Health, Washington State University, Pullman, USA

10:30 Novel H-NS-like Protein MucR Coordinates Virulence Gene Expression During Host- Association in *Brucella* spp. Through Silencer/Counter-Silencer Interactions
Ian S. Barton
Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, North Carolina, USA

10:45 Structural insights into the NyxA/B effector family
Laurent Terradot
Laboratory of Molecular Microbiology and Structural Biochemistry, Centre National de la Recherche Scientifique, Université de Lyon, France

11:00 **Coffee Break**

AGENDA

Saturday, 17 September 2022

SESSION 4
Immunology

Chair: **PAOLO PASQUALI**
Istituto Superiore di Sanità, Roma, Italy

11:30 **Keynote Lecture:** Internal affairs: defining how cytosolic receptors sense *Brucella* and contributes to host defense
Sergio Costa Oliveira
Federal University of Minas Gerais, Belo Horizonte, Brazil

12:00 Antibody response elicited by *Brucella abortus* strain RB51 vaccine in young water buffaloes (*Bubalus bubalis*) using a triple dose and the WOAH vaccination schedule. A long term trial
Flavio Sacchini
WOAH and National Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

12:15 Antibody response elicited by *Brucella abortus* strain RB51 vaccine in young water buffaloes (*Bubalus bubalis*) using a triple dose and the vaccination schedule authorised in Caserta province, Italy
Fabrizio De Massis
WOAH and National Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

12:30 Use of brucellin skin test to identify water buffaloes (*Bubalus bubalis*) vaccinated with the live attenuated vaccine *B. abortus* strain RB51
Flavio Sacchini
WOAH and National Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

12:45 Effects of age on immune response induced by *Brucella abortus* s19 or RB51 vaccination in heifers
Elaine M. S. Dorneles
Federal University of Lavras, Brazil

13:00 TIRAP exon 5 polymorphisms influence the outcome of *B. melitensis* infection in goats
Carlos Rossetti
National Institute of Agricultural Technology (INTA), Buenos Aires, Argentina

13:15 PMNs supports *Brucella* dispersal with reduced immune recognition
Elías Barquero-Calvo
Research Program in Tropical Diseases, School of Veterinary Medicine, National University, Heredia, Costa Rica

13:30 **Lunch Break / Poster Session (Session 3 e 4)**

14:30 International Brucellosis Society Business Meeting

AGENDA

Saturday, 17 September 2022

SESSION 5
Vaccination

Chair: **JOSÉ MARIA BLASCO**
Agrifood Institute of Aragón(IA2) CITA- University of Zaragoza, Spain

15:30 **Keynote Lecture:** The bumpy road to a *Brucella* vaccine: the near hits and good catches
Angela M. Arenas-Gamboa
Texas A&M University, College Station, Texas, USA

16:00 Registered Influenza Viral Vector Based *Brucella abortus* Vaccine for Cattle in Kazakhstan: Age-Wise Safety and Efficacy Studies
Kaissar Tabynov
Kazakh National Agrarian Research University (KazNARU), Almaty, Kazakhstan

16:15 Improving Rev1 vaccine safety in pregnant ewes
Pilar M. Muñoz
Department of Animal Science, Center for Research and Agrifood Technology of Aragón-IA2, Zaragoza, Spain

16:30 Screening for vaccinal candidates in *Brucella canis*: A genomic based strategy for selection of potential DNA target regions
Ana M. Zúñiga-Pereira
CIET, Microbiology Faculty, University of San José, Costa Rica

16:45 BM Delta-pgm, a superior vaccine for the control of brucellosis in small ruminants
Diego Comerci
Biotechnolgical Research Institute, National University of San Martin (IIB-UNSAM-CONICET), Buenos Aires, Argentina

17:00 Pathogenesis of *Brucella ovis* in pregnant mice and protection induced by the candidate vaccine strain *B. ovis* ΔabcBA
Renato de Lima Santos
Department of Veterinary Clinic and Surgery, School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil

17:15 Identification by Transposon-sequencing of essential genes for chronic infection by *Brucella* in mice
Emeline Barbieux
Research Unit in Biology of Microorganisms (URBM), NARILIS, UNamur, Belgium, Laboratoire de Parasitologie, Université Libre de Bruxelles and ULB Center for Research in Immunology (U-CRI), Gosselies, Belgium

17:30 *Brucella abortus* RB51 Vaccine Strain and Raw Milk Consumption: An Emerging Public Health Risk
Rebekah Tiller
NCEZID/DHCPP/BSPB Zoonoses and Select Agent Laboratory, Atlanta, Georgia, USA

