Statement of use/total expenditure report

General information on the project

Funding number:	2817LEAP01
Beneficiaries:	University of Hohenheim, Department of Infection and Environmental Hygiene in Farm Animals, PD Dr. Wolfgang Beyer
Project title:	Multisectoral strategy to combat brucellosis in East Africa
Duration of the project (reporting period):	August 2018 - December 2021
Cooperating partners:	• Prof Dr Joseph Erume, Makerere University, Kampala, Uganda • PD Dr. Wolfgang Beyer, University of Hohenheim, Stuttgart • Prof Dr Ignacio Moriyon, University of Navarre, Pamplona, Spain • Prof Dr Jose Blasco, Centro de Investigacion y Technologica Alimentaria de Aragon, Zaragoza, Spain • Prof Dr Lilly Bebora, Faculty of Veterinary Medicine University of Nairobi, Kenya
Associated partners:	• Animal Health Research Institute (AHRI), Mansoura, Egypt • Faculty of Veterinary Medicine, Benha University, Egypt
Further cooperation partners	• Friedrich Löffler Institute, Jena • Federal Institute for Risk Assessment (BfR) Berlin

Please insert a map of the target region:

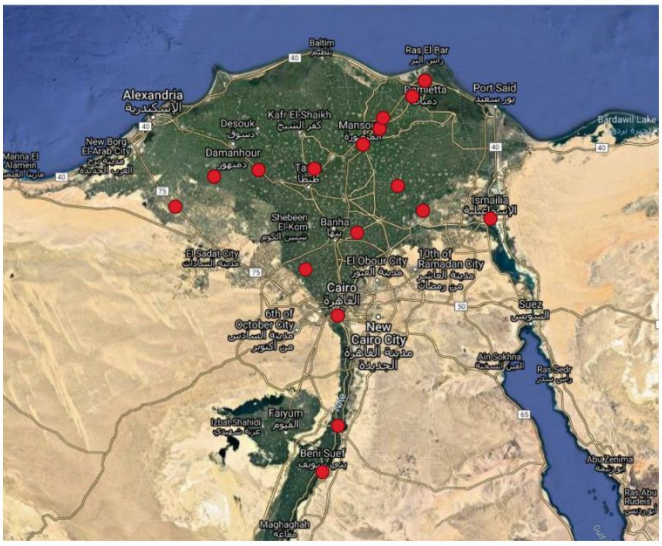

Fig. 1: Sample origin area Egypt (map taken from: Holzer et al. (2021), Microorganisms, 9, 1942.

1. Subject matter and objectives of the project

1.1. Please explain the subject matter and objectives of the project (with reference to the objectives of the specific notice or call for tenders).

Livestock farming in many African countries, including Egypt, is a very high economic factor and is of great importance for nutrition and health in these countries.
For Uganda and Kenya, the disease threatens the population of about 30.4 million cattle, 42.4 million goats, 21.1 million sheep and 5.3 million pigs. The majority of animal husbandry takes place in small farms and pastures. The mixed keeping of different animal species is the rule. Livestock farming is central to the food supply of the population in these countries and secures an income for 70-80% of the population. The impact of animal diseases such as brucellosis on food security, socio-economic development and population health is correspondingly high (Ducrotoy et al., 2017). Despite evidence of a high prevalence of brucellosis in the livestock populations of Uganda and Kenya, there are currently no government control programs there. The reasons for this are a lack of institutional and technical prerequisites as well as insufficient training and further education of the personnel required for control programs in agriculture, medicine and veterinary medicine. In addition, there is a certain ignorance and misinformation based on traditional peculiarities as well as state mismanagement.
In Kenya, up to 25%, in Uganda up to 56% and in Egypt up to 38% of farm animals are considered Brucella-infected. Therefore, the original programme aimed to examine samples from these countries in particular.
Despite the implementation of a government control program, In 1981, brucellosis is still endemic to Egypt and the incidence is rising continuously, including in the human population. It is 64 to 70 per 100,000 inhabitants.

