

WOAH Reference Laboratory Reports Activities 2023

Activities in 2023

This report has been submitted : 1 juillet 2024 09:00

Laboratory Information

Name of disease (or topic) for which you are a designated WOAHO Reference Laboratory:	Brucellosis
Address of laboratory:	FLI, Naumburger Str. 96a, 07753 Jena, Germany
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E-mail address:	falk.melzer@fli.de; heinrich.neubauer@fli.de
Website:	https://www.fli.de/de/startseite/
Name (including Title) of Head of Laboratory (Responsible Official):	Dr. Falk Melzer
Name (including Title and Position) of WOAHO Reference Expert:	Prof. Heinrich Neubauer
Which of the following defines your laboratory? Check all that apply:	Governmental

TOR1: DIAGNOSTIC METHODS

1. Did your laboratory perform diagnostic tests for the specified disease/topic for purposes such as disease diagnosis, screening of animals for export, surveillance, etc.?
(Not for quality control, proficiency testing or staff training)

Yes

Diagnostic Test	Indicated in WOAHO Manual (Yes/No)	Total number of test performed last year	
		Nationally	Internationally
Indirect diagnostic tests			
RBT		72	106
CFT		72	0
ELISA		221	222
Direct diagnostic tests			
PCR		460	48
MALDI		18	14
Sequencing		1	12

TOR2: REFERENCE MATERIAL

2. Did your laboratory produce or supply imported standard reference reagents officially recognised by WOAHO?

No

3. Did your laboratory supply standard reference reagents (nonWOAHO-approved) and/or other diagnostic reagents to WOAHO Members?

Yes

TYPE OF REAGENT AVAILABLE	RELATED DIAGNOSTIC TEST	PRODUCED/ PROVIDE	AMOUNT SUPPLIED NATIONALLY (ML, MG)	AMOUNT SUPPLIED INTERNATIONALLY (ML, MG)	NO. OF RECIPIENT WOAHO MEMBER COUNTRIES	COUNTRY OF RECIPIENTS
Pos/neg serum	CFT, RBT, ELISA	14x1ml	3x1ml	1	1	SIERRA LEONE,

antigen	RBT	20ml	20ml	1	1	PAKISTAN,
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4. Did your laboratory produce vaccines?

No

5. Did your laboratory supply vaccines to WOAHO Members?

No

TOR3: NEW PROCEDURES

6. Did your laboratory develop new diagnostic methods for the designated pathogen or disease?

No

7. Did your laboratory validate diagnostic methods according to WOAHO Standards for the designated pathogen or disease?

No

8. Did your laboratory develop new vaccines for the designated pathogen or disease?

No

9. Did your laboratory validate vaccines according to WOAHO Standards for the designated pathogen or disease?

No

TOR4: DIAGNOSTIC TESTING FACILITIES

10. Did your laboratory carry out diagnostic testing for other WOAHO Members?

No

11. Did your laboratory provide expert advice in technical consultancies on the request of an WOAHO Member?

Yes

NAME OF THE WOAHO MEMBER COUNTRY RECEIVING A TECHNICAL CONSULTANCY	PURPOSE	HOW THE ADVICE WAS PROVIDED
KUWAIT	control of brucellosis in dairy cattle farms	In loco

TOR5: COLLABORATIVE SCIENTIFIC AND TECHNICAL STUDIES

12. Did your laboratory participate in international scientific studies in collaboration with WOAHO Members other than the own?

Yes

Title of the study	Duration	PURPOSE OF THE STUDY	PARTNERS (INSTITUTIONS)	WOAHO MEMBER COUNTRIES INVOLVED OTHER THAN YOUR COUNTRY
Genotype diversity of brucellosis agents isolated from humans and animals in Greece based on whole-genome sequencing	2021-2023	Increase knowledge about the genomic diversity of brucella in Greece	Veterinary Research Institute, ELGO-DIMITRA, Campus of Themi, Themi 57001, Thessaloniki, Greece Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van 65090, Turkey 5 Microbiology Department, Faculty of Veterinary Medicine, Harran University, Sanliurfa 63200, Turkey	GREECE TURKEY
Whole-genome sequencing (WGS) analysis of Brucella suis biovar 2 isolated from domestic pigs in Egypt for epidemiological and genetic diversity tracing	2022-2023	genetic diversity of Brucella suis strains by WGS to determine their global lineage and existence of antimicrobial resistance and virulence-associated genes	Benha University, Moshtohor, Toukh 13736, Egypt; Agricultural Research Center (ARC), Animal Health Research Institute (AHRI), Cairo, Egypt; Kafrelsheikh University, Egypt	EGYPT

