Brucellosis Prevention and Control in the Mediterranean and Middle East Regions

D. Tabbaa, A. Seimenis
Introduction

• Brucellosis is a major public health problem in the Middle East and Mediterranean regions.

• The assumed high burden of the disease, particularly in low-income countries, is not matched by the attention it receives from health systems worldwide and as a result brucellosis has been included in the **WHO’s list of Neglected Zoonotic Diseases.**

• Brucellosis is receiving increasing attention in the Middle East; some countries such as Egypt and Oman are implementing vaccination programmes for small and large ruminants whereas others, e.g. Iran, Iraq Palestine and Syria are adopting mass vaccination of small ruminants.

Tabbaa, Seimenis 2019
The Concept of One Health

Healthy People

Healthy Environment

Healthy Animals

Tabbaa, Seimenis 2019
Epidemiology

• *Brucella melitensis* and *B. abortus* have been identified in most countries in the Middle East, supporting the notion of widespread presence of *Brucella spp.* especially *B. melitensis* across the region.

• Brucellosis is responsible for significant economic losses to livestock production due to abortions, reduced milk yield and infertility in addition to the public health burden.

• The disease is transmitted to humans via the consumption of unpasteurized milk and dairy products from infected animals and through direct contact with afterbirth and aborted materials.

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**Brucella** species characteristics

- Small, Gram-negative, facultative coccobacilli, most lacking a capsule, endospores, or native plasmids.

- Intracellular within the host organism, and show environmental persistence outside the host.

- The intracellular trafficking includes two or three main steps, starting with endosomal vacuoles, then endoplasmic reticulum-derived compartments and finally vacuoles having several markers of atypical autophagy.

- They survive extremes in temperature, pH, and humidity, and in frozen and aborted materials. They infect many species, but with some specificity.

- Belongs to the *Rhizobiales* group, in the *Alphaproteobacteria* class.
Phylogenic Tree of *Brucella* sp.
**Table 1.** *Brucella* species by host. Zoonotic potential is classified as pathogenicity and virulence in human hosts. Original citation indicates the original publication where the species was characterized.

<table>
<thead>
<tr>
<th>Species</th>
<th>Natural host</th>
<th>Zoonotic Potential [8]</th>
<th>Original Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. melitensis</em></td>
<td>Sheep, goats, and camels</td>
<td>Yes – High</td>
<td>[5]</td>
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<tr>
<td><em>B. abortus</em></td>
<td>Cattle, elk, and bison</td>
<td>Yes – High</td>
<td>[6]</td>
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<tr>
<td><em>B. suis</em></td>
<td>Pigs, hare, reindeer/caribou</td>
<td>Yes – High</td>
<td>[122]</td>
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<tr>
<td><em>B. canis</em></td>
<td>Dogs (domestic and wild)</td>
<td>Yes – Moderate</td>
<td>[123]</td>
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<tr>
<td><em>B. ovis</em></td>
<td>Sheep</td>
<td>No reported infections</td>
<td>[124]</td>
</tr>
<tr>
<td><em>B. neotomae</em></td>
<td>Desert wood rats</td>
<td>No reported infections</td>
<td>[125]</td>
</tr>
<tr>
<td><em>B. ceti</em></td>
<td>Cetaceans</td>
<td>Yes – Low</td>
<td>[126]</td>
</tr>
<tr>
<td><em>B. pinnipedialis</em></td>
<td>Pinnipeds</td>
<td>Yes – Low</td>
<td></td>
</tr>
<tr>
<td><em>B. microti</em></td>
<td>Red foxes and common voles</td>
<td>No reported infections</td>
<td>[127]</td>
</tr>
<tr>
<td><em>B. inopinata</em></td>
<td>Unknown</td>
<td>Yes – High</td>
<td>[2,128]</td>
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<tr>
<td><em>B. papionis</em></td>
<td>Non-Human Primates</td>
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<td>[129,130]</td>
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<td><em>B. vulpis</em></td>
<td>Red fox</td>
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<td>[131,132]</td>
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<td><em>Brucella</em> <em>NFXXX</em></td>
<td>Australian rat</td>
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<td>[133,134]</td>
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<tr>
<td><em>B. unnamed</em></td>
<td>Blue dotted ray</td>
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<td><em>B. inopinata-like 09RB8471</em></td>
<td>African bullfrogs and Big-eyed tree frog</td>
<td>No reported infections</td>
<td>[2,135]</td>
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<tr>
<td><em>Brucella UK8/14</em></td>
<td>White’s tree frog</td>
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The diagram illustrates a workflow for bioinformatics interactome learning. It begins with experimental pathology, followed by bioinformatics interactome learning, and concludes with experimental confirmation.

