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## Tracking brucellosis – a re-emerging disease

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**Abstract.** Brucellosis caused by members of the genus *Brucella* is of major concern for animal and public health and is recognized as a re-emerging zoonotic disease. Brucellosis causes flu-like symptoms like fever, sweats, weakness, pain in muscles, joint and back, with some symptoms persisting for longer time periods. Infections occur through consumption of unpasteurized dairy products or undercooked meat, inhalation, and contact with animals. Human-to-human transmission is rare. Surveillance of this disease in animals and humans and prevention of infection risks factors are the most effective strategies to prevent brucellosis. With the progress in sequencing technologies, whole genome sequencing (WGS) has become an effective tool in surveillance, tracking of pathogens and in outbreak investigation. WGS allows identification of the source of infection and to elucidation of transmission chains, which enables authorities to implement timely and appropriate interventions.

### 1. Introduction

Members of the genus *Brucella* are Gram-negative, aerobic, non-spore-forming spherical to rod-shaped bacteria. *Brucella* spp. cause brucellosis in a wide range of animals and humans. The clinical symptoms of brucellosis are infertility and abortion in wild and domestic animals [1], and flu-like illness including fever, sweats, weakness and pain in muscles, joint and back in humans [2]. Bacterial cells can colonise virtually all organs and tissues, can persist for years intracellularly and may cause recurrent infections, that – if untreated – can progress to a chronically incapacitating disease with severe focal complications such as spondylitis, neurobrucellosis or *Brucella* endocarditis [3]. Four out of 12 currently known *Brucella* sp. [4], namely *B. melitensis* (sheep and goat), *B. abortus* (cattle), *B. suis* (pig), and *B. canis* (dog), are pathogenic for humans [5]; *B. melitensis* and *B. abortus* are responsible for most of the reported clinical human cases [6].

With an estimated incidence of 500,000 cases per year, brucellosis is a frequent and widespread zoonotic disease [7]. Travelling, globalization, illegal food imports and the occurrence of new variants can be considered as driving factors for the re-emergence of brucellosis in so far brucellosis-free countries [7,8,9,10,11,12]. *Brucella* can be transmitted from infected animals and contaminated tissues to humans through inhalation or through skin contact, so is a high occupational risk hazard for hunters, farmers, veterinarians and abattoir workers. For these occupational groups, where close contact with



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animal carcasses cannot be avoided, appropriate personal protective measures are required. However, the major public health risk is due to the consumption of contaminated raw milk and raw dairy products, and the consumption of raw or undercooked meat [2,6]. Pasteurization or sterilization of raw milk before marketing or further processing into dairy products and sufficient cooking of meat are the most effective measures to prevent *Brucella*. Most European Union (EU) member states are free of bovine and ovine/caprine brucellosis, and brucellosis has become a rare zoonotic disease due to a strict eradication program. However, more than 380 cases of human brucellosis, mainly caused by *B. melitensis*, were reported in the EU in 2017, and more than 60% of patients required hospitalization [6].

*Brucella* is able to survive frozen storage conditions and can grow between 6°C to 42°C, between pH 4.5 to pH 8.8 and in up to 4% NaCl. In addition, foods with a high fat content have a protective effect, allowing a longer survival of *Brucella* [13,14]. Heating at 72°C for 15 seconds (or equivalent) is sufficient to inactivate *Brucella* [13,14].

## 2. Whole genome sequencing (WGS) based surveillance

Austria is officially recognised as being free from bovine brucellosis (OBF) and of small ruminant brucellosis, but a few human cases are still reported every year [15]. As *B. melitensis* infections in Austria are rare and usually imported, collecting as many details as possible is essential to better understand patterns of transmission. This includes epidemiological information on patient's history but also microbiological data on bacterial strains.

Whole genome sequencing (WGS) has been frequently used to identify outbreaks for various diseases. For such investigations, it is worth implementing molecular techniques with the highest possible discriminatory power, because they provide a higher probability that two isolates with similar genetic profiles are indeed related. In the case of brucellosis, a retrospective study in Portugal was able to identify likely "missed outbreaks" using WGS, as isolates with no documented links clustered together [16]. In Austria, WGS was used to investigate a cluster of brucellosis cases detected in May 2018 [10]. Following an epidemic of miscarriages, *B. melitensis* was isolated from aborted bovine material and milk. Two veterinarians and the farmer's child were hospitalized and diagnosed with brucellosis. The genetic similarity between the cattle and human isolates (<2 allelic differences in core genome multi locus sequence type (MLST)) confirmed that they all belonged to the same outbreak. This outbreak was the first proved zoonotic transmission of *B. melitensis* in Austria in more than 15 years.

WGS can also support clinical management of brucellosis patients. An Austrian patient diagnosed with brucellosis in 2017 developed a second episode of brucellosis in 2019. To determine if the second episode was a relapse or a reinfection, the two *B. melitensis* isolates were sequenced. The 2019 isolate showed only one allelic difference in core genome MLST to the 2017 strain, supporting the hypothesis of a relapse.