13. In exercising your activities, have you identified any regulatory research needs* relevant for WOAHO?

No

TOR6: EPIZOOLOGICAL DATA

14. Did your Laboratory collect epidemiological data relevant to international disease control?

Yes

IF THE ANSWER IS YES, PLEASE PROVIDE DETAILS OF THE DATA COLLECTED:

Little is known about the genetic diversity and distribution of brucellae in Iran. Therefore, forty *Brucella* spp. strains (*B. abortus* and *B. melitensis*) isolated from animals and humans were analyzed by whole genome sequencing (WGS) technology using single nucleotide polymorphism (SNP) analysis and core genome multilocus sequence typing (cgMLST). *Brucella* isolates were obtained from lymph nodes (cows and camels), milk (cows, camels and sheep), and aborted foetus samples (sheep and goats), as well as cerebrospinal fluid and blood of humans. The isolates were originating from thirteen provinces of Iran and isolated between 2015 and 2020. According to in-silico MLST, ST8 and ST2 were the most frequent sequence types in *B. melitensis* and *B. abortus*, respectively. Based on phylogeographic reconstruction using cgSNP analysis, the investigated Iranian *B. melitensis* strains belonged to the American and Mediterranean lineages of the *B. melitensis* phylogeny. Furthermore, cgSNP analysis revealed a similarity between Iranian *B. abortus* isolates and strains from Iraq and Egypt. Therefore, the origin of the Iranian strains can be suggested to be strains from neighboring and Middle East countries. Moreover, cgMLST analysis showed that the Iranian *B. melitensis* strains were closely relative to strains recovered from sheep and humans in Iraq, Afghanistan, Syria, Turkmenistan, and Pakistan. In the current panel of strains, cgMLST and cgSNP analysis provided an appropriate and accurate tool for effective traceback analyses for *Brucella* spp. from Iran. The results of cgSNP and cgMLST helped to understand the geographic distribution and interspecies transmission of Iranian strains and highlight the importance of specific brucellosis control measures in Iran with regard to the One-Health approach.

15. Did your laboratory disseminate epidemiological data that had been processed and analysed?

Yes

IF THE ANSWER IS YES, PLEASE PROVIDE DETAILS OF THE DATA COLLECTED:

Although the occurrence of brucellosis must be reported to the authorities, it is believed that the disease is under-reported in Greece, and knowledge about the genomic diversity of brucellae is lacking. Thus, 44 *Brucella* isolates, primarily *B. melitensis*, collected between 1999 and 2009 from humans and small ruminants in Greece were subjected to whole genome sequencing using short-read technology. In silico genotyping revealed that the isolates belonged to three of the known sublineages of the East Mediterranean genotype. In addition, a novel subgenotype was identified that was basal to the other East Mediterranean sublineages, comprising two Greek strains. The majority of the isolates can be assumed to be of endemic origin, as they were clustered with strains from the Western Balkans or Turkey, whereas one strain of human origin could be associated with travel to another endemic region, e.g. Portugal. Further, nucleotide substitutions in the housekeeping gene *rpoB* and virulence-associated genes were detected, which were characteristic of the different subgenotypes. One of the isolates originating from an aborted bovine foetus was identified as *B. abortus* vaccine strain RB51.

16. What method of dissemination of information is most often used by your laboratory? (Indicate in the appropriate box the number by category and list the details in the box)

a) Articles published in peer-reviewed journals:

