

Brucellosis Surveillance Jordan Newsletter 2021



**Evaluating the Impact of Enhanced Laboratory-Based Surveillance
of Animal and Human Brucellosis in Jordan.**

Introduction

Brucellosis is a contagious bacterial zoonotic disease caused by various *Brucella* species, which mainly infect cattle, goats, sheep, swine, and dogs. Humans generally acquire the disease through ingestion of unpasteurized dairy products or close contact with infected animals and their tissues, manifesting as undulant fever with joint and gastrointestinal involvement. Species of brucella are known to infect cattle causing significant economic impact to infected herds. It is a highly infectious zoonoses for humans and is one of the top ten pathogens at the wildlife-livestock interphase. The highest incidence rates of brucellosis have been reported in Middle Eastern countries, such as Jordan. However, recent studies have revealed that the disease is still under-reported in the country. In addition, there is little information on the risk factors and geographical patterns of brucellosis in Jordan.

Hence, as of May of 2019, EMPHNET and U.S. Centers for Disease Control and Prevention (CDC) in collaboration with Ministry of Health (MOH), Ministry of Agriculture (MOA) and the Royal Medical Services (RMS), have been implementing the activities for a project titled, "Evaluating the Impact of Enhanced Laboratory-Based Surveillance of Animal and Human Brucellosis in Jordan" in three areas, Mafraq, Karak and East Amman, aiming to assess the true burden of the disease in the country, identify its associated risk factors, and improve lab capacities needed for its surveillance.

This is the 4th newsletter covering project activities to be produced, it reports updated data regarding the numbers of animal and human cases detected until August 2021. This includes the laboratory results of the Rose Bengal test (RBT), Serum Agglutination test (SAT), Enzyme-linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) analysis results identifying the brucella species distribution among the human cases, and the RBT and fluorescence-polarization assay (FPA) results of the animal samples. Previous editions provided the rationale and design of the project, highlighted the improvements added to the lab testing facilities in the study governates, and reported the surveillance data of animal and human brucellosis cases from May 2019-Dec 2020 and the challenges brought by the COVID-19 pandemic.

This issue covers activities and updates that took place between January and August of 2021. During this period, a meeting was conducted on the 20th of February in coordination with RMS representatives for the three working teams from King Hussein Medical Center,

King Talal and Prince Ali Ben Al Hussein Military Hospitals, to discuss project activities, challenges, and solutions, and to emphasize the importance of effective engagement and proper implementation.

Five awareness sessions were also conducted by EMPHNET, targeting members from animal health directorate including private veterinarians, para-veterinarians, farmers, women who produce milk and dairy products, and undergraduate vets to raise awareness on brucellosis symptoms, treatment, and prevention, and to raise awareness on safety and hygienic precautions required in milking animals and producing dairy products.

An abstract of the project's preliminary results, up to March 2021, was accepted for a rapid-fire talk at the World Microbe Forum 2021, a joint virtual conference of the annual American Society of Microbiology (ASM) Microbe and Federation of European Microbiological Societies (FEMS) meetings. The project findings were presented there on the 23rd of June as part of the Clinical and Public Health Microbiology (CPHM) track.



Moreover, EMPHNET organized regular follow-up visits to each of the study sites in Mafraq, Karak, and East Amman and held several meetings with each site's study coordinators to ensure return to normal workflow post easing of COVID-19 restrictions and address challenges they had faced with data collection. In addition, as part of the project's aims of assessing genetic diversity of circulating subspecies in the country, BioNumerics Multi-Locus Variable Tandem Repeat Analysis (MLVA) software was provided to Princess Haya Biotechnology Center (PHBC) and training sessions were provided by the CDC on the analysis of genetic diversity of collected samples, which is currently underway.

Additionally, with the aim of creation of spatio-temporal maps, EMPHNET, in collaboration with Information and Graphics Systems Consultants (InfoGraph), conducted a five-day training workshop on geographic information systems (GIS) and its application on zoonotic diseases. The participants were from MOH, MOA in addition to GHDIEMPHNET staff. The training covered skills of working with data formats specific to GIS platforms; geoprocessing in ArcGIS Pro, entering data into geodatabases, importing subsets of data, creating spatial and attribute queries, incorporating environmental layers with data variables, calculation of descriptive statistics, and spatial clustering analyses. At the end of the training course, the participants gained the capacity of producing spatio-temporal maps and presenting all needed variables on such maps with better data visualization.

