TRAINING MANUAL

FOR VETERINARY STAFF ON IMMUNISATION AGAINST EAST COAST FEVER

By S.K.Mbogo, D. P.Kariuki, N.McHardy and R. Payne

Revised and updated by: S.G. Ndungu, F. D. Wesonga, M. O. Olum and M. W. Maichomo

September 2016

GALVmed

Protecting Livestock – Improving Human Lives

KALRO

Kenya Agricultural & Livestock Research Organization

This publication has been supported by GALVmed with funding from the Bill & Melinda Gates Foundation and UK aid from the UK Government. GALVmed, BMGF and the UK Government do not make any warranties or presentations, expressed or implied, concerning the accuracy on safety of the use of its content and shall not be deemed responsible for any liability related to the practices described in this manual.

Contents

Figures

Introduction

The KARI-ODA Tick-borne Diseases Project which was based at Muguga between 1993 and 1998, was given the task of developing the method of immunization against East Coast Fever (ECF) known as the "infection and treatment" system and training staff from the Veterinary Department to apply it throughout Kenya. This training course is intended to give you a useful level of background information on all aspects of ECF and its transmission, and then to train you in the methods we have developed for application of the process in the field. Under the direction of the Director of Veterinary Services, your role will then be to immunize cattle throughout your area. We at VRI, VRC Muguga will continue to conduct research aimed at improving the method and we will keep you informed of these developments, so that you can apply them in the field.

The system is basically very simple, but it does require some understanding of the background to ECF and to immunization if you are to apply it correctly and to its best effect. It is also important that you can explain the system to farmers and inform them of its benefits. You will also need to be able to guide them on how they should care for their cattle when they have been immunized, particularly with regard to tick control, to avoid their animals suffering from other tick-borne diseases and suffering from the direct damage which can be caused by ticks themselves.

As you probably know, research on the infection and treatment system has been conducted at VRI, VRC Muguga for many years. The staff here are experts in the method so you will benefit from working with them and learning first had how best to apply it. We feel that the title "the infection and treatment method of immunization against East Coast Fever" is rather clumsy, so we have registered a short trademark for the system. This is "ECFiM". You will see also the logo for ECFiM that we have developed. This is intended to get the system known widely, and to simplify your discussion of the subject.

The present ECFiM is effective only against true East Coast Fever; it is not yet effective against the related condition "corridor disease," in which the infected ticks pick up their infection from buffalo. It is important that you know the reasons for this, so a separate section (Section 4) of this manual gives you information on buffalo-derived theileriosis.

The training course will take the form of a series of illustrated talks and practical demonstrations, in which you will practice the methods you will be using in the field. We will then take you to one of our field trial sites to see how the system works on the farm, and to meet some of the farmers whose animals we have immunized. At the end of the course we hope that you will feel completely confident in the system and that you will be able to immunize cattle in your area and keep the farmers fully informed on how to protect their cattle against ECF and other tick-borne diseases.

We hope you will enjoy the course, join the discussions and then return to your own area to the benefit of your local farmers.

KARI; Kenya Agricultural Research Institute, ODA; Overseas Development Agency, United Kingdom

1. What is East Coast Fever?

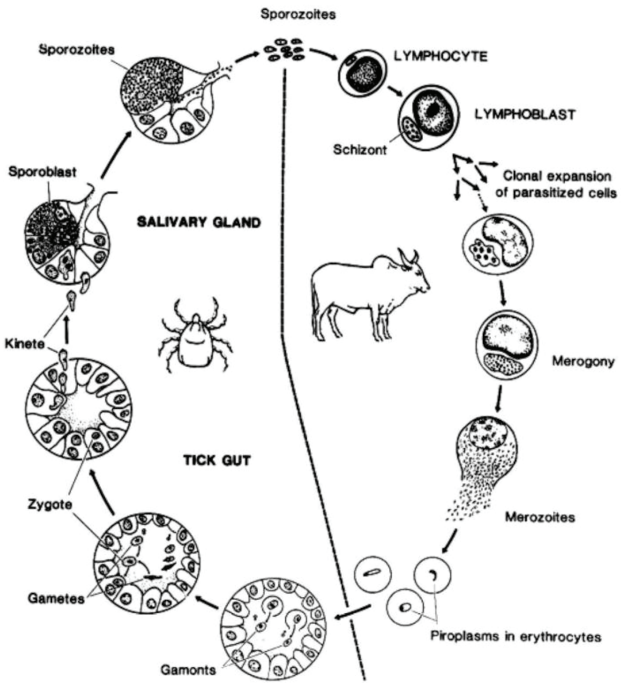
- East Coast fever is a disease of cattle which kills around 100,000 animals of all ages in Kenya each year. It is probably, therefore, the most important disease of cattle in the country.
- It is called East Coast fever (ECF) because it occurs only in countries on the Eastern side of Africa.
- ECF is cause by a protozoan parasite called *Theileria parva.*
- ECF is transmitted to cattle by the tick *Rhipicepalus appendiculatus* ('the brown ear tick') infected with *T.parva* when it attaches to the cattle and begin to suck blood. The parasites are injected into the cattle in the saliva of the tick.

The Life Cycle Of T. Parva In The Vector Tick, R. Appendiculatus

1. Tick picks piroplasms from erythrocytes of cattle or buffalo
2. In the tick gut lumen lysis of erythrocytes occurs and piroplasms are released
3. Piroplasms differentiate into male and female gametes
4. Syngamy occurs to form a zygote
5. The zygote enters gut epithelial cells
6. During moulting the zygotes develop into motile kinetes
7. The kinetes penetrate epithelial cell wall and enter the haemolymph
8. They invade type III acini and enter type e cells of the newly developed salivary glands and form sporonts.
9. The sporont undergoes meiosis (sporogony) when the tick feeds to form sporozoites (haploid)
10. Sporozoites enter mammalian host through tick saliva.

[1] acinar cell is estimated to contain between 40-50 thousand sporozoites.

Diagrammatic representation of *T. parva* in the tick vector and the cattle host



- When they enter the animal's body, the parasites quickly enter lymphocytes, the cells in the body which usually protect the animal against infections. Over the next week or so they develop inside the lymphocyte, and cause the lymphocyte to change its form from a small round cell with only a little cytoplasm to a much bigger cell, the lymphoblast, which has far more cytoplasm. In stained smears taken from a lymph node of an infected animal at this time, the lymphoblasts are easy to recognize because their enlarged cytoplasm stains blue and their nucleus is also bigger than the nucleus of an unchanged lymphocyte.

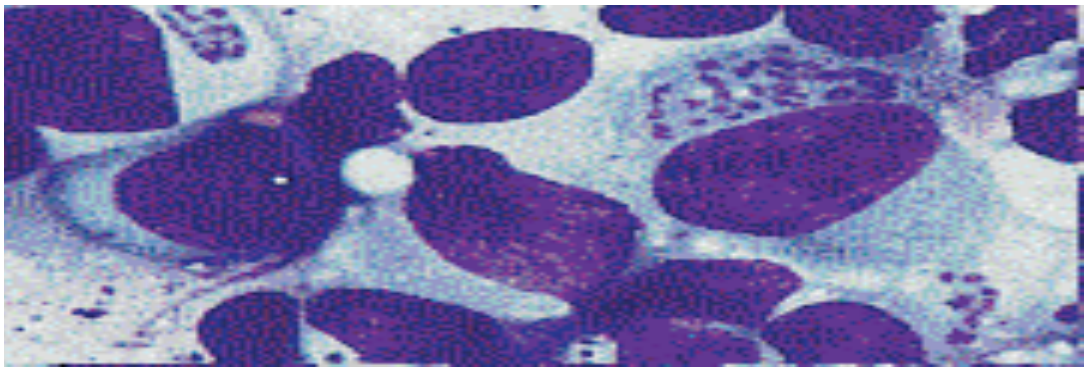
- The other main way in which lymphoblasts differ from lymphocytes is that they repeatedly divide into two new lymphoblasts. Lymphocytes do not divide. Very soon, therefore, stained smears show large number of lymphoblasts. The lymph node begins to enlarge, and is said to be "active". Enlarged lymph nodes are often the first recognizable sign that an animal is infected with ECF.

- About a week or ten days after the animal is infected with ECF, the *Theileria* parasites first become visible in stained lymph node smears. They appear in the cytoplasm of the lymphoblasts as small groups of pinkish staining dots surrounded by an area of relatively clear cytoplasm. This is the early "macroschizont" (Koch's blue bodies, KBBs) stage of the parasite.

- Over the next few days the number of macroschizonts increases, and so does the number of lymphoblasts. The average size of the macroschizonts also increases, and each one may now contain more than ten pink staining particles. The pink particles are the nuclei of the developing parasites. They are not separated from each other by cell membranes, but share a single body of cytoplasm. In advanced cases, some schizonts may contain more than 50 nuclear (or "chromatin") particles.

- Macroschizonts do not increase in number by infecting new lymphoblasts. Instead, they stimulate the cell that they are in to divide into two new cells and this "host" cell divides, so the macroschizont divides with it. In this way, both the two new lymphoblasts contain a *Theilieria* macroschizont.

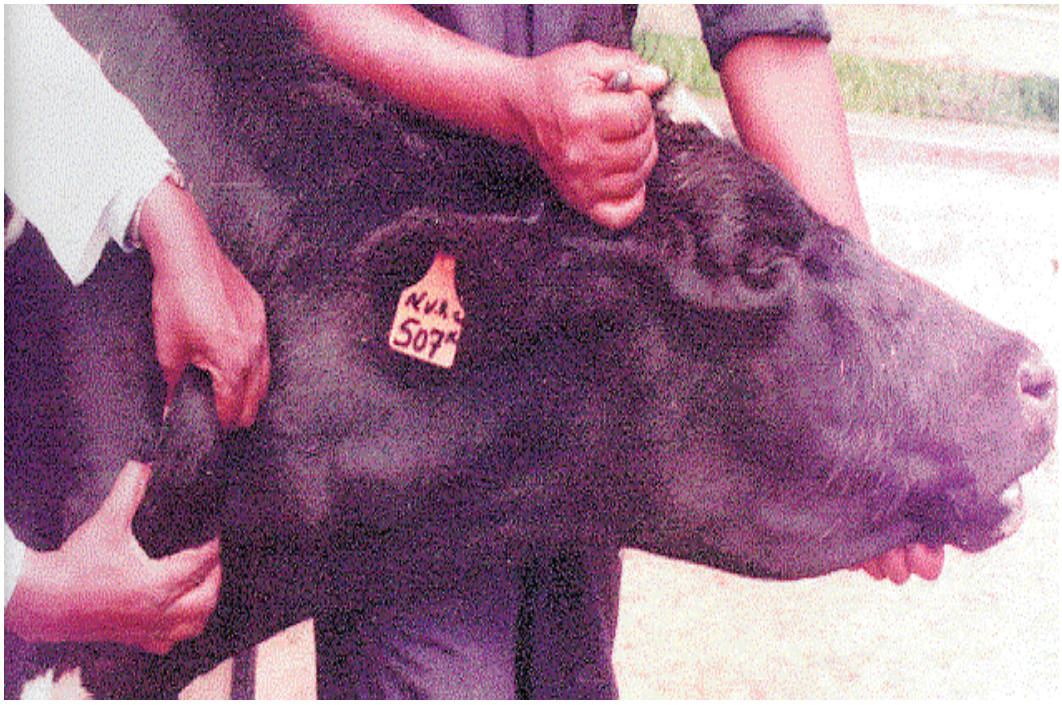
T. parva macroschizonts (KBBs)



- At about this time, perhaps 10 to 15 days after the animal became infected, the animal's temperature rises, the normal temperature of cattle is around 38 – 38.5 degrees C, (though it may be significantly higher on a hot day). In the animal infected with ECF the temperature may rise above 41 degrees C, and sometimes to 42 degrees C. A temperature of 39.5 degrees C or above is regarded as being significantly raised above normal levels.

- As the ECF infection develops, the animal shows progressively more severe symptoms. If it is a milking cow, milk production is likely to fall dramatically and this may be the first sign that the farmer notices. The animal will probably also begin to eat less, and may begin to show signs of dullness. The farmer will probably suspect ECF because then he will have noticed the swollen lymph nodes, which are likely to be very clearly enlarged at this time, particularly the ones in front of the should (prescapulars) and below the ears (parotids). The nodes on the flanks (precrurals) also enlarge.

Swollen lymph nodes (prescapular and parotid)



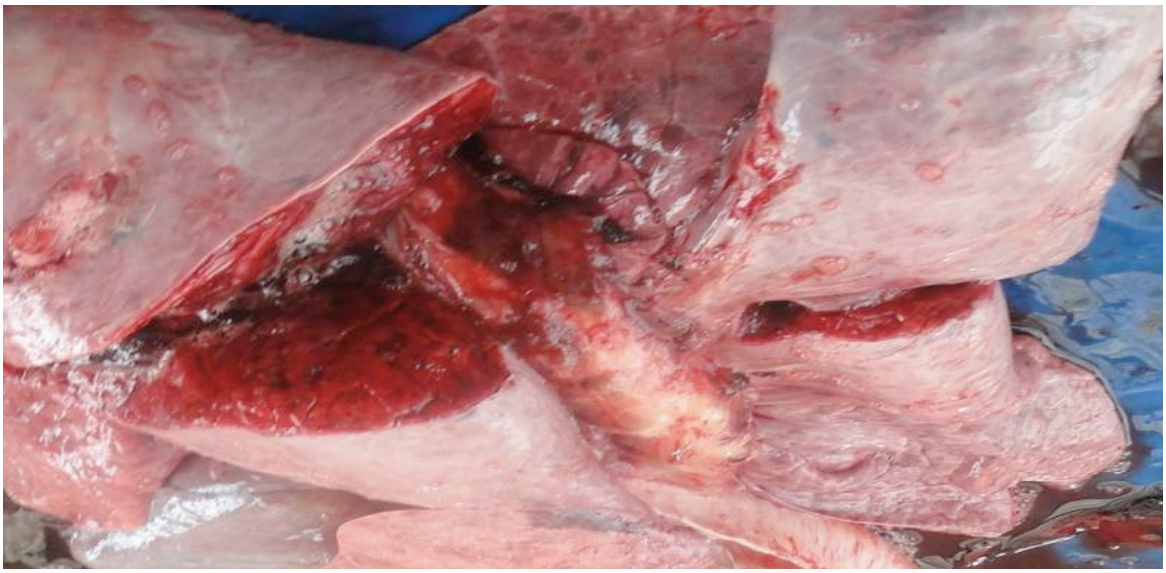
- Meanwhile, the schizonts continue to multiply causing even more severe ECF reaction. Some of the schizonts begin to change their appearance as they transform into the next stage of the parasite life cycle, the microschizont. The first sign of this change, under the microscope, is that the nuclear particles begin to become less defined, almost like small streaks of pink made with a paint brush. This usually happens to the biggest of the macroschizonts, containing more than 20 particles. Over the next day or so, the schizont takes the form of mature microschizont. This contains a large number, sometimes more than a hundred, round more darkly staining particles. The host lymphoblast often bursts at this stage and the particles spill out. Microscopic examination of the particles reveals that they are surrounded by a small ring of blue cytoplasm. These are the micromerozoites.

- The micromerozoites then enter the red blood cells where they form piroplasms. Sometimes more than one piroplasm is seen in a single red blood cell. The number of infected red blood cells increases, sometimes very quickly, so that after a few days more than 50% of them may contain a piroplasm. The percentage of the red blood cells which are infected is described as the "percent parasitaemia".

- When a new tick attaches to the infected cow and sucks up this infected blood, it becomes infected with the *Theileria* parasites and after it has moulted, it can infect susceptible cattle.

- Another result of all of this activity by the parasite is that large numbers of lymphoblasts and lymphocytes are destroyed. Even uninfected cells are killed. This is what causes the main symptoms of ECF. There are two main reasons for this. Firstly, the contents of the broken cells, which include various enzymes and other chemicals, attack healthy issues, causing them to break down. This is seen most clearly in the lungs, which leak fluid into the airways. The first symptom of this is that the animal begins to cough. In the advanced stages of ECF the lungs become full of fluid, breathing becomes very difficult, and finally froth fills the airways and the animal dies of a mixture of choking and drowning, probably the most easily recognized sign of ECF at *post mortem* examination. The cells lining the gut are also affected, causing digestive upsets, and "cigarette burn" ulcers appear in the rumen. These are often very noticeable at post mortem although ECF is not the only condition in which they are seen. The gut, particularly the colon, may show "zebra striping" as pigment is released as the cells of the gut wall are destroyed. The surfaces of the kidneys may show white spots (lymphoid infarcts), which extend down into the kidney tissue. Various other symptoms may be seen, but the ones described are the easiest to recognize.

Oedematous lungs



Stages of the ECF syndrome

The second effect of the destruction of lymphoid cells is that the animal's immune system is partly destroyed. This is likely to make the animal more susceptible to other diseases, particularly other tick-borne diseases such as babesiosis (red water) and anaplasmosis (gall sickness). This often makes correct diagnosis, and therefore treatment, much more difficult, and may be the reason for failure of some treatments for ECF. Other diseases, particularly bacterial infections, may also become apparent in cattle suffering from ECF. We will return to this subject later.

- In the later stages of ECF the animal is likely to lose condition, often rapidly. The farmer will certainly notice this. However, sometimes loss of condition is not very marked. The reason for this variability is not clear but some cattle die of ECF while they still appear to be in good condition, while some become very thin before they die.

- Not all cattle which develop ECF die. Indigenous cattle (the *Bos indicus* breeds) are far less likely to die than exotic breeds (*Bos taurus*) and their crosses. It is generally accepted that, if untreated, between 20% and 50% of indigenous cattle infected with ECF are likely to die, while up to 100% of exotic cattle may die. Cross breed, including grade cattle, show an intermediate degree of susceptibility.

- An animal which recovers from ECF may do so very slowly, or in some cases very quickly. Again the reasons for this variability are not simple and may depend on the severity of the infection, the plane of nutrition of the animal and the quality of the nursing care it receives, or the virulence of the strain of ECF that affected it. The rate at which its damaged immune system, may also play a part.

- There is some, not very convincing, evidence that young calves are more resistant to ECF than older cattle. The reasons for this, if true, are not known. In any case, many calves die of the diseases, causing very severe losses to the farmer.

- Even in a group of cattle of similar age and the same breed, infected with ECF, the severity of the infection may be very variable. There may be several reasons for this. Some may have more effective immune system than others, so are better able to combat the infection. Some will receive heavier infections from the ticks than others, either through having different numbers of ticks feeding on them or because a higher proportion of the ticks are carrying *Theileria* parasites.

- If an animal does recover from ECF or it is cured with drugs, it develops a very strong immunity to re-infection, at least with the same strain of *Theileria*. The immunity lasts for several years, although it is probably boosted by re-infection throughout the animal's life.

  This last point, that cattle which recover from ECF are strongly resistant to re-infection, is the basis of the ECFiM system of immunization.

On the next page of the manual is a series of questions on the information you have just read. We suggest that you answer them as a way of checking that you have understood and absorbed this basic introduction, before you go on to the next section which describes how East Coast fever is transmitted to cattle by ticks. You will find a similar set of questions at the end of each section of the manual. You might like to complete the answers in pencil so that you can test yourself again in the future, so that you can be sure that you always remember the most important points in the training course.

Questions on EAST Coast fever

Question 1 Approximately how many cattle die of ECF in Kenya each year?

Answer _____

Question 2 When the *Theileria* parasites enter the cow, what kind of cells do they enter?

Answer _____

Question 3 Name the first stage of the parasite which can be seen under the microscope.

Answer _____

Question 4 How long after the animal becomes infected are the first clinical symptoms of ECF likely to be seen?

Answer _____

Question 5 What are the first two symptoms that you are likely to see in a milking cow?

Answer _____

Question 6 What is the normal temperature of a cow and what range of temperature is likely to be seen in a cow suffering from ECF?

Answer _____

Question 7 Name the three lymph nodes that you are most likely to notice as swollen an animal suffering from ECF.

Answer _____

Question 8 What mortality rates can be expected for ECF in a) indigenous b) exotic breeds of cattle?

Answer _____

Question 9 Is an animal which recovers from ECF likely to be immune to re-infection?

Answer _____

Question 10 A trademark has been registered for the Muguga system of immunization against ECF. What is it?

Answer _____

2. Transmission of ECF – The role of the tick

- In Kenya, there is only one species of tick which is important in the transmission of ECF. This is the brown ear tick, *Rhipicephalus appendiculatus*. Its "predilection site" of attachment to cattle (i.e. its favourite site) is in the ear, hence its name.

Engorged ticks in the ear

- It also attaches to other parts of the head, particularly around the eyes, and on other parts of the body.

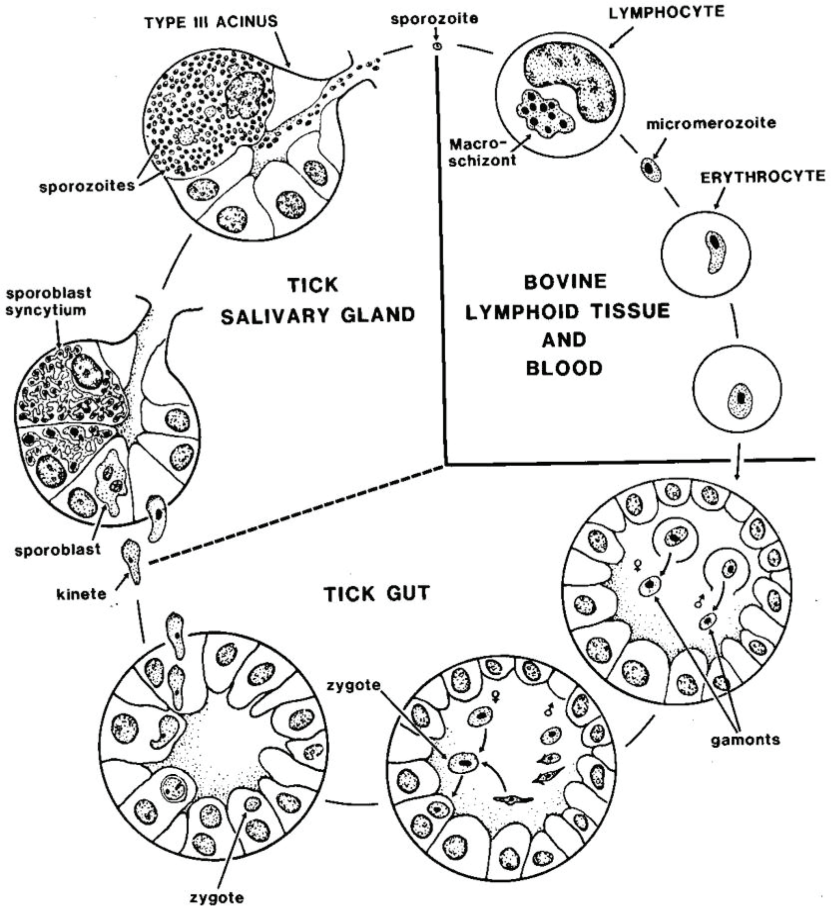
- The brown ear tick is a three-host tick. This means that the three stages in its life cycle, the larva, nymph and adult attach to different cattle. When the larva hatches from the egg, it attaches to one animal, feeds (engorges) and drops off the animal onto the ground. It then buries itself and moults into the nymph stage. The nymph finds another animal, feeds on it and again drops off to moult into an adult. The adult attaches to a third animal, feeds, drops off after a few days and lays a large number of eggs on or under the ground. When these hatch into larvae, the whole cycle is repeated. It is important that you know this in order to understand how ECF is transmitted.

- The eggs of the brown ear tick are not infected with *Theileria* parasites, so the larvae which hatch from them cannot transmit the disease. If the larva feeds on an animal with *Theileria* parasites (piroplasms) in its red blood cells, it will become infected. The animal does not need to be suffering from a clinical attack of ECF for this to happen since recovered animals still carry the parasites in low numbers, and so can infect ticks. When the larva moults to a nymph, the nymph will already be infected, so it can transmit the infection to another animal. Similarly, if a nymph feeds on an infected cow, it will become infected and the adult which develops from it will be able to transmit ECF. So nymphae and adults of the brown ear tick can transmit ECF but larvae cannot. When nymphae and adults feed on an uninfected cow they inject all of the parasites from their salivary glands, so they cleanse themselves of the infection. When these cleansed nymphs moult into adults, therefore, they cannot transmit ECF. Cleansed adults lay their eggs and die, so they cannot transmit either. However, if the infected nymphs feed on an infected cow, they will pick up a new infection and they will be infective when they moult into adults.

- Not all ticks will feed on infected cattle, so not all ticks are infected with ECF. Even some of the ticks which do feed on an infected cow will fail to become infected. Generally, around 2-10% of ticks in an ECF endemic area are infected with *Theileria* parasites. This means that the presence of brown ear ticks on a susceptible animal does not necessarily mean that the animal will develop ECF. However, if there are more than a few ticks on the animal, then the risk of infection is increased. Remember, it only takes one infected tick to transmit ECF.

- Ticks can survive for long periods on the ground without feeding on cattle. They may survive for up to two years. This means that even if cattle are dipped or sprayed regularly, and all ticks which attach are killed, it can take up to two years to eliminate ECF infection from the ticks on a farm. Even then, cattle on the farm are likely to be carriers of ECF if they have recovered from the disease, so ticks which feed on them at any time will probably become infected. This means that farmers must continue to be aware of the risk of ECF, particularly among new calves and any other cattle that have been added to the herd, even when there have been no cases of ECF on the farm for more than a year.

Life cycle of R.appendiculatus



- As stated in the last section, there are many strains of *Theileria* in Kenya. Some are more virulent than others, and some may be very mild. Therefore, some animals which do become infected with Theileria may not develop ECF, particularly if the infecting strain was mild or if the infection level in the tick was very low. Cattle which develop mild or in apparent ECF infections will be immune to re-infection with the same strain, but they may be susceptible to other strains, so they remain at some risk of developing clinical ECF at a later date.

- When a tick feeds on an animal infected with *Theileria* piroplasms the parasites go through a complex life cycle in the tick before they finally reach the salivary glands and become infective "sporozoites". The details of this cycle need not concern us, but the parasite life-cycle is completed before the tick moults to the next stage.

- When an infected tick climbs onto a new animal it takes some time to reach its predilection site for attachment. It then attaches and begins to feed. This process stimulates parasites in the salivary glands to become mature and infective, so that when they are injected into the cow with the tick's saliva, they are able to establish an ECF infection. The period between the time that the tick attaches to the time when it actually transmits ECF is usually 2 - 4 days. It is important that you remember this when you give advice to farmers.

- In the past, the only way to be sure of protecting cattle against ECF was to dip them frequently so that no ticks were attached for long enough to transmit the disease. Acaricides were not particularly long-acting so that in high risk areas cattle had to be dipped perhaps twice per week at times of high tick activity. This was very expensive, required a lot of labour and probably caused significant production losses through stress. Some acaricides now give protection for more than a week but they are very expensive and farmers tend not to use them as effectively as is necessary to prevent transmission of ECF.

- In areas or on farms where most of the cattle are of indigenous breeds it may be uneconomical and unnecessary to dip frequently because potential losses due to ECF and other tick-borne diseases may be less than the cost of controlling ticks intensively. With less intense systems of tick control in these areas some losses from disease are likely to occur, but these may be acceptable.

- Another very important benefit of reduced tick control in these areas is that cattle may become infected with ECF and other tick-borne diseases while they are still calves. Calves are less likely than adults to develop severe or fatal disease. They usually recover and become immune. Indeed exposure of calves to ticks has been common practice in parts of Kenya for many years.

- Conversely, an important effect of intensive tick control is that cattle are totally prevented from becoming infected with any tick-borne diseases so they develop no immunity. If dipping fails, or acaricides are not available, these animals are completely susceptible to infection, and losses may be catastrophic.

- It follows, therefore, that a few ticks on cattle need not be a problem. The core of the ECFiM system is to immunize against ECF, the most important tick-borne disease, and then to allow a few ticks, including *Boophilus* and *Amblyomma*, to infest immunized cattle so that they become infected with anaplasmosis and babesiosis and perhaps heartwater while they are still young enough to resist these diseases. Immunised cattle are also likely to have their immunity to all tick-borne diseases boosted by repeated infections. The implications of this policy are addressed in the next section of this manual.

- One of your principal tasks in using the ECFiM system will, therefore, be to advise farmers on the need for tick control, how often to treat their animals, and to explain to them why a few ticks on immunized cattle can help to stimulate and maintain immunity.

Transmission Of East Coast Fever

FIRST HOST	SECOND HOST	THIRD HOST	
UNINFECTED COW	**ECF NOT TRANSMITTED**	**ECF NOT TRANSMITTED**	
Larvae feed and moult into nymphs →	→ Nymphs moult into non-infected adults	Non-infected adults feed	Eggs hatch into larvae
Nymphs feed on susceptible cow	→ Adults feed on susceptible cow	Adults drop and lay eggs	Eggs
HEAVILY INFECTED COW	**ECF TRANSMITTED**	**ECF NOT TRANSMITTED**	
Larvae feed and moult into nymphs →	→ Infected nymphs	→ Non-infected adults	Eggs
Nymphs feed and moult into adults →	Nymphs feed and moult into adults		Eggs
	→ Infected adults		
	Adults feed		
CARRIER	**ECF MAY BE TRANMITTED**	**ECF NOT TRANMITTED**	
Larvae feed →	→ Nymphs may be infected	Adults	Eggs
	Nymphs feed		
Nymph feed →	→ Adults may be infected	Adult feed	Eggs
	Adults feed		

Questions on ticks and East Coast Fever

Question 1 Only one tick is important in the transmission of ECF in Kenya. Give a) its common name b) its scientific name.

Answer _____

Question 2 Two stages of the tick can transmit ECF. Which are they?

Answer _____

Question 3 On a farm, approximately what percentage of brown ear ticks are likely to be infected with ECF?

Answer _____

Question 4 How long after an infected tick attaches is it likely to start to transmit ECF?

Answer _____

Question 5 Which other important diseases are transmitted to cattle by ticks?

Answer _____

Question 6 Is tick control necessary if only indigenous breeds of cattle are kept? Why?

Answer _____

Question 7 Why might it be good to allow calves to be infested with a few ticks?

Answer _____

Question 8 Give two reasons why limited tick control might benefit owners of cattle which have received ECFiM immunization.

Answer _____

Question 9 Can ticks become infected when they feed on an animal which is immune to ECF?

Answer _____

Question 10 For how long can brown ear ticks survive without feeding on cattle?

Answer _____

3. Immunity to East Coast fever

- Cattle which recover from ECF are strongly immune to re-infection, at least with the strain of the parasite with which they were infected. This is the basis of the ECFiM system of immunization. In the ECFiM system we infect cattle with a low dose of live *Theileria* parasites, which we prepare from infected ticks, and at the same time we inject them with oxytetracycline, which controls the infection so that the cattle do not become sick. About four or five weeks after immunization the cattle become immune to ECF. The oxytetracycline does not kill the parasites but it prevents them growing and multiplying in the body, and it prevents the lymphocytes which contain the parasites transforming and dividing, so that the infection cannot spread through the body. The cow, therefore, can develop an immune response to ECF without suffering from a severe attack of the disease.

- Once we have immunized an animal, it is strongly immune and the immunity lasts for several years. In the field it is likely that the immunity will be boosted by repeated infections from infected ticks. We therefore expect that cattle will have to be immunized only once in their lifetime.

- Immunity conferred by ECFiM is as strong in exotic cattle as it is in indigenous cattle. This is also true in animals which have recovered naturally or after drug treatment.

- Immunity to ECF is "cell mediated". This means that the immune animal is protected by immune cells, including lymphocytes (remember them, they are the same cells that the *Theileria* parasite infects) and macrophages. Although large amounts of antibody are produced against the *Theileria* parasites, antibody does not seem to have any effect in controlling ECF. Because antibody gives no protection against ECF, mothers are not able to pass their immunity on to their calves in the colostrums. Calves born to immune dams are, therefore, fully susceptible to ECF.

- Immunity does not develop as soon as the animal becomes infected. Immunity develops over a period of several weeks and is fully developed only after four or five weeks.

- Cattle cannot be immunized against ECF by injecting them with killed *Theileria* parasites. Protective immunity can only be stimulated by a living infection. Attempts to attenuate *Theilera* as the basis of a live vaccine have also been unsuccessful. Genetically engineered vaccines that have been tried so far have shown only limited efficacy.

- There are many different strains of *Theileria*. Different parts of Kenya have different strains, but several different strains may be present in a single area. This is probably because cattle have been brought into the area from other parts of Kenya and they have brought their local strains with them and local ticks have picked up the "foreign" strains.

- Immunity to one strain of *Theileria* does not necessarily confer immunity to any other strain. So, if immunized cattle are moved to another area they may be completely susceptible to the strains which exist there. Similarly, cattle which have recovered from ECF, or have been cured with drugs, may be fully susceptible to the strains of ECF in the area to which they are moved.

- One of the benefits of immunization may be that farmers will be able to produce and raise enough cattle locally that there is no need to buy animals from outside their own area. Part of your job will be able to advise them of the dangers of buying cattle in and to tell them that they should not sell their immune cattle in another area because they may not be immune to the strains, of ECF that they will encounter there.

- A major problem is that there is no simple test to show whether an animal is immune to a particular strain of *Theileria*, or whether any one strain will protect against another. The only way this can be done is immunizing cattle with one strain, curing it and "challenging" it with the second strain. We do this at VRI, VRC Muguga, but it is very expensive, and requires much time and expertise.

- It appears that a small proportion of cattle are not able to develop immunity to ECF, even when treated with ECFiM, and they will remain susceptible. The reasons for this are not known.

- Immunity to ECF does not protect against any other diseases.

Questions on Immunity to East Coast fever

Question 1 How long does immunity to ECF last, following ECFiM immunization?

Answer _____

Question 2 What type of immunity gives protection against ECF?

Answer _____

Question 3 Which types of cell are responsible for immunity to ECF?

Answer _____

Question 4 Is immunity to ECF stronger in exotic or indigenous cattle?

Answer _____

Question 5 Does antibody help to protect cattle against ECF?

Answer _____

Question 6 Does immunity produced by ECFiM protect against all strains of ECF?

Answer _____

Question 7 If an animal is protected by ECFiM in one area, will it be immune if it is moved to another area?

Answer _____

Question 8 Can killed vaccines protect against ECF?

Answer _____

Question 9 Are calves born to immune dams immune to ECF?

Answer _____

Question 10 Does immunity to ECF confer protection against anaplasmosis or babesiosis?

Answer _____

4. Buffalo – derived theileriosis – "Corridor disease".

- In many parts of Kenya buffalo share grazing with cattle or migrate across cattle grazing land. Buffalo carry low levels of infection with *Theileria parva*, though they do not usually suffer any clinical symptoms of theileriosis. However, brown ear ticks which feed on these bufflalo become infected with *Theileria* and when they have moulted, they can transmit the infection to cattle. The disease which develops in the cattle is similar to ECF, but there are some important differences, it therefore has a separate name, "corridor disease". This derives from the fact that it is associated with buffalo "corridors" which cross farming land.

- Clinically, corridor disease and ECF are fairly similar, and are difficult to distinguish. However, the disease may develop more rapidly than ECF and mortality rates may be higher. Frequently, the eyes become opaque due to invasion by lymphocytes, and the animal may go blind. (This happens far less frequently in ECF). If the animal recovers, sight is usually restored as the lymphocytes leave the eyes.

- Greater differences are seen in stained lymph node and blood smears. In lymph node smears there are usually far fewer schizonts than in an ECF infection and the average number of nuclear particles in each schizont is significantly smaller, though some large ones are seen. Similarly in blood smears the piroplasm parasitaemia is very low, seldom being more than 1%. Often the animal dies before any piroplasms are seen. This rarely happens in ECF.

- For reasons which are not clearly understood, ticks which feed on cattle suffering from corridor disease are not able to transmit the infection to other cattle. Therefore, while corridor disease can be devastating on first transmission to cattle, it is "self – limiting". If buffalo do not pass through again it will die out within a year or two as the ticks which originally fed on buffalo either feed on cattle and so lose their infection, or they die of natural causes before they can feed on cattle.

- Perhaps the most important feature of buffalo-derived strains of *Theileria* is that cattle which recover from corridor diseases are not immune to true ECF and animals which recover from ECF are not immune to corridor disease. This is because infections in buffalo seem to include a far wider range of immunological types than occur in cattle infections and these strains appear to change over time in the same buffalo. This means that it is far more difficult to immunize against corridor disease than against ECF.

- For this reason, the ECFiM system can only be used reliably in areas which are known to be free of buffalo.

Questions on corridor disease

Question 1 Why is corridor disease so called?

Answer _____

Question 2 Which tick transmits corridor disease to cattle?

Answer _____

Question 3 How do the clinical symptoms of corridor disease differ from those of ECF?

Answer _____

Question 4 Describe the macroschizonts of corridor disease as seen on a stained smear

Answer _____

Question 5 What is the highest piroplam parasitaemia usually seen in corridor disease?

Answer _____

Question 6 Are cattle which recover from corridor diseases likely to be immune to ECF?

Answer _____

Question 7 Are cattle which recover from ECF likely to be immune to corridor disease?

Answer _____

Question 8 Does ECFiM protect against corridor disease?

Answer _____

Question 9 Can corridor disease be transmitted via ticks from cattle to cattle?

Answer _____

Question 10 How long after buffalo have been present may you see cases of corridor disease in cattle?

Answer _____

5. Other tick-borne diseases

- East Coast fever is undoubtedly the most important tick-borne diseases in Kenya, in terms of the value of animals and animal products lost due to the disease, the cost of attempting to control the disease and the disruption it causes to farming systems.

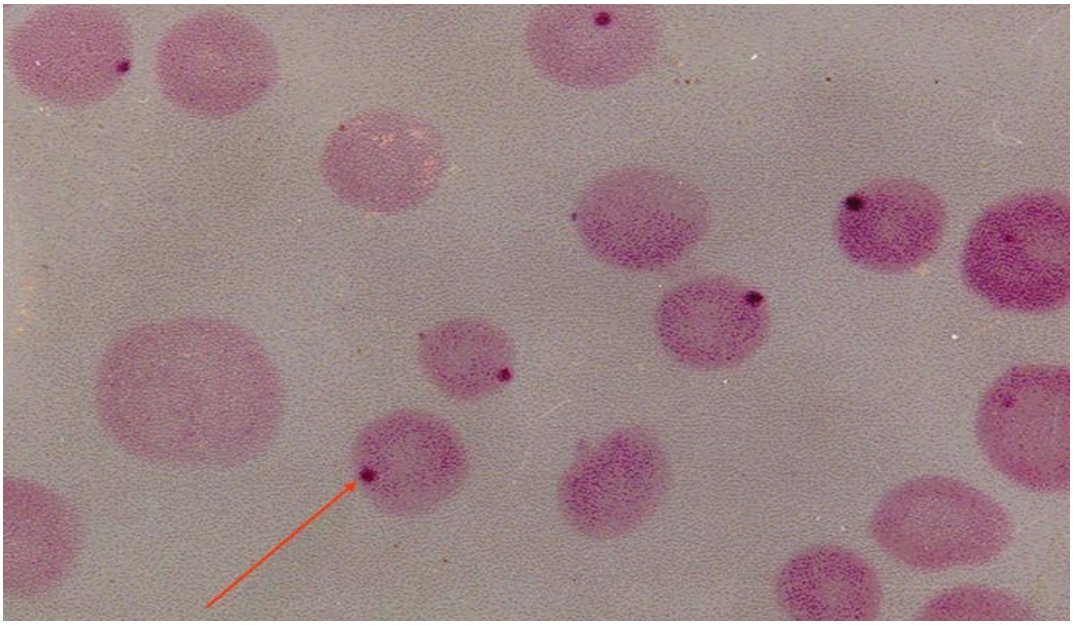
- Besides ECF and corridor disease, there are several other tick-borne diseases of varying importance in Kenya. The three most important are anaplasmosis, babesiosis and heartwater. There are also other species of *Theileria* which cause minor losses but whose greater importance may be because of the confusion they may cause in diagnosis. These include *T. mutans, T. buffeli* and *T. taurotragi.* There are also various rickettsial diseases other than anaplasmosis and heartwater, such as Ondiri disease, which may be important locally, or which may cause complications when associated with other diseases. You need to be aware of these, but a detailed knowledge is not necessary.

5.1 Anaplasmosis

- Anaplasmosis is a rickettsial disease caused by *Anaplasma marginale.* It is probably second in importance to ECF. It is transmitted by the blue tick, Boophilus decoloratus and possibly also by biting flies. It can also be transmitted by hypodermic needles, surgical instruments etc, used on more than one animal without being sterilized and in blood transfusions.

- Anaplasma is a parasite of the red blood cells, appearing as a small, dark staining dots, mostly near the margin of the cell. The principal symptoms are high temperature, anaemia, jaundice and sometimes constipation. In the advanced stages mucous membranes are pale and be icteric (yellow) due to jaundice, because the liver is unable to get rid of all the haemoglobin from the infected red blood cells, which are broken down there. Treatment is with tetracycline or imidocarb (Imizol).

Anaplasma marginale



- Calves are more resistant to anaplasmosis than are older cattle. Mortality rates in older cattle can be very high but young calves usually develop a relatively mild disease from which they recover and become immune carriers of the infection. It is therefore, important to expose calves to ticks so that they can become infected with anaplasmosis while they are still young, when they will develop immunity to the infection.

- In parts of Kenya the infection is very common and almost all cattle more than a few weeks old are carriers. This indicates that sufficient ticks have fed on them at some time for them to become infected, but they have survived the infection, often without showing any

symptoms of disease, i.e. a situation of enzootic stability exists for anaplasmosis. In other areas infection rates are far lower or absent. This indicated either that tick control has been very effective, so that no ticks have been able to feed and transmit the infection or *Anaplasma* may be absent or low incidence in ticks in the area. This indicates a situation of enzootic instability, and a reduction in tick control could result in an increase in numbers of blue ticks and hence an outbreak of anaplasmosis.

- Anaplasmosis frequently occurs at the same time as ECF. This can be for two reasons. Either tick control has failed so that both blue ticks and brown ear ticks have been able to feed on the animal, and both diseases have been transmitted as a result. However, the incubation period of anaplasmosis is usually for longer than for ECF (four to ten weeks, compared with around two weeks) so this may not be the usual reason.

- The more likely reason is that ECF has developed first and, as described in Section 2 of this manual, this has caused a depression of the animal's immune responses. This has then allowed an *Anaplasma* infection which was already present in the carrier state in the animal to flare up as clinical anaplasmosis. It may take only one week or so for this to happen.

- An animal which is suffering from both ECF and anaplasmosis is clearly likely to be very sick, because in addition to the symptoms of ECF it is likely also to be anaemic from the anaplasmosis. Prompt treatment should, therefore, be given for both diseases.

- Research at Muguga has indicated that even very low Anaplasma parasitaemia (sometimes only 2%) can cause severe symptoms in association with ECF, whereas uncomplicated anaplasmosis parasitaemia may be between 10% and 70%.

- It is therefore essential that whenever you suspect ECF you should take a lymph node smear and also a blood smear to check for the presence of anaplasmosis. It is important that the blood smear is taken before you treat the animal with either Clexon or Butalex because these drugs cause *Theileria* piroplasms to become round and dark-staining, looking very like *Anaplasma*. Examination of a blood smear taken after treatment will therefore make it very difficult to distinguish between *Anaplasma* and drug-damaged *Theileria* piroplasms.

- Once an animal becomes sick from anaplasmosis it can die very quickly, because anaemia is often very severe by this time. It is, therefore, very important that a diagnosis of anaplasmosis is make on first examination so that treatment can be given immediately. If an animal suffering from both anaplasmosis is treated only for ECF, i.e. the diagnosis of anaplasmosis was missed, then there is a risk that, when it fails to show signs of clinical recovery, it will be treated again only for ECF and is then likely to die principally of anaplasmosis. Not only will the animal have died unnecessarily but a second, expensive, dose of Clexon or Butalex will have been wasted.

- Anaplasmosis, then, is a very important disease on its own but it is even more of a risk when associated with ECF.

Other Tick –Borne Disease

Questions on anaplasmosis

Question 1 How is anaplasmosis transmitted?

Answer _____

Question 2 How would you diagnose anaplasmosis?

Answer _____

Question 3 How resistant are calves to anaplasmosis compared to older cattle?

Answer _____

Question 4 Which drugs are used to treat anaplasmosis?

Answer _____

Question 5 Why does anaplasmosis often occur at the same time as ECF?

Answer _____

Question 6 Why is it important to take a blood smear from a suspected case of ECF before you treat it?

Answer _____

Question 7 What % parasitaemia is likely to cause clinical symptoms in a) uncomplicated anaplasmosis b) anaplamosis associated with ECF?

Answer _____

Question 8 Give two reasons why mixed infections of ECF and anaplasmosis should be treated promptly with drugs specific for both diseases.

Answer _____

Question 9 In an area of low incidence of anaplasmosis why might it be dangerous to reduce the frequency of tick control?

Answer _____

Question 10 How would you recognize *Anaplasma* on a stained blood smear?

Answer _____

5.2 Babesiosis

Babesiosis is a protozoan disease caused by *Babesia bigemina* and *Babesia bovis. Babesia bigemina.* It is transmitted by the blue tick, *Boophilus decoloratus* and *Boophilus microplus.* The disease is also known as "redwater" because in advanced cases the urine is colored red by haemoglobin released from ruptured red blood cells. *Babesia bovis* is transmitted by the larval stages of *B. microplus.* Unlike *B. bigemina*, it causes nervous signs and causes no haemolytic syndrome. Other clinical signs are similar.

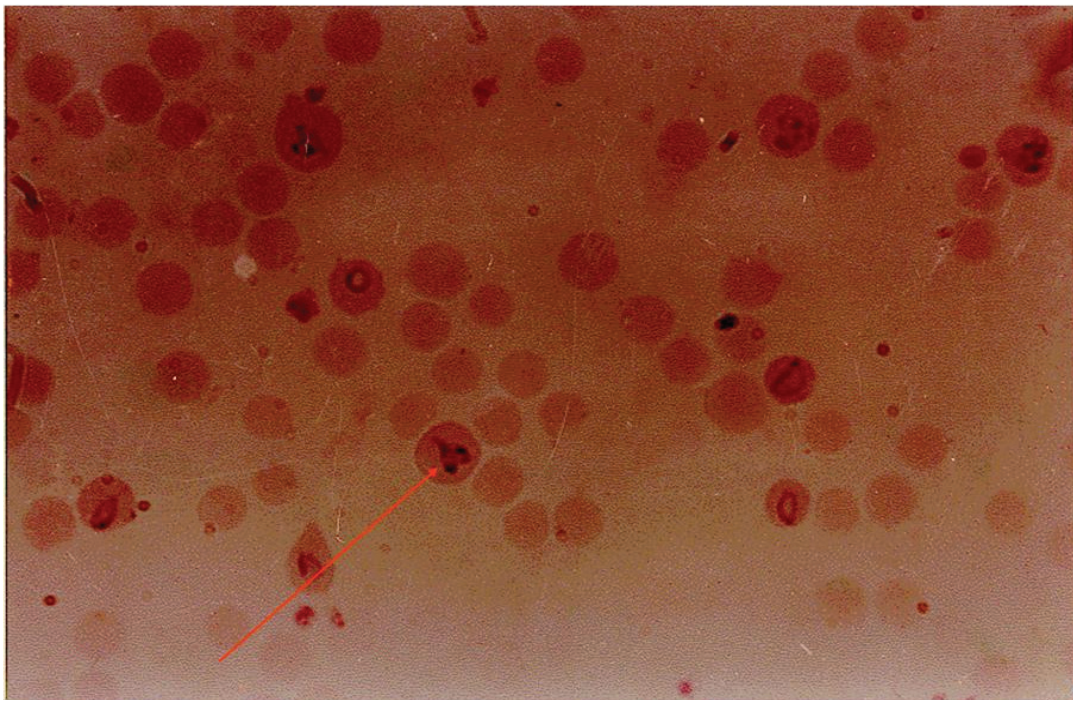
- The symptoms of babesiosis can easily be confused with anaplasmosis before the advanced stage is reached when the urine becomes coloured red. Anaplasmosis does not produce red urine. Babesiosis also causes high temperature and severe anaemia, but it may not be diagnosed until late in the syndrome, when anaemia is already very advanced and the urine is already red.

- Like anaplasmosis, babesiosis is usually less severe in young calves than in older cattle so the carrier state is likely to arise in the same way and the same warnings apply to changes in tick control policy. Babesiosis is widespread but perhaps not as common as anaplasmosis. It can be treated witt diminazene aceturate (Berenil) or imidocarb (Imizol).

- Babesiosis often occurs at the same time as ECF for the same reasons as anaplasmosis. However, because its incubation period is much shorter (typically 2-4 weeks) than for anaplasmosis, the disease occurs because immunodepression "releases" the carrier state or because a failure in tick control has allowed susceptible cattle to become infected with ECF and babesiosis at the same time.

- *Babesia* parasites are easy to recognize in stained blood smears. They are large piroplasms which may fill half of the cell and they often appear as paired parasites, hence the name *"bigemina",* which means twins. However, there are often very few parasites on a smear, even in an advanced clinical case, and it is important to search for quite a long time if you suspect babesiosis. The *B. bovis* are much smaller and resemble a signet ring. They are even more difficult to find on a blood smear.

Babesia bigemina

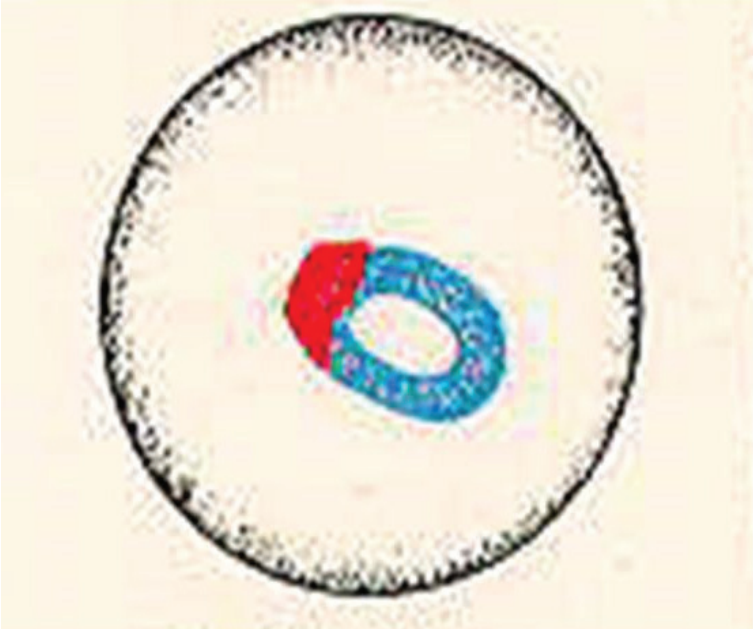


- Macrophages often take up large numbers of Babesia-infected red cells and also un-infected ones. If you see macrophages containing red blood cells when you are examining a blood smear, you should suspect babesiosis and search carefully for *Babesia* parasites to confirm diagnosis.

- As with mixed infections of ECF and anaplasmosis, mixed infections of ECF and babesiosis should be treated promptly for both infections. You should also take a blook smear before you treat. Because Clexon and Butalex have no effect on *Babesia* parasites, *Babesia* is still easy to recognize after treatment for ECF.

Diagrammatic representation of *B. bovis* and *B. bigemina*

B. bovis



B. bigemina

Questions on babesiosis

Question 1 How is babesiosis transmitted?

Answer _____

Question 2 What are the main clinical symptoms of babesiosis?

Answer _____

Question 3 Which drugs cure babesiosis?

Answer _____

Question 4 Are calves more resistant to babesiosis than older cattle?

Answer _____

Question 5 How long is the incubation period for babesiosis?

Answer _____

Question 6 Babesiosis and ECF often occur at the same time. Why?

Answer _____

Question 7 Do drugs which treat ECF have any effect on babesiosis?

Answer _____

Question 8 Describe the appearance of Babesia in a blood smear.

Answer _____

Question 9 What other sign on a blood smear could make you suspect babesiosis?

Answer _____

5.3 Heartwater

- Heartwater is the third of the important tick-borne diseases other than ECF. It is a very severe disease, usually killing the animal very quickly after the first clinical symptoms are seen. This makes it very hard to treat successfully.

- Heartwater affects sheep and goats as well as cattle.

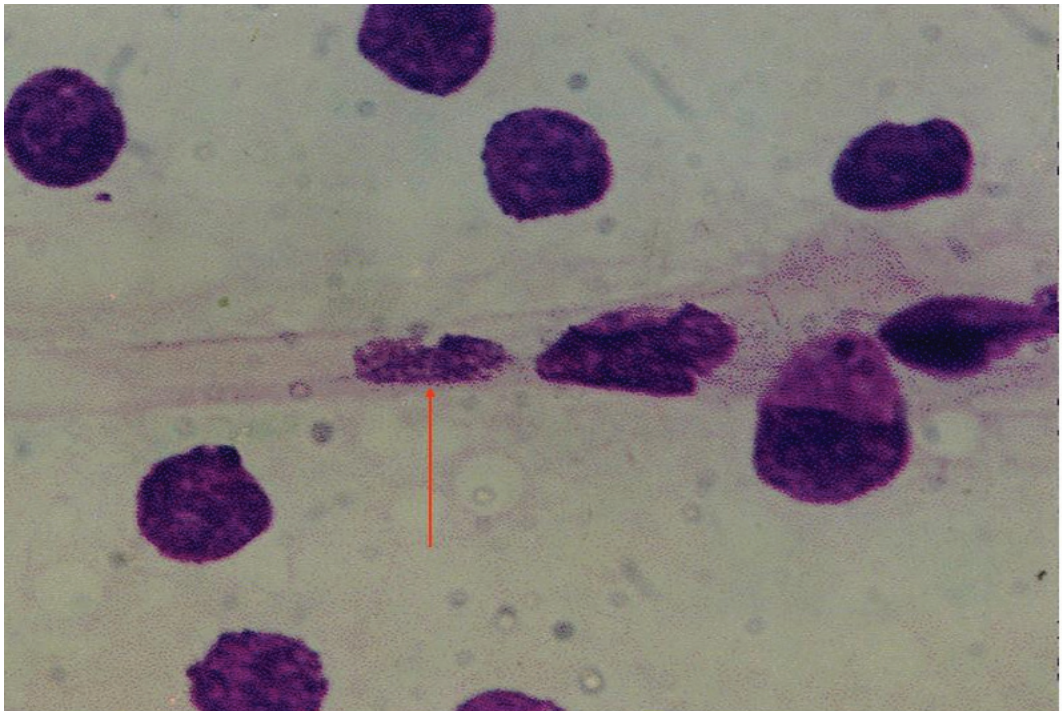
- Heartwater is a rickettsial infection *(Erhlichia (Cowdria) ruminantum)* transmitted by bont ticks of the genus *Amblyomma.* These ticks are big and brightly patterned. Farmers will usually recognize them. Their predilection sites for attachment are bare areas of skin, particularly the teats, under, scrotum and under the tail. They have very long big mouth parts and cause a lot of damage to cattle. Teats can be totally destroyed by only one or two ticks. It is therefore very important that farmers kill these ticks.

Bont tick *(Amblyoma spp)*

- The heartwater parasites infect the cells that line blood vessels and capillaries (the endothelial cells) and cause the symptoms of heartwater apparently by making them leak body fluids. The temperature also rises, sometimes approaching 42 degrees. The classical symptom of advanced heartwater is that the animal becomes uncoordinated in its movements and walks by raising its feet much higher than usual, like a horse trotting (high-stepping gait). Finally, it likes down and "paddles" its legs before dying. When these symptoms are seen it is usually too late for treatment to be effective. The main symptom of heartwater seen at post *mortem* examination is an accumulation of fluid in body cavities, such as the peritoneum surrounding the heart (hence the name heartwater) and in the chest and abdominal cavities.

- Diagnosis of heartwater is best done by eliminating other possibilities and also looking for bont ticks on the animal. You may then suspect heartwater. *Post mortem* diagnosis is done by looking for fluid in body cavities and taking a smear of tissue from the cortex of the brain, colonies of Cowdria in the endothelial cells of the capillaries. You must, of course, be careful when handling the brain in case the animal was suffering from rabies. If heartwater is diagnosed in a dead animal, this may be helpful in diagnosing further cases in the same location since heartwater often occurs in several animals in a short period.

Ehrlichia ruminantum



- The only drug that is effective against heartwater is tetracycline.
- Because heartwater is so difficult to diagnose, while bont ticks are so common, it is suspected that there is far more heartwater in Kenya than is usually diagnosed. We think that when farmers reduce the level of tick control after ECFiM immunization, heartwater may become more common, and you should look out for this.
- As with anaplasmosis and babesiosis, but not ECF, calves are more resistant to heartwater than are older cattle, so exposure of calves to a few bont ticks may allow them to become infected, recover and become immune.
- Heartwater is said to be more common in some areas of Kenya than others, for example Samburu, Narok, Ngong and similar places. The bont tick does not occur at very high altitudes so in the higher areas where ECF occurs, heartwater may not be a risk.
- Heartwater may be precipitated from the carrier state by ECF, but this is not certain.

Questions on heartwater

Question 1 Which ticks transmit heartwater?

Answer _____

Question 2 How can you recognize the bont tick?

Answer _____

Question 3 Why is clinical diagnosis of heartwater so difficult?

Answer _____

Question 4 Which drug would you use to treat heartwater?

Answer _____

Question 5 Which farm animals are affected by heartwater?

Answer _____

Question 6 Where are the predilection sites for attachment by bont ticks?

Answer _____

Question 7 In addition transmitting heartwater, why are bont ticks dangerous?

Answer _____

Question 8 Why should you be careful when taking a brain smear for heartwater diagnosis?

Answer _____

Question 9 Why might it be good to let a few bont ticks feed on calves?

Answer _____

Question 10 In which areas of Kenya are you unlikely to find heartwater?

Answer _____

5.4 Other tick-borne diseases

- Several other tick-borne diseases of cattle occur in Kenya. While none of them cause as much disease as the four already discussed, they have some relevance for the ECFiM system.

- There are three species of *Theileria* other than *parva* which you may encounter. *T. mutans* is the most common, and it occurs widely. It does not usually cause clinical disease, but sometimes it will, and ECFiM does not protect against it. *T. mutans* is often seen as piroplasms in the red blood cells of healthy calves, and it is difficult to distinguish them from those of *T. parva.* It does not produce many schizonts. If you do see them, they usually contain many particles which are like pink brush-strokes, whereas those of *T. parva* are most clearly defined. When *T. mutans* does cause clinical disease, piroplasm parasitaemia can be very high and the animal becomes anaemic, as in babesiosis. Cattle suffering from ECF do not become anaemic. Pathogenic *T. mutans* infection can, therefore, be confused with either ECF or babesiosis. *T. mutans* is transmitted by bont ticks *(Amblyoma variegatum).*

- Cattle may also be infected with *T. taurotragi*, although this is normally a parasite of the eland. It is apparently not pathogenic to cattle and the piroplasm parasitaemia is usually low. It is important to us because antibody to *T. taurotragi* (and to some extent *T. mutans)* cross –reacts in serological tests for *T.parva.* This can cause confusion in interpreting results of serological tests.

- *T. buffeli* may also be seen in blood smears from cattle. It is not pathogenic but can cause confusion in the same ways as *T.mutans* and *T. taurotragi.*

- Various other rickettsial parasites also occur in cattle. They include Ondiri disease and *Erhlichia bovis.* They are important to us only because antibodies to them give confusing results in serological tests for heartwater. It is possible that reduced levels of tick control will result in higher incidence of these diseases, so you should be aware of this possibility.

Questions on "minor" tick-borne diseases

Question 1 Name three species of *Theileria*, other than *parva*, which can affect cattle.

Answer _____

Question 2 Which of these may cause clinical disease in cattle?

Answer _____

Question 3 What is the main clinical symptom of *T. mutans* infection?

Answer _____

Question 4 Which tick transmits *T. mutans*?

Answer _____

Question 5 Which other disease does this tick transmit?

Answer _____

Question 6 Apart from sometimes causing disease, why are these species of *Theileria* important in the ECFiM system?

Answer _____

Question 7 What is the normal host of *T.taurotragi*?

Answer _____

Question 8 Name some "minor" rickettsial infections of cattle.

Answer _____

Question 9 Why are these infections significant to the ECFiM system?

Answer _____

Question 10 Describe the schizonts of *T. mutans*.

Answer _____

6. The ECFiM system of immunization against ECF.

Learning about the ECFiM system and how to use it is the main purpose of this training course. The information that you have learned in the previous sections of this manual should give you a better understanding of the background to the system and so allow you to operate it effectively and give the best possible advice to cattle owners and herdsmen. Obviously you do not need to tell them all you have learnt, but it is best that you have this detailed background.

The ECFiM system itself is based on very simple principles that have been studies for many years at VRI, VRC Muguga and elsewhere. Although the principles are simple, it has taken a lot of hard work to convert all this knowledge into a simple and effective immunization system that you should be able to use easily in the field. The techniques you will use are also very basic but must be applied carefully in accordance with instructions. When carried out correctly, the ECFiM system gives high levels of protection against ECF and it is very safe. If used wrongly, it can fail to give protection and can kill "immunized" cattle. It is very important, therefore, that you understand the system completely and that you operate it correctly. Your advice to farmers must also be sound.

This section of the manual describes the principles on which the ECFiM system is based and how to use it, and it tells you how the "vaccine" is made and tested for efficacy and safety.

Section 7 is the basis for the practical training part of the course. You will be taught how to plan an immunization campaign, how to give ECFiM to the cattle and how to explain the system to farmers.

6.1 The basis of the ECFiM system

- The ECFiM system is based on the fact that cattle which have recovered from ECF are immune to the disease. However, ECF usually kills most of the cattle that it infects so few survive to become immune. In the ECFiM system, the cattle are infected with *T. parva* parasites, and at the same time the animal is treated with a 30% oxytetracycline formulation at the recommended therapeutic dose. Oxytetraclines slow down the multiplication of infected cells. The disease elicited in this way is therefore mild and the animals recover and become immune. Immunity is stimulated as well by this mild infection as by a severe clinical infection.

- The infective material in the system is essentially a "vaccine", but it is not treated in any way to weaken, or attenuate it. It is prepared by grinding up infected ticks and separating the *T. parva* parasites (the infective sporozoite stage) from the rest of the tick material. Small doses of this material are then injected into the cattle to be immunized and at the same time they are treated with a dose of tetracycline, which slows down the development of the infection, by preventing the infected lymphocytes transforming and dividing. The animal then develops an immune response to this mild infection of ECF and cures itself. In this way the animal becomes immune to ECF.

- The dose of infective material that is injected has been carefully established in the laboratory to give a wide spectrum of protection. The exact dose is determined at VRI, VRC Muguga by giving small groups of cattle a range of dilutions of the material prepared from the infected ticks. The animals are monitored to find which dilution gives a level of infection which can be reliably controlled by tetracycline. Five weeks after the test cattle were infected and treated, they are challenged by infecting them with the same volume of undiluted original infecting material. If they survive this without showing significant symptoms of ECF, then this dilution is used as the working dose in the ECFiM system.

- Obviously, this testing system is very expensive and takes a long time. We therefore need to produce a very big batch of infective material from the ticks to make it economical. However, the "vaccine" dies very quickly after it is prepared so we have to have a way of keeping it alive while we run our tests, and for a long time after that so that you can use in the field. This is done by freezing it in liquid nitrogen, ie. it is cryo-preserved . When it is thawed, it is still alive and can be used in the ECFim system. A deep-frozen batch of

this infective *Theileria* material is called a "stabilate" – that is a, batch of material whose infectivity is stabilised.

- In any large group of cattle which are given the same amount of stabilate, most will react exactly like the one in our small laboratory test of infectivity. However, a small number may not become infected at all and some may be much more susceptible to the infection and develop a severe ECF reaction. The first group will probably not become immune but there should be very few of these. The group that reacts severely must be treated. Such animals when they recover will be immune.

- A most important element of the whole ECFiM process is the advice and information that you give to the farmer. He needs to be told, as simply and clearly as possible, what the ECFiM system does, how it works, what the risks and benefits are, and what he must do to keep his immunized cattle and any others in good health.

- The ECFiM system is obviously fairly expensive. Preparation and storage of the vaccine costs a lot of money. The tetracycline used as the "block" is expensive, and you will be using syringes and needles to administer both the stabilate and the tetracycline. Your time, transportation and cost of treatment of the rare reactor, has to be factored in the cost of the vaccine. The Director of Veterinary Services has stated that the whole system must be self-financing. This means that the farmer must pay the true cost of the process. The actual charge should be based on the weight of the animal, professional charges and the distance. Part of your job will therefore be to explain this to the farmer so that he can decide whether the risks of ECF that he faces justify the cost, and therefore, whether he wants his cattle to be immunized.

Benefits of ECFiM

- Relaxation of spraying to once every 2-3 weeks thus cutting the spraying costs by between half and 2/3.

- Immunity is life-long and boosting occurs naturally

- Reduction in the cost of drugs to treat ECF cases

- Immunised animals fetch better prices in the market than non-immunised animals

- Reduction of environmental contamination with acaricides.

- Reduced incidence of resistance development in ticks to acaricides

Questions on the basis of the ECFiM System

Question 1 What is a deep-frozen preparation of live parasites called?

Answer _____

Question 2 Which gives the stronger immunity – a mild or a severe ECF infection?

Answer _____

Question 3 Which drug is used to "block" the ECFiM "vaccine"?

Answer _____

Question 4 How strong will the immunity be if we give too small a dose of stabilate?

Answer _____

Question 5 What will happen if the stabilate dose is too high?

Answer _____

Question 6 In our laboratory tests, how long after immunization do we"challenge" the immunity of the experimental cattle?

Answer _____

Question 7 What dose of stabilate do we use for the challenge?

Answer _____

Question 8 What do we call the process in which we keep immunized cattle under observation for clinical reactions?

Answer _____

Question 9 List the elements in the ECFiM system which contribute to its cost.

Answer _____

Question 10 Will the farmer be charged for ECFiM?

Answer _____

6.2 Preparation of stabilate

- The *Theileria* stabilate used in the ECFiM system is prepared by infecting a group of cattle with the appropriate strain of the parasite and allowing nymphal *R. appendiculatus* ticks to feed on them at the time when the piroplasm parasitaemia is rising. In this way the ticks become heavily infected. When they have fed to repletion, they drop off and are collected.

- The ticks are counted and placed in an incubator at the optimum temperature and humidity for them to survive and moult into adults. This usually takes 4 – 6 weeks. Up to 20,000 ticks can be fed on a single animal. Samples of the moulted ticks from each animal are then examined to find how heavily they are infected. Their salivary glands are removed, stained and examined under the microscope to determine what proportion of them are infected and how heavy the infection is. When this has been calculated, preparation of the bulk stabilate begins.

- The remaining large batch of ticks harvested from the infected cattle are then fed on rabbis for four days to allow the parasites to complete their development into infective sporozoites. This requires a large number of rabbits since only 200 adult ticks can be fed on each rabbit. The ticks are then carefully removed from the rabbits by hand and counted again to find how many have fed. They are washed in alcohol to kill any bacteria on their surface, then repeatedly washed in water to remove the alcohol, which would also kill the *Theileria.* They are then ground up to release the parasites into a complex mixture of saline and culture medium which keeps them alive.

- This suspension is gently centrifuged to remove the debris of the tick's bodies. The supernatant liquid contains the *Theileria* sporozoites. We add glycerol, to protect the sporozoites against being damaged in the freezing process, (glycerol is a "cryoprotectant") and the liquid is loaded into "straws". The straws, each containing 0.5 ml of the parasites suspension, are frozen in liquid nitrogen to keep the sporozoites alive.

- When parasites are required for dose standardization, two straws are removed from liquid nitrogen, thawed by immersion in a water bath at 37 degrees then allowed to stand in the bath for 30 minutes. This allows the parasites to "equilibrate", that is, recover from being frozen. A series of dilutions is then made from the thawed material and volumes of 1ml are injected into small groups of cattle to check that the parasites are still alive and to find the correct dilution for immunization. This is called the "user dilution" of stabilate.

- When the stabilate is to be used for immunization, straws are thawed as before, equilibrated and diluted to the "use dilution". This dilution is usually about one in 10 or one in 20.

TITRATION OF STABILITATES *(Typical results)*

STAGE 1 **VIABILITY** – IN 2 CALVES / DILUTION

1) **1ml neat stabilate**	2) **1ml 1:10 stabilate**	3) **1ml 1:100 stabilate**
↓	↓	↓
Rapid , severe , fatalECF	**Typical , fatal ECF**	**May or may not show signs of ECF**

STAGE 2 **TITRATION** 2 CALVES : STABILATE ONLY
4 CALVES : STABILATE + TLA

Titration : Day 0				**Challenge**
STABILATE DOSE	**TLA**	**NO**	**RESULT**	Day 28, 1ml neat stabilate
1:5	–	2	2 dead	
1:5	+	4	4 reactors (cured)	No reaction
1:10	–	2	2 dead	
1:10	+	4	3 reactors (cured)	1 mild ECF 3 no reaction
1:20	–	2	2 dead	
1:20	+	4	1 reactor (cured)	1 mild ECF 3 no reaction
1:40	–	2	1 dead 1 recovered	
1:40	+	4	no reactors	2 mild ECF 2 no reaction
1:80	–	2	mild ECF	
1:80	+	4	no reactors	2 severe ECF 2 mild ECF

With this result, adilution of 1:20 would be used for field vaccination

Questions on stabilate preparation

Question 1 When preparing a stabilate, how many ticks can be fed on each calf?

Answer _____

Question 2 Which stage of the tick is fed on the calves?

Answer _____

Question 3 After being placed in the incubator, how long do they take to moult to adults?

Answer _____

Question 4 For how long are the adults fed on rabbits?

Answer _____

Question 5 Why are ticks fed on rabbits?

Answer _____

Question 6 How are the ticks washed, and why?

Answer _____

Question 7 What volume is placed in each straw?

Answer _____

Question 8 How are the straws thawed?

Answer _____

Question 9 What is the thawing process called and why is it done this way?

Answer _____

Question 10 How is the "use dilution" of the stabilate calculated?

Answer _____

6.3 Immunisation of cattle

- An immunization campaign requires a lot of planning and preparation. Farmers in the area to be immunized must be contacted and told what the system involves. The number of cattle to be immunised must be determined, stabilate and the necessary drugs, syringes, etc must be obtained and the timing of the whole process must be planned. Only then can immunization begin. Details of this procedure will be discussed in the next section.

- The first step in the process of immunization is to thaw and dilute the stabilate. You will have received the necessary number of straws in liquid nitrogen for your campaign, together with bottles of diluents which each contain sufficient diluents to make the "user dilution" from one straw. Next, you thaw one straw completely by rolling it in your warm hands. You must then cut off the end of the straw with scissors and empty the contents into the diluent. Shake the bottle gently to mix it thoroughly, then allow it to stand for 30 minutes to equilibrate. Your stabilate is then ready to use. Remember, any diluted stabilate that has not been used within one hour must be discarded, so you should plan your visits so as to waste as little as possible.

- The diluents contains nutritive medium to keep the sporozoites alive, glycerol to prevent them being damaged in the thawing process and a 'buffer' to prevent the liquid becoming acid. The diluent is amber in colour when you receive it, but it turns pink when it becomes acidic. Acidic diluent kills the sporozoites, so you must discard any stabilate which become pink. If for any reason the diluents is already pink when you receive it, you must not use it, because acidic medium kills the sporozoites very quickly.

- Because it is important that you can identify the immunised animal during the monitoring phase, you must note its number if it has one, or be absolutely certain that you and the owner can do so. Ideally, you should attach a special "ECFiM" eartag and write an identification number on it. You must then record this number and the animal's owner.

- In the immunization procedure itself, the animal to be immunised is restrained and its temperature is taken. If this is elevated, a brief clinical examination should be carried out and any necessary treatment given. The temperature should be recorded, whether it is elevated or not. You must then decide whether the animal can be immunised. Remember that the immunization process causes some stress, and if you are in any doubt, you should not immunize. If you suspect that the animal is already suffering from ECF, you should confirm your suspicions by taking a lymph node smear and a blood smear for examination. You should not immunize an animal which you suspect has ECF.

- Next you must determine the weight of the animal, so that you can calculate the correct dose of tetracycline to "block" the stabilate. You may feel sufficiently confident to merely estimate the weight, but it is much better to use a weigh-band. Remember, if you under-estimate the weight you will give too little tetracycline and so increase the risk of a clinical reaction to the stabilate while if you over-estimate; you will be wasting expensive tetracycline.

- The calculated dose of tetracycline should now be injected intramuscularly into the gluteal muscels. Remember that the correct dose is 1ml per 10kg body weight. Because there is a risk that, even with a weigh-bank the weight may be underestimated, you should give 10% more tetracycline than the calculated dose. Thus, for a 100kg animal, the calculated dose is 10ml, plus 10% overage, a total of 11ml.

- Next, you inject the stabilate. You should take up 1ml of stabilate into a 1ml syringe and inject it subcutaneously close to any superficial lymph node. Here at VRI, VRC Muguga inoculation is done above one of the prescapular lumph nodes. Other sites preferred include below and infront of the parotid lymph nodes to mimic the predilection site of the vector tick. All animals receive 1ml, regardless of their weight. You should always inject on the same side. This is important in the monitoring process so that you can take all lymph node samples from the same side, which is where the infection is likely to be most easily detected on a smear. The first phase of the ECFiM process is now complete.

- It should be noted that all the inoculation sites for the stabilate (whether pre-scapular, pre-crural or parotid) result into similar levels of immune response and hence the site of immunisation against ECF has no influence on the efficacy of the vaccine.

Steps in ECF immunization and post-immunization monitoring

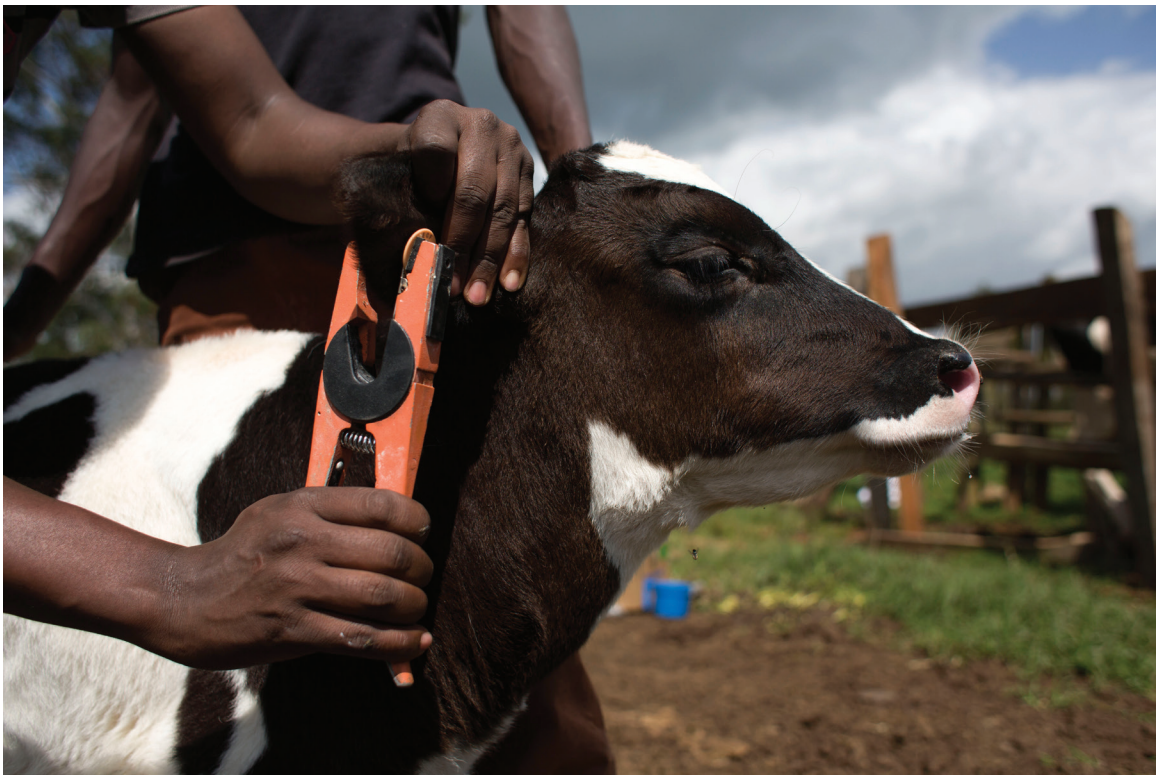
Step 1: Preparation. Make sure that everything is available by having a check list of all the items required for the exercise. These include stabilate, diluent, blocking agent, atropine or adrenaline, needles, syringes, serum tubes, weigh band, ear tags, tagger, thermometers, ice, scissors, cool box, glass slides and note books. The diluent must be kept in a freezer (-20^{0}C). Remove the diluent from the freezer and keep it in a cool box full of ice. The stabilate must be kept in liquid nitrogen until use.

Step 2: Full clinical examination of the animal to be immunized. Do a full clinical examination of the animal before immunization. The following should not be immunized:

a. Animals showing symptoms of ECF
b. Animals with a fever
c. Animals in poor body condition
d. Calves less than one month old
e. Animals in the last month of pregnancy
f. Animals within a farm with an outbreak of viral diseases such as FMD and LSD

Note: It has been observed that animals that have been treated with the anthelmintic Levamisole have a higher risk of becoming reactors following immunization. Avoid immunizing animals that have been treated with this anthelmintic in the last one month if you are able to establish.

Step 3: Ear tag the animal. Ear tag the animal and record the ear tag number.



Step 4: Take the weight of the animal. Estimate the weight of the animal using a weigh band and record the weight.

Step 5: Thaw the diluent. The diluent can be thawed by leaving the bottle at room temperature or in warm water. After thawing the diluent should be kept on ice throughout the immunisation period.

Step 6: Thaw the stabilate. Take out one straw from the liquid nitrogen and thaw it by rolling it between the hands for 1-2 minutes. One straw is used for one diluent bottle. All the doses of the vaccine from one straw must be used within 4 hours after reconstitution. When recruiting the animals to be immunized this fact must always be borne in mind. More than one straw can be used at once as long as all the vaccine is used within 4 hours.

Step 7: Reconstitute the vaccine. Clip the straw with a pair of scissors at the base of the plug on one end of the straw and let the contents flow into the bottle containing the diluent. Mix the contents by gently rolling the bottle.

Step 8: Allow the reconstituted vaccine to equilibrate. Place the bottle on ice for 30 min. for the vaccine to equilibrate. The vaccine is now ready for use. The reconstituted vaccine must be used within four hours.

Step 9: Inject the blocking agent first. First inject the 30% Oxytetracyline-Long acting (OTC-LA) (1ml/10kg) intramuscularly. Determine the dose based on the weight obtained with the weighing band. Calves below 50 kg receive a standard 5ml of the drug.

Step 10: Inject the stabilate. Inject 1ml of the reconstituted vaccine subcutaneously close to the parotid gland or in front of the prescapular lymph node.

Step 11: Observe immunized animals for 20 minutes. Monitor the immunized animal for about 20 minutes for possible allergic reactions. If any animal shows signs of such reactions (skin rush, lachrymation, salivation, swollen eyelids, rapid breathing) treat it with adrenaline, antihistamine or atropine at the manufacturer's recommended dosage. Any vaccine that remains after the last animal has been immunized should be discarded.

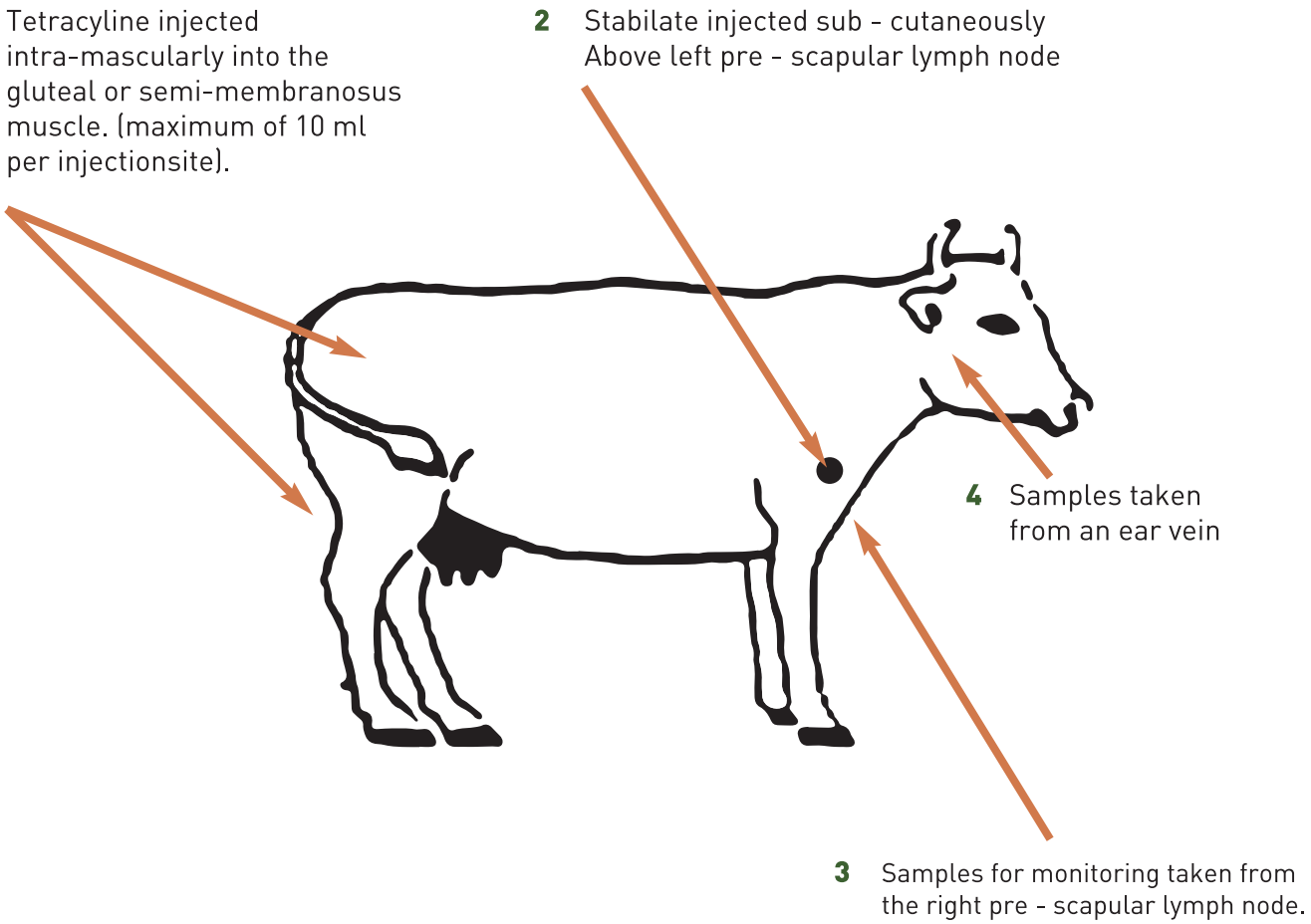
Step 12: Post immunization monitoring. The farmer should be advised to monitor immunized animals closely for one and half months when reactors are expected. A small proportion (←1%) of immunized animals may develop clinical signs of ECF after immunization. Any animal showing signs of ECF for 2 days or more must be treated.

It is important to confirm the diagnosis by taking a blood and lymph node smear.

Step 13: Tick control after immunization. Tick control can be reduced to once every 3 weeks two months after immunization in areas where there are no *Amblyoma* sp ticks. (Note immunization prevents only ECF and not all tick-borne diseases). Reducing tick control allows animals to develop immunity against the other tick-borne diseases. Even if there is a slight increase in the number of cases of the other tick-borne diseases initially, in the long run they will become fewer when calves are allowed to be infected by ticks when they are still young. Reducing tick control allows immunity against ECF to be boosted and removes the need to repeat immunization.

Ticks are parasites in their own right. It is therefore necessary to control them when their numbers increase.

Diagram:Injection and sampling sites



Questions on the immunization process

Question 1 What are the main stages in planning an immunization campaign?

Answer _____

Question 2 For how long must you equilibrate the thawed stabilate before using it?

Answer _____

Question 3 For how long after equilibration can you use a stabilate?

Answer _____

Question 4 Why must you discard any stabilate or diluents which is pink?

Answer _____

Question 5 What must you do if an animal has a high temperature?

Answer _____

Question 6 Give two reasons why accurate estimation of the animal's weight is important.

Answer _____

Question 7 What volume of tetracycline would you give to an animal weighing 70kg?

Answer _____

Question 8 What volume of stabilate would you give to a) a 40kg calf, b) a 400kg cow?

Answer _____

Question 9 Where, and by what route, would you inject stabilate?

Answer _____

Question 10 Where, and by what route, would you inject the tetracycline?

Answer _____

6.4 Monitoring

- You will remember that it is that a small proportion of the animals may develop clinical ECF reactions between 14 and 28 days after you immunize them. This may be because some animals will receive slightly more stabilate than others, and some may have less efficient immune systems. There is a risk that these reactors will die of ECF if they are not treated. The purpose of the monitoring process is to detect reactors as early as possible so that they can be treated. It is therefore important for the farmer to keep you informed of any clinical cases in the immunized animals.

- If you find an animal with a raised temperature (39.50 C or more) you should make a detailed clinical examination for symptoms of ECF or any other disease. No matter what your initial diagnosis, you should take a smear from the prescapular lymph node on the side into which you injected the stabilate. This is the most likely place to find schizonts. You should also take a blood smear. You may not see *Theirleria* piroplasms at this stage, but you may see *Anaplasma* or *Babesia*, and these may need to be treated.

- If you are absolutely certain that the animal is suffering from clinical ECF, you may treat it immediately. Otherwise you should examine the smear in the laboratory and treat only when a positive diagnosis has been made.

- It may not be necessary to treat every animal in which you find schizonts, because some may recover by themselves and to treat them would be an expensive waste. In general, you should only treat a reactor which is showing clinical symptoms of the disease, or when schizonts have been detected in its lymph node smear for more than two days, or both.

- You should treat reactors in exactly the same way that you would treat a field case of ECF because once the disease is established in immunised cattle, they are just as likely to die. When you have treated an animal, you must keep it under observation for at least a week, because it may need a second treatment. You should take a second lymph node smear three or four days after treatment and examine it to confirm that treatment has been successful.

- Do not forget that anaplasmosis and babesiosis can be precipitated by an episode of ECF. You must look for these two parasites on blood smears from reactors, and if necessary, treat these infections too.

Questions on monitoring

Question 1 If you find an animal with a high temperature, what must you do?

Answer _____

Question 2 Which parasites should you look for in the blood smear?

Answer _____

Question 3 You may not need to treat all animals showing signs of an ECF reaction. Why?

Answer _____

_____

Question 4 What must you do after you have treated a reactor?

Answer _____

_____

6.5 Planning an immunization campaign

Preparation

- The ECFiM system is intended to give a service to individual farmers. Strategies for planning an ECFiM immunization campaign therefore differ from those used for e.g. an FMD or rinderpest campaign. With ECFiM you will probably be treating only selected animals on a farm or in an area, not the whole cattle population.

- The first thing you must do is select the area where immunization is to be offered to farmers. This will probably be an area where ECF is common and there is a high proportion of exotic or grade cattle owned by smallholder dairy farmers. You must tell the farmers that you intend to immunize cattle in the area against ECF then visit the area to tell the farmers about the ECFiM system. You will need to open the discussion by talking about ECF, other tick-borne disease, tick control and the farmer's attitudes and experiences. You should try to find out how common ECF is in the area and whether the farmers recognize the disease easily and how sick their cattle actually are when they notice that they are sick. You could then discuss how they can improve their diagnosis and nursing of sick cattle, and advise them to call the vet as early as possible because this will improve the chances of him curing the animal and it is likely to reduce the cost of treatment because fewer doses of drug will be needed. i.e., get a "feel" for the area.

- Only then will you begin to describe and discuss ECFiM. Tell the farmers, in very simple terms about the system and what its advantages are, and what the risks are. You should also explain the need for careful monitoring after immunization has been carried out.

- Do not forget to tell them that they will be charged for the service, and tell them what that charge will be. You must also tell them how the charge will be collected from them. You can expect that, at first, this whole task will be difficult but, with time, farmers in an area will hear about the system from neighbours whose animals you have already immunised. They will already understand some of the benefits and risks, and they will know that they must pay for the immunization.

- Explain that cattle of all ages can be immunized, except calves of less than four weeks.

- So far the system of charging has not been agreed, but by the time you start to immunize in the field, this should be known. There are two main possibilities. Either a single price will be charged for all animals, regardless of size, or bigger animals will be charged more because they require more tetracycline. If the second system is used, you must explain the cost for a range of animal weights, and why immunization of bigger animals will cost more.

- Allow the farmers to ask questions, and give them honest, simple answers.

- When you are sure that the farmers understand what you are offering, make a list of the cattle that they would like you to immunize, who owns them and where they are kept. If possible, arrange for all the cattle in an area to be brought to a central place for immunization. This will simplify the process and allow you to immunize far more cattle in a day.

- You must obtain the agreement of the farmers to immunize their animals and then agree with them when you will immunise their cattle.

The immunization process

- You must now calculate quantities of all materials you will need for the campaign. This includes a liquid nitrogen container, stabilate, diluents (which must not be pink), drugs, syringes and needles, thermometer, weigh-band, slides, etc. You must then obtain all of these supplies, and arrange for transport. You should also take a notebook and pen, so that you can record all the details of the animals and any other useful information. Don't forget to take a rope, and don't forget the list of animals to be immunized!

- You can always expect that more animals will be presented for immunization on the day than you were told about, so always take a bit extra of everything.

- Check carefully that you have all the equipment that you will need before you set out. You should prepare a check – list to help you in this. There is nothing more embarrassing and irritating than to arrive at a farm to find that you left your thermometer behind. On the next page you will find a suggested checklist, which you may find helpful.

- Keep all your equipment tidily in a box. This not only looks more professional, but it reduces the risk of leaving anything behind on the farm. You are now ready to begin your day's immunization.

- When you arrive at the immunization site, talk to each owner to ensure that they remember what you told them before, check that the right animals are being presented and that the farmers still agrees that you should immunize his cattle.

- You must explain again the need to keep immunized cattle under observation for any reactions. They must continue tick control until the animal is immune, about five weeks after you immunise it, because cattle can become infected with a "field" strain during the time that they are developing immunity.

- It is most important that you remind them of the need for monitoring for reactors from this day 14 until at least day 28 after immunization. You must make clear arrangements for this to be done and arrange who will do it. If you are to do it yourself, then there should be no problems. However, if someone else is to do the monitoring, you must be sure that they get the smears to you and that you examine them immediately. You must then go to the sick animal and treat it as soon as possible. Remember that a farmer it as soon as possible. Remember that a farmer is likely to be more upset if his animal dies because of an immunization reaction than from a naturally acquired case of ECF.

- Sometime you will find an animal with clinical symptoms of ECF when you start to monitor on day 14. This animal is almost certainly suffering from a natural ECF infection which it contracted either before or just after immunization. You should treat this case. Immunized cattle almost never develop clinical symptoms before day 14.

- If an animal does die despite treatment, make every effort to diagnose why. Carry out a post mortem examination if possible, and take smears of lymph nodes and blood for examination in the laboratory. If you see symptoms of any other disease, take samples to help your diagnosis. Remember, the farmer will want to know why his animal died, and it will help us in improving the ECFiM system if you can give us a full report of any failures.

Questions on planning an ECFiM campaign

Question 1 How does an ECFiM campaign differ from one for FMD or rinderpest?

Answer _____

Question 2 Why must you visit the campaign area before you intend to immunize there?

Answer _____

Question 3 What are the most important things you must tell the farmers at your meetings?

Answer _____

Question 4 What ages of cattle cannot be immunized?

Answer _____

Question 5 Why might the charge for immunizing a cow be more than for a calf?

Answer _____

Question 6 If you have 50 cattle for immunization, average weight 70kg, a) how much tetracycline should you take? b) How many doses of stabilate should you take?

Answer _____

Question 7 If an animal dies despite treatment, what should you do?

Answer _____

Question 8 Give two reasons why you should keep your equipment tidy.

Answer _____

Question 9 For how long after immunization should you advise the farmer to maintain strict tick control, and why?

Answer _____

Question 10 For how long after immunization should you advise the farmer to maintain strict tick control, and why?

Answer _____

Check-list of equipment

1. Liquid nitrogen container, containing straws of stabilate.
   Cool box, containing diluents and melting ice.
   Beaker or small lunch box, to maintain equilibrated stabilate in melting ice.

2. Equipment box:-

Weigh-bank (1)

Ropes (2)

Ear tags, applicator and pen

Thermometer (2)

Syringes:- 1ml (5)
10ml (10)
20ml (10)

Hypodermic needles:-

18G – for injecting stabilate and taking lymph node and blood samples (18G could be used equally well)

16G – useful for injecting big or wild cattle.

Calculate the number of each size of needle required, remembering that a new needle must be used for each animal treated, and for each sample taken.

Microscope slides (1 box)

Spreaders

Diamond or wax pencil (for making slides)

Long-acting tetracycline injection (take 50% more than your calculated needs)

Treatment for clinical ECF (Butalex, Clexon or Terit)

Other drugs, e.g. Imizol, pen-strep inject, antihistamine, Catasol, etc. to treat any clinical diseases that you may diagnose. This is not strictly part of the immunization procedure, but it is good PR.
Vacutainer tubes, needles and holders (if samples are requested by VRI, VRC Muguga)
Note-book (which should contain instructions for immunization and monitoring)

Immunisation record sheets

Pens and pencils.

Sticky labels to identify samples

Box or tray to store prepared slides.

Muslim gauze (for polishing slides before use)

Scissors (to cut stabilate straws, and for general use)

Cotton wool and spirit (to swab injection and sampling sites)

Sealable box (to store used needles, etc.)

Box to store used syringes, etc.

ECFiM Immunisation Record Sheet

1. Identification data

Farmer's Name _____

Farmer code _____

Farm location _____

Date immunized _____ No. immunized _____

Stabilate used (identification no. and dose) _____

Monitoring dates _____

Grazing system _____

2. Animal data

Animal No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Weight (kg)										
Temperature										
TLA dose (ml)										
Breed										
Sex										
Age										
Reaction										
Treatment										
Result										
Comments:										

7. Practical Work

- The main purpose of this training on the ECFiM system is for you to learn how to operate the system successfully and efficiently. It is essential that you fully understand the way in which it works, and why you must do everything very carefully. Sections 1 to 6 are intended to give you this basic knowledge. Section 7 shows you how to do it.

- We now come to the practical side of the Training Course. It will consist of three phases. Firstly, you will learn how to take samples from cattle, and how to examine and interpret them. Next, you will learn the actual techniques of immunization and monitoring then you will be trained in how to present the system to the farmer.

- The techniques may be very similar to one you already use, every day. However, because it is most important that the ECFiM system is carried out precisely according to our instructions every time, we will show you all the techniques you will use. You will probably see some important differences from the way you do things in other aspects of your job.

- You will also be show the stages in preparation of a stabilate, and various other specialized techniques which are carried out at VRI, VRC Muguga, but you will not need to learn these yourselves.

7.1. Taking samples and making smears.

7. 1.1 General

- A good smear, whether of blood or lymph, is essential if you are to detect infections easily, accurately and as quickly as possible.

- The two most important points in making good smears are to have clean slides and to have a good spreader. Slides taken straight from the box are likely to be greasy and dusty. They must thoroughly polished with a piece of clean cotton cloth before use. The spreader is made from a clean slide by breaking off one corner so that the end used to spread the film is about 80% of the width of the slide, you ensure that you will have two clear edges to the smear, away from the edges of the slide.

- When making you smear, put the spot of blood, or lymph about one third away from one end. This leaves you enough clear space to write on the slide. Next, hold the slide on a firm surface and place the spreader in front of the sample and move it back until it just touches it. Wait briefly for the sample to spread across the whole width of the spreader, then move the spreader slowly along the slide. The more slowly you move the spreader, the thinner and more even the smear will be. A good slide has a single layer of cells all over it, and it is very easy to read. Wave the slide in the air to dry it quickly. This will avoid distortion of the cells. Do not simply leave in the sun to dry as this may also distort the cells and flies are likely to feed on it, making it difficult to read. In very humid weather, dry the slide by warming it on the inside of your wrist.

- Do not "fix" slides in the field. There are two reasons for this. Firstly, it is essential that fixing is done with alcohol that is completely free of water. A bottle of alcohol in the field will be opened many times and will take up water from the atmosphere. Poorly fixed slides are difficult to read because they do not take up the stain well. Secondly, fixed smears deteriorate more quickly than unfixed ones and soon become difficult to stain well. A dried, unfixed smear will stain well when fixed and stained more than a year later. A fixed smear may stain poorly after only a few days.

7.1.2 Blood smears

- Blood smears should be taken from a vein on the back of the ear. We prefer to take blood from the ear rather than from the tip of the tail because ear blood is always circulating whereas tail blood may be "sludged", that is, it may have settled there because of poor circulation, and so may not be typical of the general circulation.

- With the animal restrained, take hold of an ear and examine the back for the location of a vein. Grip the ear beyond the place you are going to take blood from son that you stop the flow of blood. Prick the vein with a hypodermic needle so that blood comes out as a small droplet. Pick up this droplet with your spreader, and make your smear immediately. This is preferable to taking the droplet up on the needle for two main reasons. Firstly, it is quicker and more direct, so there is less chance of the blood partly drying before you make your smear and secondly, there is less risk of the droplet spreading away through the hair by capillary attraction. A smaller drop can be picked up with the spreader than with the side of a needle.

- Sometimes it is difficult to get blood from an ear vein. If so, take a smear from the tail, but note on the slide that it is tail blood.

7.1.3 Lymph node smear

- Lymph node smears are made from biopsy material taken from a lymph node with a hypodermic needle. Once you have taken your sample, the smear is prepared exactly as for a blood smear.

- Lymph nodes of an animal with ECF are usually greatly enlarged and taking a sample from them is usually far easier than from a small, normal node. However, sometimes the enlarged node is very oedematous (contains fluid) which makes it difficult to obtain good lymph.

- The easiest node from which to take a sample is the prescapular. It is large and can usually be gripped fairly easily. The parotid node, below the ear, is often sampled, and is easy when enlarged, but more difficult when of normal size. It may be important to sample this node in field cases because it drains the main site of attachment of the brown ear tick. So it is the place you are most likely to find schizonts.

- Grip the node firmly so that the skin is tight above it. Then insert an 18-gauge needle into it. Lymph will sometimes flow into the needle with no further help, but often you will need to move the needle a bit while at the same time covering and uncovering the top of the needle with the thumb to create suction pressure. When withdrawing the needle, the top of the needle should be covered with the thumb to prevent the material in the needle from flowing out. Uncover the top of the needle to let the biopsy material to run onto the glass slide. Do not "stir up" the node. This will simply make it haemorrhage so that you do not get a good sample of lymph. You will get a blood sample instead.

  It will also make it more difficult next time you sample the node and can cause a focus for bacterial infection. When you think you have some lymph in the needle, withdraw it and expel the lymph onto the slide either by tapping the end of the needle on the slide, or by blowing down it. Make the smear exactly as for a blood smear. If there is no lymph in the needle, try one more time, but do not keep on trying to sample the same node. Try the one on the other side of the animal, if necessary. It is better to fail to get a good lymph slide for one day than to destroy the node by repeated efforts to obtain a sample.

7.1.4 Giemsa staining: Thin blood smears (or lymph smears)

Preparation of Merk's Giemsa stain

Reagents

- Glycerol (Analar) 540 mls, Methanol 840 mls Merk's Giemsa powder 10g Azur II 0.2g/100mls=2.75 g.

Method

- Grind the Giemsa powder in a large glass mortar with some glycerol.
- Pour into a large flat-bottomed flask.
- Wash the mortar with the rest of glycerol and pour into the flask.
- Leave flask in water bath @ 60oC for 1 hr.

- Cool to room temperature.
- Add 840 mls methanol and shake well.
- Put on stirrer overnight.
- Add Azur II.
- Stir overnight. Filter (Whatmans No 4 filter paper) and store in a dark bottle.
- This is the stock Giemsa stain.

Giemsa buffer

- Giemsa buffer tablets GURR 65500.
- 1 tablet per 1 litre of distilled de-ionized water.
- Use the buffer to dilute the stock stain.

Staining

- Fix the smears in absolute methanol for 1-2 minutes.
- Stain with 5% Giemsa stain in a staining jar for 40 minutes (10% for 20 minutes, 20% for10 minutes).
- Remove slides from jar and rinse with the Giemsa buffer or tap water.
- Place the slides on a drying rack to dry.
- Observe the smears under a microscope.

7.1.5 Taking a blood sample

- You will not normally need to take blood samples for your part in the ECFiM system, but VRI, VRC Muguga may ask you to take them for their research purposes. This is likely to be either for serology or for isolation of parasites into culture or for freezing as blood stabilates, or we may need to do haematological studies on it, for example counting the number or red or white blood cells.

- Blood is taken from the jugular vein, using a vaccutainer. You will only be trained in this technique if there is a strong likelihood that you will be asked to take samples for us. In any case, instructions are printed on the vacutaner box.

- There are two types of vacutainer commonly used at VRI, VRC Muguga. One contains an anticoagulant, to prevent the blood clotting. This is used when we want to isolate or preserve parasites, or conduct haematological tests.

- The second type contains no anticoagulant. When blood is taken into this tube it clots and after a few hours the clot "retracts" and the serum separates from it. We use the serum to conduct serological tests to detect antibody to the parasites.

7.1.6 Taking a brain smear

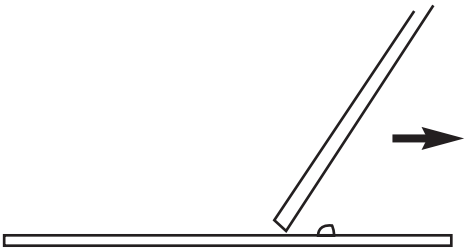
- We sometimes need to take a brain smear to look for *Cowdria*, the causative organism of heartwater. We use two techniques for this. At *post mortem* examination, we cut the skin over the skull, then split the skull open to take a smear from the cerebral cortex. Alternatively we can drill a small hole in the skull and withdraw the sample with a syringe. Occasionally we take a brain sample from a live animal by this second method (after injecting a local anaesthetic). These techniques will be demonstrated to you if a suitable animal is available. We will also demonstrate the specialized techniques form making a brain smear.

MAKING SMEARS

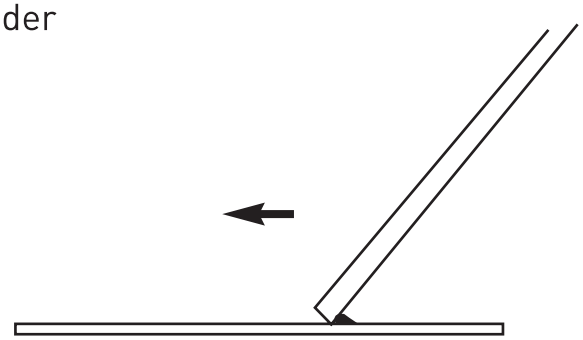
1. Clean slides
2. Take sample
3. Put small drop on slide

4. Spreader

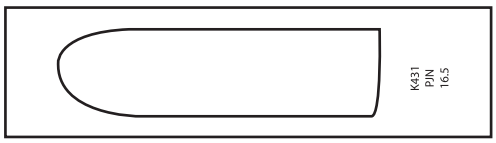
5. Place slide on firm surface
6. Bring spreader up to drop



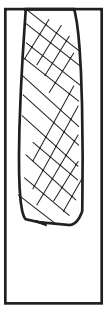
7. Wait for sample to flow across spreader



8. If sample is too big, discard some
9. Move spreader slowly and smoothly along slide at 45 degrees
10. Dry smear by waving in air, then label it
11. Good smear - thin, even and with clear edges

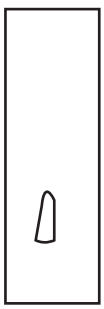


12. Bad smears:-



Sample too big

Spreader moved too fast



Sample too small

Questions on taking samples and making smears

Question 1 Why should you use a "spreader" when making "smears"

Answer _____

Question 2 Describe a "good" smear

Answer _____

Question 3 Give two reasons why a smear should be dried quickly.

Answer _____

Question 4 Why should you not "fix" slides in the field?

Answer _____

Question 5 Where is it best to take a blood sample from to make a smear?

Answer _____

Question 6 Why might it be best to take a lymph smear from the prescapular node of an ECFiM reactor, but from the parotid (ear) node of a field case of ECF?

Answer _____

Question 7 Give two reasons why you should not "stir up" a "difficult" lymph node.

Answer _____

Question 8 Describe the two types of vacutainer you may be asked to use.

Answer _____

Question 9 For what would VRI, VRC Muguga use the blood samples from the two types of vacutainer?

Answer _____

Question 10 Why might you take a brain smear?

Answer _____

7.2 The ECFiM System

- This is the most important part of the whole training course. You will be shown all the methods we use at VRI, VRC Muguga to prepare stabilates, test samples and, of course, how we immunise and monitor the cattle. This is the real practical part of the programme. You will be asked to do each of the procedures that you will use, and we will help you to do these properly. You will meet our veterinary, scientific, technical and support staff, who routinely perform these tasks at VRI, VRC Muguga. Get to know these people so that you can contact them in future if you need further help.

- Some of the techniques will simply be demonstrated to you. You do not need to be able to do them yourselves, but a knowledge of how they are done will help you to understand the ECFiM system. You may like to take your own notes on these techniques. They include:

- Preparation of stabilates – You will be shown all the stages in the process from attachment of ticks to cattle to final freezing of the stabilate.

- Serology – You will see how we test serum samples for the presence of antibody to the parasites.

- Cell culture – We will show you how we grow parasites in culture, and some of things we use these cultures for.

- Haematology Laboratory – You will see how we stain smears, count red and white blood cells, measure packed cell volume etc.

- Tick Laboratory – Here you will see how we culture, identify and examine ticks for the presence of parasites, and we will show you what the stages of *Theileria* in the ticks look like.

- Next we will demonstrate the techniques you will need to be able to perform in order to operate the ECFiM system in the field.

They include:-

- Taking blood and lymph samples and making smears.
- Using vacutainers.
- Making a clinical examination of an animal for all four tick-borne diseases.
- Staining slides.
- Examining slides under the microscope, identification of each of the para-sites you may see, and the different stages in the life-cycle of each of them.
- Thawing, diluting and equilibrating stabilate.
- Injection of stabilate and tetracycline.
- What to look for, and what to do in the monitoring process.
- Treatment of ECF and the other tick-borne diseases.
- Record keeping – an essential part of the system.

7.3 Explaining the ECFiM system to farmers

- Explaining the ECFiM system to farmers is a very important part of the programme. It is essential that they understand what the system can do, what they must do to obtain the greatest benefit from it, and what it will not do. They must also be told what it will cost, and how this cost is made up. You will be able to operate the system best, within a minimum of problems, if you explain it well to the farmers. Remember that the farmer is operating a small business and that he is an expert on this subject, just as you are an expert on immunization. Treat him with respect, because he has a lot of farming experience and he is very familiar with ECF.

- You will be asked to prepare the presentation you would give at a farmer's briefing and then use it in front of a group of VRI, VRC Muguga staff, who will pretend to be farmers. (Some of them will be farmers). They will ask you questions that they think the farmers you will meet are likely to ask, and you must handle these questions. The whole exercise is designed to help you to plan a good immunization campaign. The audience will be asked to make the meeting as realistic as possible, so you can expect some difficult questions and some surprises. However, there will be no tricks, and the role of the audience is to help you, not to criticize you. Please prepare yourself carefully for this exercise. It is very important.

7.4 Socio-economic aspects of ECFiM

- An important part of the work at VRI, VRC Muguga is to assess the socio-economic impact of ECFiM. We need to know, accurately, what the farmers think of the system, what they see as its benefits and difficulties, and we need to measure the economic impact of the system. This allows us to make the system more user-friendly, more effective and sustainable. The socio-economic team will probably want to meet you so that they can arrange to monitor the progress, success and benefits of the ECFiM system in your area, with your help.

- You will be given a short talk on the work and findings of the socio-economic team, followed by a discussion of the whole topic.

7.5 Professional code of ethics

The veterinary profession is a noble profession. Humanity over the ages and all over the world has bestowed on the veterinary profession the obligation and responsibility to protect and promote the health and welfare of all animal species, whether in the home or in the wild. Humanity uses animals as part of its sources of wealth, income, food, draught power, companionship, security and recreation. In Kenya, animals in the wild make a notable contribution to the economy through tourism. Inedible animal products are used by man in the areas of clothing, soil fertilization and in the manufacturing of other valuable commodities. Animals are a major component of biodiversity and their protection and welfare ensures its continuity, which in turn guarantees human survival.

The veterinary profession is obligated to apply its stock of scientific knowledge and principles to protect and assure the health of humans in their interaction with animals and in their consumption and utilization of animal products. The veterinary profession plays its noble role in assuring the health of animals, humans and the environment, which are all interdependent, through application of the principle of "One Health".

The Code of Ethics is the written means by which the conduct of veterinary surgeons and veterinary paraprofessionals, in maintaining the dignity and high calling of the veterinary profession, is measured and regulated. This Code calls on veterinary professionals and paraprofessionals to maintain professional standards and portray themselves in a manner and appearance that projects a professional image to the general public. This Code contains disciplinary measures in case of breach, and provides an avenue for complaints from members of the public. The Code is in addition to any other national law relevant to veterinary surgeons and veterinary paraprofessionals, the World Organization for Animal Health standards, guidelines and recommendations and the International Health Regulations.

Veterinary surgeons and veterinary paraprofessionals fulfill their professional responsibilities by acknowledging, observing and maintaining the following five tenets of conduct and practice which are elaborated in the Code:

1. Professional competence
2. Honesty, dignity and integrity
3. Independence and impartiality
4. Client confidentiality and trust
5. Professional accountability

It is important that ECF vaccinators familiarize and adhere to the code of ethics in order to uphold professionalism

7.6 Closing discussions

- We hope that you will enjoy this course, and that you will feel that it has been useful. You will not be part of the ECFiM team. No doubt there will still be things that you would like us to clarify, and this final session will give you the opportunity to raise them. The discussion will cover all aspects of the training course, and situations you are likely to meet in the field, when you are operating the system. This is your opportunity to discuss all of these issues before you return them home to use the ECFiM system yourself.

You are a trained ECFiM operator! You must now go into the field and apply what you have learned. You will find that there is much more to learn as you use the system, and you will continue to improve your technique. Remember that the team at VRI, VRC Muguga is available to help you at all times, so contact us if you need our help or advice.

GOOD LUCK AND WELCOME TO THE WAR AGAINST ECF
YOU ARE NOW A TRAINED MEMBER OF TEAM ECFIM!

Suggestions For Further Reading

1. Progress towards the control of East Coast fever (theileriosis) in Kenya. (1990). Published by KARI, Editors:- A.S. Young, J. J. Mutugi, and A. C. Maritim.
This is probably the most useful reference of all. It comprises a series of papers by the staff of VRI, VRC Muguga and others, and reviews all aspects of ECF immunization, epidemiology and tick control. It is available from VRI, VRC Muguga.

2. The epidemiology of theileriosis in Africa. (1992). Norval, R. A. I., Perry, B. D. and Young, A. S. Published by Academic Press.
This book is more advanced, and covers all aspects of control, epidemiology and economics of theirleriosis.

3. Babo Martin,S., Di Guilio,G., Lynen,G., Peters,A. and Rushton, J. (2010). Assessing the impact of East CoastFever immunization by the infection and treatment method in Tanzania pastoralistsystems. *Preventive Veterinary Medicine*, 97: 175 – 182.

4. De Castro, J. J., Young, A. S., Dransfield, R. D. Cunnignham, M. P. and Dolan, T. T. (1985). Effects of tick infestation on Boran (Bos indicus) cattle immunised against theileriosis in an endemic are of Kenya. *Research in Veterinary Science* 39, 279-288.

5. Dolan, T. T., Injairu, R., Gisemba, F., Maina, J. N., Mbadi, G. Mbwiria, S. K., Mulela, G.H.M. and Othieno, D. A. O. (1992). A clinical trail of buparvaquone in the treatment of East Coast Fever. *The Veterinary Record* 130, 536-538.

6. Gachohi,J., Skilton, R., Hansen, R., Ngumi,P and Kitala, P. (2012). Epidemiology of East Coast fever (*T.parva* infection) in Kenya:Past,Presentandthefuture. *Parasitesand vectors,* 5:194.

7. Irvin, A. D. (1987). Characterisation of species and strains of *Theileria. Advances in Parasitology* 26, 146-197.

8. Kariuki, D.P.K. (1988). Current status of theileriosis immunization by infection and treatment in Kenya. *Kenya Veterinarian* 12, 2-5.

9. Kariuki, D.P.K., Young, A. S., Morzaria, S. P., Lesan, A. C., Wafula, J. and Molyneux, D. H. (1994). Theileria parva carrier state in naturally infected and artificially immunised cattle. Tropical *Animal Health and Production* (in press).

10. Maritim, A. C., Young, A. S., Lesan, A. C., Ndungu, S. G., Mutugi, J. J. and Stagg, D. A. (1989). *Theileria* parasites isolated from carrier cattle after immunization with *Theileria parva* by infection and treatment. *Parasitology* 99, 139-147.

11. McHardy, N., Wekesa., L. S., Hudson, A. T. and Randall, A. W. (1985). Antitheilerial activity of BW720C (buparvaquone) : a comparison with parvaquone. *Research in Veterinary Science* 39, 29-33.

12. Morzaria, S. P. and Nene, V. (1990). Bovine theileriosis: Progress in immunization methods. *International Journal of Animal Sciences* 5, 1-14.

13. Mutugi, J. J., Young, A. S., Maritim, A. C., Ndungu, S. G., Mining, S. K., Linyonyi, A., Ngumi, P. N., Leitch, B.L., Morzaria, S. P. and Dolan, T. T. (1989). Immunisation of cattle against theileriosis in Coast Province, Kenya. *Research in Veterinary Science* 47, 170-179.

14. Ochanda, H. Young, A. S., Mutugi, J. J., Mumo, J. and Omwoyo, P. L. (1988). Effect of temperature on the rate of transmission of *Theileria parva parva* infection by its tick vector, Rhipicephalus appendiculatus. Parasitology 97, 239-245.

15. Pegram, R. G., Tatchell, R. J., de Castro, J. J., Chizyuka, H. G. B., Creek, M. J., Moran, M. C. and Nigarura, G. (1993). Tick control: new concepts. *World Animal Review* 74/75, 1-14.

16. Rumberia, R. M., Eley, R. M., Young, A. S., Rowland, A. C. and Watson, E. D. (1993). The effect of high and low Theileria parva infection on the reproductive function of Boran/Friesian cross heifers. *Theriogenology* 40, 977-986.

17. Rumberia, R. M., Rowland, A. C., Watson, E. D., Eley, R. M., and Young, A. S. (1993). The effect of immunization against theileriosis on the reproductive function of Boran/Friesian cross heifers. *British Veterinary Journal* 150.

18. Spooner, P.B. (1990). The effect of oxytetracycline on *Theileria parva in vitro.* Parasitology 100, 11-17

19. Young, A. S., Groocock, C. M. and Kariuki, D. P. (1988). Integrated control of ticks and tick-borne diseases of cattle in Africa. *Parasitology* 96, 403-432.

20. Young, A. S., Leitch, B. L., Dolan, T. T., Mbogo, S. K., Ndungu, S. G. and de Castro, J. J. (1990). Evaluation of infection and treatment methods of immunization of improved cattle against theileriosis in an endemic area of Kenya. *Veterinary Parasitology* 35, 239-257.

21. Wanjohi, J.M., Rumberia, R.M. and Kamau, M.M. (1995). Different stabilate inoculation sites in East Coast fever immunization. In: Annual Scientific Conference, NVRC. 6-8th December 1995. Pp 41-43. Eds S.W.Wanyangu, D.P. Kariuki and S.K. Mbogo.

22. Wesonga, F.D., Ndungu, S., Muraguri, G., Rumberia, R., Ngeranwa, J.J., Mbgo, S.K. and Kariuki, D.P.(2000). Strategic tick control following immunization of cattle against East Coast fever in the central Rift Valley of Kenya. *Bulletin of Animals Health and Production in Africa* 48, 63-70

23. Wesonga,F.D (2013). Assessment of major livestock diseases and associated production constraints in a smallholder production system in Machakos County, Kenya pp 148 – 170. PhD Thesis,University of Nairobi, Kenya.

24. Wanjohi, J.M., Ngeranwa, J.N., Rumberia, R.M., Muraguri, G.R. and Mbogo, S.K. (2001). Immunisation of cattle against East Coast fever using Theileria parva (Marikebuni) and relaxation of tick control in the North Rift, Kenya. *Onderstepoort Journal of Veterinary Research* 68, 217-223.

25. Ndungu, S.G., Ngumi, P.N., Mbogo, S.K., Dolan, T.T., Mutugi, J.J. and Young, A.S. (2005). Some preliminary observations on the susceptibility and resistance of different cattle breeds to Theileria parva infection. *Onderstepoort Journal of Veterinary Research* 72, 7-11

26. Ndungu, S.G., Brown, C.G.D. and Dolan, T.T. (2005). In vivo comparison of susceptibility and disease resistance between Bos indicus and Bos Taurus cattle types to Theileria parva Muguga infection. *Onderstepoort Journal of Veterinary Research* 72, 13-22.

Veterinary Reasearch Institute, Muguga North
P.O Box 32 - 00902 Kikuyu,
Tel: 020 - 2524616
Email: Director.vri@kalro.org