9

- Brangsch H, Sandalakis V, Babetsa M, Boukouvala E, Ntoula A, Makridaki E, Christidou A, Psaroulaki A, Akar K, Gürbük SE, Jamil T, Melzer F, Neubauer H, Wareth G. Genotype diversity of brucellosis agents isolated from humans and animals in Greece based on whole-genome sequencing. *BMC Infect Dis.* 2023 Aug 14;23(1):529. doi: 10.1186/s12879-023-08518-z
- Dadar M, Alamian S, Brangsch H, Elbadawy M, Elkharsawi AR, Neubauer H, Wareth G. Determination of Virulence-Associated Genes and Antimicrobial Resistance Profiles in *Brucella* Isolates Recovered from Humans and Animals in Iran Using NGS Technology. *Pathogens.* 2023 Jan 3;12(1):82. doi: 10.3390/pathogens12010082
- Dadar M, Brangsch H, Alamian S, Neubauer H, Wareth G. Whole-genome sequencing for genetic diversity analysis of Iranian *Brucella* spp. isolated from humans and livestock. *One Health.* 2023 Jan 3;16:100483. doi: 10.1016/j.onehlt.2023.100483
- Wareth G, Abdel-Hamid NH, Hamdy MER, Elmonir W, Beleta EIM, El-Diasty M, Abdel-Gil MY, Melzer F, Neubauer H. Whole-genome sequencing (WGS) analysis of *Brucella suis* biovar 2 isolated from domestic pigs in Egypt for epidemiological and genetic diversity tracing. *Vet Microbiol.* 2023 Feb;277:109637. doi: 10.1016/j.vetmic.2022.109637
- Brangsch H, Horstkotte MA, Melzer F. Genotypic peculiarities of a human brucellosis case caused by *Brucella suis* biovar 5. *Sci Rep.* 2023 Oct 3;13(1):16586. doi: 10.1038/s41598-023-43570-4
- Djokic V, Freddi L, de Massis F, Lahti E, van den Esker MH, Whatmore A, Haughey A, Ferreira AC, Garofolo G, Melzer F, Sacchini F, Koets A, Wyllie S, Fontbonne A, Girault G, Vicente AF, McGiven J, Ponsart C. The emergence of *Brucella canis* as a public health threat in Europe: what we know and what we need to learn. *Emerg Microbes Infect.* 2023 Dec;12(2):2249126. doi: 10.1080/22221751.2023.2249126
- Aurich S, Schneider J, Brangsch H, Koets A, Melzer F, Ewers C, Prenger-Berninghoff E. *Brucella suis* biovar 1 infection in a dog with orchitis in Germany. *Front Vet Sci.* 2023 Aug 3;10:1233118. doi: 10.3389/fvets.2023.1233118
- Linde J, Brangsch H, Hölzer M, Thomas C, Elschner MC, Melzer F, Tomaso H. Comparison of Illumina and Oxford Nanopore Technology for genome analysis of *Francisella tularensis*, *Bacillus anthracis*, and *Brucella suis*. *BMC Genomics.* 2023 May 12;24(1):258. doi: 10.1186/s12864-023-09343-z
- Zeeshan MA, Ali S, Ahmed I, Rehman AU, Rafique MK, Nasir A, Khan AU, Kashif M, Mertens-Scholz K, Arshad MI, Ehtisham-Ul-Haque S, Neubauer H. Sero-epidemiological study of zoonotic bacterial abortifacient agents in small ruminants. *Front Vet Sci.* 2023 Jul 28;10:1195274. doi: 10.3389/fvets.2023.1195274 Titel anhand dieser DOI in Citavi-Projekt übernehmen. Erratum in: *Front Vet Sci.* 2023 Sep 14;10:1287165. PMID: 37576834

b) International conferences:

3

Gamal Wareth: *Epidemiological situation of brucellosis in North Africa: Webinar on Brucellosis, 23rd November 2023, belonging to STOR-REMESA, Palermo, Italy*
Brangsch H, Linde J, Elschner MC, Melzer F (2023) *Genotyping and epidemiological analyses of bacterial isolates at the FLI. SHARP WP7 Technical Meetings. Rom, Italien. 10.05.2023.*

Melzer F (2023) *News about brucellosis. Conference: Biotechnology And Its Role In Ensuring Human And Animal Health. Kyiv 20.12.2023.*

c) National conferences:

2

Wareth G (2023) *Brucellosis in Tropical countries. Course at faculty of medicine, Jena university belonging to Tropical Medicine course, Germany*

Aurich S, Ewers C, Melzer F, Hoffmann C, Prenger-Berninghoff E (2023) *Canine Brucellosis - Isolation and Differentiation of five Brucella canis and Brucella suis isolates in a German Veterinary Microbiological Diagnostic Laboratory. Tagung der DVG-Fachgruppe "Bakteriologie und Mykologie" 2023, Berlin 22.5.-24.5.2023.*

d) Other (Provide website address or link to appropriate information):

1

Wareth G (2023) *Humboldt-Kolleg zu "Repositioning Science and Technology to Tackle Food Insecurity, Environmental and Health Challenges through Research Collaboration and Capacity Building for Sustainable Development". Lagos, Nigeria. 01.-05.05.2023.*

Melzer F (2023) *"Brucellose": 7. AVID-Themenabend <http://avid.dvg.net/index.php?id=2788>*

