PILOTING NOVEL DESIGNS FOR EVALUATION OF THE SAFETY, EFFICACY AND EFFECTIVENESS OF THE SUBOLESIN BASED ANTI-TICK VACCINE

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Presentation outline

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Background

- **Ticks and tickborne diseases** cause extensive losses - mortalities, low productivity, high costs of treatment and control resulting into a loss of approximately UGX 2.6 - 3.8 trillion annually in Uganda

- Ticks cause blood loss (anaemia), tick worry, lower the quality skin and hide, tick paralysis and most importantly transmit economically important hemo-parasites

- NARO has developed the Subolesin based Anti-tick vaccines because of universal conservation of the genes

- In Uganda genetically modified organisms must be confined during research - UNCST

Important Tick and Tickborne diseases (TTBDs) affecting cattle

	Tick species		Disease pathogens transmitted
1	Brown ear tick *R. appendiculatus - RA*		East Coast fever (ECF)
2	Blue tick - *R. (Boophilus) decoloratus - RD*		Babesiosis (Red Urine) and Anaplasmosis (Gall sickness)
3	Bont tick *A. variegatum - AV*		Heartwater & Dermatophilosis
4	**NaLIRRI maintains a sterile tick colony of these three tick species**		

NARO

The history of tick control

1. Acaricide applications (spray, dipping, pour-on)

2. Fencing, stall fed and creation of bare ground to block tick movement

3. Breeding for resistance and controlled to TTBDs (Bos indicus cattle such as Boran, Short horn Zebu, Ndama)

4. Vaccines for ticks and tickborne diseases

5. Bush-burning to destroy free living stages for ticks

6. Handpicking of ticks

The NARO Anti-tick Vaccine Journey

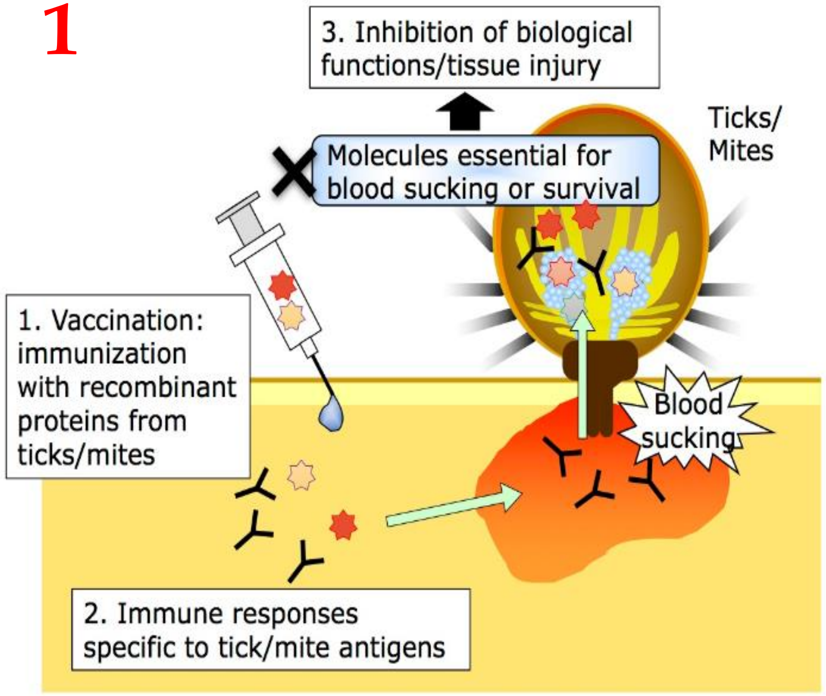
NARO

Period	Milestones
2014 -17	▪ Proof of concept ▪ Immunization of cattle and infestation with tick larvae
2018 - 20	▪ Product formulation batch and On-station clinical trials (injectable and oral) ▪ Bio-informatics, genomics, immunogenetics, anti-body screening
2020 - 22	▪ UNCST and NBC guided the research into a regulatory realignment of the project (audits) and trainings ▪ Product formulation for confined field trials
2021 - 23	▪ Permits for Conducting of Confined Field Trials with partners ▪ Construction and equipping of the vaccine research and production facility