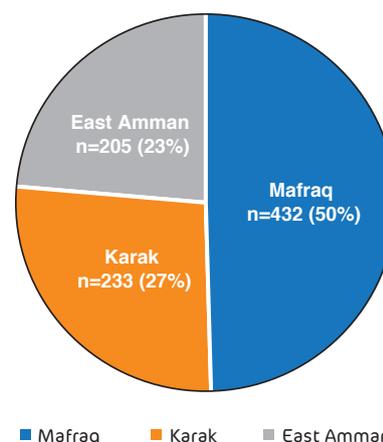


Brucellosis surveillance findings since project initiation in May 2019 through August 2021

Laboratory findings of human cases

Overall, a total of 11,000 suspected cases arrived at the designated study sites in Mafraq, Karak and East Amman, serum samples were collected from them, and Rose Bengal testing (RBT) was performed as a screening method for brucellosis. Of those, 870 (7.9%) individuals had positive RBT results. The distribution of RBT positive cases in each governorate varied greatly, with the majority (n= 432, 49.7%) being in Mafraq, followed by Karak (n= 233, 26.8%) and East Amman (n= 205, 23.6%) (Figure 1).

Figure 1. Distribution of human cases of brucellosis in Mafraq, Karak, and East Amman governorates in Jordan from May 2019-August 2021 (N=870)



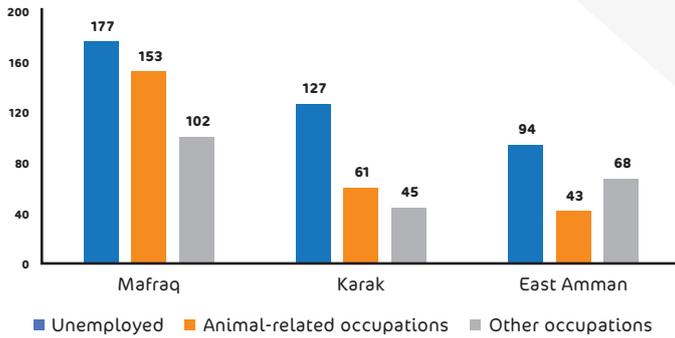
All the RBT positive samples underwent additional serum agglutination testing (SAT) and enzyme-linked immunoassay (ELISA, IgM, and IgG) testing. The results of the SAT and ELISA tests seemed to be more specific than the RBT, since only 82.3% of the RBT positive samples (n=716) were positive by SAT, 62.1% (n=540) had positive ELISA IgM results, and 69.5% (n=605) had positive ELISA IgG (Table 1).

Table 1. Lab testing results of human brucellosis cases in Mafraq, Karak, and East Amman governorates of Jordan from May 2019-August 2021

Governorate	RBT n (%)	SAT n (%)	ELISA IgM n (%)	ELISA IgG n (%)
Mafraq	432 (49.7%)	383 (44.0%)	282 (32.4%)	369 (42.4%)
Karak	233 (26.8%)	160 (18.4%)	137 (15.6%)	140 (16.1%)
East Amman	205 (23.6%)	173 (19.9%)	121 (13.9%)	96 (11.0%)
Total	870	716 (82.3%)	540 (62.1%)	605 (69.5%)

For this study, patients' occupations were considered as well. All the participants were asked if their occupations involved contact with animals or their tissues; 257 (29.5%) in total had animal-related occupations (such as being livestock owners, butchers, dairy makers, veterinarians, and farm laborers), 398 (45.7%) were unemployed or underage, and 215 (24.7%) had other unrelated occupations. The highest number of participants who had animal-related occupations were from Mafraq, with 153 patients, followed by 61 from Karak and 43 in East Amman (Figure 2).

Figure 2. Distribution of human cases by occupation type and governate (N=870)



Animal farm investigation findings

To date, 258 farms were investigated by the study team, the majority being in Mafraq, with 121 farms, followed by 77 farms in East Amman, and 56 in Karak (Figure 3). A total of 2,110 samples were collected from sheep (n=1633, 77.4%), goats (n=400, 19.0%), cows (n=61, 2.9%), and camels (n=15, 0.7%) (Figure 4).