17:45 **Coffee Break / Poster Session (Session 5)**

19:00 End of the second day

AGENDA

Sunday, 18 September 2022

SESSION 6
Diagnostics

Chair: **FALK MELZER**
Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Jena, Germany

09:00 **Keynote Lecture:** The diagnosis of brucellosis

John McGiven
Brucellosis Reference Laboratory, Animal and Plant Health Agency (APHA), Bacteriology Department, Addlestone, United Kingdom

09:30 Investigation into efficacy of rLPS based serodiagnostic antigens

Laurence Howells
Brucellosis Reference Laboratory, Animal and Plant Health Agency (APHA), Bacteriology Department, Addlestone, United Kingdom

09:45 Cross-reaction comparative evaluation of five enzyme-linked immunosorbent assay and Golden Standard methods for porcine Brucellosis diagnosis

Acacia Ferreira Vicente
EU, WOAH & National Reference Laboratory for Animal Brucellosis, Animal Health Laboratory, Paris-Est University/ ANSES, Maisons-Alfort, France

10:00 Machine Learning for MALDI-TOF MS identification of *Brucella*

Teresa Romualdi
WOAH and National Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

10:15 Swab types and storage conditions affect *Brucella* recovery and DNA detection

Luca Freddi
EU, WOAH & National Reference Laboratory for Animal Brucellosis, Animal Health Laboratory, Paris-Est University/ANSES, Maisons-Alfort, France

10:30 Proteomics-based identification of immunodominant *Brucella canis* proteins as candidates for serodiagnosis and vaccine development

Dirk Hofreuter
Department of Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany

10:45 Preliminary study of interferon gamma test used for diagnosis of brucellosis in the water buffalo

Lorena Schiavo
National Reference Centre for Hygiene and Technology of Breeding and Buffalo Production, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Salerno, Italy

11:00 **Coffee Break**

AGENDA

Sunday, 18 September 2022

SESSION 7
Human Brucellosis

Chair: **IGNACIO MORIYÓN**

Emeritus Professor, Institute for Tropical Health and Department of Microbiology, University of Navarra, Pamplona, Spain

11:30 **Keynote Lecture:** Challenges in human brucellosis

Nicholas J. Beeching
Liverpool School of Tropical Medicine, Liverpool, UK

12:00 Brucellosis in a farming community in central South Africa: A longitudinal cohort study (2015-2018)

Jennifer Rossouw
National Institute for Communicable Diseases, Johannesburg, South Africa

12:15 First analysis of antimicrobial resistance in *Brucella* strains isolated in Italy

Katiuscia Zilli
WOAH and National Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

12:30 Whole Genome Sequencing for Tracing Geographical Origin of Imported Cases of Human Brucellosis in Sweden

Tara Wahab
Department of Microbiology, Public Health Agency of Sweden, Solna, Sweden

12:45 Screening of FDA approved Drugs to treat Brucellosis

Nammalwar Sriranganathan
Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, Virginia, USA

13:00 Human brucellosis exposure in confirmed cutaneous anthrax cases, Dien Bien, Vietnam with an update on human prevalence regionally

Jason Blackburn
Spatial Epidemiology & Ecology Research Laboratory, Department of Geography, University of Florida, Gainesville, Florida, USA

13:15 Going chronic: about a complicated case of pediatric brucellosis being the first 'endemic' incident of human brucellosis in Belgium (2021-2022)

Marcella Mori
National Reference Centre for Brucella spp., Sciensano, (Brussels), Belgium

13:30 **Lunch Break / Poster Session (Session 6-7)**

AGENDA

Sunday, 18 September 2022

SESSION 8
Epidemiology, Control and Eradication

Chair: **PAOLO CALISTRI**
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

15:30 **Keynote Lecture:** Control of Brucellosis in Domestic Ruminants. Yes we can!
Bruno Garin-Bastuji
French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Maisons-Alfort, France

16:00 T-Racing: a contact tracing tool for supporting epidemiological investigation during livestock disease outbreaks
Luca Candeloro
WOAH and National Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

16:15 Follow up investigations on non-infected dogs adopted from the *B. canis* italian outbreak
Fabrizio De Massis
WOAH and National Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

16:30 Risk factors for the persistence and spread of Brucellosis in buffaloes in the province of Caserta (2015-2020)
Maria Ottaiano
Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italia

16:45 Outbreak of bovine brucellosis in the Bargy mountain: special feature of *B. melitensis* infection in cattle
Claire Ponsart
EU, WOAH & National Reference Laboratory for Animal Brucellosis, Animal Health Laboratory, Paris-Est University/ANSES, Maisons-Alfort, France