1. **Experimental Pathology**
   - Known Protein Binding Domain

2. **Bioinformatics Interactome Learning**
   - Known and Predicted Interaction Knowledge Base
   - Predicted Host-Pathogen Protein-Protein Interaction
   - Interactome Network Learning

3. **Experimental Confirmation**
   - High confidence disease phenotypes

The process involves:
- Finding domain based on sequence similarity if positive.
- Checking if the domain is present in both known and predicted interaction knowledge bases.
- If not, looking for known protein binding domains.
- If no known domains are found, the process is repeated in silico for Brucella.

Key interactions include:
- Domain A interacts with Domain B.
- Brucella interacts with Host.
- In vivo and in vitro approaches.

The diagram is sourced from Tabbaa, Seimenis 2019.
Global occurrences of brucellosis in humans.

The highest incidences are recorded in Syria (1603.4 incidences per 1M people per year), Mongolia (605.9 incidences per 1M people per year), and Kyrgyzstan (362.2 incidences per 1M people per year).

Tabbaa, Seimenis 2019
Heat map of Human incidence and animal outbreaks of Brucellosis 2014

Tabbaa, Seimenis 2019
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*OIE data base: zoonoses in humans and EFSA-EU–EFSA Journal 2015; 13: 4329.  ** Data not available
Available at: www.oie.int/wahis2/wash/action7en.php / www.efsa.europa.eu/efsajournal
Map of the Middle East showing ruminant brucellosis infection and vaccination status in different countries of the region, data obtained from OIE, 2013. N, Disease not reported; NV, no vaccination programme.
Diagnosis

• *B. melitensis* biovars 1, 2, 3 and *B. abortus* biovars 1, 2, 3, 7 and 9 were the most frequently isolated *Brucella spp.* in the majority of the Middle Eastern countries and recently, molecular work in Egypt has shown the presence of *B. suis* biovar 1 in milk and lymph node samples from cattle.

• Frequent isolation of *B. melitensis* from cattle in the Middle East raises questions on the role of cattle in disease maintenance and transmission, which needs further investigation.
Detection of B.melitensis in infected animals

Historically

• Direct visualization (Stamp’s staining),
• Isolation and identification of the causative agent,
• Indirect measurement of either humoral (antibodies) or cell-mediated immune (CMI) responses.

• Polymerase Chain Reaction (PCR) assays (including real-time format) are useful additional techniques for direct detection of an organism
Serological test in Animals

• RBT and Complement fixation tests (CFT) are the most widely used for small ruminants.
• iELISA or, to a lesser degree, cELISA using various antigens, with a high content of smooth lipopolysaccharide.
• Native-hapten (NH) gel precipitation tests have been developed and tested to allow for differentiation of infected versus vaccinated sheep.
• Fluorescence Polarisation Assays (FPA) are an official test for the diagnosis of bovine brucellosis.
• CMI-based brucellin skin-test used in unvaccinated flocks.
• Time-resolved fluorescence energy transfer (TR-FRET).
Serological tests in humans

• RBT,
• Tube serum agglutination (SAT) with and without 2-mercaptoethanol,
• Coombs,
• Brucellacapt,
• iELISA,
• cELISA,
• lateral flow immunochromatography
• FPA.
**Multiple Cross Displacement Amplification (MCDA)**

A novel isothermal amplification technique for Brucella diagnosis

Confirmation and verification of *Brucella*-MCDA products.