Anti -Tick Vaccines – Mode of action

- Anti-tick vaccines were first tested in 1939 as crude tick extracts
- Once administered, trigger antibody production in the body, disable tick feeding, growth, reproduction and immunity thus lower infestations and tick borne pathogens (TBP). Vaccines are safe and more sustainable means of tick control

1



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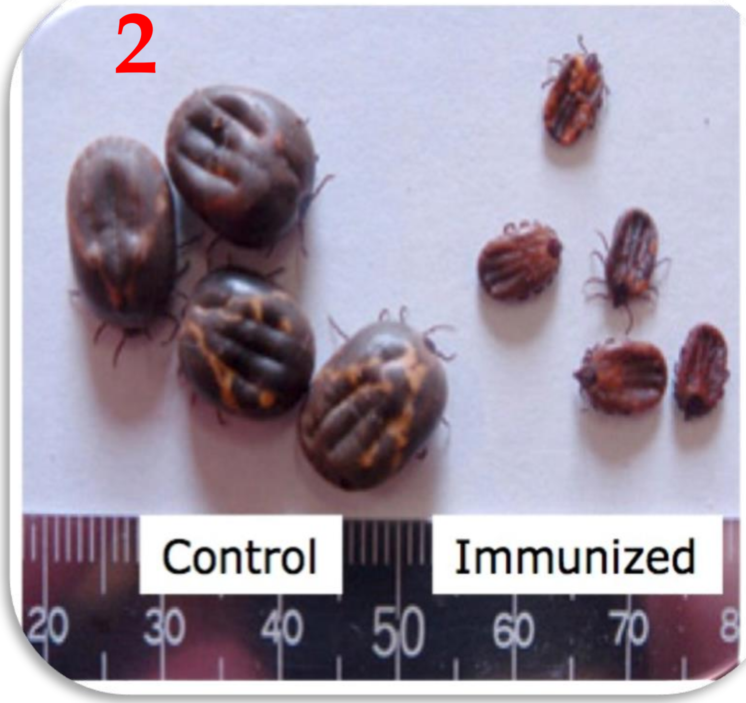


3. Anti-tick vaccines lack the knock down effect compared to acaricides chemicals

Stage of the Anti-tick vaccine development cycle – where are we as NARO

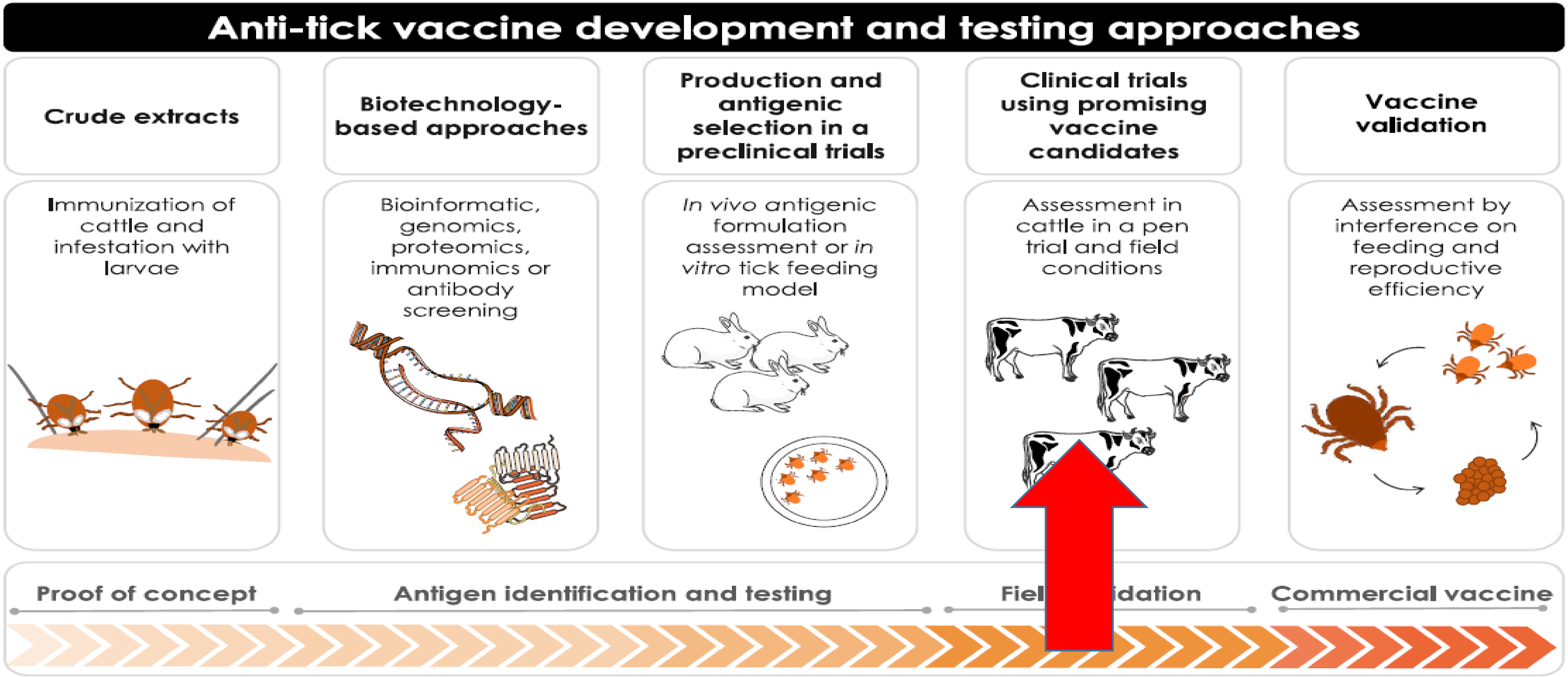
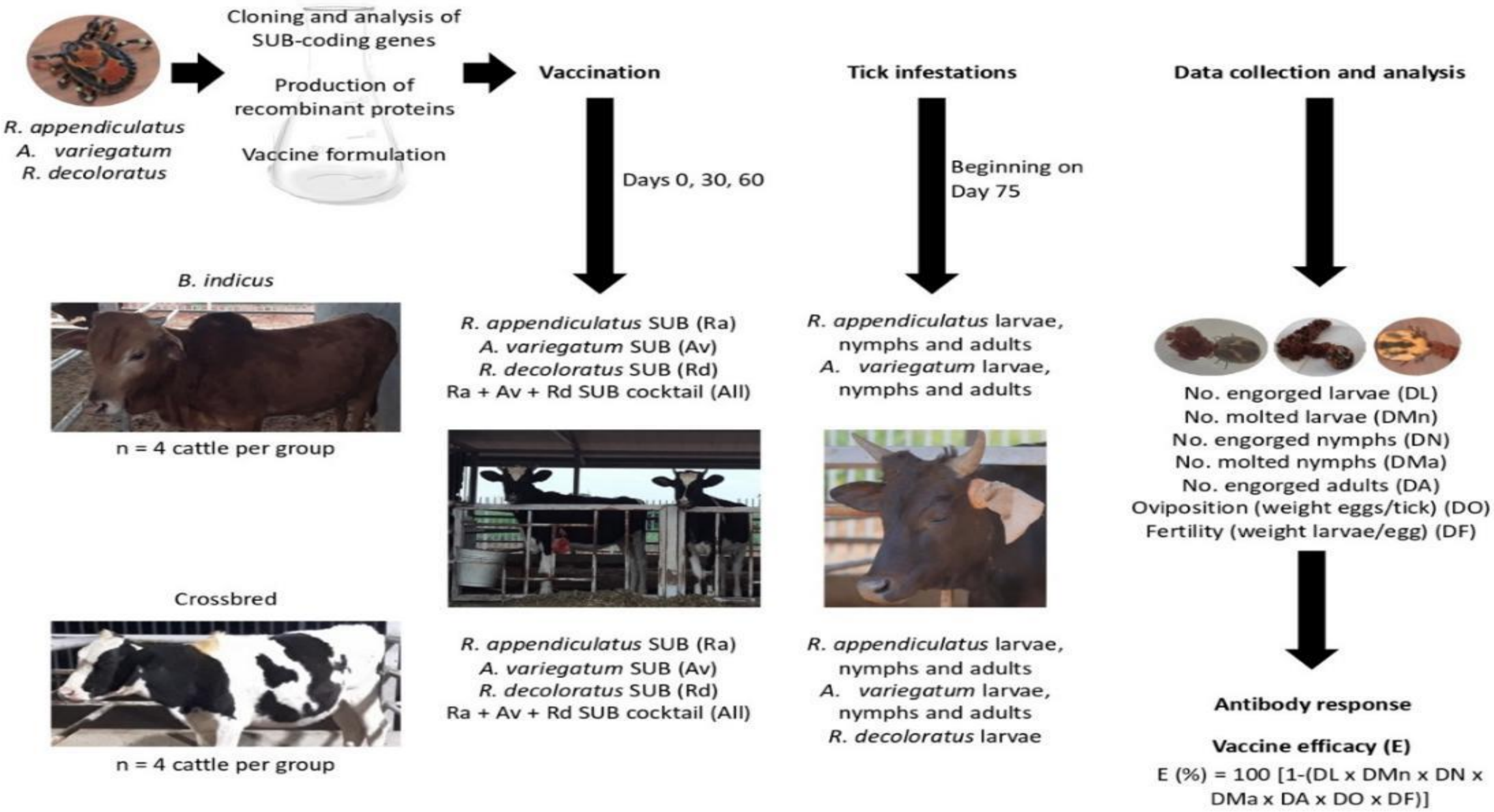