Finally, WGS can also be used to support the investigation of isolated and imported brucellosis cases. Indeed, identifying importation routes is of crucial importance to take adapted control measures. When the epidemiological investigation does not succeed, genomic data can be used to identify the most likely geographical origin of the cases. This can be achieved using environmental or animal samples, as done in a study from Germany [9]. They collected *B. melitensis* strains from Turkish sheep and were able to link these animal isolates to clinical isolates from travellers returning from Turkey or Turkish immigrants, suggesting that these cases were indeed imported from Turkey. When neither food isolates nor animal isolates are available, *B. melitensis* strains can be compared with each other, analysing how strains of unknown origin cluster with strains of known origin. Previous studies from Germany and Italy used this method, and were successful thanks to a large number of isolates with known origin [17,18]. When lower numbers of isolates are available, published strains for which the country of infection is reported can be used, as was done in Malta, Belgium, Norway and Sweden [19,20,21,22]. In Austria, we were able to identify a cluster of 12 Austrian patients. *B. melitensis* strains isolated by Serbian partners in animals grouped within this cluster, supporting the hypothesis that all cases of this cluster were infected in Balkan countries.

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## References

- [1] Lapaque N, Moriyon I, Moreno E and Gorvel J P 2005 *Brucella* lipopolysaccharide acts as a virulence factor. *Curr. Opin. Microbiol.* **8** 60–6
- [2] WHO 2020 Brucellosis *Fact sheets* 2020
- [3] Adone R and Pasquali P 2013 Epidemiology of brucellosis. *Rev. Sci. Tech.* **32** 199–205
- [4] Głowacka P, Żakowska D, Naylor K, Niemcewicz M and Bielawska-Drózd A 2018 *Brucella* - virulence factors, pathogenesis and treatment. *Pol. J. Microbiol.* **67** 151–61
- [5] Hull N C and Schumaker B A 2018 Comparisons of brucellosis between human and veterinary medicine. *Infect. Ecol. Epidemiol.* **8** 1500846
- [6] European Food Safety Authority and European Centre for Disease Prevention and Control 2018 The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J.* **16** 5500
- [7] Pappas G, Akritidis N, Bosilkovski M and Tsianos E 2005 Brucellosis. *N. Engl. J. Med.* **352** 2325–36
- [8] Mailles A, Rautureau S, Le Horgne J M, Poignet-Leroux B, d'Arnoux C, Denetière G, Faure M, Lavigne J P, Bru J P and Garin-Bastuji B 2012 Re-emergence of brucellosis in cattle in France and risk for human health. *Euro Surveill.* **17** 20227
- [9] Gwida M, Neubauer H, Ilhan Z, Schmoock G, Melzer F, Nöckler K, Janczyk P, Tomaso H, Rösler U and Al Dahouk S 2012 Cross-border molecular tracing of brucellosis in Europe. *Comp. Immunol. Microbiol. Infect. Dis.* **35** 181–5
- [10] Blaschitz M *et al.* 2019 Use of whole genome sequencing in an outbreak investigation of bovine brucellosis due to *Brucella melitensis*, Austria, 2018. *Int. J. Infect. Dis.* **79** 84
- [11] Schaeffer J *et al.* 2021 Tracking the origin of Austrian human brucellosis cases using whole genome sequencing. *Front. Med.* **8** 635547
- [12] Spicic S *et al.* 2021 New *Brucella* variant isolated from Croatian cattle. *BMC Vet. Res.* **17** 126
- [13] ICMSF 1996 *Brucella* Microorganisms in Food 5: Microbiological Specifications of Food Pathogens (London: Blackie Academic and Professional) pp 36–44
- [14] FSANZ 2006 A risk profile of dairy products in Australia. Food Standards Australia New Zealand Report, Canberra <http://www.foodstandards.gov.au/code/proposals/documents.pdf>
- [15] Much P, Arrouas M and Herzog U 2018 Zoonoses and Zoonotic Agents in Austria - Report 2018. Austrian Agency for Health and Food Safety (AGES)
- [16] Pelerito A, Nunes A, Nuncio M S and Gomes J P 2020 Genome-scale approach to study the genetic relatedness among *Brucella melitensis* strains. *PLoS ONE.* **15** e0229863
- [17] Georgi E, Walter M C, Pfalzgraf M-T, Northoff B H, Holdt L M, Scholz H C, Zoeller L, Zange S and Antwerpen M H 2017 Whole genome sequencing of *Brucella melitensis* isolated from 57 patients in Germany reveals high diversity in strains from Middle East. *PLoS ONE.* **12** e0175425
- [18] Janowicz A *et al.* 2018 Core genome multilocus sequence typing and single nucleotide polymorphism analysis in the epidemiology of *Brucella melitensis* infections. *J. Clin. Microbiol.* **56** e00517–18
- [19] Muchowski *et al.* 2015 Using molecular tools to identify the geographical origin of a case of human brucellosis. *Epidemiol. Infect.* **143** 3110–3
- [20] Hanot Mambres D *et al.* 2017 Imported human brucellosis in Belgium: bio and molecular typing of bacterial isolates, 1996-2015. *PLoS ONE.* **12** e0174756
- [21] Johansen T B, Scheffer L, Jensen V K, Bohlin J and Feruglio S L 2018 Whole-genome sequencing and antimicrobial resistance in *Brucella melitensis* from a Norwegian perspective. *Sci. Rep.* **8**

8538

- [22] Sacchini L, Wahab T, Di Giannatale E, Zilli K, Abass A, Garofolo G and Janowicz A 2019 Whole genome sequencing for tracing geographical origin of imported cases of human brucellosis in Sweden. *Microorganisms* **7** 398