Figure 3. Distribution of investigated farms by governate (N=258)

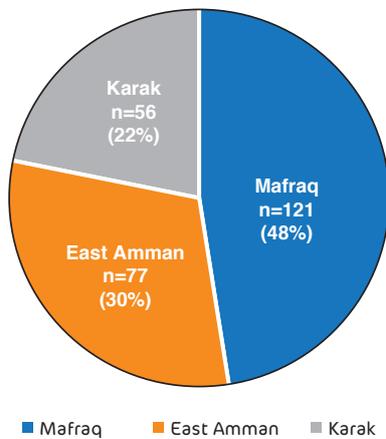
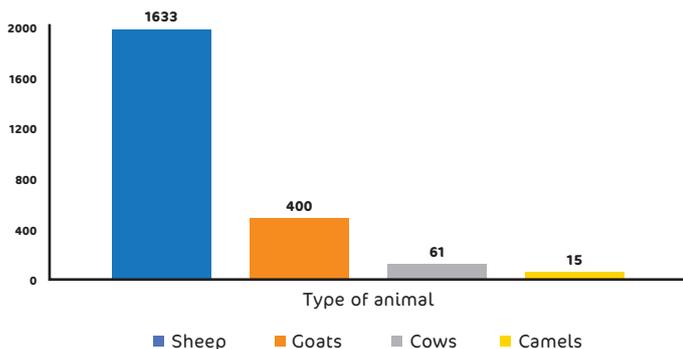
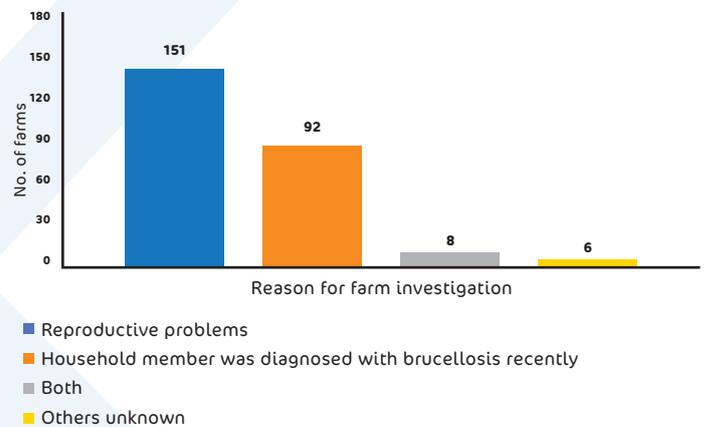


Figure 4. Distribution of collected animal samples by type of animal (N= 2110)



In accordance with the study design, the method of selecting farms to investigate was by asking all patients included in this study if they owned or were in close contact with animals, and if so, the MOA was notified of the location of the farm or herd that the person revealed. After obtaining the consent of the owners, blood and milk samples were collected from the animals of the suspected farm. MOA representatives also investigated the animals of any farms in East Amman, Mafraq, and Karak whose owners voluntarily asked for examination due to apparent symptoms of the disease (Figure 5).

Figure 5. Number of farms visited by cause for investigation (N=258)



RBT and fluorescence-polarization assays (FPAs) were conducted in tandem for the blood samples collected from the suspected animals (2,098 samples), while only FPA was carried out for the collected milk samples (759 samples). The portion of blood samples positive for brucella was higher with FPA (38.7%) than with RBT (31.5%). On the other hand, 30.4% of milk samples were positive. The highest number of FPA-positive blood samples was observed in Mafraq, followed by East Amman and Karak (Table 2).