Fig. 1. A schematic representation toward the development of vaccines for the control of tick infestations.

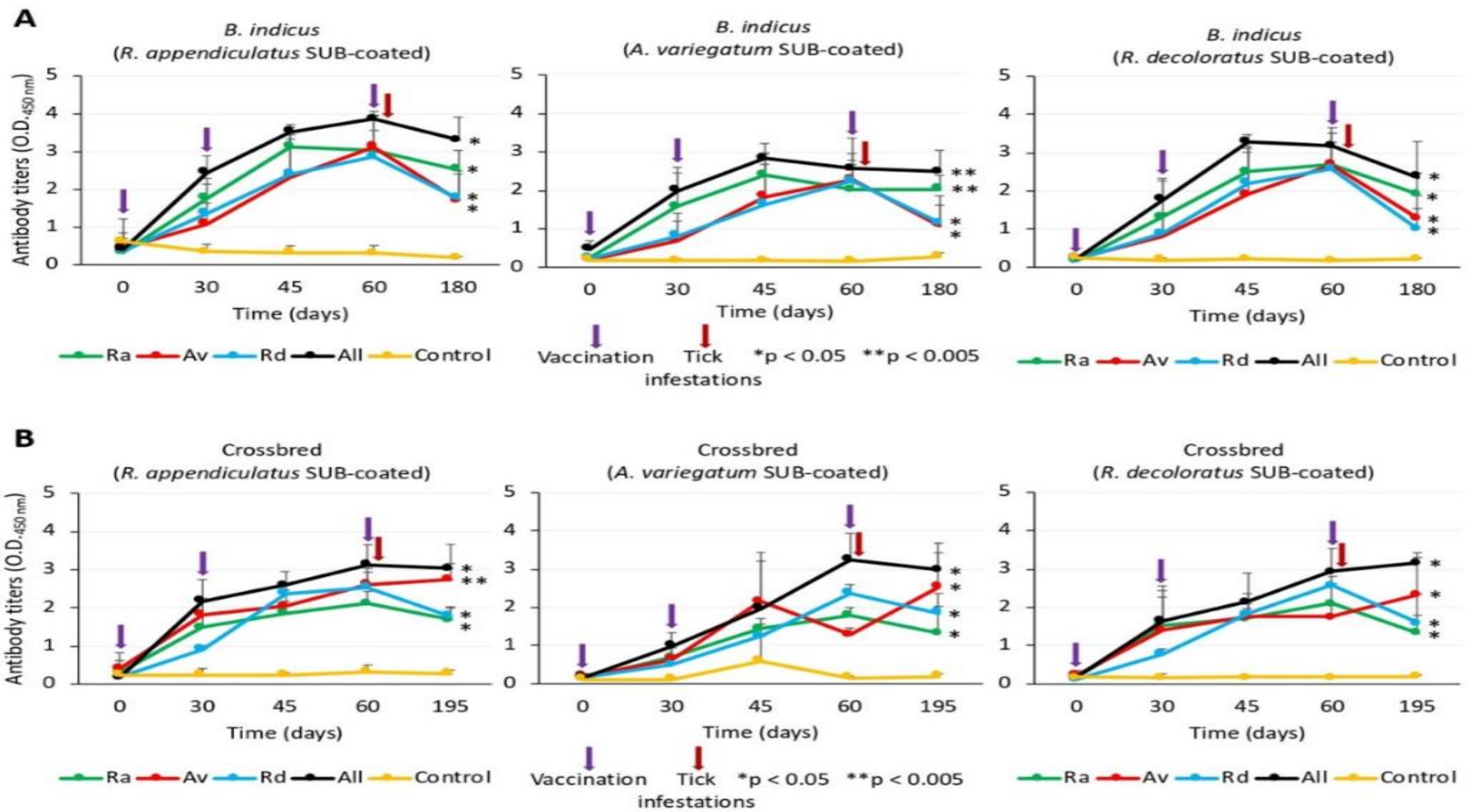
Regulation of our research

- NARO Institutional science committee (SC)
- NARO Institutional Animal Care Use Committee (IACUC)
- NARO Institutional Biosafety committee (IBC)
- Uganda National Council for science and Technology (UNCST)
- National Drug Authority (NDA)

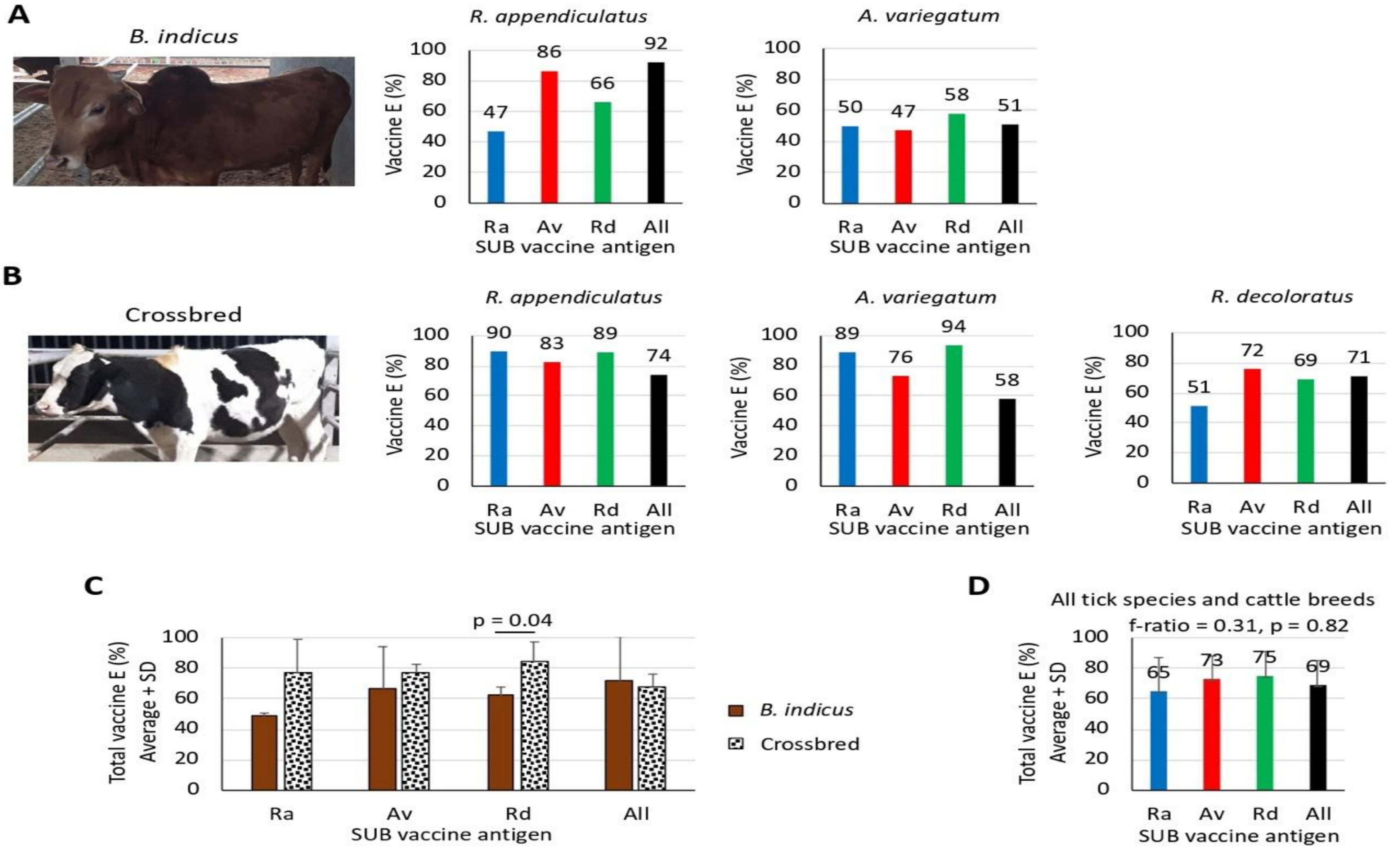
On-station clinical trials - Experimental design



Antibody titre measurements determine the booster dose timing



On-station clinical trial Results: Analysis of vaccine efficacy (E)



Contribution to scientific knowledge

vaccines

MDPI

Article

Vaccination with Recombinant Subolesin Antigens Provides Cross-Tick Species Protection in *Bos indicus* and Crossbred Cattle in Uganda

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Abstract: Cattle tick infestations and transmitted pathogens affect animal health, production and welfare with an impact on cattle industry in tropical and subtropical countries. Anti-tick vaccines

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Oral vaccine formulation combining tick Subolesin with heat inactivated mycobacteria provides control of cross-species cattle tick infestations

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CONFINED FIELD CLINICAL TRIALS

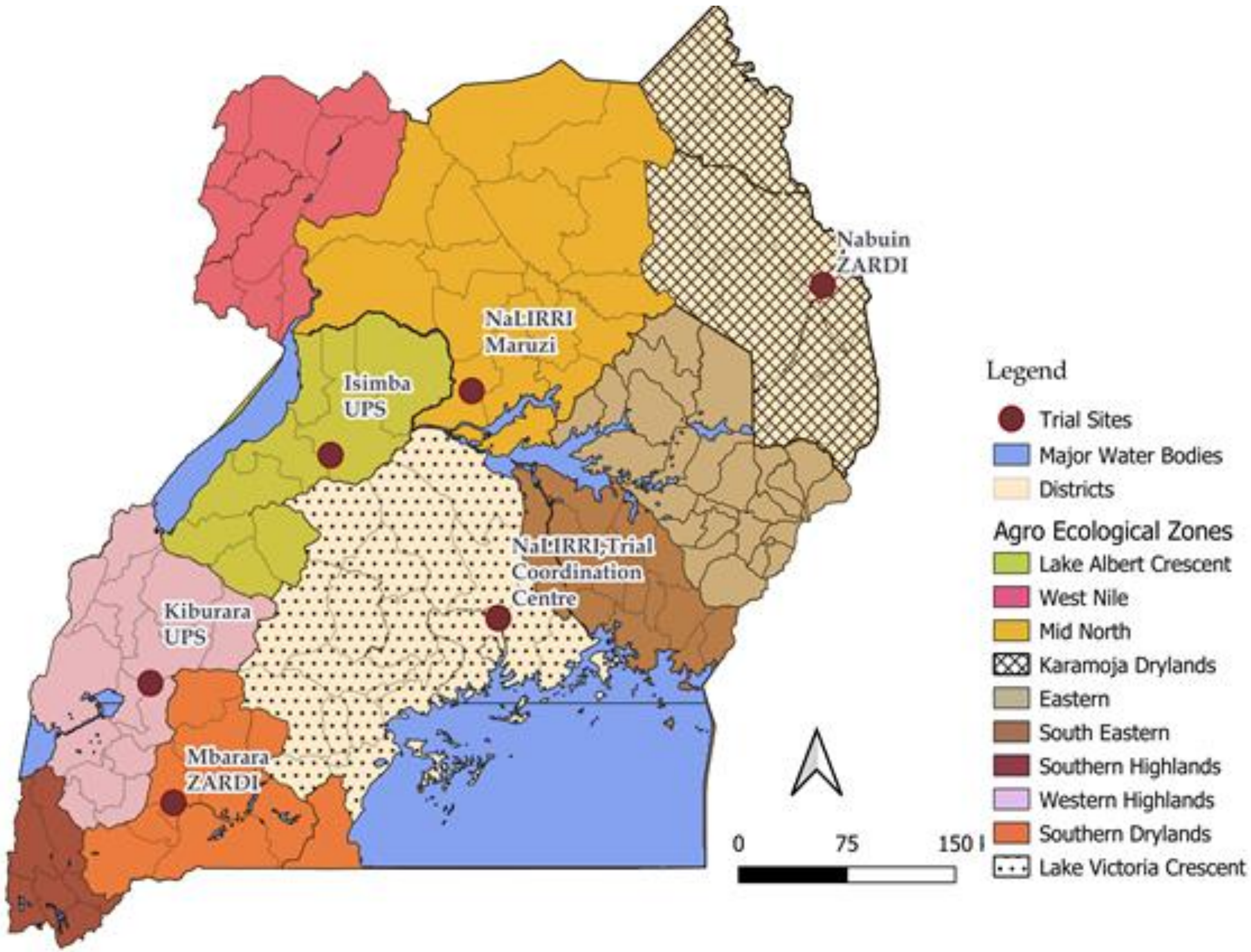
Overall and Specific objectives of the field trial

General Objective: Evaluation of the Safety, Efficacy and Effectiveness of Subolesin based Anti-tick Vaccines for control of ticks

Specific objectives

- To determine the Safety of the injectable Subolesin based Anti-tick vaccine for control of tick infestations under confined field conditions.
- To determine the Efficacy of the injectable Subolesin based Anti-tick vaccine for control of tick infestations under confined field conditions.
- To determine the Effectiveness of the injectable Subolesin based Anti-tick vaccine for control of tick infestations under confined field conditions.

Location of experimental cattle sites and reasons for their selection



1. AEZs – at least two as per NDA ecto-paracitocide regulation

2. Cattle breeds (Friesian, EASZ, Boran, Ankole) adopted to their sites

3. Tick species abundance/ burden/challenges/ distribution

4. Availability of cattle for 365 days

5. Institutional farms for reduced cost – platforms for dissemination

6. International vaccine trial designs

How do we achieve our Specific objectives

- **Safety of the IVP:** Clinical parameters after administration of the IVP (body temperature, heart rate, liver function tests, vaccine residues in organs, milk and engorged ticks), Insect, soil, plants sampling

- **Efficacy of the IVP : Tick** counts of engorged, development (moulting from larvae to nymphs and adults) oviposition and hatchability, antibody titre dynamics

- **Annual Booster doses**: Tick counts and Anti-body titres beyond 180 and 195 up to 365 days

- **Effectiveness of the IVP :** The data on morbidity and mortality associated tickborne diseases

Experimental design – Key considerations

1. Random selection of experimental study subjects
2. Continuous confinement of experimental subjects
3. Double blinded trial (ethical reasons)
4. Administration of the IVP (TP - 33 and CP - 33) = 66 sample size
5. Mix the TP and CP treated cattle to achieve herd/population health
6. Collection of samples (randomly)
7. Standardise tick control to avoid biases based on tick control using acaricides (Duodip)

8. Standard of care (diagnostic and treatment) shall be maintained uniform unblinding the trial

Experimental cattle site components

- NARO - Maruzi, Nabuin, Mbarara ZARDI, UPF - Isimba and Kiburara
- Cattle (health, all age groups, both sexes)
- Personnel (AV, SRV, herdsmen, security)
- Paddocks for confinement (observations, enhance care - TLC)
- Office – Records, Laboratory – sample maintenance and Store - Drugs
- Feed and water (clean, adequate and adlibitum)
- Restraint facility (crush, night boma, working shed)

Experimental subjects - rights – welfare and ethics – key points of Good Clinical Practice (GCP)

- Freedom from suffering – Confinement using paddocks
- Freedom from disease and injury – Attending Veterinarian
- Freedom from hunger and thirst – Provision of water and feed ad libitum
- Freedom from loneliness (socialise) – Male and Females
- Freedom from fear and anxiety – Herdsmen and security

Vaccine administration

- Use a specialised semi-automatic syringes with gauge 16 needles
- Intramuscular injection
- Neck region is priority, secondly gluteal muscle
- Administer Prime dose at day 0
- Administer booster dose at day 28 – 30

- Monitor tick attachment and feeding (all species and stage of development) on 10 cattle

- Booster dose at day 180 ??

Key activities at the cattle trial sites

1. Trial coordination centre

a. Account for the trial product

b. Receive field sample (blood, serum, tissues, milk and ticks)

c. Analyse and compile results

d. Archive samples for future reference

e. Release results to DG

2. Experimental Cattle sites

a. Keep good records

b. Maintain experimental cattle (health, confinement, feed and water)

c. Collect sample and forward them for testing

Staff at the trial sites

- Trial site coordinator -1
- Attending veterinarian - 1
- Assistant veterinarian - 2
- Herdsmen - 2
- Security - 2
- Laboratory assistant – 1
- Total = 8 to 9

Termination of experimental cattle subject and disposal

- Trial cattle subjects can terminated due to grave sickness, severe injury as determined by the PI
- Trial cattle may die due to disease which will require a thorough post-mortem examination
- Carcases will be buried at 4 feet deep or burn to avoid excavation
- Laboratory diagnosis through submission of blood and tissue
- At termination of the trial (365 days), experimental cattle will be examined for any peculiar for 6 months

Packages of the IVP

Recombinant Anti-tick Vaccine development and production line - Spain

- Technical expertise _ Prof. Jose de la Fuente and other scientists
- Training institution at international level

Study team composition

Name	Discipline	Role
Dr. Fredrick Kabi	Epidemiologist and tick Immunologist	Project management
Dr. Patrick Emundong - PLT	Acarologist	Trial manager
Dr. Moses Matovu - SRO	Food Biotechnologist	Safety of beef from vaccinated cattle
Dr. Moses Dhikusooka - SRO	Epidemiologist and Biotechnologist	Vaccine administration, Field data collection/ entry
Paul Kasaija - PLT	Molecular microbiologist	Vaccine design, Data collection/ entry and analysis
Dr. Susan Kerfua -SLT	Molecular Biotechnologist	Laboratory manager – Samples analysis
Dr. William Nanyeenya SRO	Socio-economist	Economics of using vaccines for TTBDs control
Dr. Nelson Muwereza - RO	Microbiologist	Vaccine administration, Field data collection/ entry
Dr. Godfrey Nsereko - RO	Vet Epidemiologist	Vaccine administration, Field data collection/ entry
Mr. Juma Ndhokero	Bio-statistics	Sample size determination, efficacy, effectiveness
Dr. Swidiq Mugerwa	Livestock Nutritionist	Overall administration
Jose de la Fuente (Prof)	Molecular Biotechnologist and immunologist	Anti-tick vaccine design and production

Acknowledgement

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- NDA
- IREC

Thank you for listening