The aim of the project was to record the phylogeographic diversity and distribution of *Brucella* spp. as well as the determination of possible current and historical distribution channels. It should be examined to what extent genotyping with different methods, MLVA and SNPanalyse, makes the source and distribution of outbreak strains comprehensible. These data can form the basis for targeted control strategies in the countries.

For the MUSBCEA project, the following questions were important:
1) What is the prevalence of brucellosis in livestock in Uganda, Kenya and Egypt?
2) What are verifiable distribution pathways between livestock and from animals to humans?
3) Which outbreak strains occur and can their spread be tracked (biovars/genotypes)?
4) Which vaccines promise sustainable vaccination success?
5) Which infrastructural and personnel capacities are necessary for brucellosis management on site, how can they be provided and focused?
6) How can the attention of the population, especially livestock farmers, be increased?

The task of the German project partner in the project network was the isolation, characterization and genotyping of *Brucella* spp. from the partner countries. Using genetic and epidemiological metadata, phylogeographic maps of the spread and spread of brucellosis outbreak strains in the countries of origin should be established. The genotyping methods used followed, where possible, a hierarchical scheme, using canSNP analysis (for *B. melitensis*), fragment analysis (Bruce-ladder

PCR, Multilocus Variable Number of Tandem Repeats Analysis (MLVA) and whole genome sequencing followed by core genome SNP (cgSNP) analysis. The sequence and MLVA data should be fed into the international databases and compared with existing data.

Literature:
Ducrotoy M, Bertu WJ, Matope G, Cadmus S, Conde-Álvarez R, Gusi AM, Welburn S, Ocholi R, Blasco JM, Moriyón I (2017): Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. Acta Tropica 165:179–193.

1.2. Name the scientific and technical status that was followed up at the beginning of the project.

According to the conventional diagnostic scheme, *Brucella* spp. grown from clinical field samples using a selective culture medium and then biochemically characterized.
After preparation of genomic DNA, the species can also be determined by means of a diagnostic multiplex Bruce-ladder PCR (Lopez-Goni et al., 2008). These classical diagnostic methods allow a differentiation of *Brucella* Isolates up to biovar. However, they do not allow differentiation of outbreak strains and are therefore unsuitable for the analysis of outbreak sources and the spread of outbreak strains.
More recently, methods for analysing the prevalence and diversity of *Brucella* spp., as well as many other pathogens, shifted from serological and laboratory-cultural phenotypic methods to genotypic methods of differentiation and characterization, such as SNP analysis for high-resolution differentiation of genotypes (Janowitz et al., 2018) and their phylogenetic relationship. A common method of genotypic differentiation is MLVA. The molecular subtyping of Brucella using MLVA has been described several times better (Al Dahouk et al., 2003; Le Fleche et al., 2006; Whatmore et al. 2006; Al Dahouk et al., 2007; Whatmore 2009, Maquart et al, 2009; Ferreira et al., 2012; Garafolo et al. 2013, Borrielo et al., 2013). However, laboratory-based techniques suffer from poor repeatability, almost impossible interchangeability of data between different laboratories, and a lack of phylogenetic information. Despite high discriminatory capacity, they are unsuitable for defining outbreak strains and tracking them. Unfortunately, this has not been sufficiently taken into account in the previous literature.
The project therefore pursues not only the improvement of the laboratory-based MLVA by means of an in silico analysis of the VNTR markers but also the comparison of the MLVA data with those from the in silico Coregenome SNP analysis.

Literature:
1. Abdel-Hamid, N.H.; El-Bauomy, E.M.; Ghobashy, H.M.; Shehata, A.A. Genetic variation of Brucella isolates at strain level in Egypt. *Vet. Med. Sci.* **2020**, *6*, 421–432, doi:10.1002/vms3.260.
2. Al Dahouk S, Le Flèche P, Nöckler K, Jacques I, Grayon M, Scholz HC, Tomaso H, Vergnaud G, Neubauer H (2007): Evaluation of Brucella MLVA typing for human brucellosis. J Microbiol Methods, 69:137-145.
3. Al Dahouk, S., Tomaso, H., Nöckler, K., Neubauer, H., Frangoulidis, D. (2003): Laboratory-based diagnosis of brucellosis—a review of the literature. Part I: techniques for direct detection and identification of Brucella spp. Clin. Lab. 49, 487–505.
4. Ducrotoy M, Bertu WJ, Matope G, Cadmus S, Conde-Álvarez R, Gusi AM, Welburn S, Ocholi R, Blasco JM, Moriyón I (2017): Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. Acta Tropica 165:179–193.

5. Garofolo G, Di Giannatale E, De Massis F, Zilli K, Ancora M, Cammà C, et al (2013): Investigating genetic diversity of Brucella abortus and Brucella melitensis in Italy with MLVA-16. Infect Genet Evol.,19:59–70.
6. Janowicz, A.; Massis, F. de; Ancora, M.; Cammà, C.; Patavino, C.; Battisti, A.; Prior, K.; Harmsen, D.; Scholz, H.; Zilli, K.; et al. Core Genome Multilocus Sequence Typing and Single Nucleotide Polymorphism Analysis in the Epidemiology of Brucella melitensis Infections. J. Clin. Microbiol. 2018, 56, doi:10.1128/JCM.00517-18.
7. Khan, A.U.; Melzer, F.; Sayour, A.E.; Shell, W.S.; Linde, J.; Abdel-Glil, M.; El-Soally, S.A.G.E.; Elschner, M.C.; Sayour, H.E.M.; Ramadan, E.S.; et al. Whole-Genome Sequencing for Tracing the Genetic Diversity of Brucella abortus and Brucella melitensis Isolated from Livestock in Egypt. *Pathogens* **2021**, *10*, 759, doi:10.3390/pathogens10060759.
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10. Wareth, G.; El-Diasty, M.; Melzer, F.; Schmoock, G.; Moustafa, S.A.; El-Beskawy, M.; Khater, D.F.; Hamdy, M.E.R.; Zaki, H.M.; Ferreira, A.C.; et al. MLVA-16 Genotyping of Brucella abortus and Brucella melitensis Isolates from Different Animal Species in Egypt: Geographical Relatedness and the Mediterranean Lineage. *Pathogens* **2020**, *9*, doi:10.3390/pathogens9060498.
11. Whatmore, A.M. (2009): Current understanding of the genetic diversity of Brucella, an expanding genus of zoonotic pathogens. Infect. Genet. Evol. 9, 1168–1184.
12. Whatmore, A.M., Murphy, T.J., Shankster, S., Young, E., Cutler, S., Macmillan, A.P. (2005): Use of amplified length fragment polymorphism to identify and type Brucella isolates of medical and veterinary interest. J. Clin. Microbiol. 43, 761–769.

1.3. Explain the methods used.

With the exception of defined reference strains, all samples used come from field isolates, which could be assigned to an outbreak in animals (cattle, sheep, goat, camel, dog) and humans. The samples were provided from the laboratories of AHRI and Benha University. Pure cultures from our own isolation or killed culture material were available for DNA preparation. The whole genome sequencing was awarded to the company Eurofins. For the analysis of the raw data, the Linux-based bioinformatics pipeline WGSBAC (v.2.1.) of the IBIZ Jena was used. The species determination was carried out by bruce-ladder PCR in silico with the help of Geneious v.11.1.5 or after fragment analysis in capillary electrophoresis. Bionumerics v.8.0 was used for cluster analysis and the creation of phylogenetic family trees. MlVA with 16 markers was performed using MISTReSS in silico or as fragment analysis in capillary electrophoresis. Phylogeographic maps were created using QGIS v3.12.2.

2. Results and usability of the project

2.1. Please present in detail the main results of the project.

For the analysis of the distribution and diversity of outbreak strains of *Brucella* spp. were able to collect 47 samples of *B. abortus* from 11 governorates and 137 samples from *B. melitensis* from 17 governorates of Egypt from the years 2001-2020.
Genotyping, based on differences in cgSNPs or the fragment length of VNTR markers, allowed for accurate differentiation at the isolate level. For the assignment of genotypes to outbreak strains and thus their differentiation, the epidemiological metadata, time and place as well as animal species of isolation, were used.
The previous assumption, published several times, that isolates with different MLVA strings, i.e. different MLVA genotypes, also represent different outbreak strains or vice versa identical MLVA genotypes identical outbreak strains was refuted in this project. The MLVA, which is prone to errors in comparison, can and should be reliably replaced by a cgSNP analysis.
The analyses revealed a completely new picture of the diversity and distribution of *Brucella abortus* and *Brucella melitensis* in Egypt. According to this, there are rather old outbreak strains, whose origin probably lies in older animal imports from other European countries (e.g. Italy and Great Britain) and, in addition, younger and very diverse outbreak strains, for which individual younger animal imports could be blamed.
In detail, outbreak strains with a sometimes wide distribution over up to 5 governorates could be detected. These results speak for a spread of the pathogens with live animal transport or via contaminated animal foods, especially dairy products.
These assignments are of utmost relevance for a targeted analysis of the epidemiological situation of the occurrence and the distribution pathways and causes of brucellosis in Egypt. With the methodology established here, human and veterinary medical institutions would have a tool in their hands for the establishment of targeted control measures. This approach is based on any other region with epidemic occurrence of brucellosis.

An important milestone of the project was the establishment of a bioinformatics pipeline developed at the reference laboratory Brucellosis, IBIZ FLI Jena, on the "BinAC" server in Tübingen and the establishment of corresponding external access for the processors in Hohenheim and ibiz. This lays the foundations for the evaluation of bacterial whole genome sequences, not only for *Brucella* spp., at the University of Hohenheim.

As an additional working point to the originally planned methods for genotyping, the canSNP analysis for *Brucella melitensis* after Jeffrey Foster (Foster et al. 2018) in Hohenheim. This method enables the phylogeographic classification of isolates of *B. melitensis* and thus the establishment of a hierarchical typing scheme for new field isolates of *Brucella* Spp.

As part of the workshop for the opening of the project at the Faculty of Veterinary Medicine University of Nairobi, in Kenya, from 21-29 March 2019, scientific and technical staff from the Faculty of Veterinary Medicine at the University of Nairobi received a four-day training in sample selection, sampling and vaccination of farm animals as well as laboratory diagnostics for brucellosis. On a further 4 days, the theoretical basics of bacteriology, diagnostics, epidemiology and immunology of brucellosis were taught to participants from the university, veterinarians and physicians. The originally invited participants from various ministries as well as the Zoonosis disease Unit Kenya did not participate. All lectures and demonstrations were organized by the partners from Spain, University of Navarre and CITA and the University of Hohenheim.

The workshop planned in Uganda analogous to the event in Kenya could not take place due to a lack of funding. It was not until 29 June 2020 that an online event took place at the beginning of the project, with presentation of the project to local participants of the University of Kampala. Another online event with lectures on the basics of vaccination strategies against brucellosis was organized by the partners in Uganda and Spain for Feb. 1, 2022. Participants of this event were students of the Veterinary Faculty of Makerere University.

Key statements and policy advice:

Already in a very early phase of the MUSBCEA project, Dr. Beyer had pointed out in an e-mail of 20 February 2019 to the BLE that while the work at the German partner had begun in August 2018 with the employment of a doctoral student according to the work plan, there had been serious financial problems for all other project partners, with foreseeable negative consequences for the entire project.
The midterm report of the PL of the project, Prof. Joseph Erume, Makerere University, Uganda read:
"The project is facing a number of challenges.

