



THE UNITED REPUBLIC OF TANZANIA
MINISTRY OF AGRICULTURE LIVESTOCK AND FISHERIES
VETERINARY COUNCIL OF TANZANIA

**TRAINING MANUAL ON
IMMUNIZATION AGAINST EAST
COAST FEVER FOR
VETERINARIANS AND
VETERINARY
PARAPROFESSIONALS IN
TANZANIA**

2016

**VETERINARY COMPLEX,
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ACKNOWLEDGEMENT

This manual is an outcome of consultation and collaboration with key stakeholders and colleagues interested in East Coast Fever (ECF) control. The manual is intended to guide animal health service providers and trainers in training ECF vaccinators and improving the delivery of ECF vaccine in the country. This is in recognition that, high cattle morbidity and mortality rates due to diseases such as ECF are an impediment to the livelihoods of rural small farmers in Tanzania who struggle to attain food, nutritional and economic security..

We wish to thank all people and institutions who have worked with us. We acknowledge and appreciate the crucial role played by International Livestock Research Institute (ILRI) and Global Alliance for Livestock and Veterinary Medicine (GALVmed) for facilitating the preparation of this training manual that will be used in training of ECF vaccinators in Tanzania with the aim of increasing cattle productivity through protection against ECF.

Furthermore, we wish to acknowledge the support given by the Directorate of Veterinary Services, of the United Republic of Tanzania, Stakeholders in ECF control and Private Veterinary Practitioners towards the preparation of this manual.

This work would have not been accomplished without the contribution of various stakeholders. The Veterinary Council of Tanzania (VCT) wishes to express its sincere gratitude and appreciation to all who devoted their time and expertise in the preparation of this manual, particularly Drs. Henry Mbwille and George Kiama the representatives from Ronheam Tanzania Ltd, Dr Henry Magwisha from Tanzania Veterinary Laboratory Agency, Dr Martin Ruheta from the Directorate of Veterinary Services , Dr Bedan Masuruli from the Veterinary Council of Tanzania and Dr Hamisi Nikuli from the Directorate of Aquaculture, Ministry of Agriculture Livestock and Fisheries.

It is our expectations that users of this manual will find it useful.

Dr. B. Masuruli.

Registrar Veterinary Council of Tanzania

LIST OF ACRONYMS AND ABBREVIATIONS

DNA	Deoxyribose Nucleic Acid
ECF	East Coast Fever
ELISA	Enzyme Linked Immuno Sorbent Assay
Hrs	Hours
IFAT	Immuno Fluorescent Antibody Test
I.M	Intra Muscular
ILRI	International Livestock Research institute
ITM	Infection and treatment Method
KBBs	Koch's Blue Bodies
LA	Long Acting
LN ₂	Liquid Nitrogen
LSD	Lumpy Skin Disease
MCL	Muguga Cocktail Lilongwe
OTC	Oxytetracycline
PCR	Polymerase Chain Reaction
SOPs	Standard Operating Procedures
TBDs	Tick Borne Diseases
VCT	Veterinary Council of Tanzania

INTRODUCTION

This training manual on the immunization of cattle against East Coast Fever (ECF) using the “Infection and Treatment Method” (ECF – ITM) will be used to train vaccinators on the best practice for ECF vaccination in order to achieve the best results.

This training Manual gives a background on all aspects of the ECF Disease, the field application of ECF immunization including the pre and post immunization issues that need to be understood and taken care of.

The current ECF –ITM is effective only against true East Coast Fever and not against other related conditions such as Corridor Disease where infection is picked by ticks from Buffaloes!

The training will be provided for a period of three days to vaccinators who have a minimum qualification of a certificate in Animal Health or Diploma in Animal Health or Degree in Veterinary Medicine after which they will be issued with certificates of attendance. Theoretical and practical aspects of ECF immunization will be covered during the training

THE PURPOSE OF THE MANUAL

This manual is intended to give veterinarians and veterinary paraprofessional’s knowledge and skills on all aspects of ECF disease and control through the application of the immunization process in the field. Under the direction of the Director of Veterinary Services, the role of field staff will be to immunize cattle throughout their respective areas of operation.

At the end of the training, ECF delivery agents are expected to demonstrate competency in knowledge, skills, values, attitudes, aptitudes and behaviors in the practice of East Coast Fever cost effective immunization applicable to a broad range of cattle breeds in the context of ever-changing societal expectations and life-long learning for improving the health and productivity of livestock.

WHO WILL USE THE MANUAL

This manual is intended for use by:

- i. Veterinarians who are registered with the Veterinary Council of Tanzania; or holders of
- ii. Diploma in Animal Health attained from recognized Livestock Training Institute and enrolled with the Veterinary Council of Tanzania; or holders of
- iii. Certificate in Animal Health and Production attained from recognized Livestock Training Institute and enlisted with the Veterinary Council of Tanzania; or
- iv. Any other stakeholders who are engaged in the Livestock industry who wish to promote ECF immunization in Tanzania.

1.0. EAST COAST FEVER (ECF) – THE DISEASE

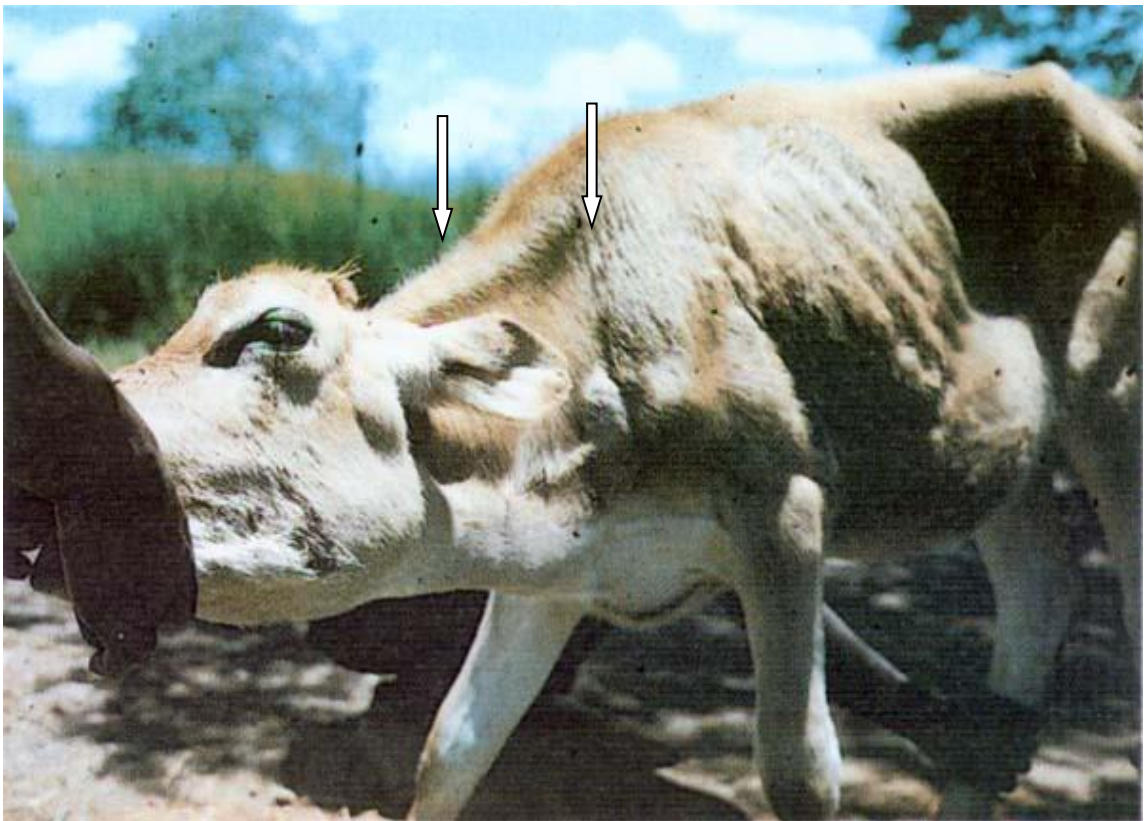
1.1 Aetiology

Is an acute protozoan disease of cattle characterized by high fever, swelling of lymph nodes, emaciation, and high mortality caused by *Theileria parva*. The disease is a very serious problem in East and Central Africa.

1.2 Clinic Signs

Key signs of East Coast Fever are:

- i) Swelling of the draining lymph node (parotid, prescapular and precrucial lymph nodes) as shown in photograph 1;



Photograph 1: Cattle with swollen Pre scapular and Parotid lymphnodes
(See arrows)

- ii) Difficult and fast breathing due to pulmonary oedema that is frequently seen as a frothy nasal discharge;
- iii) Loss of appetite (Anorexia);
- iv) Increased body temperature above 39.5°C;
- v) Marked pin point (petechial) and paint brush (ecchymotic haemorrhage) on mucous membranes of the conjunctiva and the buccal cavity; and
- vi) Drastic milk reduction (milking cow).

1.3 Other clinical signs

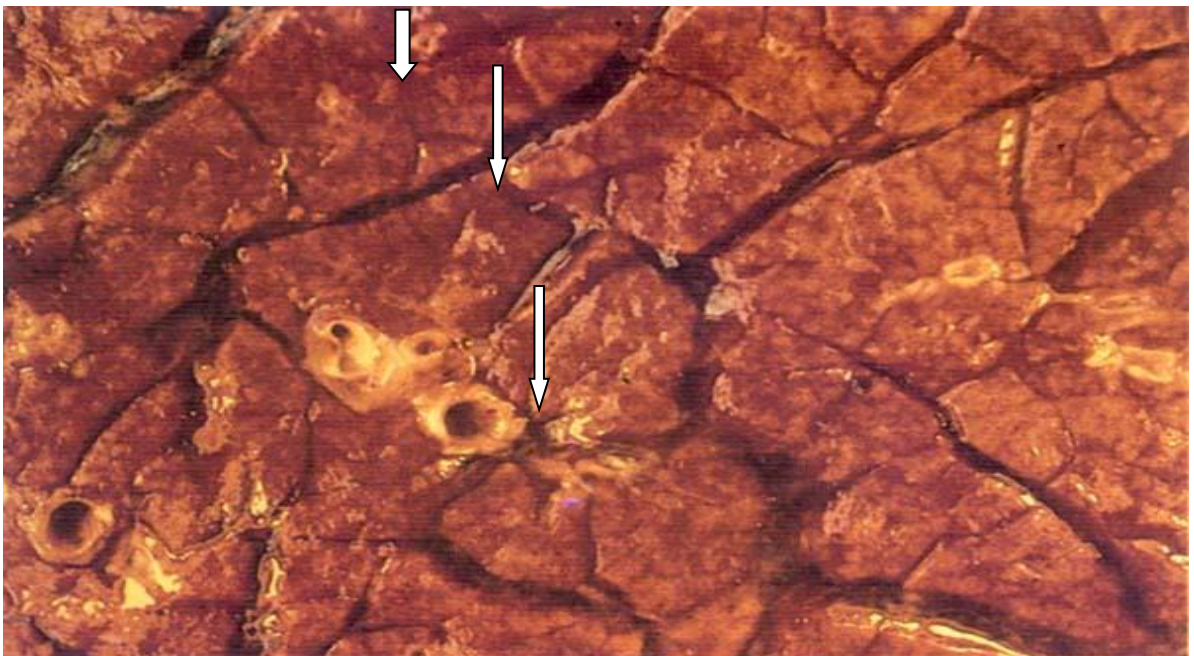
Other signs may include lacrimation, corneal opacity, nasal discharge, coughing; terminal dyspnoea, and diarrhea; pale mucous membranes (anaemia) or jaundice and mortality in exotic cattle can reach 100%;

1.4 Pathological signs

Pathological signs include frothy and oedema in the lungs as shown in photographs 2 and 3.



Photograph 2: Frothy in the cut surface of the lung (see arrow)



Photography 3: Oedema of the lung – see widening of inter-lobular space (Arrows)

Other signs are:

- “Cigarette burn” ulcers appear in the abomasum;



Photograph 4: Cigarette burn in the abomasum

- “zebra striping” in the colon; and
- Kidney infarcts (white spots).

1.5 Differential diagnosis

- **Heartwater** because of pulmonary edema and hydrothorax. Examination of brain smears and lymph node or spleen impression smears can differentiate between the two diseases.
- **Trypanosomosis** because of edema, lymphadenopathy, and anemia. Blood and lymph node smear examination will normally differentiate between the two diseases.
- **Babesiosis** and **Anaplasmosis** because of anemia. These diseases can easily be differentiated from ECF on examination of blood smears.
- **Malignant Catarrhal Fever** because of lymphadenopathy and corneal opacity. Examination of blood and lymph node smears will clearly differentiate between the two diseases.

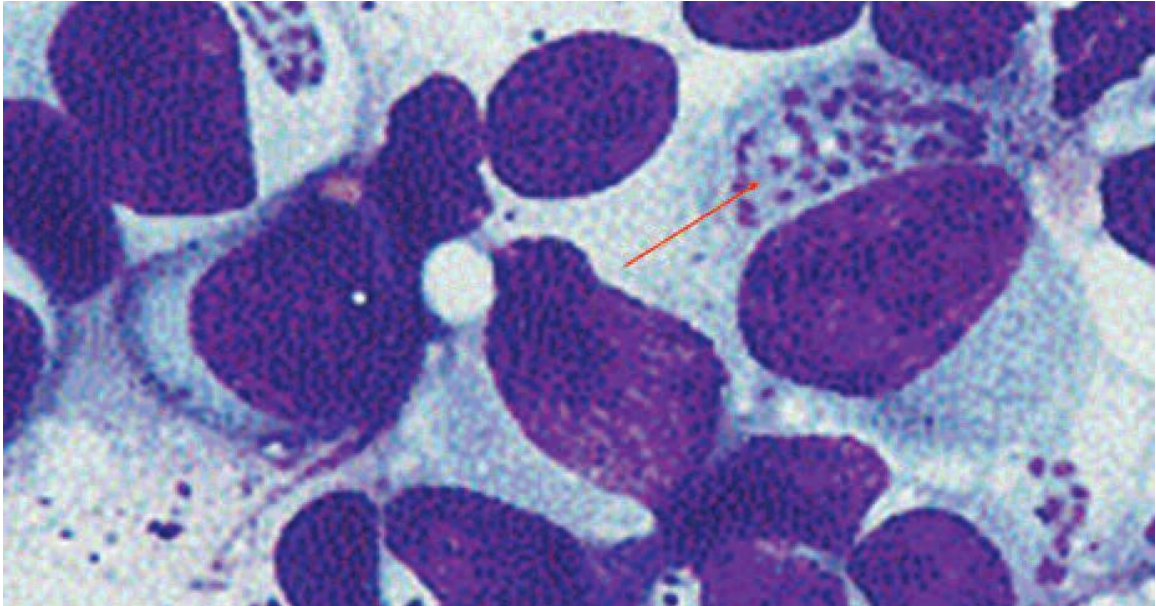
1.6 Diagnosis

Tentative diagnosis is based on:

- i. Clinical signs; and

ii. Postmortem signs.

Definitive diagnosis is through Laboratory tests by taking lymph, blood and/or impression smears. Detection of Koch's Blue Bodies is confirmatory test for ECF as shown in photograph 4.



Photograph 4: Koch's Blue Bodies

NB: Identification of schizonts in lymphoid cells is considered to be pathognomonic of East Coast Fever. (Koch's Blue Bodies).

1.7 Other laboratory diagnosis

- Polymerase chain reaction (PCR) tests and DNA probes are sometimes used to detect and identify *Theileria* species;
- Antibodies to *T. parva* and *T. annulata* can be detected with an Enzyme-linked immuno sorbent assay (ELISA), or an Indirect Fluorescent Antibody Test (IFAT); and
- Serological tests may not be sensitive enough to detect all infected cattle, and cross-reactions can occur with other species of *Theileria*.

1.8 Economic Importance in Tanzania

Deaths;

Reduced milk production; and

High treatment costs.

2.0 ECF VACCINE – “MUGUGA COCKTAIL”

2.1 Name

Muguga Cocktail (MCL01) ECF Stabilate was the first batch of ECF vaccine that was manufactured in Lilongwe. A second batch to have MCL02 is expected in the near future.

2.2 Qualitative and quantitative composition

Each dose of Muguga cocktail (MCL01) stabilate contains:

Active ingredients	Quantity per unit dose
<i>T. parva</i> Kiambu5 sporozoites	100-135 x 10 ⁵
<i>T. parva</i> Muguga sporozoites	100 -135 x 10 ⁵
<i>T. parva</i> Serengeti sporozoites	100 -135 x 10 ⁵

Excipients

Eagles Minimum Essential

Bovine serum albumin 3.5%

Glycerol 7.5%

Penicillin 150 units/ml

Streptomycin 150µg/ml

2.3 Description

Muguga cocktail (MCL01) stabilate is a golden brown tick derived suspension of non-attenuated protozoa *Theileria parva*. It is a neat concentration of sporozoites suspension that translates to 10 infected crushed tick material in 1ml of diluent. Packaging is 0.5ml transparent red straws.

2.4 Indication for use

Muguga cocktail (MCL01) stabilate contains live *Theileria parva* sporozoites. It is used for the active immunization of cattle to protect them against East Coast Fever (ECF) using the infection and treatment method of immunization.

The onset of immunity has been demonstrated to provide solid immunity against challenge by 35 days after administration. The duration of immunity after a single dose is expected to be lifelong.

2.5 Contraindications, warnings and precautions

Close monitoring of exotic breeds of immunized cattle may be required as they are more susceptible to *Theileria parva*; Do not vaccinate cattle which have had previous exposure to any strain of *Theileria parva* infection; Do not use in animals undergoing physiological stress due to starvation, injury, or concurrent infections; Do not use in animals in last trimester of pregnancy; and only vaccinate healthy animals. Do not vaccine cattle that have been treated with levamisole derivatives in last two weeks.

2.6 Adverse effects following immunization

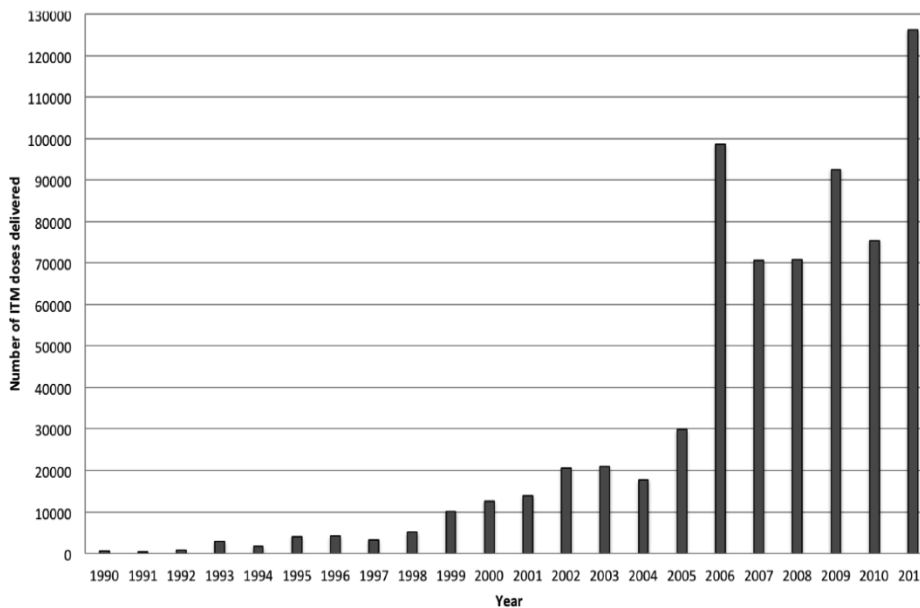
Adverse effects may occur in very small proportion of animals, in case of allergic reaction, inject adrenaline as per manufacture's recommendations.

2.7 Current Adoption

ECF immunization has been gaining acceptance in Tanzania since it was first tested in early 1990s as shown hereunder in Graph 1:

Graph 1:

The number of ITM doses delivered in Tanzania 1990-2011



It should be noted that, Immunization with a combination of *T. parva* strains appear to offer immunity that withstands challenge with heterologous strains from widely geographically separated locations.

3.0 ORGANISATION OF VACCINATIONS

Immunization against ECF will be carried out by registered veterinarians, enrolled and enlisted paraprofessionals. Conduction of vaccinations requires preparation of vaccination teams, equipment, and consumables to be used as follows:

3.1 Vaccination team and its composition

It is strongly advisable to form a team when vaccinating animals against ECF. The Vaccination Team has to constitute the following in number and order as shown in **Appendix 1**:

- i. *Recording Assistant* – record the weight and temperature of the animal;

- ii. *Animal Restrainers/Handlers* – these must be experienced so as to avoid injuries to both animals and vaccinators;
- iii. *Temperature taker* – will take the temperature of the animal to make sure only afebrile or healthy animals are vaccinated. Temperature should not be above 39.5°C;
- iv. *Operator of the Weigh- band* – who will take the measurement to determine the weight of the animal and hence the volume of OTC required as in the photograph 5 below;



Photograph 5: Measuring weight of cattle using a weigh band

- v. *Vaccinator* – must be clean and should not involve himself in restraining to maintain asepsis;
- vi. OTC Injection; and
- vii. Application of Ear Tag Assistant as shown in the photograph 6 below.



Photograph 6: Eartagging Ankole Cattle

3.2 How to reconstitute ECF vaccine

The vaccine is composed of infective sporozoite stabilate “Muguga cocktail” prepared from adult ticks and preserved in a straw placed in a canister stored in liquid nitrogen (LN₂) at -196°C as shown in the photograph 7 below.



Photograph 7: Straw placed in a canister stored in LN₂ at -196°C.

The reconstitution of the vaccine has to follow the following steps:

- I. **STEP 1:** Boil a cupful of water. Allow the water to cool to lukewarm temperature (40°C);
- II. **STEP 2:** Introduce the deep-frozen (-20°C) diluent into the lukewarm water until the diluents is completely thawed;
- III. **STEP 3:** Take the straw containing the stabilate out of the LN₂ tank. Roll the straw gently between the palms to ambient/atmospheric temperature as shown in the photograph 8 below;



Photograph 8: Rolling the straw gently between the palms

- IV. **STEP 4:** Introduce the straw into a cryo-vial. Using a new and clean razor blade, cut the straw just below the cotton plug. Make sure while you are cutting the straw, the tip of the straw is inside the cryo-vial;
- V. **STEP 5:** On cutting the straw, the stabilate will gravitate out of the straw into the cryo-vial. Taking a 1ml syringe, gently draw all the stabilate from the cryo-vial;
- VI. **STEP 6:** Gently inject the stabilate into the thawed diluents while upside down to avoid air bubbles;
- VII. **STEP 7:** Mix the stabilate with the diluent by gently mixing/ turning the diluent bottle;
- VIII. **STEP8:** Keep the re-constituted vaccine in a cool box with ice-packs at 4-8°C ready for field vaccination as shown in photograph 9 below; and



Photograph 9: A cool box with ice - packs

- IX. **STEP 9:** Allow 5-10 minutes for the vaccine to equilibrate before using the vaccine.

NOTE: Re-constituted vaccine should be used within 6 hours after re-constitution. Discard unused vaccine.

3.3 Mode of East Coast Fever vaccine delivery and vaccination location on the animal

Before embarking into a field trip for ECF vaccination make sure you have the necessary equipment as listed in **Appendix 2 and ECF immunization sheet as in Appendix 3.**

Animals presented for vaccination should be in good health condition, more than one month of age and should have not been treated with a dewormer containing Levamisole within TWO weeks prior to ECF vaccination and not to be given the same product in the next TWO weeks after vaccination .Get the herd

history from the animal owner on whether the herd was infected with Foot and Mouth Disease or Lumpy Skin Disease within the past one month. Animals in a farm with an outbreak of FMD or LSD should not be immunized.

Try to maintain cleanliness and asepsis as much as possible. The persons taking temperature and weight measurements should not handle OTC injections at the same time. A recording assistant could also be injecting OTC; or a weight taker could also do ear-tagging. Animals that have been immunized should not be put under stress.

Delivery of the vaccine has to follow the following steps:

- I. The dose for each animal (calves and adults) is 1ml only of the re-constituted vaccine;
- II. After making sure that, the animal has no fever ($\leq 39.5^{\circ}\text{C}$), 1ml of the vaccine shall be injected subcutaneously behind the ear near the parotid lymph node;
- III. After vaccine has been applied, OTC 30% will be injected into the animal;
- IV. The Dose for OTC 30% shall be 1ml/10kg Body weight – as determined by the weigh-band. One site not exceeding 20ml; Due to reaction to pain, injecting the animal with antibiotic shall be the last task performed.
- V. Ear tag will be applied and recorded as in the ECF immunization sheet;
- VI. Record the following for each animal: Breed, Sex, Age, Weight, Ear Tag No., and volume of OTC 30% used. Good records are essential for efficient Monitoring and Evaluation. A sample of a record sheet see appendix.

NOTE: For calves give antihelmintics preferably benzimidazoles, but not levamisole.

PRECAUTIONS

Allergic Reaction:

Due to tick material suspended in the vaccine, an allergic reaction could occur in sensitized animals within 10-30 minutes after injection of the vaccine. Occurrence of allergic reaction is minimal (0.06% i.e 6 animals out of 10,000).

In case of reactors, practitioners are advised to administer supportive treatment using Adrenalin.

ECF Reactors:

Animals are showing a transient fever two (2) weeks after ECF vaccination. A small percentage (0.04%) of vaccinated animals can develop clinical ECF two weeks after immunization. These animals need to be given anti-theilerial drugs at the time of clinical reaction.

3.4 Monitoring and evaluation

Monitoring and evaluation is a continuous process during and after implementing the immunization. It has to be implemented jointly involving animal owner, team member and authority to evaluate immunization process as per code of ethics.

The animal owner is advised to monitor immunized animals closely for one month. If any of the immunized animals show signs of ECF the farmer should contact the person who did the immunization immediately.

Tick control regime after immunization should not be changed for two months following immunization. After that period animal owners are advised to reduce the number acaricide applications.

4.0 CODE OF ETHICS DURING VACCINATION

Immunization of cattle against ECF is part of veterinary practice and every practitioner is required to abide to the code of ethics for veterinarians, veterinary specialist and veterinary paraprofessionals and paraprofessional assistants.

The principles of code of profession conduct include;

- Every practitioner shall have the obligation to conduct ECF immunization with highest degree of honest, skills and integrity.
- Every practitioner shall have to maintain appropriate personal, moral and ethical standards during ECF immunization and in those other aspects of his personal life which reflect upon the veterinary profession.
- The Practitioner shall promote and enhance the integrity of the profession by fostering a sense of trust and mutual responsibility between colleagues and in the event of a difference of opinion in ECF delivery.
- The Practitioner shall promote and maintain an effective alliance during ECF immunization in order to provide services that reflect credit on the profession as a whole.
- The Practitioner shall respect the professional endeavors and integrity of their colleagues and neither exploits unfair situations nor use unreasonable action to gain an advantage over them during immunization exercise.
- The Practitioner who becomes aware of professional misconduct, unprofessional conduct or discreditable conduct by a colleague during ECF immunization shall take appropriate action, which may include reporting the matter to the Veterinary Council.
- The Practitioner shall make effort to identify and avoid potential conflict of interest
- The Practitioner shall have an obligation to remain familiar with all appropriate legislation affecting the profession
- The Practitioner shall maintain a registration, or enrollment or enlistment with the Veterinary Council in jurisdiction in which they practice and undertake to abide by the decision of the Council

5.0 REFERENCES

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- Mukhebi, A.W., Morzaria, S.P., Perry, B.D., Dolan T.T., and R.A.I. (1990). Cost analysis of immunization for East Coast fever by the infection and treatment method.

Appendix 1: PRACTICAL ECF VACCINATION – VACCINATION TEAM DURING TRAINING

	DUTY	TEAM 1	TEAM 2	TEAM 3
1	<p>Vaccine Handler: TEAM LEADER</p> <ul style="list-style-type: none"> • Reconstitution • Cold –chain • Time keeper (preparation to end of immunization) • Vaccine application • Strict asepsis observation 			
2	<p>Temperature taker</p> <ul style="list-style-type: none"> • Take the temperature of the animal • Avoid febrile animals or with subclinical illness • Temp. ≤ 39.5°C 			
3	<p>Operator of the Weigh- band</p> <ul style="list-style-type: none"> • Correct weight of the animal 			
4	<p>Recording Assistant</p> <ul style="list-style-type: none"> • Time vaccine is re-constituted • Batch Number • Body Temperature and weight • Volume of OTC 30% injected • Ear Tag Number • Time Immunisation completed • Complete Immunisation Form 			
5	<p>DEWORMING PERSON</p> <ul style="list-style-type: none"> • Albendazole for calves at 2.5cc per 50kg Bwt for 10% pack 			
6	<p>Ear Tag Assistant</p> <ul style="list-style-type: none"> • Apply ear tag 			
7	<p>OTC 30% Injection Assistant</p> <ul style="list-style-type: none"> • Inject correct dose of 30% OTC depending on weight (1ml/10kg bwt) 			
8	<p>Animal Handlers</p> <ul style="list-style-type: none"> • Humane and correct restraining of animals 			

Appendix 2: CHECK LIST OF EQUIPMENT REQUIRED FOR ECF IMMUNIZATION IN THE FIELD

S/N	ITEM	QUANTITY
1.	Liquid Nitrogen Tank - with stabilate straws	Depends on the number of animals (1 straw = 40 doses)
2.	Diluent – frozen and placed in a cool box	One bottle per straw
3.	Cryo-vial	One vial per straw
4.	Cool box with enough ice packs/ice	1
5.	1ml syringe and needle	Needles 21G X 5/8 inches
6.	Razor blade – new or a pair of scissors	1
7.	Thermometer	2

8.	Weigh band	1
9.	Ear Tags	40 per straw (add slightly more)
10.	Ear Tag Applicator + accessories	1
11.	Albendazole 10% 1 Litre	1 litre per 5 straws
12.	Syringe 10 ml for Albendazole	2
13.	Alamycin - OTC 30%	350 mls per straw (take more if the herd consists of more adult bovines)
14.	Syringes – 20cc + Needles Syringes – 10cc + Needles	2 2 (depends on the number of animals)
15.	Bull nose ring (optional but helpful)	1
16.	Lukewarm water – temperature at 40°C could be prepared on site	Enough to submerge the diluents bottle.

17.	Vaccination Sheet	Depends on the number of straws
18.	First Aid Kit	1
19.	Adrenalin	1
20.	Dexamethasone	1

Appendix 3: ECF IMMUNIZATION SHEET:

Date:-----

Delivery Agent (Vaccinator)-----

Address -----

Batch:-----

Ward..... District:-----

Region: -----

	Name of Owner/Tel. No.	Temp. °C	Weight (kg)	Ear No.	Tag	OTC mls	Reactor (Signs)
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
10.							
11.							
12.							
13.							
14.							
15.							
16.							
17.							
18.							

Time: Batch Prepared: -----Hrs Signature of Vaccinator.....

Time: Immunization Finished-----Hrs Name of Vaccinator.....