17:00 Human brucellosis: First calculated estimate of global annual incidence
Christopher G. Laine
Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, Texas, USA

17:15 Insights into the seroprevalence of *Brucella canis* infection in dogs in Portugal
Ana Cristina Ferreira
National Institute of Agricultural and Veterinary Research (INIAV), Oeiras, Portugal

17:30 **Coffee Break / Poster Session (Session 8)**

19:30 **Gala Dinner**
Lectio Magistralis: **Brucellosis and OneHealth: inherited and future challenges**
Ignacio Moriyón
Professor, Institute for Tropical Health and Department of Microbiology, University of Navarra, Pamplona, Spain

AGENDA

Monday, 19 September 2022

SESSION 9
Wildlife brucellosis

Chair: **JACQUES GODFROID**
The Arctic University of Norway, Tromsø, Norway

09:00 **Keynote Lecture:** Brucellosis in wildlife and livestock
Henriette van Heerden
University of Pretoria, Onderstepoort, South Africa

09:30 Isolation of *Brucella suis* biovar 2 in a male roe deer (*Capreolus capreolus*), in central Italy
Marco Gobbi
Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy

09:45 *Brucella ceti* infection in cetaceans from Italian Seas: associated lesions and epidemiological data
Carla Grattarola
C.Re.Di.Ma., Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy

10:00 Brucellosis in vampire bats of Costa Rica: *Brucella nosferati* sp. nov.
Gabriela Hernandez-Mora
Veterinary Medical Microbiology Unit, National Animal Health Service (SENASA), Ministry of Agriculture and Livestock, Heredia, Costa Rica

10:15 Pathogenesis of *Brucella suis* biovar 1 in the armadillo (*Chaetophractus villosus*)
Luis Ernesto Samartino
University of Salvador, Pilar, Buenos Aires , Catholic University of Cuyo, San Luis, Argentina

10:30 **Coffee Break / Poster Session (Session 9)**

Closing Ceremony

Chair: **NICOLA D'ALTERIO**
Director General, IZS Teramo

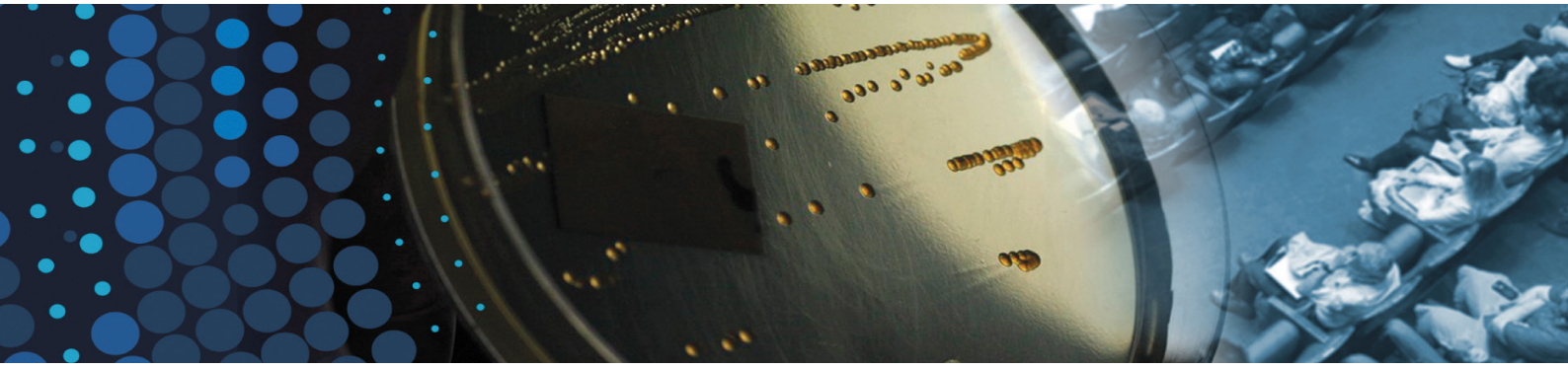
11:00 Poster Award from the International Brucellosis Society

12:00 Closing Remarks and Farewell - Closure of the Conference

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"
TERAMO

INTERNATIONAL BRUCELLOSIS SOCIETY

VETERINARIA ITALIANA
RIVISTA DI SANITÀ PUBBLICA VETERINARIA



Teramo Brucellosis 2022

INTERNATIONAL RESEARCH CONFERENCE

Including the 74th Brucellosis Research Conference

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