TOR7: SCIENTIFIC AND TECHNICAL TRAINING

17. Did your laboratory provide scientific and technical training to laboratory personnel from other WOA Members?

Yes

a) Technical visit : 3

b) Seminars : 1

c) Hands-on training courses: 7

d) Internships (>1 month) 0

Type of technical training provided (a, b, c or d)	Country of origin of the expert(s) provided with training	No. participants from the corresponding country
C	TUNISIA	2
C	ALGERIA	1
C	MALAYSIA	2
C	UKRAINE	3
C	EGYPT	5
C	PAKISTAN	2
C	IRAQ	1
A	BENIN	2
A	CENTRAL AFRICAN (REP.)	2
A	ESWATINI	2
A	ERITREA	2
A	LIBYA	2
A	MALAWI	2
A	MAURITANIA	2
A	MAURITIUS	2
A	SEYCHELLES	2
A	SIERRA LEONE	1
A	GREECE	5

A	TURKEY	4
A	NIGERIA	250
B	ALGERIA	2
B	EGYPT	2
B	FRANCE	2
B	ITALY	2
B	LIBYA	2
B	MOROCCO	2
B	MAURITANIA	2
B	PORTUGAL	2
B	SPAIN	2
B	TUNISIA	2

TOR8: QUALITY ASSURANCE

18. Does your laboratory have a Quality Management System?

Yes

Quality management system adopted	Certificate scan (PDF, JPG, PNG format)	
ISO 17025	pdf	Akkreditierungsurkunde_2022.pdf

19. Is your quality management system accredited?

Yes

Test for which your laboratory is accredited	Accreditation body
Microbiological, serological and molecular diagnosis	DAKKS

20. Does your laboratory maintain a "biorisk management system" for the pathogen and the disease concerned?

Yes

following the German law of Biostoff-Verordnung, e.g. Access control to all labs by administrative regulations, Existence of a Biorisk Committee, regularly audits by external responsible authorities

TOR9: SCIENTIFIC MEETINGS

21. Did your laboratory organise scientific meetings related to the pathogen in question on behalf of WOA?H?

No

22. Did your laboratory participate in scientific meetings related to the pathogen in question on behalf of WOA?H?

No

TOR10: NETWORK WITH WOA?H REFERENCE LABORATORIES

23. Did your laboratory exchange information with other WOA?H Reference Laboratories designated for the same pathogen or disease?

No

24. Do you network (collaborate or share information) with other WOA?H Reference Laboratories designated for the same pathogen?

Yes

NETWORK/DISEASE	ROLE OF YOUR LABORATORY (PARTICIPANT, ORGANISER, ETC)	NO. PARTICIPANTS	PARTICIPATING WOA?H REF. LABS
Brucellosis	Interlaboratory reproducibility analysis to validate ELISA kits for Buffalo sera	3	ANSES France, IIZS Italy

25. Did you organise or participate in inter-laboratory proficiency tests with WOA?H Reference Laboratories designated for the same pathogen?

Yes

PURPOSE OF THE PROFICIENCY TESTS: 1	ROLE OF YOUR REFERENCE LABORATORY (ORGANISER/ PARTICIPANT)	NO. PARTICIPANTS	PARTICIPATING WOAHP REF. LABS/ ORGANISING WOAHP REF. LAB.
Interlaboratory reproducibility analysis to validate ELISA kits for Buffalo sera	participant	3	ANSES France, IIZS Italy

26. Did your laboratory collaborate with other WOAHP Reference Laboratories for the same disease on scientific research projects for the diagnosis or control of the pathogen of interest?

No

TOR11: OTHER INTERLABORATORY PROFICIENCY TESTING

27. Did your laboratory organise or participate in inter-laboratory proficiency tests with laboratories other than WOAHP Reference Laboratories for the same pathogen?

Yes

Purpose for inter-laboratory test comparisons ¹	Role of your reference laboratory (organizer/participant)	No. participating laboratories	Name of the Test	WOAHP Member Countries
Brucellosis serology RBT	organiser	2	RBT	SIERRA LEONE, UKRAINE,
Brucella spp. realtime PCR	organiser	1	PCR	AUSTRIA,

TOR12: EXPERT CONSULTANTS

28. Did your laboratory place expert consultants at the disposal of WOAHP?

Yes

KIND OF CONSULTANCY	Location	SUBJECT (FACULTATIVE)
Expert visit	Kuwait	Diagnosis and vaccination brucellosis

29. Additional comments regarding your report: