FRIEDRICH-LOEFFLER-INSTITUT
FLI
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

Training Report

Diagnostic procedures for PPR

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CGIAR

BUILD Boosting Uganda's Investment in Livestock Development

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

Project: Boosting Uganda's investments in Livestock Development (BUILD) - PPR component

Training on cell culture maintenance, PPRV isolation on cell culture, molecular detection of PPRV genome and serological detection of PPRV specific antibodies

14 – 25 November 2022, Greifswald, Germany

Training participants (left to right): Joseph Nkamwesiga, Milovan Milovanović (Trainer), Gladys Kiggundu Nakaniako, Eugene Arinaitwe (Picture ©Fischer/FLI)

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1. Project Background

In Uganda, like most countries in sub-Saharan Africa, around 70% of all households keep at least one kind of livestock (including poultry). Livestock production is primarily a family business, but only a fraction of the food produced is used for home consumption. Most of it is sold at local markets which are mainly informal.

Pathogenic diseases are one of the constraints that limit livestock production, often resulting in death of animals and loss of income and livelihoods for livestock keepers. Zoonotic diseases such as brucellosis and Rift Valley fever threaten the health of producers, processors and consumers. Improved animal health, therefore, directly contributes towards improved livelihoods and human health through better diets and fewer zoonotic diseases.

Knowledge and awareness about animal diseases, their risks and intervention options are limited in Uganda. For many of the animal health problems, there are effective solutions at hand, for example, vaccines. However, lack of infrastructure and institutions in low- and middle-income countries does not allow last-mile delivery of solutions or implementation of disease control. Lack of awareness on the benefits of vaccines results in unwillingness to purchase them. This problem is compounded by lack of trained personnel to deliver vaccines. There is little investment in capacity building and professional development of processors, which leads to gaps in research and surveillance of transboundary and zoonotic diseases.

The research for development project Boosting Uganda's Investment in Livestock Development (BUILD) is led by the International Livestock Research Institute (ILRI), funded by the German Federal Ministry for Economic Cooperation and Development (BMZ) and implemented in partnership with the Ugandan Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), the German Federal Research Institute for Animal Health (FLI), the German Federal Institute for Risk Assessment (BfR), the Kenya Medical Research Institute (KEMRI), Freie University Berlin (FUB), the Ugandan National Livestock Research Resources Institute (NaLIRRI), and Vétérinaires sans Frontières Germany (VSFG). It aims to support existing structures by helping to scale solutions through a collaborative effort in research, extension and partnerships.

The program has four main components:
(1) Support ongoing campaigns to eradicate Peste des petits ruminants
(2) Control zoonotic diseases
(3) Control antimicrobial resistance
(4) Improve veterinary public health at the point of slaughter.

The BUILD-PPR team has developed an outbreak investigation protocol and investigated more than 20 outbreaks in Uganda since 2020. Thirty-six field veterinarians and animal health technicians in 12 districts have been trained in participatory disease surveillance and are now integrated into the project's outbreak investigation protocol. In collaboration with laboratory staff of Uganda's National Animal Disease Diagnostics and Epidemiology Centre (NADDEC), the BUILD team is analysing the samples, including genetically

characterizing the isolates. MAAIF and BUILD have jointly refurbished the lab and animal houses for vaccine experiment and outbreak investigation work. Moreover, the national PPR steering committee has been mobilized with BUILD funds and plans for a multi-stakeholder platform and regional engagement have been completed.

Ongoing activities:

1. Cost-benefit analysis of interventions; for instance, packaging the PPR vaccine with other small ruminant vaccines and identifying incentives and constrains for intervention uptake.
2. Epidemiological surveys on the spatial distribution of the virus in Uganda and study transmission pathways and rates for the prevalent virus strains in different production systems for risk-based vaccine targeting and delivery.
3. Establishing animal health service delivery partners for future production and delivery of the vaccine in countries where the project operates.
4. Promoting regional networking and capacity development through existing networks and infrastructures (e.g. Participatory Epidemiology Network for Animal and Public Health (PENAPH), Global Peste des Petits Ruminants Research Alliance (GPRA), Biosciences eastern and central Africa-ILRI hub (BecA-ILRI Hub)).
5. Conducting inventory of all national and regional PPR initiatives in Africa, including emergency and routine surveillances programs.

2. Training summary

2.1 Objective

The Institute of Diagnostic Virology and Institute of Animal Health/One Health of Friedrich Loeffler Institute (FLI) designed and organized a 10-day training on the maintenance of a cell culture, Peste des Petits Ruminant virus (PPRV) isolation, the molecular detection of the N protein gene of PPRV and serological detection of PPRV specific antibodies for colleagues from NADDEC and ILRI from Uganda. The main objective of the training was capacity building at NADDEC for cell culture maintenance and virus isolation together with improvement of molecular and serological diagnostic methods.

2.2 Expected outcomes

1. To educate and prepare the participants for independent work with cell culture at NADDEC
2. To educate and prepare the participants for independent work of PPRV isolation on cell culture using swab and tissue sample material at NADDEC
3. To improve swab sample processing and molecular detection of PPRV genome by real-time reverse transcriptase polymerase chain reaction (RT-qPCR) method

4. To improve serological analysis for detection of PPRV specific antibodies by using of commercially available PPR competition Enzyme Linked Immunosorbent Assay (ELISA) kit (ID, Montpellier, France).

2.3 Pre-training assessment

In total three participants participated on this training: Two technicians from NADDEC, i.e. Gladys Kiggundu Nakanjako and Eugene Arinaitwe, and a PhD student from ILRI Uganda, Joseph Nkamwesiga. The pre-training assessment identified that participants do not have any practical knowledge on the maintenance of cell culture and virus isolation, but that they are performing molecular (RT-PCR) and serological methods (ELISA) as a daily work routine.

2.4 Structure and methods

The training was structured as follows: introduction of the participants to working conditions in a high containment facility, theoretical and practical introduction of the participants to work with cell culture, virus isolation, molecular detection of the PPRV genome and serological detection of PPRV specific antibodies.

2.5 Topics

The following training topics were covered through PowerPoint presentations, demonstrations and practical performance. Detailed training notes are available in the Agenda below.

- Maintenance of Vero Dog cells expressing signaling leucocyte molecule (VDS): cell culture thawing, passaging/trypsinization, and freezing/cryopreservation
- PPRV isolation on VDS cells: sample selection, VDS cells preparation, PPRV adsorption to VDS cells, washing, incubation and result analysis
- Swab samples from Uganda: preparation, PPRV nucleic acid extraction, RT-qPCR and result analysis
- Serum samples from Uganda: master plate preparation, PPR Competition ELISA and result analysis

3. Agenda

Date	Module	Coverage	Trainer
14 November 2022	Theoretical	Introduction to working conditions in high containment facility on FLI	Bernd Hoffmann
		Providing the protocol for cell culture maintenance (thawing, passaging/trypsinization, and freezing/cryo-preservation) for each of the participants	Milovan Milovanović
15 November 2022	Theoretical	Short recap about cell culture thawing	Milovan Milovanović
	Practical	Cell culture thawing ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants	Milovan Milovanović
16 November 2022	Theoretical	Short recap about cell culture passaging/trypsinization and freezing/cryopreservation	Milovan Milovanović
	Practical	Passaging/trypsinization and freezing/cryopreservation of the VDS cells ➢ Preparation of necessary material and equipment for the work ➢ Demonstration of the method ➢ Supervising and guidance of each of the participants	Milovan Milovanović
17 November 2022	Theoretical	Providing the protocol for virus isolation on VDS cells for each of the participants Presentation following virus isolation protocol	Milovan Milovanović
	Practical	Sample preparation (first 91 swab samples from Uganda) ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants Nucleic acid extraction ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants RT-qPCR targeting N protein gene of PPRV ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants According to RT-qPCR results preparation of VDS cells in 6 well plates, rest of the cell's suspension was prepared in T25 cell culture flask	Milovan Milovanović

		➢ Preparation of necessary material and equipment for the work ➢ Demonstration of the method ➢ Supervising and guidance of each of the participants	
18 November 2022	Theoretical	Short recap about virus isolation	Milovan Milovanović
	Practical	Virus isolation on 24 hours old VDS cells in 6 well plate ➢ Preparation of necessary material and equipment for the work ➢ Demonstration of the method ➢ Supervising and guidance of each of the participants	Milovan Milovanović
21 November 2022	Practical	Sample preparation (second 91 swab samples from Uganda) ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants Nucleic acid extraction ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants RT-qPCR targeting N protein gene of PPRV ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants According to RT-qPCR results preparation of VDS cells in 6 well plates. Rest of the cell's suspension was prepared in T25 cell culture flask ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants Inspection of the 6 well plates after 3 days of incubation for Cytopathogenic effect (CPE) of the virus or potential bacterial or fungal contamination using stereomicroscope ➢ In parallel demonstration and supervising of each of the participants	Milovan Milovanović
22 November 2022	Practical	Virus isolation on 24 hours old VDS in 6 well plate ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants	Milovan Milovanović
23 November 2022	Practical	Preparation of serum samples from Uganda in master plates for serological testing ➢ Preparation of necessary material and equipment for the work	Milovan Milovanović

		➢ Supervising and guidance of each of the participants Passaging/trypsinization of the VDS cells ➢ Preparation of necessary material and equipment for the work ➢ Gladys independently perform the procedure	
24 November 2022	Practical	ID Screen PPR Competition ELISA ➢ Preparation of necessary material and equipment for the work ➢ In parallel demonstration of the method and supervising of each of the participants Passaging/trypsinization of the VDS cells ➢ Preparation of necessary material and equipment for the work ➢ Eugene and Joseph independently performed the procedure Virus isolation second blind passage of 6 samples from first attempt ➢ Preparation of necessary material and equipment for the work ➢ Supervising and guidance of each of the participants	Milovan Milovanović
25 November 2022	Practical	Passaging/trypsinization of the VDS cells ➢ Preparation of necessary material and equipment for the work ➢ All three participants independently performed the method	Milovan Milovanović

4. Training material

Material used for practical part of the training

Maintenance of VDS cells	PPRV isolation on VDS cells	Molecular detection of N protein gene of PPRV	Detection of PPRV specific antibodies
1. Laminar flow hood; 2. Water bath; 3. Stereomicroscope; 4. CO2 incubator; 5. T25 cell culture flask; 6. Serological pipets 5, 10 and 25 mL; 7. Pipetboy; 8. Micropipettes (20, 100 and 1000 µL); 9. Micropipettes filter tips (20, 100 and 1000 µL); 10. Cryovials; 11. Mr. Frosty; 12. -80 °C freezer; 13. Liquid nitrogen container with liquid nitrogen; 14. 15 ml centrifugal tube; 15. Centrifuge for 15 mL tubes; 16. VDS cells; 17. Culture medium (DMEM); 18. Foetal calf serum (FCS); 19. Zeozin; 20. Trypsin-EDTA; 21. Dimethyl sulfoxide (DMSO); 22. Isopropanol; 23. Microzid or 70% Ethanol; 24. Paper towel.	1. Laminar flow hood; 2. Stereomicroscope; 3. CO2 incubator; 4. 1,5- or 2-mL centrifugal tubes; 5. Micropipettes (20, 100 and 1000 µL); 6. Micropipettes filter tips (20, 100 and 1000 µL); 7. Centrifuge for 1,5- or 2- mL tubes; 8. Vortex shaker; 9. Tilt shaker; 10. 6 well cell culture plates; 11. Serological pipets 5, 10 and 25 mL; 12. Pipetboy; 13. Glassware; 14. VDS cells; 15. Foetal calf serum (FCS); 16. Culture medium (DMEM); 17. Zeozin; 18. Trypsin-EDTA; 19. Penicil/Streptomycin antibiotic combination; 20. Gentamycin; 21. Amphotericin B; 22. Vennovet 1 Super; 23. Microzid or 70% Ethanol; 24. Paper towel.	1. Laminar flow hood; 2. Vortex shaker; 3. 96 well plate shaker; 4. 96 well plate centrifuge; 5. Multipette; 6. Combi tips (5, 10 and 50 mL); 7. King Fisher 96 flex; 8. King Fisher duo; 9. Bio Rad 96 Real-Time cycler; 10. PCR box for master mix preparation; 11. PCR box for template; 12. Heat sealer; 13. BIO-RAD, 96-well PCR Plate white; 14. PE Heat sealing foil; 15. Sealing foil self-adhesive Alu; 16. NucloMag Vet kit for extraction of NA; 17. Primer probe mix for detection of N protein gene of PPRV (1); 18. Primer probe mix for detection of internal control (ß-actin) (2); 19. Sagrotan; 20. Microzid or 70% Ethanol; 21. Paper towel.	1. Laminar flow hood; 2. Vortex shaker; 3. Centrifuge for 1,5- or 2- mL tubes; 4. 96 well plates (500 µL); 5. Micropipettes (1000 µL); 6. Micropipettes filter tips (1000 µL); 7. Multichannel micropipette (200 µL); 8. Micropipette tips without filter (200 µL) 9. Multipette; 10. Combitips (5, 10 and 50 mL); 11. Sealing foil self-adhesive Alu; 12. ELISA reader (450 nm filter); 13. PPR Competition ELISA kit (ID, Montpellier, France); 14. Microzid or 70% Ethanol; 15. Paper towel.

[1] Polci, A.; Cosseddu, G.; Ancora, M.; Pinoni, C.; El Harrak, M.; Sebhatu, T.; Ghebremeskel, E.; Sghaier, S.; Lelli, R.; Monaco, F.J.T.; et al. Development and preliminary evaluation of a new real-time RT-PCR assay for detection of peste des petits ruminant's virus genome. **2015**, *62*, 332-338. https://doi.org/10.1111/tbed.12117

[2] Wernike, K., Hoffmann, B., Kalthoff, D., König, P., & Beer, M. (2011). Development and validation of a triplex real-time PCR assay for the rapid detection and differentiation of wild-type and glycoprotein E-deleted vaccine strains of Bovine herpesvirus type 1. Journal of Virological Methods, 174, 77–84. https://doi.org/10.1016/j.jviromet.2011.03.028

5. List of participants

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1	Eugene Arinaitwe	arieugene@yahoo.com	Uganda
2	Joseph Nkamwesiga	J.Nkamwesiga@cgiar.org	Uganda
3	Gladys Kiggundu Nakanjako	gladyskiggundu@gmail.com	Uganda