- Key among which is the lack of disbursement of funds by the Uganda government. This is causing a lot of frustration in the consortium and research uptake. The whole planned activities of the project are basically on hold and we are wondering as to what can be done to accomplish work as planned.
- More than half the project's life time has been lost without proper activities due to Ugandan funding situation.
- The students from Uganda and Germany already enrolled since September 2018 but up to now have not commenced their studies.
- Lack of funding from Uganda, made the European partners to support Ugandan students to attend the launch and training in Kenya in March 2019. This caused a strain in the finances of these partners since this was not planned for.
- The Consortium members in Europe are finding it very cumbersome and difficult to provide mandatory project progress reports with no activities going on.
- The project Coordinator is finding it difficult to coordinate the project activities without resources.
- The money released in Kenya is less than expected and this is constraining accomplishment of the project activities
- The procurement procedures in Kenya are too tedious and long and this is causing unnecessary delays in execution of project activities.
- In Kenya there is slow progress towards isolation of Brucella from the collected samples since up to now approval for use of KEMRI lab has not been granted."

These problems addressed in the report became increasingly acute in the course of the project. In addition, there were other structural problems associated with the corona pandemic, e.g. "lock-downs" as well as foreign and domestic travel restrictions, which continued until the end of the German part of the project in December 2021.

In the following, some of the problems and their impact on cooperation with African partners are addressed.

Cooperation with Makerere University, Uganda

According to the project leader, Prof. Joseph Erume, the project in Uganda was paid partial funding for 3 months each in mid-2020 and 2021. Of this, both MOSTI and Makerere University in Kampala retained an overhead of 15% of the funding amount each. With this completely inadequate underfunding, the planned activities could not be carried out on site, neither the necessary field research, nor corresponding laboratory work, nor the planned workshops. For this reason, the German partner provided funds from its own budget, e.g. for travel expenses of the scientists from Uganda to the opening meeting in Kenya as well as for the transport of all participants to workshops at farms, for the financing of the ethics vote for Uganda and for a corresponding order and delivery of consumables to the laboratory in Kampala, in 2020. Orders from the university's own resources were declared to be ineffective, as they were associated with considerable regulatory complications at the University of Kampala and therefore too time-consuming. Despite multiple commitments from a local company in Kampala, the delivery of the order via the University of Hohenheim was insufficiently carried out, so that the working capacity of the laboratory at Makerere University could not be sufficiently established by the end of the project.

Due to additional problems with Covid-19, Uganda was a high-risk area and was still in lock-down from May-August 2021, as well as the locust plague 2019/2020, no sampling could be planned on site after receiving the first round of financing. These difficulties also prevented the German doctoral student's originally planned 6-month research stay in Uganda. Only in the second half of 2021 could the first laboratory work take place in Uganda and by September 2021 a total of 15 Brucella strains were isolated from samples in the laboratory in Kampala, but their dispatch for genotyping could no longer be successfully organized until the end of the project in December 2021.

Cooperation with University of Nairobi, Faculty of Veterinary Medicine, Kenya

As already explained in the interim report to the BLE of 24.04.2020, the Kenyan partner was paid only 7,500 euros in 2019 instead of the planned 40,000 euros for three years of research. In 2020, no further funding of the cooperation partners took place at the University of Nairobi, Kenya. The sum was in no way sufficient for the originally planned activities for sample collection and processing by university staff. In addition, the University of Nairobi does not have its own security level 3 or level 2plus laboratory in which the *Brucella* Isolates could be cultivated. However, this would have been a sine qua non condition for all further analyses at the German partner and would also have been shown accordingly in the original of the project application. The shortcoming was not known to the MUSBCEA consortium and was first addressed during the project meeting in April 2019 at the University of Nairobi. Considerations for the short-term establishment of a corresponding laboratory in a partner faculty of the university did not lead to any result. The only possible alternative to an approved S3 laboratory was at the Kenyan Medical Research Institute (KEMRI) in Nairobi. Despite intensive contacts between Dr. Beyer and the representative of KEMRI, it was not possible to obtain an access authorization or a working time window for the MUSBCEA project from the Japanese operator of the laboratory. Nothing is known about the partner's activities at the University of Nairobi. In connection with the Covid-19 restrictions that also apply to Kenya, the aforementioned difficulties led to the fact that the research stay of the doctoral student from Germany, originally planned for 6 months, could not take place. About the insulation of *Brucella* there is no information from the Kenyan project partner from collected field samples. The corresponding funds in the budget were mainly rebooked to the increased number of whole genome sequencings or repaid to the BLE at the end of 2020.

Cooperation with AHRI, Egypt and FLI Jena

The background to the association of another partner from Egypt in the MUSBCEA project was the idea of expanding the original objectives of the project, which arose in the course of consultations with the partners from Spain, Uganda and Kenya, during the workshop in Nairobi in March 2019. The collected epidemiological and phylogenetic data should be used to develop a long-term map of the spread of brucellosis along historical trade routes both between the countries south and north of the Sahara and in North Africa, linked in time and geographically with phylogenetic markers. Such phylogeographic analyses, based on whole genome analyses and corresponding epidemiological metadata, are well known and published, for example, from anthrax research. The outlined objective is to be seen as a longer-term project, for which the current project should create the basis in the sense of cooperation between competent partners and the merging of existing and new database sets.

The expansion of the partnership to include the Egyptian partner was made possible by the previously existing long-standing cooperation between the Department of Infection and Environmental Hygiene in Farm Animals (formerly institute for environmental and animal hygiene) of the University of Hohenheim and the FLI Jena and currently the German and OIE reference laboratory for brucellosis there. A first meeting of the partners from Egypt took place during an international meeting in Palermo, June 2019, on the occasion of the closing event of the project "Brucellosis in the mediterranean countries". Our project and the scientists involved in it were given the opportunity to participate in all existing cooperations. This concerned in particular the training and further education as well as experimental work on site for the doctoral student, Ms. Katharina Holzer, as well as the joint processing of sample material from the reference laboratory in Jena.

During a research stay of PD Dr. Beyer and Ms. K. Holzer in Cairo and Mansoura, in November 2019, the following research institutions were visited and discussions were held to expand cooperation relations:

- Animal Health Research Institute, Cairo,
- Benha University, Benha,
- Animal Health Reserach Institute, Provincial Lab, Mansoura.

Ms. Holzer and Dr. Beyer gave invited lectures at the conference "Brucellosis and abortion in sheep" organized by AHRI in Mansoura to present the MUSBCEA project as well as lectures on brucellosis (species, infection in animals and humans, transmission, virulence, safety) and the genotyping of brucellosis. In addition to the doctoral student's research stay in Egypt, the project expansion included in particular the collection and processing of sample material from the institutes in Cairo and Mansoura. Corresponding budget changes were approved by the BLE. A further workshop or already planned activities in the field by the UHO in Egypt could no longer take place due to the pandemic and the associated strict travel bans. Of the 313 samples from Egypt, 191 publishable whole genome sequences could be created.

2.2. Identify the expected benefits and usability of the results. Which practice-relevant results were achieved in the project? Please explain to what extent these results are directly applicable in practice and what possibilities you see for a transfer of these results into practice.

The results achieved in the German part of the project exemplify the spread and possible distribution routes of brucellosis in Egypt over a period of about 10 years.
The established methods can be used as a blueprint for the development of government control programmes of the responsible authorities in the agricultural, human and veterinary sectors. This gives the authorities the opportunity to exert targeted influence on the import and spread of brucellosis pathogens, to trace the sources and spread of outbreaks and, if necessary, to prevent them.
For the implementation of such programs, the establishment of the methods in state-owned leading laboratories is necessary, combined with the transfer of knowledge on the scientific-medical basics as well as the technical prerequisites. This transfer was included in the original approach of the MUSBCEA project, e.g. through on-site training of employees in Uganda and Kenya or through training stays of employees from Uganda, Kenya and Egypt in the German laboratory of the University of Hohenheim. These projects could not be implemented in 2020 and 2021 due to the identified problems, the catastrophic financial undersupply of the partners in Uganda and Kenya, the lack of structural conditions in Kenya and the travel restrictions associated with Corona in and between all countries.

2.3. What recommendations can you derive from the results achieved?

With the establishment of the differentiation of Brucella genotypes by means of cgSNP analysis in conjunction with the assignment of genotypes to outbreak strains defined by epidemiological metadata, the diversity and distribution of brucellosis in Egypt could be analyzed in an exemplary manner. This methodology should be used in all future brucellosis control programmes.

2.4. What possible further questions or points of contact do you see?

The genotypic analysis of outbreak strains of brucellosis, once established in appropriate laboratories, could make a significant contribution to brucellosis control in all countries where brucellosis is endemic. Corresponding projects should therefore be launched in other countries. Basically, the spread of brucellosis on the African continent, north of the Sahara, remains an important open question. While previous programmes have mainly looked at the input and spread of brucellosis in the Mediterranean region, future projects should be extended to the south. The focus should be on the historically traditional trade routes, e.g. along the Nile.

2.5. List past and planned activities to disseminate the results.

Whole genome sequences are added to the international database on National Center for Biotechnology Information (NCBI) under BioProject number PRJNA742519 and the following (https://www.ncbi.nlm.nih.gov/bioproject/742519, accessed on 9 September 2021)if they are part of a publication. The MLVA data is completely transferred to the freely accessible MLVA database (https://microbesgenotyping.i2bc.paris-saclay.fr/databases/view/54==References== These and all other data will be published together with the respective project partners in scientific journals and at corresponding conferences.

2.6. Please provide an overview of all publications realized in the reporting period (publications, print media, newsletters, etc.). Please attach as a separate attachment.

J. Erume, W. Beyer, I. Moriyon, I. Marco, J.M. Blasco, L.C. Bebora, J.M. Figueres and P.R. Blanco: "Multi-sectorial strategy for brucellosis control in Eastern Africa"
LEAP-Agri Project's Kick-Off Meeting of ERA-Net LEAP-Agri Funded Projects
8 – 10 October 2018 | Bari, Italy

Wolfgang Beyer: "Molecular Genotyping of Isolates"; "University of Hohenheim and contribution to MUSBCEA"
Katharina Holzer: "Methods for genotyping of different Brucella species"
Lectures on the occasion of the kick-off meeting, University of Nairobi, Department of Clinical Studies
College of Agriculture and Veterinary Medicine, Upper Kabete, Nairobi Kenya, 21.-29.3.1019

Wolfgang Beyer: "Molecular Genotyping of Isolates"
Katharina Holzer: " The terroristic microorganism–molecular biology of *Brucella"*
Lectures on the occasion of the AHRI conference: "Brucellosis and Abortion in sheep", Mansoura, Egypt, 04.11.2019

Holzer, K.; El-Diasty, M.; Wareth, G.; Abdel-Hamid, N.H.; Hamdy, M.E.R.; Moustafa, S.A.; Linde, J.; Bartusch, F.; Sayour, A.E.; Elbauomy, E.M.; Elhadidy, M., Melzer F.; Beyer W.: Tracking the Distribution of *Brucella abortus* in Egypt Based on Core Genome SNP Analysis and In Silico MLVA-16. Microorganisms **2021**, 9, 1942. https://doi.org/10.3390/ microorganisms9091942.

In preparation:

Holzer, K., El-Diasty, M., Wareth G., Abdel-Hamid, N.H., Hamdy, M.E.R., Moustafa, S.A., Linde, J., Bartusch, F., Sayour A., Elhadidy, M., Melzer, F., Beyer, W.: Tracking the distribution of *Brucella melitensis* in Egypt based on core genome SNP analysis and MLVA-16. (Manuscript in preparation).

Katharina Holzer: "Investigation of the spatio-temporal spread of brucellosis outbreak strains in Egypt using cgSNP analysis and multi-locus VNTR analysis", 2022.
Dissertation on the attainment of the doctoral degree in natural sciences (Dr. rer. nat.). Faculty of Natural Sciences, University of Hohenheim.

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