(A) Color change of Brucella-MCDA tubes;

(B) LFB (nanoparticles-based lateral flow biosensor) applied for visual detection of *Brucella*-MCDA products.

Tube A1 (biosensor B1), positive amplification;

Tube A2 (biosensor B2), negative amplification (*Salmonella*);

Tube A3 (biosensor B3), negative amplification (*Bacillus cereus*);

Tube A4 (biosensor B4), negative control (DW).
Brucella Isolation

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Brucellosis Surveillance and monitoring System

• Decisions on whether to prioritize brucellosis control over other diseases should ideally be based upon estimates of the human health burden expressed as disability adjusted life years (DALYs) and measures of monetary impact, i.e. economic losses due to human illness and decreased livestock productivity

• To monitor the disease in the Mediterranean and Middle east regions a good regional, inter-sectorial, comprehensive surveillance system should be established

• OIE regional office could establish a collaborative laboratory in the region to run this system and WHO, FAO and OIE in addition to national veterinary and public health authorities, could support it.
Comprehensive and effective Brucellosis control policies
Prevention

• Infected livestock are the source of most human cases; therefore, prevention of human brucellosis is dependent on the control of the disease in livestock.

• Control the disease in Livestock has been achieved with varying degrees of success using a combination of vaccination, test and slaughter of positive animals and quarantine/animal movement controls.

• Cattle brucellosis, caused primarily by *B. abortus*, has been successfully eliminated from several countries including Japan, Canada, some European countries, Australia and New Zealand. However, the control of *B. melitensis* in small ruminants appears to be more challenging than that of *B. abortus*, potentially as a result of its higher infectivity as well as the characteristics of the livestock systems where it is endemic including increased mobility of small ruminant populations compared to large ruminants.
Control of Brucellosis

• Need for more detailed information on the frequency and distribution of infection and its associated burden to identify the most cost-effective options for control.

• Need for more comprehensive and well designed epidemiological studies to bridge the current gap in brucellosis knowledge in the Middle East; such perspective could be achieved through regional and international collaboration.

• Strategic vaccination of ruminant populations combined with sustained surveillance and monitoring systems and public health education programmes may be the most appropriate control strategy.

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Planning Control Strategy

should take into consideration several factors including

• Understanding of local and regional variations in animal husbandry practices.
• Epidemiological patterns of the disease.
• The level of infrastructure support.
• Cross-sectoral brucellosis epidemiological surveillance Coordination.
• social customs.
• community awareness.

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Control strategies

- Establishment of an efficiently-operating inter-sectoral epidemiological surveillance system.
- Introduction of extensive and effective vaccination programs for susceptible livestock (bovine, sheep, goats and, where appropriate, buffaloes and camels) (S19 or RB51 for Cattle, Rev1 for small ruminants).
- Veterinary services contribution and regular laboratory support.
- Control of Food of animal origin for *Brucella sp*. Contamination
- Detect and control human cases.
- Agricultural Extension and Public Health Education.

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Additional control strategies

• The test-and-slaughter strategy, which might gradually lead towards the elimination of infections and establishment of modern cattle farms.

• Expansion of milk pasteurization.

• Recruiting different means of mass media for running persistent, extensive and structured public health education campaigns addressing for instance, milk boiling and avoidance of raw milk or fresh white cheese consumption.

• The preservation of public health and alleviation of social and economic burdens.

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Conclusion

• Brucellosis remains a major public health problem in the Mediterranean and Middle East regions.

• Cases are likely to arise from subpopulations directly exposed to ruminants or from the consumption of unpasteurized fresh dairy products from infected ruminants, with some ruminant subpopulations in the region showing among the highest seroprevalence levels compared to other endemic regions.

• Animal movement between different countries in the region and the intense animal transfer between the Horn of Africa and the Middle East for trading represent a challenge for the control and require more collaboration at the regional and international level.

• There is a lack of reliable estimates of the frequency of disease both in humans and livestock which precludes the formulation of inter-sectorial control policies.

• There is a need for a well-designed regional inter-sectorial surveillance and monitoring system that could assess the real burden of the disease in humans and animals in order to build a comprehensive and effective Brucellosis control policies.
References:


