END OF PROJECT REPORT

Project Title	***Epidemiology of brucellosis on the livestock, wildlife and human interface***: Improving the diagnostic capacities of brucellosis disease, enhance the control strategies with special emphasis on farmers' awareness in the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan.
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Implementing partners	
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Participating countries	Uganda, Democratic Republic of Congo, South Sudan
Project cost	$63971($30000 – PGTF; $33971-University of Navarra)

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Acknowledgement

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Executive summary

Zoonotic diseases like brucellosis constitute major economic and public health challenges in many developing countries. Globally brucellosis is recognized as one of the major zoonotic diseases. In the great lakes region the disease constitutes one of the major constraints to animal production and public health. The disease is one of the seven zoonoses that has ben prioritized for control in Uganda. The prevalence and risk factors for brucellosis in cattle has been widely studied but in small ruminants and humans the situation is not well known. Moreover, the situation of caprine brucellosis at the human – livestock wildlife interface of the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan needs to be understood in order to develop suitable control strategies.

Therefore, with funding and technical support from the Perez-Guerrero Trust Fund for South-South cooperation, members of the Group of 77 and the Universidad de Navarra, Spain a project titled "***Epidemiology of brucellosis on the livestock, wildlife and human interface***: Improving the diagnostic capacities of brucellosis disease, enhance the control strategies with special emphasis on farmers' awareness in the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan" was implemented. The overall objective of the project was to gather evidence for informing viable control strategies in goats, sheep, cattle and humans. Specifically the project aimed at isolating and characterizing the infecting *Brucellae species* in goats, sheep, cattle, humans and wildlife within Bwindi-Mgahinga, Queen Elizabeth, and Murchison Falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan; increasing public awareness of animal and human brucellosis and the ways of preventing this disease in both animals and humans and improving the diagnostic capacities and awareness by health practitioners about brucellosis in domestic ruminants, wildlife and humans in Uganda, DRC and South Sudan. Twenty three (23) health practitioners from Uganda and DRC were interviewed to assess awareness on brucellosis diagnosis, prevention and control. Our findings revealed knowledge gaps that can adversely affect the judgment, decision-making and management of brucellosis by the different health

service providers. As a follow up we trained 30 health care (Including veterinarians, medical personnel, Lab technologists, animal production officers, academicians) personnel from DRC and Uganda on brucellosis diagnosis, prevention and control with technical assistance from Universidad de Navarra, Spain. To increase public awareness about the disease we conducted 2 community awareness meetings around Murchison Falls National Park (MFNP) in Uganda and 2 meetings around Virunga National Park in DRC were conducted. In total we reached out to over 247 community members in both countries. One thousand one hundred and eleven (1111) bovine sera, 943 caprine sera, and 35 ovine sera were collected from Kasese, Nakasongola, Nakaseke and Kiryandongo districts in Uganda and screened for brucella anti S/LPS antibodies using the RBT. We found 6.4% sero prevalence of brucella anti S/LPS antibodies in cattle, 0.009% sero prevalence in goats and 0.08% in sheep. No brucella isolates were recovered from milk cultures. To disseminate our work a policy brief and a short communication were developed. The policy brief is available at www.nalirri.or.ug but it has also been shared with key stakeholders. The short communication titled *"Awareness about brucellosis among health and veterinary practioners from Uganda and Democratic Republic of Congo and the implications for service delivery"* is due for publication in a suitable journal. From the foregoing work we recommend periodic CPD courses tailored to brucellosis for frontline health service providers in endemic areas to address knowledge gaps and provide information on recent advances in research on the disease. We also propose collecting postmortem samples at slaughterhouses for isolating brucellae in future studies.

Introduction

Brucellosis is an important disease among livestock and people in the great lakes region with highest incidences registered in farms with large herds compared to small ones (Kabagambe *et al.*, 2001). The disease is among the 7 priority zoonotic diseases targeted for control in Uganda (Sekamatte *et al.*, 2018). The prevalence and risk factors for infections for brucellosis in small ruminants is poorly understood since a lot of attention is paid to bovine brucellosis (Makita *et al.*, 2011; McDermott *et al.*, 2013; McDermott *et al.*, 2002). This species bias consequently affects prioritization of control activities. A study involving the Autonomous University of Barcelona (UAB), the Government of Andorra, Daktari, a local Non-Governmental Organization and Makerere University observed a widespread circulation of brucellosis in sheep and goats within the Mgahinga conservation area (Marco et al., 2016). This suggests a bigger problem that requires urgent intervention. There is need therefore, to estimate the prevalence of brucellosis in all these domestic livestock species and wild life potentially in contact and as well isolate and characterize the infecting *Brucellae* species. Brucellosis has a tremendous impact on multiple animal species and humans thus making it a high priority disease both in sub- Saharan Africa and other regions of the developing world (McDermott *et al.*, 2002). According to Kabagambe *et al.* (2001) *Br. melitensis* is the main organism infecting sheep and goats in Uganda. Therefore, if it is not studied and controlled in these species, a steady supply of infectious organisms to maintain transmission to humans, livestock and wildlife will be sustained.

It is worth noting that human cases are mainly found in areas with high prevalence of caprine brucellosis (Mishal *et al.*, 1999). This is due to the fact that, sheep and goats are the primary reservoir of *Brucella melitensis*, which is widely known for being the most pathogenic species for humans and animals (Emslie *et al.*, 2002). Furthermore, goat and sheep rearing is being practiced by majority of households irrespective of wealth rank as an important source of food for self-consumption and income generation in rural areas (Benda *et al.*, 2015). Therefore, goats and sheep have an important role in the transmission and perpetuation of brucellosis (Minas *et al.*, 2004). The relationship between humans and small ruminants can result in an increment of brucellosis cases and outbreaks among goat keepers.

Although human brucellosis is the most common bacterial zoonotic infection worldwide, it is still a regionally neglected disease (Pappas *et al.*, 2006). It is often misdiagnosed in developing countries due to poor knowledge of zoonotic diseases by medical professionals especially in rural areas, often resulting in under reporting of the cases.

Information concerning epidemiological patterns of caprine brucellosis in the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan is still scarce. This is because epidemiological studies in Uganda have been focused mainly on cattle (Magona *et al.*, 2009; Makita *et al.*, 2011; McDermott *et al.*, 2002; Mwebe *et al.*, 2011; Oloffs *et al.*, 1998). Even in the recent serological study where the sero-prevalence was studied in goats and sheep, risk factors affecting the epidemiology of *Brucella melitensis* between goats, sheep, humans and wildlife were not studied. This project aimed at gathering evidence for use in designing viable control strategies in goats, sheep and cattle.

Objectives

The objectives were;

1. To isolate and characterize infecting *Brucellae species* in goats, sheep, cattle, humans and wildlife within Bwindi-Mgahinga, Queen Elizabeth, and Murchison Falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan.
2. To increase public awareness of animal and human brucellosis and the ways of preventing this disease in both animals and humans
3. To improve the diagnostic capacities and awareness by health practitioners about brucellosis in domestic ruminants, wildlife and humans in Uganda, DRC and South Sudan.

Outputs, activities and achievements

Output 1: Capacity gaps identified and addressed

Activity1: Assess the capacity of laboratories and their personnel about awareness on brucellosis diagnosis, prevention and control.

Achievements: Twenty three (23) health practitioners from Uganda and DRC were interviewed. We found out that over 60% of the respondents demonstrated a fair understanding of the various aspects of brucella biology and epidemiology that were assessed. However, less than 50% of the respondents were knowledgeable about the various aspects of brucellosis pathology and diagnosis that were assessed. Furthermore, over 60% of the respondents were not sure of the various aspects of brucellosis prevention and control that were assessed**.** Our findings revealed knowledge gaps that can adversely affect the judgment, decision-making and management of brucellosis by the different health service providers. Periodic CPD courses tailored to brucellosis for these frontline health service providers in endemic areas can help address these gaps and inform them about recent advances in research on the disease.

Activity 2: Train 50 veterinary, wildlife and medical personnel on brucellosis diagnosis, prevention and control

Achievements: A one-week theory and practical training for 30 personnel from DRC, Uganda and South Sudan was conducted. The training was conducted with technical assistance from Universidad de Navarra, Spain

Output 2: Public awareness of animal and human brucellosis enhanced

Activity 1: Conduct community awareness meetings/workshops around 3 selected protected areas

Achievements: Two (2) community awareness meeting around Murchison falls national park (MFNP) in Uganda and 2 meetings around Virunga National Park in DRC were conducted. Over 106 community members reached out in various locations in DRC while 141 community members were reached out in Uganda.

Output 3: Brucellae isolates obtained and characterized up to biovar level

Activity 1: Collect samples for screen against brucella anti S/LPS antibodies, laboratory culture and confirmation

Achievements: We collected and screened 1111 bovine sera, 943 caprine sera, and 35 ovine sera against brucella anti S/LPS antibodies using the RBT. The samples were collected from Kasese, Nakasongola, Nakaseke and Kiryandongo districts in Uganda. We found 6.4% sero prevalence of brucella anti S/LPS antibodies in cattle, 0.009% sero prevalence in goats and 0.08% in sheep. No brucella isolates were recovered

from milk cultures. We propose collecting postmortem samples at slaughterhouses for isolating brucellae in future studies.

Output 4: Brucellosis epidemiological information around the project area disseminated

Activity1: Publish Manuscripts and other dissemination/advocacy materials

Achievements: A policy brief on Brucellosis and strategies for its prevention and control developed and disseminated. The policy brief is accessible through the institute website www.nalirri.or.ug by all stakeholders for action. The brief has also been shared with the National onehealth platform (Uganda) to guide actions geared towards brucellosis prevention and control. In addition a manuscript (short communication) titled "*Awareness about brucellosis among health and veterinary practioners from Uganda and Democratic Republic of Congo and the implications for service delivery"* has been finalized and pending submission to a suitable journal for peer review and publication.

Conclusions and recommendations

We discovered that frontline health practitioners had knowledge gaps regarding brucellosis pathology, diagnosis, brucellosis prevention and control. We also discovered low prevalence of brucella anti S/LPS antibodies in goat and sheep sera. No isolates were recovered from milk cultures. We recommend periodic CPD courses tailored to brucellosis for frontline health service providers in endemic areas to address knowledge gaps and provide information on recent advances in research on the disease. We also propose collecting postmortem samples at slaughterhouses for isolating brucellae in future studies.

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Annexes

The following documents are attached for reference

Report on training of health practitioners

Community awareness reports from Uganda (Kiryandongo, Nakasongola)

Community awareness reports from DRC

Policy Brief

Draft Manuscript

Report of sample collection and laboratory work

A REPORT OF THE TRAINING COURSE ON BRUCELLOSIS EPIDEMIOLOGY, DIAGNOSIS, PREVENTION AND CONTROL AT THE HUMAN LIVESTOCK WILDLIFE INTERFACE THAT TOOK AT THE NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI), NAMULONGE –UGANDA FROM THE 13TH TO 18TH AUGUST 2018

UNDP

Universidad de Navarra

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)

NAGRC&DB ANIMAL GENETIC SOLUTIONS

Executive Summary:

Under the one health umbrella, a multisectoral team of veterinary, medical and wildlife practitioners from Uganda, Democratic Republic of Congo, and South Sudan converged at the National Livestock Resources Research Institute (NaLIRRI) in Uganda for a one week training in Brucellosis epidemiology, diagnosis, prevention and control at the human, Livestock wildlife interface. A four-member team of Brucellosis experts from Spain (University of Navarra) and Algeria conducted the training with support from 3 scientists from Uganda. The training was aimed at improving participants understanding of the epidemiology of brucellosis in livestock, humans and wildlife in Uganda, Democratic Republic of Congo and South Sudan. The training was also aimed building the capacity of participants in brucellosis diagnosis and the various strategies for its prevention and control. The training was conducted through lectures/ presentations; discussions, laboratory sessions as well as field based practical sessions. The role of different domestic animals, wildlife and humans in the brucellosis epidemiological cycle were discussed. Several approaches to brucellosis diagnosis in livestock, humans and wildlife were discussed. Smooth and rough brucella vaccines were discussed in addition to other strategies available for prevention and control of brucellosis. At the end of the training participants were tasked to put into practice what they learnt and help mentor others at their work places. The trainers and the hosts (NaLIRRI) pledged to offer similar or more advanced training in future and to support strategies aimed at reducing the prevalence of brucellosis in Africa

DAY ONE: INTRODUCTORY REMARKS

Speech from Dr. James Bugeza

At 9:12am on the 13th August 2018, Dr. James Bugeza on behalf of the National Livestock Resources Research Institute welcomed all participants from Democratic Republic of Congo (DRC), South Sudan and Uganda. He remarked that the participants are largely veterinary, wildlife, medical and laboratory practitioners working in districts, line ministries, departments, research and teaching institutions in their respective countries. He also appreciated the trainers for sparing time to travel from Spain to come to Uganda and share with the participants their experiences about brucellosis. He thanked the partners from the University of Navarra, Catholic university of Butembo and the University of Juba for offering letters of support during the grant application process. He also thanked the Perez Guerrero Trust Fund through the UNDP country office for Uganda for funding the training. Dr. Bugeza was saddened that some colleagues from South Sudan may not manage to make it to the training due to the political instability in their country that constrains their movement.

SELF INTRODUCTIONS

Dr. Bugeza requested all participants to team up in pairs and get to know each other's names, place of work, hobbies and their nicknames. Each member was then requested to introduce their teammates by giving their details above. This was done to ensure proper rapport building and creating a ground for the training. Other participants who came in later were requested to introduce themselves at session intervals during the training.

PARTICIPANT EXPECTATIONS FROM THE TRAINING - MS. DAISY NABADDA

The following expectations were listed

1. Differentiate the four types of brucellosis (in terms of diagnosis), 2.Clearly understand what brucellosis is and how it can be avoided/prevented, 3.Understand the specific and sensitive diagnostic methods of brucellosis, 4.Explain the cause of abortion in animals, 5.Know why veterinarians mind about domestic animals leaving out wild animals, 6.Know the dangers of eating meat infected with Brucella, 7.Understand the Brucellosis treatment and control strategies available in Uganda, 8.Collaborate with different sectors through a Onehealth approach, 9. Determine the risk factors of Brucellosis disease in the great lakes region, 10.Go with some equipment to start a main lab for Brucellosis diagnosis- in Rubanda district, 11.Detect sub-clinical and clinical Brucellosis before proceeding to the laboratories or health centers (signs and symptoms), 12.Describe the common challenges in the lab diagnosis of Brucellosis in the participating countries, 13.Run a PCR, culture and other methods like ELISAs, 14.Understand differential diagnosis/ other diseases with similar characteristics, 15.Know the current diagnostic methods that can tell the species of Brucella.

PARTICIPANT FEARS DURING THE TRAINING

The following fears were listed;
1.We have people who do not understand English, how do we address this issue?, 2.The use of difficult words of science, how best can they be made understandable, 3.No administrative issues have been talked about

RESPONSE TO THE FEARS

For people who did not understand English (colleagues from DRC), two of our trainers could ably hear and speak French (JM Blasco and Mammar Khames) and the other two could ably hear French (Amaia zuniga Ripa and Ignacio Moriyon) therefore communication would be made simpler for everyone, 2.For the use of difficult words, participants were requested to ask whatever they did not understand but the trainers also promised to use the simple terms, 3.Administrative issues were addressed by Dr. Bugeza in the course of the training

OFFICIAL OPENING Speech from Dr. Fredrick Kabi

On behalf of the Director of research of National Livestock Resources Research Institute (NaLIRRI) who was away on his pilgrimage, Dr. Fredrick Kabi mentioned that this regional training was timely, as it would complement strategies to control brucellosis that are being implemented in some of the participating countries. He added that the training was a stepping-stone for future collaborations in training, research, and capacity building in the region with Uganda, DRC and South Sudan taking lead with hope of involving other countries in future.
He welcomed the participants from Uganda, DRC and South Sudan. He also welcomed the trainers from Spain and Algeria. He mentioned that NaLIRRI was willing to initiate further collaboration with all the stakeholders in future for advancement of science, promoting the health of people, livestock and wildlife in the region that will eventually lead to shared prosperity. He requested all participants and trainers to feel at home as NaLIRRI was a home far from home and requested the consortium to stay in touch even after the training to ensure sustainability. After those remarks he declared the training officially open.

PRE-COURSE ASSESSMENT

This was aimed at understanding the participants' knowledge and understanding of Brucella biology, epidemiology, diagnosis and brucella control strategies including the available vaccines. The assessment was conducted using a questionnaire, which was filled by the participants before the training commenced.

SUMMARY OF THE TRAINING SESSIONS FOR DAY 1

SESSION 1: BRUCELLOSIS IN UGANDA

Bacteria belonging to the genus *Brucella* are the causative agents of brucellosis. The disease is endemic in Uganda, many studies have been done to ascertain prevalence in cattle based on serology however, few studies have been conducted in man and other livestock. Prevalence in cattle ranges between 1- 16% (animal level) and 1.2 -100% (herd level). Brucellosis is a serious

public health threat and a public outcry has aroused national concern. Situation is worse in hard to reach pastoral areas with poor access to medical care. More research is needed to understand the role of wildlife and other livestock in the epidemiological cycle of brucellosis. Brucellosis is not a public good disease and therefore control is the responsibility of individual farmers. Some of the risky practices/conditions that expose man and livestock to Brucella include raw milk consumption in some cultures, Poor on farm biosecurity, mixed herding, low awareness among other factors which require more research to understand the complex dynamics of the disease.

Several efforts have been put in place in Uganda including drafting a 5-year national surveillance plan for brucellosis in humans and animals. The plan specifies the roles of the Ministry of Agriculture, Animal industry and Fisheries, Ministry of Health, Local governments and other stakeholder. There is however need to study brucellosis (including identifying the species and biovars) in other species including man as well as developing capacity for Brucella vaccine research and development. Continuous awareness creation among all health practitioners and at risk groups is however pivotal in the control and prevention of the disease.

SESSION 2: BRUCELLOSIS AND BRUCELLA: AN OVERVIEW (DR. IGNACIO MORIYON)

The following key issues were discussed;

The different names of brucellosis indicate a poorly defined picture.
Historical aspects of brucellosis from Malta fever to epizootic abortion of small ruminants, from infectious abortion of cattle and pigs to human disease were also discussed. The different names of brucella species according to preferential hosts were discussed too. Human brucellosis global incidence was also discussed and the fact that brucellosis is an under reported disease was pointed out. Finally animal brucellosis in Africa and Europe was also discussed.

The following is a summary of key issues learnt in this session

1. Brucella taxonomy (i.e. species denomination) has been delineated mostly according to the preferential host. This is useful but cross-infections exist in mixed systems
2. The disease lacks specific symptoms in both animals and humans
3. The disease is rarely deadly in humans yet it is very incapacitating
4. Animals not always abort.
5. 2+ 3 + 4 cause under reporting
6. There is no significant human to human transmission
7. Not all Brucella are equally virulent for humans. Yet quite often the human diseases is the best indicator of the disease in animals
8. Food hygiene (milk pasteurization) is the single most effective measure to control transmission to the general public
9. Human brucellosis is often the best indicator of the animal disease

SESSION 3: BRUCELLA PATHOGENESIS, VIRULENCE AND IMMUNE RESPONSE

The following aspects were discussed; Brucellosis is a stealthy disease i.e. it exhibits a silent behaviour especially in young animals. The route of infection include the oropharyngeal mucosa

leading to colonization of the head lymphnodes and subsequent lymphatic spread to the spleen, liver 9Kupffer cells), mammary and genital lymphnodes and placenta leading to eventual abortion in pregnant animals with concomitant shedding of the bacterium and environmental contamination. Brucella is a silent facultative intracellular parasite of many types of cells. However virulent brucella and vaccines differ in their intracellular abilities. Brucellae induce minimal levels of proinflammatory cytokines (IL-10, IL-6, TNF-α and IL-1β) in vivo. Brucellae are capable of evading innate immunity and adaptive immune response in brucella infection comes too late and is detrimental.

The strategies for the silent behaviour of brucellae include

1. Reduction of external Parasite Associated Molecular Patterns (PAMPs) and become simple
2. To make tough outer membrane (OM). OMs must be broken up for pathogen recognition receptors (PRRs) to recognize all PAMPs
3. To remodel/take advantage of ancestral structures (CβG, LPS genes and Pcholine)
4. Not to release host damaging agents that would trigger systemic alarms.

The following is a summary of key issues learnt in this session

1. In ruminants, contagion occurs mostly through the oropharynx and congenitally
2. Brucella is an intracellular parasite that escapes recognition by innate immunity during the early stages of infection. This delays effective activation of the adaptive niche (and endoplasmic reticulum derived vacuole) of dendritic cells, macrophages and other cells.
3. Carried alive in phagocytes. Brucella spreads through the lymphatic system (and blood) to lymph nodes, spleen, liver, etc. Often it becomes localized in the genital organs.
4. Despite in 2., a fraction of bacteria are killed within antigen presenting cells leading to antibody and cell-mediated immunoresponses that are however most often unable to clear the infection
5. The silent behavior of Brucella is extreme in newborn and young animals until parturition/ abortion when a massive amount of Brucella are released
6. Live attenuated vaccines, however are mostly destroyed (within 3 months in vaccinated animals) and trigger adaptive immunity. This adaptive immunity is protective in the case of S. vaccines
7. Because of the above, conjunctival vaccination (point 1) with live attenuated smooth vaccines (point 6) at the right age (point 5) is the immunopropohylactic method of choice.

DAY TWO: DIAGNOSIS OF ANIMAL BRUCELLOSIS

SESSION 1: BACTERIOLOGICAL DIAGNOSIS- DR. JOSE MARIA BLASCO

The clinical signs of brucellosis are very similar in all affected species but non-pathognomonic. Testicular palpations is only presumptive value and of limited sensitivity only 10-40% of Brucella infected rams show palpable lesions and specificity 50-95% of lesions are due to other pathogens

It is important to isolate Brucella in order to confirm field infection (strain &biovar), identify infection by vaccine strains (Rev1, S19, and RB51) as well as to differentiate FPSR (False

Positive Serological Reactions). In order to conduct a proper bacteriological diagnosis, you must have; adequate Samples conduct a proper Sample processing and prepare a proper culture media and incubation. Ultimately, once an isolate is identified as Brucella by the simple tests, it is advisable to send the isolate to a good Brucellosis Reference Laboratory for definitive species &biovar typing. It is important to conduct a bacterioscopical examination of smears of vaginal swabs of RBT positive animals (STAMP staining procedure). However, a milk culture procedure is cumbersome, dangerous (aerosols) less sensitive. A suitable sample processing using culture media is critical for adequate sensitivity. The use of ***selective*** culture media is required for avoiding the overgrowing contaminants. It is imperative to always change gloves from animal to animal to avoid cross-contamination

SESSION 2: INDIRECT TESTS: SEROLOGY- Dr. Ignacio Moriyon

The following were the key highlights of this session

Both the direct (culture, PCR and Ag detection) and the indirect (serology) techniques could be used for the detection of brucellosis.

Serological tests are good when they*;*

1. Detect many infected animals (few false negative results)
2. Do not give positive results in uninfected animals (few false positive results)

There is need not to confuse Diagnostic Se/Sp and Analytical DSe/DSp. Poor sensitivity of a test leads to a useless test while poor specificity leads to over kill. It is important to set up a control in any diagnostic test. A positive control for serum refers to sera obtained from truly infected animals, which were determined by the "Gold standard": well-performed bacteriological culture, from animals not selected by a previous positive result in a given serological test and Un-vaccinated animals. Negative control sera should be truly *Brucella*-free obtained from animals that were unvaccinated and are from *Brucella*-free areas. Such a control should come from the same area/conditions where the test is to be applied. Therefore, diagnostic Se/Sp are population specific (cut-offs have to be adjusted locally).

There are two general kinds of tests i.e. qualitative and quantitative tests

Quantitative tests measure the amount of antibody indirectly (titer, optical density, fluorescence polarization), are complex and thus require standardization of all components. In addition, such tests require an assessment of cut-off for optimal diagnostic, sensitivity/specificity as well as requiring more equipment to perform and many can be automatized. Examples of quantitative tests include; tests that use whole Brucella cells (SAT, SAT-mercaptoethanol and Rivanol, and Complement fixation test: CFT) and tests that use Brucella cell surface antigens (iELISA, cELISA and Fluorescence Polarization assay (FPA). Qualitative tests on the other hand give positive (+) or negative (-) and are usually simpler and more robust. These tests also require antigen titration for optimal diagnostic sensitivity/specificity. Examples include tests that use whole Brucella cells (Rose Bengal, card and Buffered Plate agglutination test-BPAT) and tests

that use Brucella cell surface antigens (Reverse radial: RID or double gel: DGD immunodiffusion).

Session 3: Infections by Smooth (S) and Rough (R) Brucellae

Infections by S Brucellae

The following were the key highlights of this session

1. The O-PS is immuno-dominant in the antibody response; antibodies can be detected using S bacteria, S-LPS or OPS-core extracts.
2. NH are closely related to the O-PS but have distinct immuno-precipitation properties
3. The S-LPS of some bacteria (*Y.enterocolitica* O: 9) cross-react with the S-LPS of brucellae and may cause false positive serological reactions in brucellosis tests
4. Proteins elicit antibody and DTH response but no immuno-dominant protein has been identified
5. Cross-reactivity generated by proteins has no diagnostic importance, and protein tests can be considered as specific of *Brucella* infections

Infections by (or vaccination with) R brucellae

The following were the key highlights of this session

1. R brucellae bear no O-PS and the Immunoresponse to the R-LPS is comparatively less important
2. R-LPS and S-LPS or acid-obtained O-PS-core share core epitopes. Therefore, some S-LPS tests (ELISAs or tests with O-PS) detect antibodies to R-LPS
3. The antibody response to omps is more important
4. R cell suspensions are not useful as diagnostic antigens and are substituted by heat extracts

DAY THREE: VACCINATION AND CONTROL

SESSION 1: SMOOTH AND ROUGH TYPE VACCINES – DR. JOSE MARIA BLASCO AND DR. IGNACIO MORIYON

Currently, we have *Br.abortus* RB51 (R) as the only rough vaccine while *Br.melitensis* Rev 1 and *Br.abortus* S19 as the two common smooth vaccines against brucellosis. Different merits and demerits of the several types of available vaccines were discussed under this session.

The following information is true for the above vaccines;

1. In cattle, RB51 affords significantly less protection than S19 or Rev 1 and the differences become larger when the challenge increases (endemic areas????)
2. Claims on the usefulness of RB51 are based on field observations ***WITHOUT*** appropriate controls to differentiate the effects of the vaccine from those of the management measures of control
3. In sheep, RB51 is useless
4. Extensive studies strongly suggest that the R vaccine approach will not lead to the development of good vaccines against *Br. melitensis*
5. Rough Brucella vaccine interfere in S-LPS tests where core epitopes are exposed (iELISA, cELISA and possibly FPA)

SESSION 2: HUMAN BRUCELLOSIS BY SMOOTH BRUCELLA SPECIES- Dr. Ignacio Moriyon and Dan Nyehangane

Brucellosis is a well-known zoonosis mainly caused by *Br.abortus*, *Br.melitensis*, *Br.suis* and *Br.canis*.

Clinically, the following considerations are critical for the diagnosis of human brucellosis

1. Signs and symptoms are not pathognomonic / variable (urban – rural)
   - Overlaps with malaria, tuberculosis, typhoid fever, lupus erythematosus,
   - Rheumatoid arthritis, sarcoidosis, active lymphoma, and others
   - Abortion: controversial
2. Good anamnesis is critical (high suspicion of Brucellosis)
   - Possibility (professional or not) of contact with animals
   - Ingestion of contaminated food (not only dairy).
   - Person to person: exceedingly rare

SESSION 4: CONTROL AND ERADICATION STRATEGIES OF BRUCELLOSIS IN CATTLE AND SMALL RUMINANTS- Dr. Jose Maria Blasco

Tip of iceberg. Most people focus on only the following two as the main methods for eradication of infectious diseases including brucellosis; these are; ***Diagnostic tests and Vaccines***

Below the iceberg: It is however ideal that the following are given due attention in any eradication process; ***Official intervention & budget, Quality of vet. Services, Design to real epidemiological situation and active involvement of farmers***
On the control side of the disease, minimizing disease effects by reducing prevalence to a minimum is key. However on the other side, total elimination of *B. abortus/ B. melitensis from* all animals species involved in the epidemiological cycle.

SESSION 5: BASIC REQUISITES FOR APPLYING ANY BRUCELLOSIS CONTROL STRATEGY- Dr. Jose Maria Blasco

An adequate organization of veterinary services involved; Owner & animal registration required; Ability to vaccinate the whole target population in a very short time interval (lambing-calving season/lactation/pre-breeding period); ability/funds to repeat interventions; active and effective farmer's involvement; a minimum of budget (vaccine and operative costs); a precise knowledge of epidemiological situation: The epidemiological situation is almost never homogeneous in a given country and there are different epidemiological contexts within a country or even in a region of that country; a suitable vaccine and vaccination procedure ; *Br. melitensis* Rev 1 vaccine given by conjunctival route (Sheep & Goats); *Br. Abortus* S19 vaccine given also conjunctivally (ideally) (cattle). No vaccine other that Rev 1 and S19 have been proven successful in brucellosis eradication programs

SESSION 6: STRATEGIES FOR CONTROL OF BRUCELLOSIS IN A GIVEN COUNTRY - Dr. Jose Maria Blasco

OPTION 1: Mass vaccination every 2 years (WITH or WITHOUT ear tags) (the most practical and effective). Ability to identify 100% of flocks and vaccinate 100% of animals, as well as identifying the ideal window of opportunity (usually only few weeks) to minimize vaccine side effects
OPTION 2: Mass vaccination & individual Identification the 1st year, and then vaccinating and Identifying only new replacements and untagged Animals next years. Ability to register 100% of flocks and vaccinate 100% of animals in the ideal window the first year. Ability to individually identify 100% of animals Vaccinated and to vaccinate 100% of unidentified Animals (identifying these also individually) the Next years.

DAY FOUR

SESSION 1: HUMAN BRUCELLOSIS: CLINICAL ASPECTS DIAGNOSIS AND TREATMENT

The following were the highlights of this session

The absence of pathognomonic symptoms and poor physician's awareness lead to misdiagnosis and underreporting of human brucellosis. Correct anamnesis is hence critical.
Specific diagnosis requires laboratory tests. Only culture is 100% specific but requires adequate facilities and is not always positive, with a success frequency that decreases in the chronic forms. Some serological tests are easy to perform and very informative when the results are evaluated

together with clinical picture. The Rose Bengal test allows the diagnosis of a large proportion of brucellosis cases and should be performed as the first test in any suspicious case. A titer > ¼ is highly indicative of infection. A proportion of cases need complementary tests. Antibiotic therapy is long, expensive and the best regimes require parenteral administration (low compliance). The best way to solve these problems is to control the animal disease and to implement hygienic measures (milk pasteurization in particular)

SESSION 2: PROBLEMS ASSOCIATED WITH HUMAN BRUCELLOSIS

Relapses

- Objective signs of infection
- Persistently elevated titers of IgG antibodies
- Mostly occur within 6 months after therapy is discontinued
- Not due to the emergency of antibiotic resistance strains
- Can be treated by repeating the same course of therapy

Localized infections

- Therapy may fail to eliminate a deep focus of infection (osteomyelitis, deep tissue abscesses)
- Recurrence of signs and symptoms (with or without a positive blood culture) sometimes intermittently over long periods
- Persistence of non-agglutinating IgG in serum sometimes as weak titers
- In addition to antimicrobial therapy, may require surgical intervention to drain foci of infection

Delayed convalescence

- Persistence of symptoms, without objectives signs of infection after a course of therapy, with titers of antibodies that have declined or disappeared
- Etiology unknown (some studies suggest personality disorders, often predicting the onset of brucellosis)
- Patients do not appear to benefit from repeated courses of antimicrobial therapy.

FIFTH DAY: LABORATORY SESSION

Welcome remarks from the host: Mr. Kizito Muwonge the dean of Faculty of Health Sciences hosted the team at the University of Kisubi. He mentioned that the university begun in 2004 under the support of a Brother from USA who passed on recently. The University has 14 years of service but the department of health sciences is 3.5 years old. For the last 3 years, it has been natured by social science faculty until recently when they attained faculty status. He welcomed all participants from Uganda and all over the world and more so the trainers from Spain and Algeria. He guided all the participants to the laboratories and other necessary places at the University and he was finally part of the practical training.

Six (6) practical procedures were performed during the practical sessions. The following were the major practical tests demonstrated to the participants;

SESSION 1: ROSE BENGAL TEST

A rapid and simple agglutination test performed with rose Bengal stained smooth *Brucella* cells suspended in an acid buffer. Under these conditions: (i). Prozone and blocking phenomena disappear (ii). Non-agglutinating antibodies (characteristic of long evolution brucellosis) become agglutinating and (iii). IgM and IgG are detected, the latter more efficiently (1). The test is useful for diagnosing animal and human brucellosis caused by smooth (*Br. abortus, Br. melitensis* and *Br. suis*) but not by rough (*Br. ovis* or *Br. canis*) *Brucella* species. The test is also useful to confirm successful recent vaccination with *Br. melitensis* Rev 1 and *Br. abortus* S19 (60-90% of S19 or Rev 1 vaccinated animals should be RBT positive when tested 15-21 days after vaccination).

Animal brucellosis: Because of its simplicity, good diagnostic performance and very low cost, it is highly recommended as a single test when vaccination has not been implemented

In cattle: The test is highly sensitive (99.7 %) and, in brucellosis free contexts and the absence of vaccination, highly specific (99.0%).

In sheep and goats: The test has to be modified slightly for optimal sensitivity (94%). It is highly specific (100%) in brucellosis free contexts and the absence of vaccination.

Both in cattle and small ruminants, the sensitivity and specificity is equal or better than those of more sophisticated tests like iELISA, cELISA, FPA or Lateral Flow Immunochromatography (LFiC).

In other ruminants: There is a paucity of serological studies contrasted with culture (gold standard). A study suggests that RBT is a good test in water buffaloes.

In humans: The test is highly sensitive (almost 100%) and specific. Positive reactions, however, may happen with sera from healthy persons that had been in contact with infected animals or in patients after recovery. This problem is partially solved by adapting the protocol to test serum dilutions

Potential problems: Standardization of the antigen may be a problem because of poor antigen quality (S-R dissociated brucellae) or inappropriate bacterial concentration. Thus, imperfect antigen batches may have a lower sensitivity (down to approximately 85%). It is essential to use a good quality reagent, and all batches should be tested with a panel of positive and negative reference sera.

SESSION 2: DOUBLE GEL DIFFUSION TEST WITH NH (DGD-NH)

Required reagents

Noble Agar (Difco; ref 214230), NaCl, Borate Buffer (Boric Acid -6.2 g, Potassium Chloride-7.25 g, Distilled water (or equivalent quality)-800 ml)
Adjust to pH 8.3 with 1M NaOH and bring volume up to 1000 ml with distilled water.
Antigen: The antigen is a S/LPS-NH rich extract obtained from *Br. melitensis*16M that is prepared freeze dried. The optimal titre for GD can vary (according the obtention procedure) from 0.25 to 2.5 mg/ml of gel. This titre should be determined for each antigen batch with an adequate panel of sera from either *Brucella* infected and *Brucella* free animals. A stock can be prepared in distilled H_2O and kept frozen. Freezing and melting does not affect the quality of the antigen.

SESSION 3: INDIRECT ELISA (INGENASA)

INgezim Brucellosis Bovina 2.0 is an immunoenzymatic assay based on an indirect ELISA technique, which uses a monoclonal antibody (Mab) specific for bovine immunoglobulins.

Technical basis

1. Plates are coated with inactivated *Brucella abortus* antigen (LPS). Samples are added to the wells and incubated
2. If the sample contains antibodies to *Brucella abortus*, they will bind to the antigen
3. When a MAb-PO specific for bovine IgG is added, it will bind to the IgG of the sample previously bound to the antigen. This binding is detected by the development of a colorimetric reaction after the addition of the substrate

Validation of the test: The test is considered valid when;

- OD value of the positive control is ≥ 1.0
- OD value of the negative control is ≤ 0.2

SESSION 4: BRUCELLACAPT (VIRCELL)

Principle of the test: The test consists of U-bottom well strips coated with anti–human immunoglobulins. After addition and dilution of serum, the antigen is added, and strips are incubated for 24 hours until agglutination takes place.
This assay allows the detection of both agglutinating and incomplete antibodies, which only could be measured by means of the Coombs test.



SESSION 6: MOLECULAR CHARACTERISATION (MULTIPLEX PCR (INGENASA))

The INgene Bruce-ladder V kit is used for the molecular typing of *Brucella* from isolated colonies. It allows identifying all *Brucella* species and some biovars as well as the common vaccine strains.

SIXTH DAY: FIELD WORK

The participants were taken for field visit in Western Uganda at Nshaara ranch near Lake Mburo national Park under NAGRIC&DB. The farm is located in Nyabushozi county of Kiruhura district, established in 1968 after the eradication of Tsetse flies that invaded the area in 1920, which resulted in to death of thousands of cattle due to trypanosomiasis.

The 28square mile ranch has a total of 2010 cattle as of 2017. A total of 106 goats are also present at the farm. At the farm, the participants were introduced to conjunctival vaccination of both cattle and goats using a placebo to represent the actual vaccine.

Session 1: Conjunctival vaccination

Procedure for vaccine reconstitution and vaccination

1. Wearing gloves and goggles, take blue solvent with a syringe, inject it in the flask with the lyophilized vaccine and homogenize by gentle rotation (do not shake or invert!).
2. Wait for 10 min for full rehydration ***(very important).***
3. Replace metallic cover and cap. (Do not get in contact with the vaccine! Do not shake or invert the vial).
4. Wearing gloves and goggles (both vet and assistant restraining the animal), put a drop on the eye and release the lid.
5. Keep the animal in this position for a few seconds to allow absorption of the liquid
6. Dispose vaccine vials and pipette tips in a container with disinfectant (household bleach diluted to 1% in water.


DAY 7: OFFICIAL CLOSING

Closing remarks: The training was crowned up on the 18th August 2018 at Kasangati resort hotel. Representatives from the Organizers, participants and trainers were called upon to give their final remarks as we were closing the ceremony.

Remarks from Dr. James Bugeza: He started with thanking the trainers from Spain and requested them to always make Uganda their second home. He also thanked the collaborators from DRC as well as Ugandan colleagues for taking off time to attend the precious training**.**

He stated that brucellosis is with us and it is not going to go away soon, therefore everyone has equal responsibility in their respective communities to apply what we know and what we have learnt to ensure we curb down the problem. Dr. Bugeza mentioned that the director NaLIRRI was away in Saudi Arabia for his pilgrimage but his blessings are with us. He wished everyone a safe journey back home.

Remarks from a representative of the Participants: The participants were first and foremost impressed with the methodology and teaching of the trainers. They mentioned that their knowledge on the bacteriology and epidemiology of brucellosis had been widened. In addition, different methods of diagnosis, control, and vaccination were improved. They stated, there is need for a multi-sectoral approach in combating zoonoses. The team leader requested all the participants to go back home and amplify whatever they have learnt.
They thanked the organizers for the job well done and finally requested for a joint effort to fight diseases without borders.

Remarks from the trainers: "It is very difficult to express feeling in a language that is not mine", said Prof. Ignacio. What is the target of all this? Motivation comes from the previous experiences in our countries. May be not the young generation, human brucellosis is very important because it affects the young and farmers.
Our gratitude to the hosts and organizers, we are happy. We are sorry that we did not fully teach in French but we tried to cover up. Part of the team is likely to come back to Uganda next year. Let us keep in touch.

Remarks from Dr. Benda: Thank you the participants plus our dear trainers. It has been such a successful training where we have acquired the knowledge and skills.
After this training, our next activities are going to be awareness creations in the project areas, measuring prevalence, community dialogues, radio-talk shows, as well as developing a policy brief. Thank you all.

Remarks from Dr. Dhikusooka: On behalf of the director, I would like to thank the organizers, trainers as well as the participants for making this happen**.** When you go back home, do not forget us.

There are several gaps in diagnosis that we have in the region but with this training are we competent enough to run all these tests? We thank UNDP for the financial support, University of Kisubi for their support with the laboratories; finally we need to work out modalities to ensure we get the right vaccines from Spain.

HAND OVER OF CERTIFICATES
On behalf of the director of research NaLIRRI, Dr. Dhikusooka Moses and Dr. Ignacio Moriyon handed over the certificates to the participants

ANNEXES

Annex 1: Protocol for (Rose Bengal Test) RBT

Materials

1. Flat glossy white ceramic tiles (these are optimal; glass plates can be used but readings are not so clear). They can be cleaned by rinsing/scrubbing in clean water after use, and then dried with a utility wiper or simple cuisine paper.
2. An automatic pipette delivering 25 to 200*µl* (microliters) and plastic tips (cones). Tips can be rinsed in clean tap water, dried and reused many times.
3. Antigen. Available commercially (see below). Antigen should be stored at 4 °C (not frozen).
4. Tooth picks (or similar; a glass rod that is cleaned alter each mixing [see below] can also be used).
5. Control sera. A positive control serum that gives a minimum positive agglutination reaction should be tested before each day's tests are begun to verify the sensitivity of the test conditions. This serum should be stored frozen in small aliquots and brought to room temperature before use. A negative control serum should be included also and stored in the same way.

Standard protocol for cattle sera

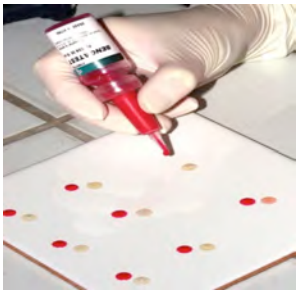
1. Bring the serum samples and antigen to room temperature (22°C); only sufficient antigen for the day's tests should be removed from the refrigerator. Always homogenize the antigen suspension by gentle shaking just before use
2. Using the automatic pipette and a clean tip, place 25µl of serum[a] on the glossy side of the tile (several serum samples can be tested alongside)
3. Make sure that the antigen removed from the refrigerator is a uniform suspension (homogenize again the amount removed if necessary); then dispense 25µl of antigen besides each drop of serum using the automatic pipette (some manufacturers include ready to use droppers; see Figure 1)
4. Immediately, mix antigen and serum with a tooth pick (or similar), and rock the plate gently clockwise and counterclockwise for exactly 4 minutes (if available use a laboratory buzzer to indicate the lapse of 4 minutes)
5. In a well-illuminated place, read the results immediately after the 4-minute rocking period (if several sera are tested on the same plate [Figure 1], take into account the dropping/mixing time delay between serum samples).

Interpretation (See Figure 2)

Negative: uniform pink mixture, no clumps and no rim.

Positive: Any perceptible agglutination (from fine clumps and some rim formation to coarse clumping and definite clearing).

[a] Fibrin in plasma causes false negative results. Thus, the test cannot be used with plasma, and blood samples must be let to clot completely, serum removed and clarified by centrifugation.



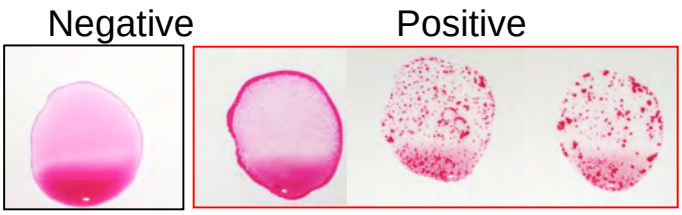


Figure 2. Different degrees of agglutination in RBT

Figure 1. Performing

Important

1. Although several sera can be tested alongside on the same plate, the number should not exceed that resulting in evaporation problems and times of mixing/rocking different from 4 minutes for the different sera. A number of 9 sera per plate is probably the most adequate.
2. Although some authors score the results using crosses (+, ++, and +++) to describe the intensity of agglutination and subsequently interpret this as low to high positivity, this is not correct, it is misleading and should be avoided. The intensity + to +++ relates not to antibody levels, but to properties of the immunoglobulins (IgM and subtypes of IgG) involved.

Modified Rose Bengal test for small ruminant sera

For these sera, the sensitivity is optimized by increasing the amount of serum to be tested to 75 *µl* (instead of 25 *µl*) but maintaining the amount (25*µl*) of antigen.

Protocol for human serum samples

The diagnosis of human brucellosis by serology must take into account that there are persons that develop antibodies upon contact with the bacterium but do not become infected. Indirect evidence suggests that infection is more easily acquired from sheep or goats (*B. melitensis*) and pigs (*B. suis*) than from cattle (*B. abortus*). However, in mixed breeding conditions typical of low income countries, cattle can be infected by *B. melitensis*, being this a serious risk of infection for humans. A thorough clinical examination and the presence of symptoms compatible with brucellosis are essential to interpret the results of any brucellosis serological test, RBT included. The antigen is the same as that used above for animal brucellosis.

Standard test

Perform the test as described for animal brucellosis (25+25µl; standard protocol). The interpretation of the agglutination is the same.

False negative results are rather unlikely (sensitivity is over 99%).

False positive results:

These are unlikely in non-endemic areas (specificity is over 99% in these areas).

In endemic areas, positive results could result from contacts with the pathogen but no infection (absence of clinical symptoms). These results can be analyzed further using the modification of RBT-serum dilutions protocol described below.

Protocol for RBT-serum dilutions

1. Dispense four 25*µl* drops of saline (0.85% NaCl) on the tile
2. To the first saline drop, add 25*µl* of the positive plain serum and mix thoroughly by aspirating and expulsing the mixture several times with the pipette

3. Rinse the pipette tip with saline and transfer 25*µl* of the first dilution to the second saline drop
4. Mix again as in 3 and transfer 25*µl* of the second dilution to the third drop
5. Mix again as in 4 and transfer 25*µl* of the second dilution to the fourth drop
6. Mix again, take 25*µl* and discard them
7. Test each drop (serum dilution) with 25*µl* of the RB reagent as described above for the plain serum.
8. The RBT results are expressed as serum titers

Last sample positive	**RBT titer**
Plain serum	½
First drop	¼
Second drop	1/8
Third drop	1/16
Fourth drop	1/32

Titers equal to or higher than 1/8 indicate active brucellosis; titers 1/2 and 1/4 must be considered with care taking into account the presence/absence of clinical symptoms and the epidemiological risk (8, 9).

Important: Although all suppliers follow OIE and EU guidelines, there might be variations among batches. Therefore, it is advisable that each antigen batch be validated (using a suitable collection of gold standard reference sera) by a reference laboratory.

Annex 2: Double gel diffusion test with NH (DGD-NH)

Reagents

1. Noble Agar (Difco; ref 214230).
2. NaCl
3. Borate Buffer
4. Boric Acid - 6.2 g.
5. Potassium Chloride-7.25 g.
6. Distilled water (or equivalent quality)-800 ml.
7. Adjust to pH 8.3 with 1 M NaOH and bring volume up to 1,000 ml with distilled water.

Antigen: The antigen is a S/LPS-NH rich extract obtained from *B. melitensis* 16M that is prepared (see reference 1 for exact details) freeze dried. The optimal titre for GD can vary (according the obtention procedure) from 0.25 to 2.5 mg/ml of gel. This titre should be determined for each antigen batch with an adequate panel of sera from either *Brucella* infected and *Brucella* free animals. A stock can be prepared in distilled H_2O and kept frozen. Freezing and melting does not affect the quality of the antigen.

Preparation of gel:

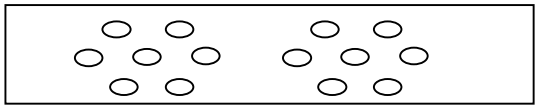
- Noble Agar-1 g
- NaCl-10 g
- Borate Buffer-100 ml

Dissolve the agar by boiling under continuous stirring. Once the suspension is uniform, it can be used immediately or stored at 4ºC and melt again by boiling when needed.

Preparation of gel plates/slides: On a flat surface, place a standard microscope glass slide (precleaned or cleaned with 1:1 ethanol-ether)

Using a glass pipette pour slowly 3.5 ml of molten gel starting from the center of the slide (see Figure below) to obtain a gel layer of about 2.5 mm thick.

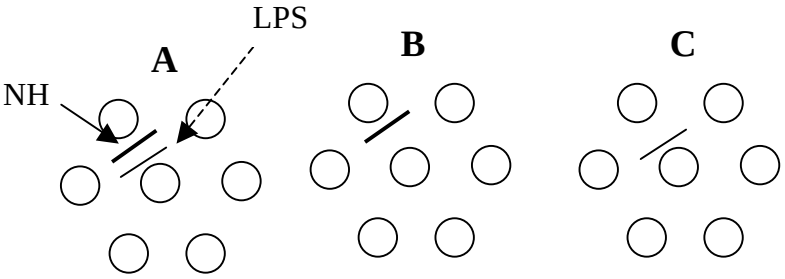
Once the gel is formed, punch 6 holes of 4 mm diameter 4 mm apart from each other forming hexagonal figures around a central hole (see pattern below).[2] Remove the agar cuttings with the help of a needle (or a pipette Pasteur connected to a gentle vacuum source). It is essential that the gel surrounding each well remains firmly attached to the slide so that sera/antigen will not leak in

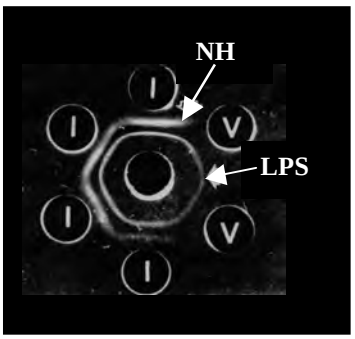


underneath when dispensed.

Procedure: Place 15-20 µl of problem sera in the outer wells and 15-20µl fill of antigen solution in the central well. Exact volumes depend on the volume of the wells and have to be standardized for successive tests.
Incubate at room temperature in a humid chamber (such as a Petri dish with a wetted cotton).
Read after 24 and 48h. at room temperature. Ideally, to remove unspecific precipitation lines before lecture, plates should be immersed in a 5% solution of sodium citrate in water for 1-2 hours.

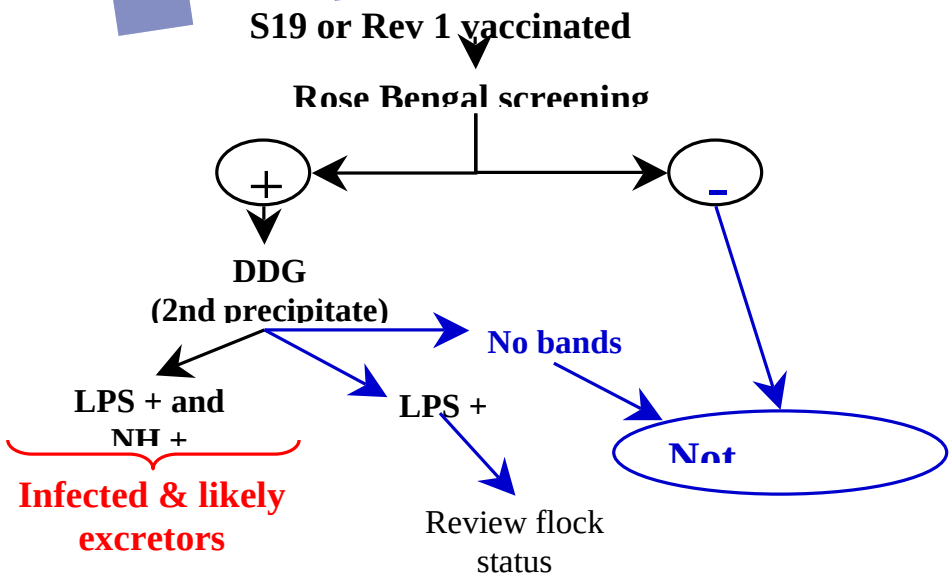
Interpretation:





No precipitin lines: not infected.

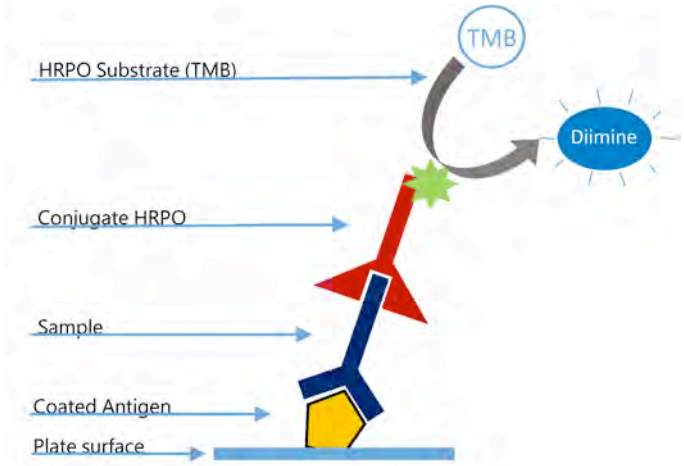
Precipitin lines: A. Double band (NH, closer to serum well; LPS, close to antigen well): infected and most likely excreting brucellae (sometimes more than 2 lines appear which indicate antibodies to proteins and do not modify the interpretation). Some very recently vaccinated animals –i.e. one month after vaccination- can produce the double precipitin line characteristic of infected animals.
B: Single NH band: infected and most likely excreting brucellae. C: Single LPS band: not infected (vaccinated animals transiently show this precipitin line).



Annex 3: Protocol for Indirect ELISA **(Ingenasa)**
INgezim Brucellosis Bovina 2.0 is an immunoenzymatic assay based on an indirect ELISA technique, which uses a monoclonal antibody (Mab) specific for bovine immunoglobulins.

Technical basis

1. Plates are coated with inactivated *Brucella abortus* antigen (LPS). Samples are added to the wells and incubated.
2. If the sample contains antibodies to *Brucella abortus*, they will bind to the antigen.
3. When a MAb-PO specific for bovine IgG is added, it will bind to the IgG of the sample previously bound to the antigen. This binding is detected by the development of a colorimetric reaction after the addition of the substrate.



Preparation of reagents

Washing solution: Dilute one part of the concentrate washing solution provided in the kit with 24 parts of distilled or deionized water (40 ml of the concentrated solution in 960 ml of water)
Conjugate: Immediately before use dilute the needed quantity of conjugate 1/100 with diluent
The necessary quantity of conjugate for a complete plate is 110*µl* of conjugate in 11 ml of diluent
The necessary quantity of conjugate for an 8 wells-strip is 10*µl* of conjugate in 1 ml of diluent.
Shake very well the solution before use. ***Controls:*** Ready to use, do not dilute.

Test procedure (with serum dilutions)

3. All reagents (except conjugate) must be allowed to reach room temperature before use. It is recommended to warm the diluent previously for 20-30 min at 37ºC
4. Add 90µl of diluent and 10µl of sera to the wells of the first column (Sera 1:A1, sera 2: B1, sera 3: C1, sera 4: D1, etc.). Add 100µl of + control (ready to use) to one well and 100µl of – control (ready to use) to a second well (wells with controls are not diluted)
5. Add 50µl of diluent to the wells of columns 2-12 in the rows dedicated to problem sera.
6. Make double serial dilution by passing 50µl from A1 to A2, A2 to A3 etc.
7. Discard 50µl from the well A12
8. Do the same with the rest of the rows dedicated to problem sera (if a multichannel pipette is available you can perform the serial dilution simultaneously for all rows)
9. Incubate 1h at room temperature (20-25ºC)
10. Wash 5 times. The washing steps could be done using an automatic washing machine or a multichannel pipetting device suitable for dispensing 300µl on each well. The washing steps must be done following these instructions
11. Throw out the content of the plate by a brusque turn over of the plate to avoid the possible mixture of the content from one well to another
12. Dispense a volume of 300µl of washing solution on each well

13. Shake delicately the plate, avoiding the contamination between wells
14. Turn over the plate brusquely to empty the wells
15. Repeat the process as many times as indicated
16. Prior to empty the content of the last washing step, verify that the next reagent is ready. Do not maintain the plate on dry more time than strictly needed
17. After the last step of washing, shake the plate turned over an absorbent filter paper
18. Add 100µl of conjugate to each well
19. Incubate 30 min at room temperature (20-25ºC)
20. Wash 5 times
21. Add 100µl of substrate to each well
22. Keep the plate in darkness for 10 min at room temperature (20-25ºC)
23. Add 100µl of stop solution to each well
24. Read the OD of each well with a spectrophotometer at 450 nm within 5 min after the addition of the stop solution

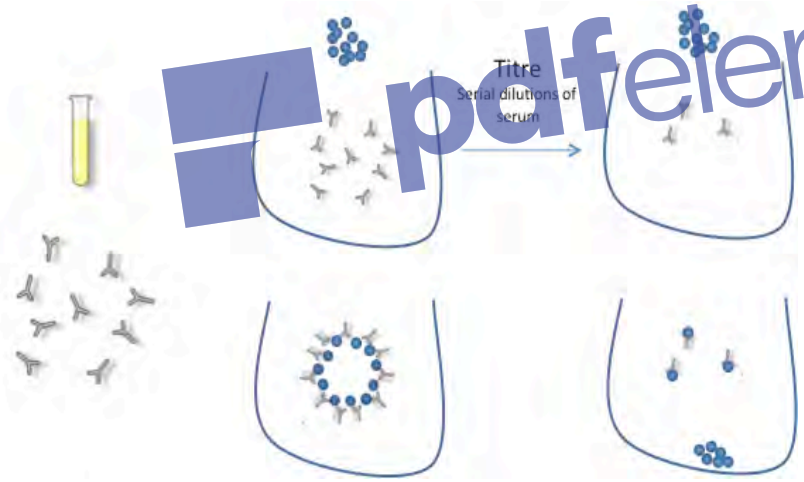
Validation of the test

The test is considered valid when;

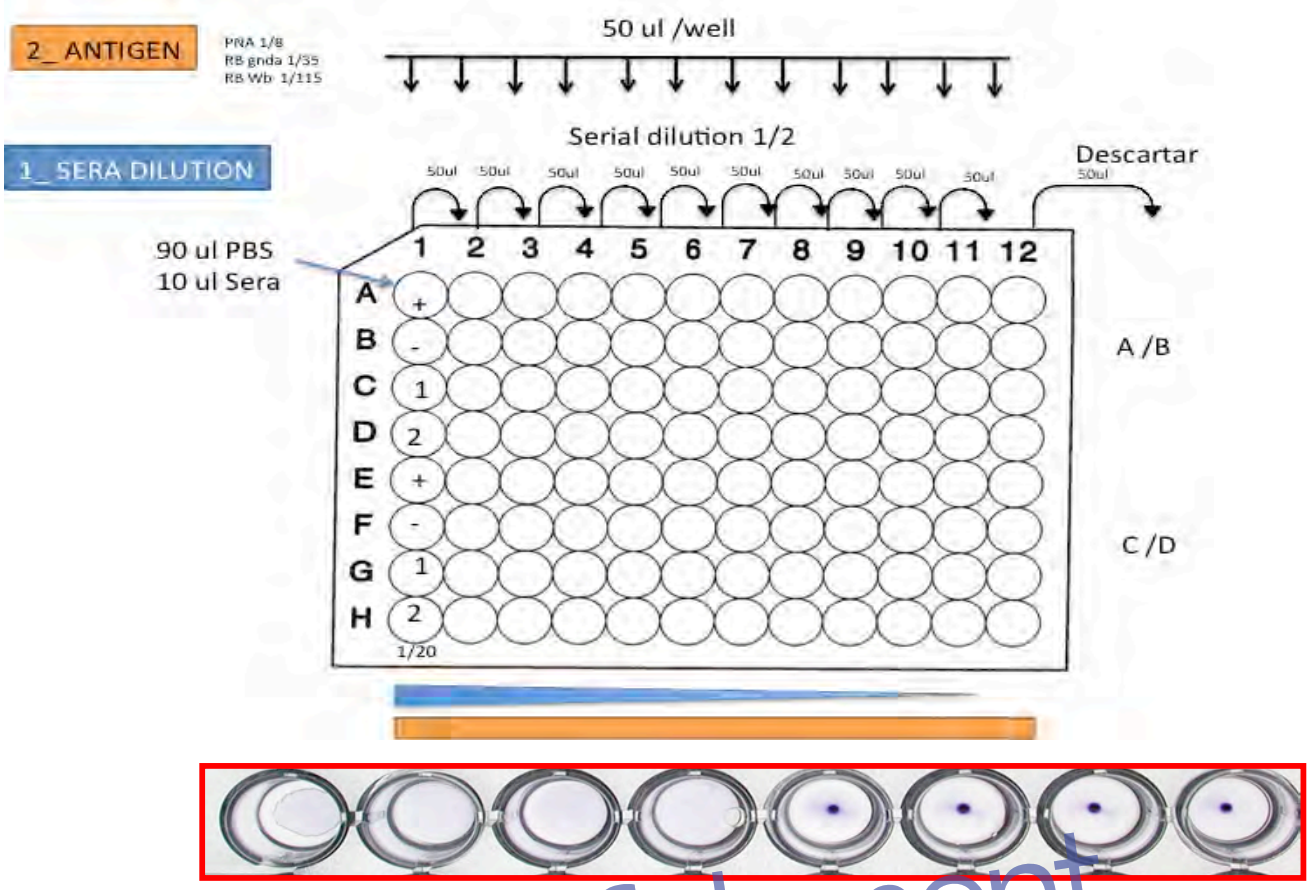
OD value of the positive control is ≥ 1.0

OD value of the negative control is ≤ 0.2

Annex 4: Protocol for SAT (Serum agglutination test)



1. Add 90µl of PBS and 10 µl of the problem sera to the wells of the first column (Sera 1:A1, sera 2: B1, sera 3: C1, sera 4: D1 etc.)
2. Add 50ul of PBS to all the wells of the plate except for the first column
3. Make double serial dilution by passing 50µl from A1 to A2, A2 to A3 etc.
4. Discard the 50ul from the well from A12.
5. Do the same with the rest of the rows. (If a multichannel pipette is available you can perform the serial dilution simultaneously for all rows)
6. Add 50ul of the previously titred bacterial suspension (can be home made from inactivated bacteria or commercially available: e.g. derived from RBT, ask for protocol if interested) to all the wells
7. Mix the suspension with a pipette
8. Incubate for 24 hours at room temperature



***Annex 5: Protocol for BRUCELLACAPT* (VIRCELL)**

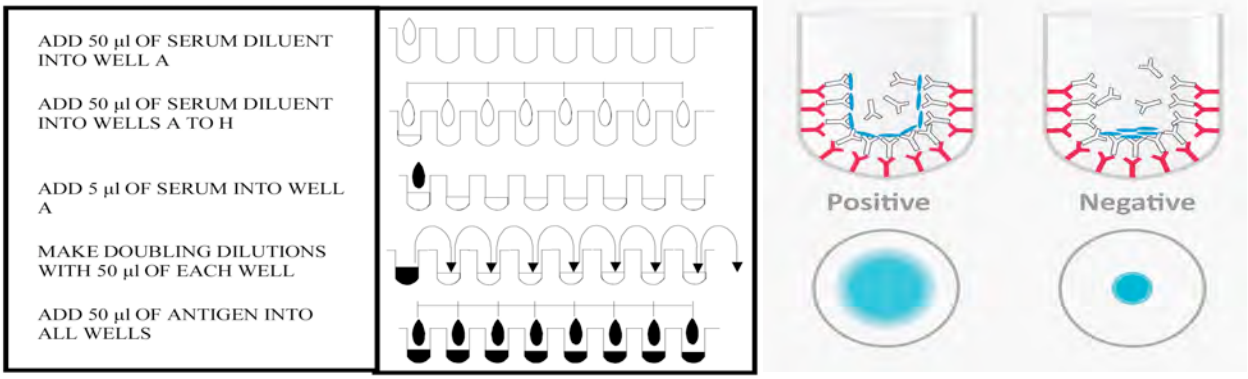
Principle of the test: The test consists of U-bottom well strips coated with anti–human immunoglobulins. After addition and dilution of serum, the antigen is added, and strips are incubated for 24 hours until agglutination takes place.

This assay allows the detection of both agglutinating and incomplete antibodies, which only could be measured by means of the Coombs test.

Assay procedure

4. Bring all reagents to room temperature before use. Remove as many well strips (1) as necessary for the samples to be processed plus one strip each for negative (5) and positive (4) controls
5. Add 50µl of serum diluent (2) into well A. Add 50 µl of serum diluent into all wells from A to H. Add 5µl of each serum and positive (4) and negative (5) controls into well A. Make doubling dilutions with 50µl of each well from A to H
6. Add 50µl of the bacterial suspension (3), previously homogenized by vigorous shaking, into all wells
7. Seal with adherent tape and incubate for 24 hours at 37ºC, in a humid chamber protected from light exposure
8. Read results taking into account that titers will be: 1/40 for row A, 1/80 for row B, 1/160 for row C, 1/320 for row D, 1/640 for row E, 1/1280 for row F, 1/2560 for row G and 1/5120 for row H

SUMMARY OF THE ASSAY PROCEDURE:



Validation protocol for users

Positive and negative controls must be run with each test. It allows the validation of the assay and kit. The titers of positive and negative controls must be the indicated in the corresponding label.

Annex 6: MOLECULAR CHARACTERISATION: MULTIPLEX PCR (INGENASA)

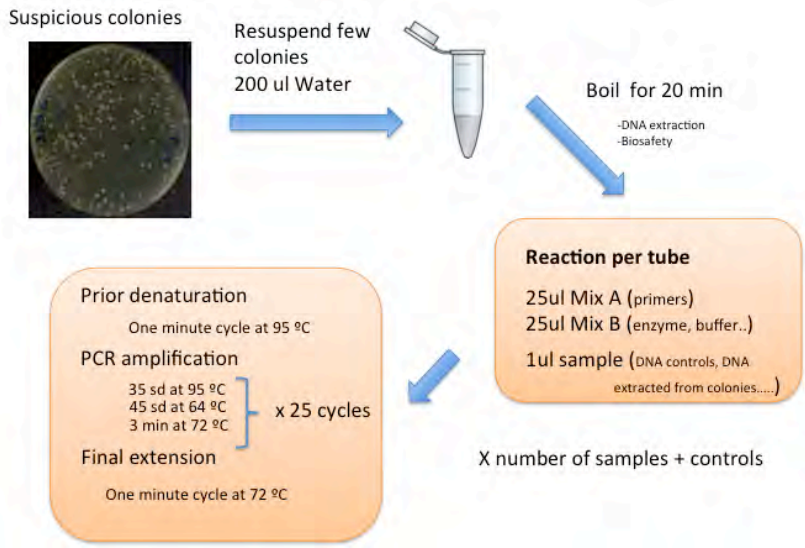
The INgene Bruce-ladder V kit is used for the molecular typing of *Brucella* from isolated colonies. It allows identifying all *Brucella* species and some biovars as well as the common vaccine strains. The PCR Bruce-ladder has been recommended by the OIE (World Organisation for Animal Health) in the chapter about bovine brucellosis 2009.

Genetic material extraction: Bacteria that have been grown in solid medium must be used as starting material. Resuspend one or few bacterial colonies with a seeding inoculation loop in 200 µl of sterile ultra-pure water. Boil the suspension in a bath at 100°C for 20 min. Cool in crushed ice for 5 min. Use 1-2 µl of said suspension in the amplification mixture for PCR (material given).

Genetic material amplification:

1. Remove reagents A and B from the refrigerator and allow them to reach room temperature before using them.
2. Separate and mark the number of PCR tubes that are expected to be used, counting one for each sample analysed plus four additional tubes for the positive controls and the negative control.
3. Prepare the mixture of reagent A and reagent B necessary for carrying out the number of expected samples and controls, in the following measurement and proportion:
4. *(12.5µl of reagent A + 12.5µl of reagent B)* X number of samples and controls*
5. Before preparing the mixture, carefully stir each of the reagents by means of manual stirring in order to achieve the correct homogenisation of its components.
6. Prepare the mixture in a tube kept in crushed ice (once the mixture has been carried out, it is convenient to keep the polymerase inactive until the time the reaction is started).
7. Homogenise the mixture carefully before using it.
8. Introduce the marked tubes in the container with crushed ice and distribute in each of them 25µl of the previously prepared mixture.
9. Add 1-2µl of the sample to each of the tubes, 1µl of distilled water to the tube reserved as a negative control and 1µl of positive controls to each of the tubes reserved for positive controls.

10. Gently mix the contents of each tube and verify that the contents of the tube are at the bottom. If this is not so then centrifuge. Program the thermal cycler according to the following conditions
11. Prior to denaturation: one 7 minute-cycle at 95°C
12. PCR amplification: 35s at 95°C, 45s at 64°C, 180s at 72°C (FOR 25 CYCLES)
13. Final extension: one 6 minute-cycle at 72°C
14. Keep the samples at 4°C until they are removed from the thermal cycler.



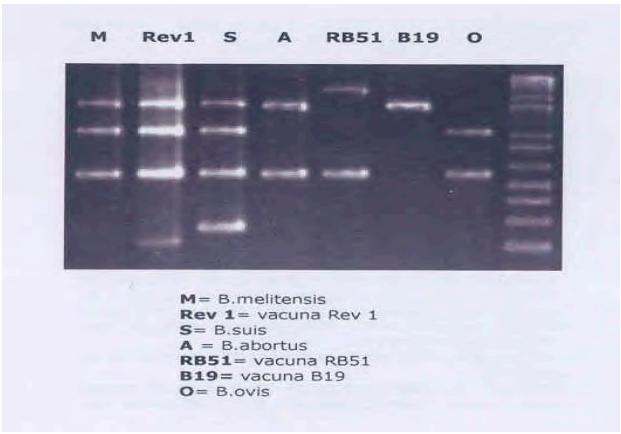
Detection in gel & interpretation of results

1. A 1.5% agarose gel in TBE (1.5 gr/100ml 0.5% TBE) stained with ethidium bromide is prepared (alternatively it can be stained after running).

2. Once it has dissolved, allow the Agarose gel to cool and avoid of being in the presence of breathing in the vapour once the bromide has been dispensed.

3. Load 15µl of PCR sample and carry out electrophoresis at 100V (40mA) for one hour. Develop with UV light transilluminator.

4. The assay is validated if the positive control II is visualised with four bands of: 1682, 1071, 587, 272bp and the negative control is 'clean'. Depending on the obtained pattern, the positive samples will be a *Brucella* species.



Annex 7: Training Program

	Timetable	**Lecturer**
Day 1,13th August		
Arrival and registration of participants Self-introduction Pre-course knowledge assessment and expectations	09:00 – 09:30 09:30 – 09:45 09:45 – 10:30	Daisy Nabadda
Tea break	10:30 – 11:00	Doreen Nankya
Current situation of brucellosis in Uganda Brucellosis on the Livestock/Human/Wildlife interface Official Opening of the course by the guest of honor	11:00 – 11:30 11:30 – 12:00 12:00 – 13:00	Dr. James Bugeza Dr. Katali K. Benda Dr. Frederick Kabi
Lunch break	13:00 – 14:00	Doreen Nankya
Basic aspects Brucellosis and *Brucella*: an overview Brucella pathogenesis, virulence and immune response Epidemiology	14:00 – 17:00	 Dr. Ignacio Moriyón Dr. Ignacio Moriyón Dr. José María Blasco
Day 2, 14th August		
Diagnosis of animal brucellosis Direct diagnosis 1. Bacteriological diagnosis (including biosafety) 2. Molecular tests Indirect tests: 1. Antigens of diagnostic significance 2. Current Serological tests: Bovines (*B. abortus*) and sheep & goats (*B. melitensis*) Porcine (*B. suis*) Infections by rough brucellae (*B. ovis* and *B. canis*)	09:00 – 13:00	 Dr. José María Blasco Dr. Amaia Zúñiga-Ripa Dr. Ignacio Moriyón Dr. Ignacio Moriyón Dr. José María Blasco Dr. José María Blasco
Lunch break	13:00 – 14:00	Doreen Nankya
Laboratory practical session I (*DDG, SAT, Brucellacapt*)	14:00 – 17:00	
Day 3, 15th August		
Vaccination and control The classical live smooth vaccines The rough vaccines Control and eradication strategies of brucellosis in cattle and small ruminants	09:00 – 13:00	 Dr. José María Blasco Dr. Ignacio Moriyón Dr. José María Blasco
Lunch break	13:00 – 14:00	Doreen Nankya
Laboratory practical session II (*Results reading, RBT, ELISA*)	14:00 – 17:00	
Day 4, 16th August		
Human brucellosis: clinical aspects, diagnosis and treatment	09:00 – 11:00	Dr. Ignacio Moriyón
Human brucellosis in Western Uganda: prevalence, risk factors and Diagnosis	11:00 – 12:00	Dan Nyehangane (MSF/Epicentre/MbararaU.)
Laboratory practical session III (*Results reading, PCR*)	12.00 – 13:00	

Lunch break	13:00 – 14:00	Doreen Nankya
Laboratory practical session III (*Results reading, PCR*)	14:00 – 17:00	
Day 5, 17th August [FIELD WORK]		
Departure of participants from Kampala to Sanga Field Station Arrival and de-briefing Animal sampling Conjunctival vaccination of ruminants	06:00	
Day 6, 18th August		
Wrap up and Post course evaluation Award of certificates	9:00	Daisy Nabadda Director of Research
Lunch break		Doreen Nankya
Closure and departure		Director of Research & James Bugeza

Copy of the certificate

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Marín,C.M., Moreno,E., Moriyón,I., Díaz,R., Blasco,J.M., 1999. Performance of competitive and indirect enzyme-linked immunosorbent assays, gel immunoprecipitation with native hapten polysaccharide, and standard serological tests in diagnosis of sheep brucellosis. Clin.Diagn.Lab.Immunol. 6, 269-272

PROJECT NAME: BRUCELLOSIS AT THE HUMAN – LIVESTOCK WILDLIFE INTERFACE

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE

Date 13TH AUGUST 2018

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PROJECT NAME: BRUCELLOSIS AT THE HUMAN – LIVESTOCK WILDLIFE INTERFACE

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE

Date 15TH Aug. 2018

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25	Dr KAMBERE KAVULIKIRWA	UG/DRC	UCG.	
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PROJECT NAME: BRUCELLOSIS AT THE HUMAN – LIVESTOCK WILDLIFE INTERFACE

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE

Date 14TH Aug 2018

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PROJECT NAME: BRUCELLOSIS AT THE HUMAN – LIVESTOCK WILDLIFE INTERFACE

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE

Date 16TH Aug. 2018.

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5	Bwambale Jeremia	uganda	Kagando Hospital	[signature]
6	KAMBERE KAVULIKIRWA	DR CONGO	UCG	[signature]
7	KATEMBO NGIKE	DR Congo	UCG	[signature]
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NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE P.O.BOX 5704, WAKISO UGANDA

BACK TO OFFICE REPORT ON COMMUNITY AWARENESS MEETING/WORKSHOP ON BRUCELLOSIS AT THE LIVESTOCK, HUMAN, WILDLIFE INTERFACE

Introduction

Brucellosis is an important disease among livestock, humans and wildlife in the great lakes region with highest incidences registered in farms with large herds compared to small ones (Kabagambe et al., 2001). The prevalence risk factors for infections by brucellosis and the available options for its prevention and control are poorly understood by the affected communities. Consequently this affects prioritization of control activities by the affected communities. A study conducted by the Autonomous University of Barcelona (UAB), the Government of Andorra, Daktari, a local Non-Governmental Organization and Makerere University observed a widespread circulation of brucellosis in sheep and goats within the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan. This has serious implications for human health since animals and animal products are the source of infection for man. Therefore some funding was received from the PGTF to implement a project entitled *"Epidemiology of brucellosis on the livestock, wildlife and human interface: Improving the diagnostic capacities of brucellosis disease, enhance the control strategies with special emphasis on farmers' awareness in the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan"*. Part of the funds were used to conduct public mobilization and awareness meetings or workshops around selected communities neighboring conservation areas in the 3 partner countries.

Activity location in Uganda and justification

The activity was conducted around communities surrounding the Murchison falls National Park (MFNP) in Uganda. The Murchison falls conservation area in Uganda forms part of the vast Congo – Sudan- Uganda Albertine ecosystem which is the world's largest reservoir of the most dangerous known pathogens. This area is also home to several species of wildlife. The inhabitants of this area are mostly livestock/crop farmers but also hunters to a large extent. The above scenario suggests a very close interaction between Humans, livestock and wildlife, which is potentially suitable for zoonotic disease transmission

Objective (s) of the meeting/workshop

The objective of the meeting or workshop was to create public awareness on the disease burden and recommend practices for its prevention and control.

Approach

The activity involved 3 day physical visits to farms and households of livestock keepers and deep interactions and discussion about the disease and how it can be prevented. In addition a 1-day workshop was conducted for selected livestock farmers to generally discuss the issue of rampant zoonotic disease outbreaks and how they can be avoided in affected communities but focusing on brucellosis in particular.

Farm Visits

Up to 12 farms in different locations were visited.
The owners and locations were as follows;

No.	Name	Village
1	Byaruhanga Hamis	Kitaleeba
2	Barigye Moses	Masindi Port
3	Abaho David	Katuugo
4	Kazoora Jacob	Wakisaanyi
5	Kiiza Godwin	Kimooka
6	Sipaka .R. John	Katamarwa
7	Munubbi James	Biroora
8	Rubareeta Caleb	Kituuza
9	Kankiriho Geofrey	Kikaito
10	Tayebwa George	Katuugo
11	Byaruhanga.J.	Myeba
12	Kiza George	Mayaba

Pictorial of some farm visits

		
Dr. Bugeza and DVO inspecting a herd of goats	The team with Mr. Kizza inspecting his cattle	With the DVO interacting with another cattle farmers who experiences abortions in his herd.
		
Dr. Bugeza and DVO inspecting a herd of goats with cases of abortion	With Dr. Nsereko at spots where wildlife cross over from MFNP to interact with livestock in adjacent communities.	Interacting with one of the farmers who stays adjacent to the National park

The workshop

The workshop was conducted on the 25th/10/2018 at Savannah guest house, Kigumba
The workshop program was as follows;

No.	Activity	Responsible person	Remarks
1	Arrival and registration	Dr. Komugisha Mariam	
2	Opening prayer	Dr. Komugisha Mariam	
3	Self-introduction	DVO Kiryandongo	
4	Opening remarks from DVO	DVO Kiryandongo	
5	Official opening by chairman LCV Kiryandongo District	DVO Kiryandongo	
6	Objectives and overview of zoonotic diseases	Dr. Bugeza James	
7	Control of ticks and tick borne diseases e.g. Crimean Congo hemorrhagic Fever	Dr. Nsereko Godfrey	
8	Brucellosis epidemiology	Dr. Bugeza James	

9	Lunch		
10	Brucellosis in humans	Dr. Bugeza James	
11	Brucellosis in wildlife	DVO Kiryandongo	
12	Questions and answers (way forward)	All Facilitators	
13	Closure and departure	-	

Arrival and registration of participants

Dr. Komugisha Mariam registered participants on arrival

Opening prayer

Mr. Kiza George gave the chairman of cattle farmers in Masindi port town gave the opening prayer.

Opening remarks from the DVO Kiryandongo

The DVO Kiryandongo, Dr. Wabwire Tonny welcomed all the farmers who responded at short notice and turned up for the meeting.

He encouraged them to always turn up for such trainings because getting a team of experts to talk to them on livestock issues was expensive and a rare opportunity. He told participants that the issues of zoonotic diseases were seriously affecting farmers in terms of income, health and animal production. He thanked NaLIRRI staff for considering communities in Kiryandongo district and he pledged to mobilize farmers to work with the institute in all future endeavors. He encouraged farmers to ask questions on all aspects of livestock production because the experts were veterinarians capable of addressing all issues of livestock production. He ended by inviting the representative of the chairman LCV to give his opening remarks.

Remarks from the chairman LCV, Kiryandongo District

On behalf of the chairman LCV Kiryandongo District, Mr. Godwin Kanongyire welcomed all participants and thanked them for all the efforts they are putting forward to improve household incomes, nutrition and food security. He encouraged them to always heed the advice given them by professionals like the one from NaLIRRI because they are the ones who have up to date information on all aspects of animal production. He encouraged them to abide by the present quarantine restrictions imposed on the district because of the Foot and Mouth Disease epidemic. He thanked the organizers for choosing Kiryandongo district. He however said that in addition to zoonotic diseases some of which have been reported in the district, the problem of tick resistance to acaricides had adversely affected cattle farmers. He therefore encouraged the trainers to also save

some time to answer farmer's questions regarding tick resistance to acaricides. He ended by declaring the workshop open and wishing the team a good training and to always return when requested.

Objectives and overview of zoonotic diseases

Dr. Bugeza James from NaLIRRI welcomed all the participants and thanked them for turning up in large numbers at short notice. He mentioned that the objective was to create awareness among livestock farmers on the silent threat of zoonotic diseases that are potentially dangerous to human health and adversely affect livestock production.

He mentioned that NaLIRRI is an institute under the National Agricultural Research Organization (NARO) mandated to conduct research in Livestock health, Livestock Breeding and Livestock Nutrition. He said that work of the institute involves generating technologies, inputs and management practices as well as disseminating them to the farmers. He therefore said that since farmers have expressed interest in asking questions outside zoonotic diseases the team would allow some time to discuss those issues. On the issue of zoonotic diseases Dr. Bugeza said that over the past 3 years the country has experienced outbreaks of Rift Valley Fever, Anthrax, Highly Pathogenic Avian Influenza, Crimean Congo Hemorrhagic Fever (CCHF), Rabies, Marburg and Brucellosis among others. He also talked about the current outbreak of Ebola Virus Disease (EBV) in neighboring Congo, which can potentially spill over, to Uganda. Specifically he mentioned that Kiryandongo District had experienced outbreaks of CCHF and that 3 people were reported affected. He mentioned that wildlife that was abundant in their district was a reservoir of most of the mentioned diseases. He talked about various drivers of disease emergence and reemergence that include, destruction of ecosystems, climate change, rapid international travel, wars and change in production methods. He mentioned that because of the importance of the diseases to the economy and public, government had prioritized the management and control of 7 of these diseases. He therefore said that the public must be aware about these diseases and take precaution because they are a real threat to life yet communities are not aware about them. He concluded by saying that since Brucellosis is the commonest zoonosis world wide the training would focus much on that particular disease because there is a public outcry about this disease, which we ought to address.

Control of ticks and tick borne diseases

Dr. Nsereko noted that the country is experiencing an unprecedented tick resistance against acaricides especially in cattle corridor districts. He regretted the enormous losses that farmers were incurring in form of expenses on drugs, loss of livestock and loss of income. He also noted that because of tick resistance to acaricides, outbreaks of CCHF were registered in several cattle corridor districts. He re-emphasized that CCHF was tick transmitted and the animals are the reservoirs of the viruses that cause the disease. On what the government is doing to address this challenge, Dr. Nsereko said that two new acaricides are being tried out and if they are found effective they will be released to the market. In addition he said

countrywide farmer sensitization on integrated control of ticks and tickborne diseases is being conducted together with MAAIF and Makerere University.

On what NaLIRRI is doing about the tick resistance challenge he said that the institute is in advanced stages of developing an anti-tick vaccine with partners from Spain, a bio-acaricide and fungal acaricide but hastened to add that these may take another five years to attain commercial status. He also said that there are efforts to improve the current Muguga cocktail vaccine to include local strains of the *Theileria parva.* At this point some farmers said that they have seen fellow farmers using herbicides imported from Tanzania as acaricides. They said that the people who market it claim it is both an acaricide and an insecticide. On this note Dr. Bugeza advised that as long as the chemical was not formulated for veterinary use then farmers should not use it for that purpose because they risk using their animals. Dr. Nsereko ended by advising farmers to ensure their farms are fenced, to observe recommended dilution rates for acaricides, avoid mixing acaricides, observing recommended application intervals, obtaining acaricides from registered stockists, following veterinary advise on acaricide use and where possible immunizing their cattle as part of an integrated program for control of ticks and tickborne diseases on their farms. He also advised those keeping goats, sheep and dogs to always consider them in the tick control program otherwise they would act as the reservoir for ticks.

Brucellosis epidemiology

On this subject Dr. Bugeza highlighted on the following. He said that the disease is the most common zoonosis worldwide claiming about 500,000 lives annually.

He said the disease is a major problem in low-income countries e.g. Uganda, DRC, South Sudan. He noted that the disease is highly contagious and is largely a neglected zoonosis but causes significant production losses in livestock. He said that despite this fact most communities are unaware of how the infection is acquired, transmitted and how it can be prevented or controlled. He told participants that animals are the source of the disease for humans and that this is the reason why the disease is found in countries where animals are kept especially in pastoral settings and around protected areas where animals freely mix with wildlife.

He however noted that some few developed countries especially in Europe and North America had succeeded in

eradicating the disease. He told participants that the causative agent is species specific. He told participants that goats, cattle, pigs and dogs are the source of zoonotic brucella species although the virulence of these species to man vary with *Brucella canis* being most mild and *Brucella melitensis* being most severe. He told farmers that seropositive animals have higher rates of abortion, stillbirth, infertility and kid mortality, as well as reduced growth and longer kidding intervals. He said that large amounts of bacteria are shed through vaginal secretions thereby contamination pastures and water sources. Clean animals therefore get infected through feeding and drinking from contaminated pastures and water. He said that although the venereal route is not the main route of transmission especially in cattle like most farmers believe. He also told farmers that dogs and carrion feeders play an important role in disseminating the bacteria and that farmers should ensure deep burial or burning of aborted fetuses. Dr. Bugeza told the farmers that some species of wildlife are a potential source of brucellosis for both humans and their livestock and cautioned those who hunt to be extra careful when preparing their game and to ensure that the meat is thoroughly cooked before consumption. He however noted that there is need for more research to clearly understand the role of wildlife in the epidemiological cycle of brucellosis. He advised farmers always screen their animals for brucellosis, buying replacement stock from disease free herds and considering a program for immunization of their livestock using the conjuctival vaccine with in a given locality if they are to control the disease.

Brucellosis in Humans

Dr. Bugeza told participants that man acquires the disease through consumption of raw or undercooked livestock products, during assistance in case of difficult birth, through abraded skin in case of butchers or flayers, through conjuctival splashes, through inoculation with vaccines and laboratory exposure for veterinarians. He told that the disease manifests as an undulant fever with an incubation period ranging from 5 days to 3 months. He told the farmers that any organ or organ system may be affected and that the disease may progress to a chronic illness with arthritis, spondylitis, orchitis (Can lead to infertility), chronic fatigue, neurological disorders (5% cases), ocular and cardiovascular complications. Dr. Bugeza told participants that there is a general public outcry in the country about this disease and that even traditional healers had taken advantage of the situation and were claiming to cure the disease. Dr. Bugeza ended the session by advising farmers to always use protective clothing like gloves, when assisting animals in case of difficult birth, always preparing meat and milk before consumption. He also advised that those who suffer from chronic fevers should visit health facilities but should advise the clinicians about their occupation or on the recent place they visited because these may help the clinician to suspect brucellosis and screen for it. He told participants that once diagnosed with the disease they should commence treatment and make sure they complete the dose even though the treatment regime is long to avoid relapses.

Brucellosis in wildlife

Dr. Wabwire Tonny informed participants that research has established that several species of wildlife like buffaloes, elk, antelopes, hares suffer from and are involved in the dissemination of brucellosis. He informed them those wild animals like foxes and other wild canids can also play a role in dissemination of brucellae by dragging aborted fetuses for long distances thereby contaminating pastures. He said that the clinical signs in wildlife are similar to those in domestic animals. Abortions, debilitation and death are common. Hygromas have been observed in buffaloes in chronic brucellosis. He informed participants that wild animals have

been implicated in contaminating pastures and water sources from where domestic animals acquire the infection. He cautioned hunters to take extra caution when preparing their game to avoid accidental exposure to the bacteria.

Questions answers and way forward

The farmers asked several questions to which the training team responded. The following however were the main issues raised;

1. Some people are using a herbicide from Tanzania as an acaricide and those who market it claim it is both an acaricide and an insecticide. The farmers wanted to know what our advice was on the above. Dr. Bugeza advised that so long as the chemical was not formulated for veterinary use then the farmers should not use it as such because they risk loosing their animals. He also advised farmers to only buy acaricides from registered outlets and always seek advise from veterinarians before using the chemicals.

2. Selective vaccination of cattle is bad and all farmers should vaccinate all their animals. Dr. Bugeza requested the DVO to mobilize farmers in future so that all animals are immunized against brucellosis. The farmers pledged to comply.

3. Privatization of veterinary drug trade is detrimental to the livestock industry. Dr. Bugeza informed that the privatization policy was part of the structural adjustment programs adopted in the 1990's. He noted that the policy meant that government divested from doing business and private enterprises took over most of the businesses. He noted that because of this policy farmers can readily get inputs at competitive prices. The monitoring and supervisory role of government should however be strengthened to ensure that only genuine products are available on the market. He advised farmers to buy drugs only upon prescription by a veterinarian and to ensure they follow the instructions strictly.

4. Can someone get infected with Brucella through breathing or through urine splashes? Dr. Bugeza responded that the main route is the oropharyngeal mucosa and through abraded skin. He said that there is no evidence that man can be infected through breathing but contaminated urine splashes in the mouth and conjuctival mucosa can be infective.

5. Can people who do not keep livestock suffer from brucellosis? Dr. Bugeza responded that if such people eat raw or undercooked livestock products or get exposed to live vaccines or through lab exposure then it is possible to get infected.

6. Are brucella vaccines safe? Dr. Bugeza advised that current vaccines are not safe on account of the possibility of causing abortion, virulence to humans and interference with serological tests. He however said that the subconjuctival vaccine, which is not yet on the Ugandan market, is free from all the above-mentioned challenges.

7. How often should we immunize against brucellosis? Dr. Bugeza advised that within a well thought out vaccination program in a given epidemiological unit if all farmers agree

to immunize their livestock once very year, then in a period of 5 years all animals would be safe from brucellosis

Closing remarks from the representative of the chairman LCV Kiryandongo

Mr. Kanongyire Godwin thanked the team from NaLIRRI for sparing time to teach farmers in Kiryandongo.

He also thanked them for choosing Kiryandongo among all districts in Uganda and should return again when invited to follow up on their recommendations. He advised the farmers to practice what they had learnt during the training and give feedback to the team. He requested the team to leave behind their telephone contacts so that farmers can always consult them. He thanked the DVO for always being available to teach the farmers and thanked everyone for attending the training. He then declared the training closed and wished everyone a safe journey back home.

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Venue: AL HAPEHA ALGA

Date: 25/01/2018

Attendance list

No.	Name	Village	Gender	Occupation	Signature
1	KIRA GEORGE	MAYABA	M	FAMER	
2	MUGEHANABO K. FRED	KITADABA	m	"	
3	Mugisa Charles	Kigumba	M	Famer	
4	Barigye Moses	Masindi port	M	Famer	
5	Kabuhende Jesca	Masindi Port	F	Famer	
6	Nahwera Fred	Masindi port	M	Peasant Farmer	
7	Gawera Richard	Wakisanya	M	Farmer	GAWERA
8	Baguma Musa	Masindi port	M	Farmer	
9	Kagisha Edward	Masindi port	M	Farmer	
10	RWIVUNGU STEPHEN	KITALEBA	m	"	
11	Titia	KIKAITO	m	Famer	
12	BYABAHANGA AMSI	KITALEBA	m	Famer	
13	SUNDAY EDWARD	Masindi port	m	famer	
14	KIIZA GODFREY	KIMOGA	M	Resi. Councillor	
15	Kafirita Steven	Wakisanya	m	famer	
16	RWAMASindi ROOBt	KATUGU		FAMER	
17	David Abaho	Katugo	m	Farmer	
18	Rubuugo Richa	Kitaleba	m	Farm	
19	Bigaririyo Varisito	M/PORT	M	Farm	
20	Bigomba Erick	Kitalebe	M	Farmer	
21	Kazoora Jacob	Wakisanya	M	Farmer	
22	Galimaka James	M/port	M	Farmer	

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Venue: LAPIKA ALEX

Date: 25th/Oct/2018

Attendance list

No.	Name	Village	Gender	Occupation	Signature
1	Kanongyire G	Masindi port	M	Farmer	KANO
2	SIPALA R JOHN	KATAMBARWA		FARMER	
3	MWESIGYE GW	WAKISHA	M	FARMER	
4	MUGISHA JIMMY	WAKISHA	M	FARMER	
5	ATIMANLE ROBERT	WAKISHA	M	FARMER	
6	NKAMUHABWA	FRED	M		
7	KIZA WILSON	WAKISHA	M	FARMER	
8	BAGUMYA PETER	KITUKUZA	M	11	
9	KAMANZI JB	Kigumba	M	11	
10	KALEGIRIHO GEORGES	KIKIATO	M	11	
11	MUNUBI JAM	BIYOSA	M	11	
12	Yebafe God	KIMANOKA	M	11	
13	MULANGIRA EDWARD	Kigaseka	M	Farmer	
14	Senganyge John	Kigaseka	M	11	
15	KADEMMA God	KATUKUKU	M	11	
16	KAKYABA STEVEN	KITADABA	M	11	
17	RUGHSIDA Chrispus	KATUKUKU	M	11	
18	RUBARETA CAREB	KITUUZA	M	11	
19	KAMANYA JAMES	KATUKUKU	M	11	
20	Sarah M Kyaddede		F	Farmer	
21	ARIGUNA MARIA		F	Farmer	
22	Mbagga Lifazi		M	11	

Remove Watermark Now

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Venue: Lapi wa Alex

Date: 28/10/2018

Attendance list

No.	Name	Village	Gender	Occupation	Signature
1	Rucunyadana J.	Katuugu	m	Farmer	[signature]
2	Byanihanga J	Myebo	m	Farmer	[signature]
3	Shubi Fred	Kaduukuti	m	" "	[signature]
4	Tayebwa George	Katugu	m	Farmer	[signature]
5	Mugisha K.K.	Wakisangi	m	F.	[signature]
6	Sunday S	Wakisangi	m	Farmer	[signature]
7	Munyelero F.	Katugu	m	Farmer	[signature]
8	Sunday K. Robert	Wakisangi	m	Farmer	[signature]
9	Rwamafonano	Wakisangi	m	Farmer	[signature]
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BACK TO OFFICE REPORT ON COMMUNITY AWARENESS MEETING/WORKSHOP ON BRUCELLOSIS AT THE LIVESTOCK, HUMAN, WILDLIFE INTERFACE

AN INTERFACE WITH FARMERS IN NAKASONGOLA DISTRICT, UGANDA

Introduction

Brucellosis is an important disease among livestock, humans and wildlife in the great lakes region with highest incidences registered in farms with large herds compared to small ones (Kabagambe et al., 2001). The prevalence risk factors for infections by brucellosis and the available options for its prevention and control are poorly understood by the affected communities. Consequently this affects prioritization of control activities by the affected communities. A study conducted by the Autonomous University of Barcelona (UAB), the Government of Andorra, Daktari, a local Non-Governmental Organization and Makerere University observed a widespread circulation of brucellosis in sheep and goats within the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan. This has serious implications for human health since animals and animal products are the source of infection for man. Some funding was received from the Perez Guererro Trust Fund (PGTF) to implement a project entitled *"Epidemiology of brucellosis on the livestock, wildlife and human interface: Improving the diagnostic capacities of brucellosis disease, enhance the control strategies with special emphasis on farmers' awareness in the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan"*. Part of the funds were used to conduct public mobilization and awareness meetings or workshops around selected communities neighboring conservation areas in the 3 partner countries.

Activity location in Uganda and justification

The activity was conducted around communities surrounding the Murchison falls National Park (MFNP) in Uganda. The Murchison falls conservation area in Uganda forms part of the vast Congo – Sudan- Uganda Albertine ecosystem that is the world's largest reservoir of the most dangerous known pathogens. Nakasongola district is one of the districts in close proximity to the MFNP conservation area. The district is also lies in the cattle corridor with a high concentration of livestock and free ranging wildlife. Nakasongola district is also home to Ziwa Rhino sanctuary. The above scenario suggests a very close interaction between Humans, livestock and wildlife, which is potentially suitable for zoonotic disease transmission.

Objective (s) of the meeting/workshop

The objective of the meeting or workshop was to create public awareness on the disease burden and recommend practices for its prevention and control. In addition the meetings were intended to create and foster partnerships for zoonotic diseases control.

Approach

The activity involved half-day physical visits to farms and households of livestock keepers and deep interactions and spot on discussion about brucellosis and other zoonotic diseases and how they can be prevented with communities. In addition a one-day workshop was conducted for selected livestock farmers to generally discuss the issue of rampant zoonotic disease outbreaks and how they can be avoided in affected communities but focusing on brucellosis in particular.

Farm Visits

Up to 10 farms in different locations were visited.
The owners and locations were as follows;

No.	Name	Village
1	Kanzira Emmanuel	Kalungu
2	Kugonza Isac Ntalo	Kyangogolo
3	Twesigye David	Karubanga
4	Mutunda Patrick	Karubanga
5	Wasswa Wilson	Kalengedde
6	Muwanga Fred	Mulonzi
7	Nsubuga David	Waddundulya
8	Manegule John	Kagiyo
9	Buhangire Yosamu	Migera
10	Wanzala Allan	Katuba

Pictorial of some farm visits and community engagements

		
A hard of beef cattle with reported abortion cases in Migera, Nakasongola district	A farmer on spot discussion a bout Brucellosis in Kasozi Nakasongola district	A beef herd in Mulonzi. The farmers complains of still births
		
An on spot discussion with farmers in Mulonzi about brucellosis	A herd visited in Kyamukonda, Nakasongola district	An on spot discussion with farmers in Kyamukonda, Nakasongola district
		
A brief discussion session about brucellosis in Kagiyo, Nakasongola district	One of the farms visited in Namizo with suspected cases of brucellosis	With one of the farmers who reportedly suffered from brucellosis in one of the villages visited.

The workshop

The workshop was conducted on the 13th/07/2019 at Jyra services center, Migera, Nakasongola.
The workshop program was as follows;

No.	Activity	Responsible person	Remarks
1	Arrival and registration	Mr. Amon Kibalikoba	
2	Opening prayer	All	
3	Self-introduction	Dr. Bugeza James	
4	Opening remarks from DVO	DVO Nakasongola	
5	Official opening by Town ClerK Migeera TC, Nakasongola District	DVO Naksongola	
6	Objectives and overview of zoonotic diseases	Dr. Bugeza James	
7	Control of ticks and tick borne diseases e.g. Crimean Congo hemorrhagic Fever	Dr. Kabi Fredrick	
8	Brucellosis epidemiology	Dr. Bugeza James	
9	Lunch		
10	Brucellosis in humans	Dr. Bugeza James	
11	Brucellosis in wildlife	DVO Nakasongola	
12	Questions and answers (way forward)	All Facilitators	
13	Closure and departure	-	

Arrival and registration of participants

Mr. Amon Kibalikoba registered participants on arrival

Opening prayer

Mr. Byaruhanga Steven one of the participants gave the opening prayer.

Opening remarks from the DVO Nakasongola

On behalf of the DVO Nakasongola, Dr. Mukumbya Isac welcomed all the farmers who responded at short notice and turned up for the meeting. He thanked the team from NaLIRRI for considering Nakasongola district for the training adding that brucellosis was one of the diseases hampering livestock production in the district but also a serious public health concern in the recent past.

He encouraged the farmers to always turn up for such trainings because getting a team of experts to talk to them on livestock issues was expensive and a rare opportunity. He told participants that the issues of zoonotic diseases were seriously affecting farmers in terms of income, health and animal production. He encouraged farmers to ask questions on all aspects of livestock production because the experts were veterinarians capable of addressing all issues of livestock production. He ended by inviting the town

clerk of Migera town council the to give his opening remarks.

Remarks from the town clerk of Migera town council, Nakasongola District

On behalf of the town clerk, Mr. Kabaseke Steven welcomed all participants and thanked them for all the efforts they are putting forward to improve household incomes, nutrition and food security. He encouraged them listen attentively to professionals like the one from NaLIRRI because they are the ones who have up to date information on all aspects of animal production especially regarding zoonotic diseases.

He told them that the current quarantine imposed on the district due to foot and mouth disease outbreak was about to be lifted and encouraged them to continue observing the regulations governing the quarantine until it was lifted. He thanked the organizers for choosing Nakasongola district but requested them to also partly cover the problem of tick resistance to acaricides because it was one of the major constraints affecting cattle farmers. He ended by declaring the workshop open and wishing the team a good training and to always return when requested.

Objectives and overview of zoonotic diseases

Dr. Bugeza James from NaLIRRI welcomed all the participants and thanked them for turning up in large numbers at short notice. He mentioned that the objective was to create awareness among livestock farmers on the silent threat of zoonotic diseases that are potentially dangerous to human health and adversely affect livestock production.

He mentioned that NaLIRRI is an institute under the National Agricultural Research Organization (NARO) mandated to conduct research in livestock health, livestock breeding and livestock Nutrition. He said that work of the institute involves generating technologies, inputs and management practices as well as disseminating them to the farmers. He therefore said that since farmers have expressed interest in asking questions outside zoonotic diseases the team would allow some time to discuss those issues. On the issue of zoonotic diseases Dr. Bugeza said that over the past 3 years the country has experienced outbreaks of Rift Valley Fever, Anthrax, Highly Pathogenic Avian Influenza, Crimean Congo Hemorrhagic Fever (CCHF), Rabies, Marburg and Brucellosis among others. He also talked about the current outbreak of Ebola Virus Disease (EBV) in neighboring Congo, which had spilled over, to Uganda. Specifically he mentioned that the neighboring district of Nakaseke had experienced outbreaks of CCHF and that 1 person was reportedly affected. He mentioned that wildlife that was abundant in their district was a reservoir of most of the mentioned diseases. He talked about various drivers of disease emergence and reemergence that include, destruction of ecosystems, climate change, rapid international travel, wars and change in production methods. He mentioned that because of the importance of the diseases to the economy and public, government had prioritized the management and control of 7 of these diseases. He therefore said that the public must be aware about these diseases and take precaution because they are a real threat to life yet communities are not aware about them. He concluded by

saying that since brucellosis is the commonest zoonosis world wide the training would focus much on that particular disease because there is a public outcry about this disease, which we must to address.

Control of ticks and tick borne diseases

Dr. Kabi Fredrick noted that the country is experiencing an unprecedented tick resistance against acaricides especially in cattle corridor districts. He regretted the enormous losses that farmers were incurring in form of expenses on drugs, loss of livestock and loss of income. He also noted that because of tick resistance to acaricides, outbreaks of CCHF were registered in several cattle corridor districts. He re-emphasized that CCHF was tick transmitted and the animals are the reservoirs of the viruses that cause the disease although they are asymptomatic.

On what the government is doing to address this challenge, Dr. Kabi said that two new acaricides namely Vectoclor and Eprinomectin were being tried out and if they are found effective they will be released to the market. In addition he said countrywide farmer sensitization on integrated control of ticks and tickborne diseases is being conducted together with MAAIF and Makerere University.

On what NaLIRRI is doing about the tick resistance challenge he said that the institute is in advanced stages of developing an anti-tick vaccine with partners from Spain, a bio-acaricide and fungal acaricide but hastened to add that these may take another five years to attain commercial status. He also said that there are efforts to improve the current Muguga cocktail vaccine to include local strains of the *Theileria parva.* At this point some farmers said that they have seen fellow farmers using herbicides imported from Tanzania as acaricides. They said that the people who market it claim it is both an acaricide and an insecticide. On this note Dr. Bugeza advised that as long as the chemical was not formulated for veterinary use then farmers should not use it for that purpose because they risk using their animals. Dr. Kabi ended by advising farmers to ensure their farms are fenced, to observe recommended dilution rates for acaricides, avoid mixing acaricides, observing recommended application intervals, obtaining acaricides from registered stockists, follow veterinary advise on acaricide use and where possible immunizing their cattle as part of an integrated program for control of ticks and tickborne diseases on their farms. He also advised those keeping goats, sheep and dogs to always consider them in the tick control programs otherwise they would act as the reservoir for ticks.

Brucellosis epidemiology

On this subject Dr. Bugeza highlighted on the following. He said that the disease is the most common zoonosis worldwide claiming about 500,000 lives annually.

He said the disease is a major problem in low-income countries e.g. Uganda, DRC, South Sudan. He noted that the disease is highly contagious and is largely a neglected zoonosis but causes significant

production losses in livestock. He said that despite this fact most communities are unaware of how the infection is acquired, transmitted and how it can be prevented or controlled. He told participants that animals are the source of the disease for humans and that this is the reason why the disease is found in countries where animals are kept especially in pastoral settings and around protected areas where animals freely mix with wildlife. He however noted that some few developed countries especially in Europe and North America had succeeded in eradicating the disease. He told participants that the causative agent is species specific. He told participants that goats, cattle, pigs and dogs are the source of zoonotic brucella species although the virulence of these species to man vary with *Brucella canis* being most mild and *Brucella melitensis* being most severe. He told farmers that seropositive animals have higher rates of abortion, stillbirth, infertility and kid mortality, as well as reduced growth and longer kidding intervals. He said that large amounts of bacteria are shed through vaginal secretions thereby contamination pastures and water sources. Clean animals therefore get infected through feeding and drinking from contaminated pastures and water. He said that although the venereal route is not the main route of transmission especially in cattle like most farmers believe. He also told farmers that dogs and carrion feeders play an important role in disseminating the bacteria and that farmers should ensure deep burial or burning of aborted fetuses. Dr. Bugeza told the farmers that some species of wildlife are a potential source of brucellosis for both humans and their livestock and cautioned those who hunt to be extra careful when preparing their game and to ensure that the meat is thoroughly cooked before consumption. He however noted that there is need for more research to clearly understand the role of wildlife in the epidemiological cycle of brucellosis. He advised farmers always screen their animals for brucellosis, buying replacement stock from disease free herds and considering a program for immunization of their livestock using the conjuctival vaccine with in a given locality if they are to control the disease.

Brucellosis in Humans

Dr. Bugeza told participants that man acquires the disease through consumption of raw or undercooked livestock products, during assistance in case of difficult birth, through abraded skin in case of butchers or flayers, through conjuctival splashes, through inoculation with vaccines and laboratory exposure for veterinarians. He told that the disease manifests as an undulant fever with an incubation period ranging from 5 days to 3 months. He told the farmers that any organ or organ system may be affected and that the disease may progress to a chronic illness with arthritis, spondylitis, orchitis (Can lead to infertility), chronic fatigue, neurological disorders (5% cases), ocular and cardiovascular complications. Dr. Bugeza told participants that there is a general public outcry in the country about this disease and that even traditional healers had taken advantage of the situation and were claiming to cure the disease. Dr. Bugeza ended the session by advising farmers to always use protective clothing like gloves, when assisting animals in case of difficult birth, always preparing meat and milk before consumption. He also advised that those who suffer from chronic fevers should visit health facilities but should advise the clinicians about their occupation or on the recent place they visited because these may help the clinician to suspect brucellosis and screen for it. He told participants that once diagnosed with the disease they should commence treatment and make sure they complete the dose even though the treatment regime is long to avoid relapses.

Brucellosis in wildlife

Dr. Mukumbya Isac informed participants that research has established that several species of wildlife like buffaloes, elk, antelopes, hares suffer from and are involved in the dissemination of brucellosis. He informed them those wild animals like foxes and other wild canids can also play a role in dissemination of brucellae by dragging aborted fetuses for long distances thereby contaminating pastures. He said that the clinical signs in wildlife are similar to those in domestic animals. Abortions, debilitation and death are common. Hygromas have been observed in buffaloes in chronic brucellosis. He informed participants that wild animals have been implicated in contaminating pastures and water sources from where domestic animals acquire the infection. He cautioned hunters to take extra caution when preparing their game to avoid accidental exposure to the bacteria.

Questions answers and way forward

The farmers asked several questions to which the training team responded. The following however were the main issues raised;

1. How can we get the ocular vaccine for brucellosis? Dr. Bugeza advised them to work with the DVO so that arrangements can be made to deliver the vaccine.
2. Can we immunize only part of the herd against brucellosis? Selective vaccination of cattle is bad and all farmers should vaccinate all their animals. Dr. Bugeza requested the DVO to mobilize farmers in future so that all animals are immunized against brucellosis. The farmers pledged to comply.

3. Can someone get infected with Brucella through eating meat? Dr. Bugeza responded that the main route is the oropharyngeal mucosa and through abraded skin. He said that if meat is not properly cooked and the carcass was infected then there are chances that one can get infected.

4. Can people who do not keep livestock suffer from brucellosis? Dr. Bugeza responded that if such people eat raw or undercooked livestock products or get exposed to live vaccines or through lab exposure then it is possible to get infected.

5. Are brucella vaccines safe? Dr. Bugeza advised that current vaccines are not safe on account of the possibility of causing abortion, virulence to humans and interference with serological tests. He however said that the subconjuctival vaccine, which is not yet on the Ugandan market, is free from all the above-mentioned challenges.

6. How often should we immunize against brucellosis? Dr. Bugeza advised that within a well thought out vaccination program in a given epidemiological unit if all farmers agree to immunize their livestock once very year, then in a period of 5 years all animals would be safe from brucellosis

Closing remarks from the representative of the town clerk Migera town council

Mr. Sekimuli Bob thanked the team from NaLIRRI for sparing time to teach farmers in Nakasongola. He also thanked them for choosing Nakasongola among all districts in Uganda and should return again when invited to follow up on their recommendations. He advised the farmers to practice what they had learnt during the training and give feedback to the team. He requested the team to leave behind their telephone contacts so that farmers can always consult them. He thanked the field veterinary officers for always being available to teach the farmers and thanked everyone for attending the training. He then declared the training closed and wished everyone a safe journey back home.

UNIVERSITE CATHOLIQUE DU GRABEN

U.C.G/BUTEMBO/DRC

P.O. BOX 29 BUTEMBO

www.ucgraben.org

FACULTY OF VETERINARY MEDECINE

Butembo, November 26, 2018

RE: Transmission of the report of DRC team field activities

To the Director of research of the National Livestock Resources

Research Institute (NaLIRRI) Uganda, P O. Box 5704 WAKISO/UGANDA

Dear Sir,

On behalf of the Faculty of Veterinary Medicine, Université Catholique du Graben, I am pleased to submit the report of the community sensitization activity for public awareness creation on brucellosis around the Parc National des Virunga in DRC (Lubiriha and Kyavinyonge towns) as part of the activities included in the project we are carrying out in partenarship with your organization.

Despite all environmental problems related to the special situation in Congo that you may now, the team did its best to make sure the goal is achieved.

All the requested documents being attached on this report, feel free to get in touch if i can be of any help. I thank you for collaboration and I'm looking forward to hearing from you.

Yours sincerely,

REPUBLIQUE DEMOCRATIQUE DU CONGO
UNIVERSITE CATHOLIQUE DU GRABEN
FACULTE DE MEDECINE VETERINAIRE
B.P 29, BUTEMBO
PROVINCE DU NORD-KIVU

Prof Dr. KATUNGU KIBWANA Denise

Vice-den in charge of research, Faculty of Veterinary Medicine,

Mail: rosebeni27@gmail.com

Phone: +243977032575; +243898141781

NATIONAL AGRICULTURE RESEARCH ORGANISATION/UNIVERSITE CATHOLIQUE DU GRABEN

RAPPORT OF THE FIELD ACTIVITIES: PUBLIC AWARENESS CREATION IN BRUCELLOSIS ARROUND PARC NATIONAL DES VIRUNGA IN DEMOCRATIC REPUBLIC OF THE CONGO

REPORT OF THE FIELD ACTIVITIES

DRC TEAM

20/11/2018

TABLE OF CONTENTS

ABBREVIATION AND ACRONYMS

- AGRIPEL: Agriculture pêche et élevage
- ACDC: Association Congolaise pour le Développement et la Communication
- Km: Kilometer
- NARO: National Agriculture Research Organization
- PNVi: Parc National des Virunga
- UCG: Université Catholique du Graben
- USD: United States Dollar

I. **INTRODUCTION**

Our project is intitled « Epidemiology of brucellosis on the livestock, wildlife and human interface: Improving the diagnostic capacities of brucellosis disease, enhance the control strategies with special emphasis on farmers' awareness in the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan » one of the activities scheduled in the project is community awareness creation on brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

In our case, we have selected two sites that are very close to the Virunga National Park and where almost all the goats and sheep live in wandering and therefore likely to have contact with the wildlife. Otherwise, because of the security situation of the area, we decided to conduct our activities in the areas of the PNVi where we were supposed to work safely.
Thus our work consisted of Raising awareness on the general epidemiology of brucellosis, the prevention and control of brucellosis at the human-domestic animal-wildlife interface.

II. CONDUCT AND CHRONOGRAM OF ACTIVITIES

The field activities lasted one (1) week, from Monday, November 12 to Saturday, November 17, 2018.
The implementation schedule was organized as follows:
Our activities were spread out over a period of six (6) days in two selected areas (Lubiriha and Kyavinyonge) located around the Virunga National Park
Three (3) public awareness days dedicated to the population of Lubiriha, an town just on the border with Uganda.
Three (3) additional days were spent at Kyavinyonge, another town located between Mount T'Shiabirimu / Virunga in the West and Lake Edward in the East.

III. PRESENTATION OF THE WORKING ENVIRONMENT

As mentioned beyond, our activities took place in two agglomerations in Beni territory, including Lubiriha and Kyavinyonge. These two agglomerations having been chosen for their closer neighborhood to the Virunga National Park and the fact that livestock and essentially small ruminants are kept in divagation. Our field of activities is presented on the following map:

Fig n°1: Location map of Lubiriha and Kyavinyonge in relation to PNVi

III.TEAM

The scientific team was composed of five (5) people who participated in the training organized in Uganda from 13 to 18 August 2018. All five (5) researchers are agents of the Université Catholique du Graben in the faculties of Veterinary Medicine and Biomedical Sciences including:

1. Dr. Obed KASEREKA MBUSA, Veterinarian
2. Dr. Emmanuel KATEMBO NGIKE, Veterinarian
3. Dr. Olivier KAMBERE KAVULIKIRWA, Veterinarian
4. Dr. Moïse VALIMUNGIGHE, Medical doctor
5. MWANZO WA VINDU KAZI, Laboratory Technician

This scientific team was supported by a technical team on ground constituted by the driver who was hired by the ACDC, organization which provided us the vehicle, for each site, two agents of the local official veterinary service and 2 personal protocol for services during ceremonies.
The table below presents all the scientific and technical staff with their respective responsibilities:

Table I Scientific and Technical Staff Used During Field Activities

N°	NAME	GENDER	INSTITUTION	RESPONSABILITY
1	Dr. Obed KASEREKA MBUSA,	M	UCG	Presentation of the animal brucellosis
2	Dr. Emmanuel KATEMBO NGIKE,	M	UCG	Presentation on the ONE HEALTH aspect of brucellosis
3	Olivier KAMBERE KAVULIKIRWA	M	UCG	Presentation on the general epidemiology of brucellosis
4	Dr. Moïse VALIMUNGIGHE,	M	UCG	Presentation of the human brucellosis
5	MWANZO WA VINDU KAZI,	M	UCG	Presentation of the lab diagnosis of brucellosis
6	DELPHIN MUTHONDA	M	AGRIPEL	Local facilitator Lubiriha
7	JOHN KAMALIIRO	M	AGRIPEL	Local facilitator Lubiriha
8	KAVUGHO VASAMYA	F	Local Farmers Association	Protocol
9	MASIKA MALISAWA	F	Local Farmers Association	protocol
10	KASEREKA SIKULI	M	AGRIPEL	Local facilitator

				Kyavinyonge
11	KAVUGHO KATEMBO	F	Local farmers' association	Protocol
12	KAMABALE KYANGETSE	M	AGRIPEL	Local facilitator
13	MASIKA NZILAMBA	F	Local Farmers Association	Protocol

IV. METHODOLOGY

The activities were planned a week before our field trip. Contacts have been made with local leaders as well as the official veterinary services for the preparation of the local population. These people considered as focal points played a big role in the organization of the meetings made on the ground in particular to identify the meeting places, the audio-visual material, the housing and the awareness of the community.
Two days before the session, we sent out announcements to local radios as well as invitations to certain personalities to allow the circulation of information in relation to the meetings with the population, the announcement containing the subject to be treated, the day, the date and the meeting place.

V. REALIZATION OF DIFFERENT ACTIVITIES

A. LUBIRIHA

We left Butembo on Monday 12 November / 2018. Upon arrival, we took care to present our civilities and our cover letter to the politico-administrative authorities of the place. After this step, we were allowed to start our activities the next day.

Tuesday, November 13th:

- Opening of the session by the official veterinarian of the place
- Training itself: Five (5) modules were developed by the team starting with (i) animal brucellosis, (ii) General epidemiology of brucellosis, (iii) Human brucellosis, (iv) Laboratory diagnosis, (v)) Prevention and control of brucellosis.
- Several questions were asked to which we answered
- At the end of the session, refreshment was provided to the attendees and their list is in **annex II**
- Started at 9:00, the meeting ended at 5:00 pm with the satisfaction of all.
- Return to the hotel for rest

Wednesday, November 14:

- From 9:00 a.m, evaluation of the previous work
- At 12:00, meet with the local authority to say goodbye
- At 13:00, departure from Lubiriha to Kyavinyonge
- At 17:00, arrival at Kyavinyonge and check-in at the hotel.

B. KYAVINYONGE

Thursday, November 15:

- At 9:00, presentation of the team to the politico-administrative authorities (Agglomeration head, Police, intelligence service)
- Presentation of the cover letter.
- At 14:00, return to the hotel and rest

Friday, November 16th:

- At 9:00, opening of the session by the official veterinarian of the place.
- Training itself: Five (5) modules were developed by the team starting with (i) animal brucellosis, (ii) General epidemiology of brucellosis, (iii) Human brucellosis, (iv) Laboratory diagnosis, (v)) Prevention and control of brucellosis.
- Refreshment
- Questions answers
- Pooling and rest.

Saturday, November 17th:

- At 09:00 departure from Kyavinyonge to Butembo
- At 14:00, arrival in Butembo

IV. USE OF FINANCIAL RESOURCES

The following table shows how the financial resources made available to us were used in accordance with the budget lines proposed by NARO. Receipt are attached to this report for proof.

TABLE II: Allocation of Financial Resources to Expenditures

DESIGNATION	NOMBRE	COAST(USD)	COMMENT
Venue hire	2	80	
Vehicle hire	1	822.9	The driver and the fuel were included in the cost of the vehicle
PA System/visual aids Syst	1	64.06	
Radio anouncements	2	75.5	
Refreshments	2	193.3	
Stationary and printing	1	64.06	
Allowances for field staff	13	688.5	List Annex
Accomodation for field staff	5	300	
TOTAL AMMOUNT		**2288.32**	

ANNEX

ANNEX I Supporting justificative documents

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: Community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Date: November 19, 2018

Payment Schedule (Allowances for field staff)

No.	Name	Designation	Amount	Signature
1	Emmanuel KATEMBO	Premium	113,7 USD	
2	Obed KASERE ICA	Premium	113,7 USD	
3	Olivier KAMBERE KAVULIKIRWA	Premium	113,7 USD	
4	MWANJO WAVAGO	Premium	113,7 USD	
5	Moise VALISUMGIGHE	Premium	113,7 USD	

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: Community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Date: November 14, 2018

Payment Schedule (Allowances for field staff)

No.	Name	Designation	Amount	Signature
1	DELPHINA MUTHONDA	Premium	15 USD	
2	MASIKA MALISAWA	Premium	15 USD	
3	JOHN KAMACIBO	Premium	15 USD	JO. KAM.
4	KAVUGHO BASAMBYA	Premium	15 USD	
5				
6				
7				
8				

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: Community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Date: November 16, 2018

Payment Schedule (Allowances for field staff)

No.	Name	Designation	Amount	Signature
1	KANUGHO KATEMBO	Premium	15 USD	
2	MASIKA NZILAMBA NOELLA	Premium	15 USD	
3	KASEREKA SIKULI	premium	15 USD	
4	KAMBALE ZYANGETSE	Premium	15 USD	
5				
6				
7				
8				

ANNEX II Presence forms for attendance

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: Community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Venue: LUBIRIHA

Date: November, 13th 2018

Attendance list

No.	Name	Village	Gender	Occupation	Signature
1	SAFARI NYAKURA	Lubiriha	M	Eleveur	
2	KAPOSO-JOSEPH	LUBILIHA	M	ELEVEUR	
3	KASEREKA KASOLENE	LUBIRIHA	M	ELEVEUR	
4	MBUSA KAIAVU	LUBIRIHA	M	VET. SOAV	
5	KASEREKA VIGHUMA	KALEMIA	M	ELEVEUR	
6	KAMBALE ISAMBIRO	KASITU	M	VET.	
7	DAVID - BATIHDE	LUBERO	M	VET	
8	MAHAMBU NZIWA	LUBERO	F	VET	
9	KYAKUHAIRE KASEMIRE	BOGA	F	VET	
10	KAVIRA TOWALOOD	NGELEZA	F.	VET	
11	KARULE				
12		KATHINA		K.T.G.ST	
13	KATEMBO KASIKI	KOMBA	M	ELEVEUR	
14	KAMBALE LUSULI	LUBIRIHA	M	VET	
15	KATEMBO ISAAC	LUBIRIHA	M	DJ	
16	FAWILI MURAMYA	LUBIRIHA	M	VET. PROF.	
17	MULESU-KASEREKA	" "	M	ELEVEUR	
18	GUERSHOM BAYISURA	" "	M	ELEVEUR	
19	MUKIRI BAORA	LUBIRIHA	M.	ELEVEUR	
20	MALEGHA VAKUYAMBA	KASINDI	M	AGRONOME	
21	MUHINDO SAFARI	LUBIRIHA	M	Eleveur	
22	DY. N'SIMBA SINDANO	KASINDI	M	REG. VET.	

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: Community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Venue: LUBIRIHA

Date: November 13, 2018

Attendance list

No.	Name	Village	Gender	Occupation	Signature
1	KATEREZ NESWAMBWA	KANGACIKA	M	VETERINAIRE	
2	DIBELPHIN MUTHONDA	LATIN	M	MED. VET	
3	DENISTE KYOVE	VUTHALEVEKWA	M	DENTISTE	
4	KASEREKA Muhomaya Vikish				
5	KAMBALE MUKE	KALEMIA	M	ELECTRICIEN	
6	PALUKU WALUBA	LUBIRIHA	M	ELEV A CULTIVAT	
7	VANUELA MUSAYI	LUBIRIHA	F.	INFIRMIERE	
8	MBAMBU SYAKIMBA	LUBIRIHA	F	INFIRMIERE	
9	KAMBALE KAVUMO	LUBIRIHA	M	INFIRMIER	
10	KAMBALE KALIEMI KASINOT		M	ELEVE	
11	ALULASI MUTASA	LUBIRIHA	F	MEDECIN	
12	TSONGO SAWASAWA	NYAMIRIMA	M	VETERINAIRE	
13	KASEREKA KAMALIRO	LUBIRIHA	M	ELEV/ENSEIGNANT	
14	SANUARY LWZANGU	LUBIRIHA	M	PHARMACIE	
15	MOBIRA SEMIDAH	11	F	PHARMACIENE	
16	NICOLE MASAMI	11	F	VETERINAIRE	
17	MUHINDO KALIZOKA	11	M	ELECTRICIEN	
18	MUHINDO VAGHENI	11	M	VETERINAIRE	
19					
20					
21					
22					

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: Community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Venue: LUBIRIHA

Date: November 13, 2018

Attendance list

No.	Name	Village	Gender	Occupation	Signature
1	KISSA BWIRA	Kasinoli	M	Traffic Paroneta tic	
2	KASEREKA KAVUTHI	KASINDI	M	VET	
3	KAMBALE-KAITENGA	KASINDI	M	Enseignant VET	
4	PALUKU KALISYA	KASINDI	M	ASS VET	
5	DR JOHN KAMALIRO	KASINDI	M	Gérant de l'abattoir	
6	KAMORE NGONGI	KASINDI	M	ETUD. ISTCR	
7	KAKULE WGESERA	KASINDI	M	INFIRMIER	
8	KAVIRA ALDEGONDE	KASINDI	F	INFIRMIERE	
9	KYAKIMWA ESPERANCE	KASINDI	F		
10	DARLOSE KIKUNDI	KASINDI	M		
11	KIZA KITHAMERERA	LUBIRIHA	M	BOUCHER	
12	KAVIRA ALEXANDRINE	KASINDI	F	Infirmière	
13	MUYISA MUTOMBO	KASINDI	M	MASSEUR	
14	MUMBERE ARISTOTE	KASINDI	M	ELEVER	
15	BESISA SAGRAKI	LUBIRIHA	M	Boucher	
16	WALUBA-BETHY	LUBIRIHA	M	ELEVEUR	
17					
18					
19					
20					
21					
22					

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: Community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Venue: ICYAVINYONGE

Date: November 15, 2018

Attendance list

No.	Name	Village	Gender	Occupation	Signature
1	NZIGAMBA NOELLA	KYAVINGONGE	F	ÉLEVE	
2	KASEREKA ZEPHANI	KYAVINYONGE	M	ÉLEVE	
3	MUHINDO [illegible]	KYAVINYONGA	M	VETERINAIRE	
4	MUHINDO KISEMBE	KYAVINYONGE	M	ÉLEVE	
5	KAVUGHO [illegible]	KYAVINYONGE	F	ÉLEVE	
6	KAVUGHO KASOMBOLWA	KYAVINYONGE	F	ÉLEVE	
7	KAMBALE KAMATHE	11	M	11	
8	KAVUGHO KAZEMBO	KYAVINYONGE	F	ÉLEVE	
9	AUGISTIN KIKALA	KYAVINYONG	M	ÉLEVE	
10	SADIKI	KYAVINYONGE	M	CHAUFFEUR	
11	KAKULE [illegible]	KYAVINYONGE	M	ETUDIANT VET	
12	KAMBALE [illegible]	[illegible]	M	DCH	
13	KAKULE WILEMA	M	M	PCHERE	
14	KATEMBO [illegible]	KYAVINYONGE	M	DIRECTEUR	
15	BALUKU DAKODA	KYAVINYONGE	M	PRESIDENT ELEVEUR	
16	KASEREKA SIKULI	KYAVINYONGA	M	VETERINAIRE	
17	KAKULE-MATEMBELA	KYAVINYONGE	M	PECHEUR	
18	KAMBALE MBAHINGANA	KYAVINYONGE	M	PECHEUR	
19	KAMBALE TSENGE	KYAVINYONGE	M	PECHEUR	
20	BALUKU - PEPE	KYAVINYO	M	COMA	
21	KAMBALE LYANGETSE	KYAVINYONGE	M	PROF	
22	TABUNIKAEUSENE	KYAVINYONGE	M	ELEVE	

NATIONAL LIVESTOCK RESOURCES RESEARCH INSTITUTE (NaLIRRI)
P.O. Box 5704, WAKISO

LIVESTOCK HEALTH PROGRAM

Re: Community awareness creation on Brucellosis epidemiology, prevention and control at the human, livestock wildlife interface

Venue: KYAVINYONGE

Date: November 15, 2018

Attendance list

No.	Name	Village	Gender	Occupation	Signature
1	KAKULE MATHINA	KYAVINYONG	M	ACHETEUR DU Poiss	
2	CHADRACK MBAKU	KYAVINYONG	M	PECHEUR	
3	KAKULE MASINDA	KYAVINYONG	M	-	
4	MUHINDO - TSONGO	KYAVINYO	M	ETUDIANT	
5	MUHINDO - KYAVU	KYAVINYONG	M	PROFESSEUR	
6	KAKULE - KASUSU	KYAVINYONG	M	Trafiquant	
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ANNEX III : Supporting pictures

Presentation of meeting

Meeting at Lubiriha

Meeting at Lubiriha

Meeting at Lubiriha (Questions-Responses)

Meeting at Lubiriha (Questions-Responses)

Meeting at Lubiriha (Questions-Responses)

Meeting at Lubiriha

Meeting at Lubiriha

DRC team with veterinarians officers

Meeting at Kyavinyonge (Presentations)

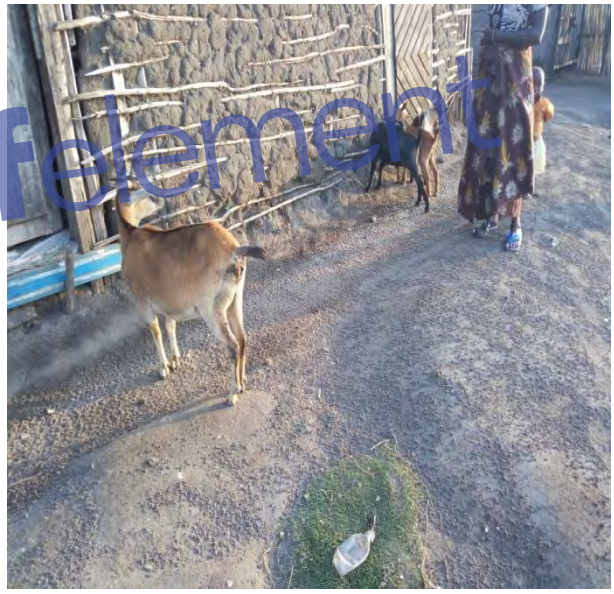
Meeting at Kyavinyonge

Meeting at Kyavinyonge (Questions-Response)

Goats in divagation (Lake Edward)

Meeting at Kyavinyonge

Goats in divagation (Lake Edward)



A sick goat in divagation at Kyavinyonge

REPORT FOR BRUCELLOSIS SAMPLE COLLECTION AND ANALYSIS

Introduction

Brucellosis is an important disease among livestock, humans and wildlife in the great lakes region with highest incidences registered in farms with large herds compared to small ones (Kabagambe et al., 2001). A study conducted by the Autonomous University of Barcelona (UAB), the Government of Andorra, Daktari, a local Non-Governmental Organization and Makerere University observed a widespread circulation of brucellosis in sheep and goats within the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan. This has serious implications for human health since animals and animal products are the source of infection for man. Some funding was received from the Perez Guererro Trust Fund (PGTF) to implement a project titled *"Epidemiology of brucellosis on the livestock, wildlife and human interface: Improving the diagnostic capacities of brucellosis disease, enhance the control strategies with special emphasis on farmers' awareness in the Bwindi-Mgahinga, Queen Elizabeth, and Murchison falls conservation areas in Uganda, Parc National des Virunga (République Démocratique du Congo) and Nimule wildlife conservation area, South Sudan"*.

One of the planned activities involve collection of samples for serology and laboratory culture and confirmation from areas around protected areas in Uganda, Democratic republic of Congo (DRC) and Republic of South Sudan.

Objectives

The objective was to further establish the sero prevalence especially in goats and sheep and to identify the infecting brucella up to biovar level.

Output (s)

The expected output was Brucella isolates obtained and characterized up to biovar level and seroprevalence of brucellosis further established in the target areas.

Methodology

Collection and processing of blood samples for detection of brucella anti-S/LPS antibodies

Blood samples were collected randomly from goat and cattle herds from communities around QENP and MFNP. Five milliliters (5ml) of venous blood was aseptically taken from the Jugular vein of cattle and goats into clearly labeled clot activation tubes (CAT). Blood was kept at room temperature until clotting was complete. Serum was extracted using 3ml Pasteur pipettes into labeled cryo-vials. These were transported on ice and refrigerated at -20°C until testing was done. Accompanying data was also captured on a pre-designed form.

Bovine serum was screened for brucella antibodies using the routine Rose - Bengal Plate Test (30µl of serum plus 30µl of antigen) as recommended by Greiner *et al.* (2009) while caprine serum was screened using the modified Rose-Bengal plate test i.e. 75µl of serum plus 25µlof antigen as described by Ferreira *et al.* (2003). The RBT antigen was obtained from Universidad de Navarra (Spain). Procedurally, serum was retrieved from the freezer and allowed to thaw at room temperature. Only the required amount of antigen was aliquoted from the bulk solution into a test tube and allowed to stand at room temperature. The tests were performed at room temperature using a white tile onto which the serum and antigen mixture were placed and rocked gently for 3 to 4 minutes. Agglutination **denoted** a positive test and non-agglutination denoted a negative test.

Collection and processing of milk samples for Brucella isolation

Milk samples were collected from female lactating animals that gave a positive result on rose test. Milk samples were collected aseptically after washing and drying the whole udder and disinfecting the teats. We ensured that all quarters were milked so that the final sample contained milk from the entire udder. Ten (10 ml) of milk was taken from each teat, changing or disinfecting the gloves from one animal to the next to avoid cross-contamination of the samples. The first streams of milk were discarded and the sample was milked directly into well labeled sterile falcon tubes. The samples were kept on ice and dispatched to the lab for processing. At the lab the milk samples were centrifuged at 1000rpm and the supernatant discarded. The cream and deposit were spread on solid selective medium.

For each sample about 0.5ml of the homogenate was smeared onto clearly labeled Modified Thayer-Martin (developed for isolation of *Br.melitensis* and *Br.ovis*) medium and Farrell's media (developed for isolation of *Br.abortus*) plates (Stear, 2005). Two plates of each medium were used per sample to increase sensitivity. The plates were incubated under adequate conditions (5% CO_2, high humidity) for 4 to seven days. For samples targeting *Brucella melitensis* the requirement for CO_2 under incubation was excluded. Colony morphology, staining with crystal violet, oxidase and Urease tests were used to identify the *Brucella* species obtained.

Results

District	No. of blood samples collected by species			No. RBT +ve			No. of milk samples collected			No. Culture +ve		
	Bov	**Cap**	**Ov**	**Bov**	**Cap**	**Ov**	**Bov**	**Cap**	**Ov**	**Bov**	**Cap**	**Ov**
Kasese	163	251	19	8	0	3	4	0	2	0	0	0
Nakasongola	728	436	0	45	6	0	7	3	0	0	0	0
Kiryandongo	97	167	11	12	2	0	3	0	0	0	0	0
Nakaseke	123	89	5	6	1	0	2	1	0	0	0	0
Total	**1111**	**943**	**35**	**71**	**9**	**3**	**16**	**4**	**2**	**0**	**0**	**0**
Prev estimate	6.4	0.009	0.08	-	-	-	0	0	0	-	-	-

Discussion and recommendations

We discovered a very prevalence of anti-S/LPS brucella antibodies in all the 3 species. Highest prevalence was found in cattle (6.4%) followed by sheep (0.08%) and lastly goats (0.009%). The possible explanation is that goats are browsers and therefore less prone to infection from contaminated pastures compared to cattle and sheep. No positive cultures were obtained. This is perhaps due to the fact that there is less localization of brucellae in the udder. Since we were taking samples from live animals the milk sample was the only available sample we came across. We did not succeed getting abortion material.

We therefore recommend taking tissue samples at slaughterhouse level in subsequent studies to increase the chances of isolating the organisms from other tissues. This will also improve on the catchment area since animals brought to the abattoir originate from diverse areas.

Some pictures for field sample collection

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ANIMAL AND HUMAN BRUCELLOSIS IN UGANDA: A LATENT THREAT TO LIVESTOCK PRODUCTION AND PUBLIC HEALTH

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Summary

Brucellosis has been listed as one of the zoonotic diseases of major economic and public health concerns in Uganda. Economic losses arise from low herd fertility, long calving interval time, loss of replacement stock, and reduced milk production. The public health effects result from the sickness, a chronic disease that, although seldom deadly, results in incapacitating sequelae and requires a long and costly antibiotic treatment..

Here, we examine the current situation of animal and human brucellosis in Uganda, provide useful insights into its diagnosis and we propose strategies for its control.

Introduction

Organisms belonging to the genus *Brucella* are the cause of brucellosis in animals or Undulant fever in humans. Three main species namely *B. abortus*, *B. melitensis* and *B. suis* that infect cattle, goats and pigs, respectively, are highly pathogenic to humans. *B. canis* is also mildly pathogenic. Brucellosis in animals is a problem and, studies have shown herd prevalence of cattle brucellosis to range between 2 to 100% in Uganda. Prevalence in pigs, goats, sheep, dogs, wildlife and humans is not well known. Yet all these species can play an important role in the epidemiological cycle of brucellosis. A control strategy involving the use of suitable vaccines requires that the role of the above species in the Brucellosis epidemiological cycle be understood.

Recommendations

1. Mandatory collection and archiving of positive and negative animal and human reference sera for validation of serological tests.
2. Enhancement of core competencies of all health practioners in brucellosis diagnosis and control.
3. Discourage the use of the Febrile Antigen and ensure availability of quality Rose Bengal antigen on the Ugandan Market for both human and animal brucellosis serology.
4. Isolation, typing and archiving of *Brucella* species involved in the epidemiological cycle in Uganda.
5. Studies should be undertaken to understand the role of domestic animals and wildlife in the epidemiological cycle of brucellosis in Uganda.
6. Creation of public awareness about the disease and the options for prevention and control
7. Mass conjunctival vaccination of all domestic ruminants in defined epidemiological units using the S19 (female cattle) and Rev1 (sheep and goats, both male and female) vaccines (they should be registered in Uganda) every two years to reduce prevalence to a minimum.

CURRENT SITUATION OF ANIMAL AND HUMAN BRUCELLOSIS IN UGANDA

Brucellosis is one of the re-emerging but largely neglected zoonoses. In the last 5 years there has been an increase in public outcry arising from losses in livestock production and human health effects attributed to the disease in Uganda. As a result, Brucellosis has been included on the list of priority zoonotic diseases in Uganda. The disease has attained an endemic status with herd prevalence in cattle ranging between 2 to 100% according to published studies. However, the disease situation in other farm animals (pigs, goats, dogs, sheep) and wildlife is largely unknown.

Similarly, in humans the disease situation is not well understood. However, although their findings cannot be generalized to the entire country, studies conducted by Nabukenya *et al.*, 2012 and that of Nyehangane *et al.*, 2017 (unpublished) put the prevalence of human brucellosis between 10 and 15%. In humans, the disease is commonly found in high-risk populations like animal keepers, handlers, abattoir workers, veterinarians etc. The symptoms are non-specific, and the clinical picture closely resembles that of malaria.

Diagnosing the disease in both animals and humans is one of the critical areas that need improvement. Whereas numerous tests are available for this purpose, not all of them are suitable for resource poor settings like Uganda. Moreover, there is proof that simple tests like the Rose Bengal (RBT) (provided the test is adequately standardized and validated) that can be performed under resource poor settings have been used in brucellosis eradication programs in most countries of the world. The use of tests like iELISA or cELISA as confirmatory test for RBT positive samples is of no value as the diagnostic performance of RBT is equal or even better than that of the ELISA. Provided that the cut-offs are well proven for each situation, the ELISA only has value for comparison purposes or when automatization is required. Validation of serological tests both in livestock and humans is needed to have reliable diagnostic results. This is rarely done in Uganda. Validation requires that positive and negative reference sera (gold-standard) be collected and archived at the national livestock and human reference laboratories, respectively.

In humans, a combination of the afore mentioned occupational factors, a clinical picture compatible with brucellosis and a positive serology using suitable antigen should raise strong suspicion by the clinician. Optimally, a positive blood culture should be performed as it is the only uncontestable proof of brucellosis infection. The culture should be typed and archived at the human reference laboratory. The current use of the Febrile Antigen for serological diagnosis of human brucellosis is contestable as it yields many false positive serological reactions and should be discouraged as such. .

Similarly, a positive serology in animals should be accompanied by a demonstration of brucellae in culture at least at herd level for the confirmation of the disease. Identifying, typing and archiving the brucellae species at the animal reference laboratory should be mandatory to clarify the epidemiological cycle of the disease. In Uganda, the circulating strains are largely unknown except for *B. abortus* biovar 1, 3 and 7 reported by Mugizi *et al.*, 2017.

The control of brucellosis in humans largely depends on its control in animals. The vaccines S19 and Rev1 have been used successfully to control brucellosis in cattle and small ruminants in some European countries like France and Spain. However, the RB51 vaccine currently used in Uganda is less effective than S19, has not proven successful in any brucellosis eradication program anywhere and does not solve the problem of the interference of vaccination in serological diagnosis. Moreover, a control strategy involving the use of vaccines requires that the complete epidemiological picture of the disease be clearly understood. Studies should be undertaken to understand the role of domestic animals and wildlife in the epidemiological cycle of brucellosis in Uganda.

Nevertheless, mass conjunctival vaccination of cattle (S19), goats and sheep (Rev1) every two years has been proven beneficial in the control brucellosis in resource poor settings like Uganda. However, this strategy requires (i) an active farmer involvement, (ii) identifying both the target population and the minimum epidemiological unit of intervention, (iii) assessing mean herd/flock prevalence, (iv) precise knowledge of the animal species involved, (v) access to vaccines of good quality at reasonable cost and (vi) a vaccination procedure with adequate organization of the veterinary services. Finally, continuous public awareness campaigns and training of health practitioners are also of paramount importance.