Table 2. Lab testing results of blood and milk samples collected from animal farms in Mafraq, Karak, and East Amman governorates of Jordan from May 2019-August 2021

Governorate	Blood samples (N=2098)		Milk samples (N=759)
	RBT n (%)	FPA n (%)	FPA n (%)
Mafraq	344 (52.0%)	370 (45.6%)	8 (3.5%)
Karak	181 (27.4%)	200 (24.7%)	28 (12.2%)
East Amman	136 (20.6%)	241 (29.7%)	194 (84.3%)
Total	661	811	230
Positivity Rate	(31.5%)	(38.7%)	(30.4%)

PCR testing and whole genome sequencing results

Overall, PCR testing was completed for 630 human samples that were positive for brucellosis by ELISA (IgG or IgM). Only 73 (11.6%) of those samples were positive for brucella species. The most common subtype to be identified was *B. melitensis*, which accounted for 36 (49.3%) of the samples. The other species to be identified was *B. abortus* with 27 (37%) samples. Seven were undefined (*Brucella* spp.), while 3 samples produced a mixed result of *both B. melitensis and B. abortus* species, for which the PCR analysis will be redone to confirm the results (Figure 6). For isolates identified as *B. melitensis* by PCR, whole genome multilocus sequence typing analysis (wgMLST) was additionally conducted. The resulting allele clusters were grouped with other clusters of *B. melitensis* from the Eastern Mediterranean region by their allelic relationships (Figure 7).

Figure 6. Results of PCR subtyping of human samples (N=630)

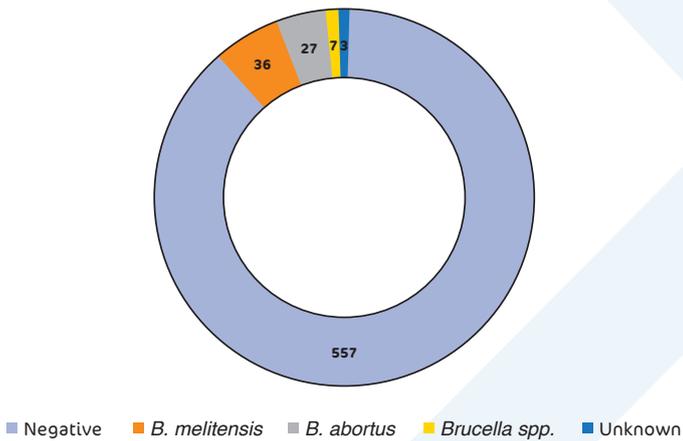
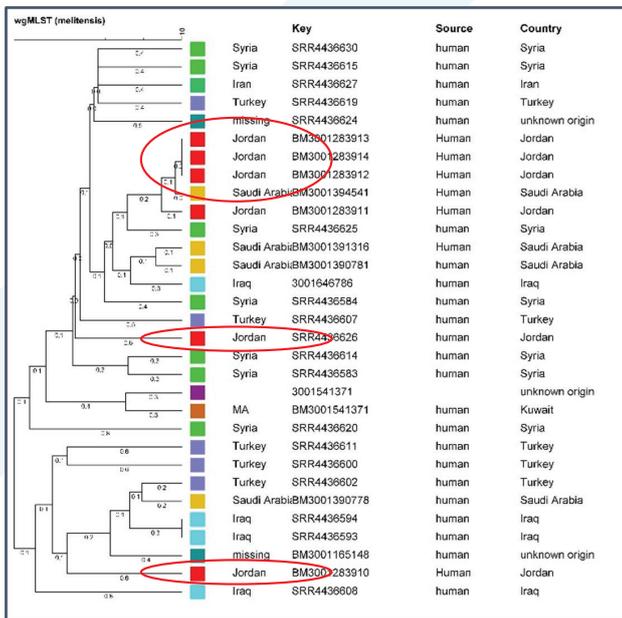


Figure 7. Whole genome multilocus sequence typing analysis (wgMLST) of *B. melitensis* isolates from Jordan



Conclusion

The activities under this project introduced to the participating bodies new lab detection methods, such as ELISA and FPA, that had never been used in this capacity in Jordan, in addition to providing them with GIS analysis and data presentation skills. As we enter our third year of the project, it is evident that participating centers are now confident in using these methods and analyzing and reporting the results. Also, in keeping with the 'One-Health' approach, we were able to initiate and facilitate a communication channel between the agricultural and health sectors that was instrumental in successfully portraying the human and animal elements of this zoonosis, and that can be utilized in future projects tackling surveillance of other zoonotic diseases. The use of whole genome multilocus sequencing and Multiple Locus Variable-number Tandem Repeat Analysis (MLVA) genotyping methods will provide new and valuable insights of brucellosis epidemiology in the country.



